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BOOK OF PROCEEDINGS

IX International Scientific Agriculture Symposium
"Agrosym 2018"
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PREFACE

A Word from the Editor-in-Chief

Dear colleagues,

In your hands are the Proceedings of the 9th International Scientific Agricultural Symposium "AGROSYM 2018" held on 4-7 October 2018 in Jahorina, Bosnia and Herzegovina. The Symposium gathers about 1200 participants from 85 different countries and organizers received over 1200 abstracts/full papers. Symposium themes covered all branches of agriculture and were divided into seven sessions: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environmental protection and natural resources management, 5) Animal husbandry 6) Forestry and Agro-forestry, and 7) Rural Development and Agro-economy.

In the plenary lectures was presented the importance of new information and communication technologies for agriculture in the 21st century and biological protection in plant production. Furthermore, a particular attention was devoted to avoiding knowledge waste through networking and partnership.

Agriculture has a complex relationship with natural resources and the environment, thus attributing specific environmental effects to agriculture is difficult and not fully understood. Today, it is obvious that conventional methods of agricultural production, in addition to providing sufficient food and other products, have led to a number of negative impacts, including direct or indirect effects on human health. Excessive use of agrochemicals can cause various disorders in the biological equilibrium of agroecosystems and beyond. These negative impacts raise serious questions about long-term sustainability of high-input agriculture. Measures to protect soil and water in agriculture include comprehensive and complex undertakings and pre-planned measures. These problems are a constant reason for 'popularisation' of all ecological trends in agriculture (e.g. organic agriculture, permaculture, biodynamic agriculture, conservation agriculture, regenerative agriculture, integrated farming, agroecology, etc.). Meanwhile, there are also calls for a genuine, deep transformation of agro-food systems that goes beyond 'ecologisation' of agricultural production. All these developments in agricultural research field, as well their implications on farmers' fields, were discussed during the 4 days of AGROSYM 2018.

All papers included in the Proceedings were peer-reviewed. Full texts of the accepted contributions are available in electronic form on AGROSYM website (http://agrosym.unssa.rs.ba).

I hope that the Proceedings will be useful to many agriculturalists and to those engaged in related fields and enable better collaboration of scientists, researchers and producers.

Many thanks to all the authors, reviewers, session moderators and colleagues for their help in editing the Proceedings "AGROSYM 2018". Special thanks go to all co-organizers for their unselfish collaboration and comprehensive support.

East Sarajevo, 07th October 2018

Prof. Dušan Kovačević, Editor-in-Chief

CONTENT

PLANT PRODUCTION

STUDY ABOUT SOME ECOLOGICAL ASPECTS OF GRAIN OAT CULTIVARS (Avena sativa L.) IN ALBANIAN CONDITIONS

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Abstract

Concentrate oat is a very important such as forage crop for animals and food for people. It is a plant with a very wide spreading range and with a high degree of eligibility to ecological conditions. Oat is a crop with high protein content, carbohydrates, fats, vitamins and other mineral elements, which make it a high-value biological food especially for animals but also for humans. It compose the food base for many ruminant animals as there is a balanced content of the main nurient elements. But recently it is being successfully used as human food by replacing other cereals and this has actually become a tradition as it is a healthy, balanced, and high-value biological food. The oat represent a high agronomic performance compare to many other cereals till it is a plant that plays an important role in cereals cultivation technology, such as plant rotation, plant mixtures and as the main single crop cultivation. It is a resistant plant to biotic and abiotic stresses and as such exhibits good adaptability to climate and soil conditions and to climate change. Today when society has become more vulnerable to environmental problems, a change in the situation of the agriculture sector and sustainability is more than required. An important factor in actual situation is the change in the structure of cultivars. But despite the importance of this crop, the collection of cultivars in production in Albania is relatively poor, although a large number of hybrids with high production potential have entered from other countries. The objective of this study was to compare differnet oats cultivars as "Këmishtaj" and "Lushnja x Kamza", native cultivars and some other cultivars coming from some Western countries as "Mansholt I", "Makte Haver Oversalt", "Torpan" and "Bendo" (Holland); "Abed Minor" (Denmark) and "Argus" (Sweden), which are widespread in production in these countries and have been successful in various environmental conditions for their biomorphological aspects, food quality, production levels, adaptation abillity, resistance and resilience. This study evidence that the "Këmishtaj" cultivar has yielded an average of 2,55 t/ha^{-1}, the "Torpan" cultivar 2,5 t/ha^{-1} and "Argus" and "Abed Minor" yielded respectively 2,3 t/ha^{-1} and 2,1 t/ha^{-1}, which shows that they are well adapted to the conditions of Albania and can be successfully used in the production.

Keywords: *oat (Avena Sativa L.), cultivars, productivity, hybride, adaptation*

Introduction

Oat *(Avena Sativa L.)* is a crop that is part of cereals group even it is cultivated cultivated less than the others. It is a herbaceous plant belonging to the graminaceae family. From the large number of its species in cultivation there are found only two species *Avena Sativa L.* and *Avena Byzantina L*. (Suttie et al., 2004). Te oat is cultivated at ancious time. It is cultivated in Egypt and in Europe. According to De Candolle (1883) the cultivated cultivated originated from Europe. Vavilov had found many varieties of *A. Sativa* in Mongolia and in China (Vavilov 1920-1940).

Oat is widely cultivated all over the world, even though there has been a decrease in the cultivated area and its production over the years. The main production country's is the EU with 7,999 thousand tons followed by Russia with 4,027 tons and then Canada 2,812 tons

compare to 21,391 thousand tons of total worlds production (USDA, United States Department of Agriculture, 2013). Researchers have identified the oat as a healthy diet crop for humans and animals (McDonald et al., 1992). It is cultivated mainly as forage crop for cattle nutrition which is used as concentrated food for animals or as a biomass for direct grazing (Suttie et al., 2004) since it is considered as high-quality food for livestock animals (Givens and Brunnen, 1987).

During recently years are the society is changed nutritional behaviors and the oat has tremendously increased for widely used as a dietary diet for human, thanks to the balanced content of its elements. It is highly valued for its nutritional values in human nutrition (Redaelli et al., 2005).

Oat is rich in protein (15-24%), fats (9%), fibber (12%) and carbohydrates. It contains many mineral salts such as calcium, potassium, magnesium and iron. This makes it successfully used as food for people in order to regulate their metabolic processes. (Bellato, 2013). It is considered as a food with potential free gluten content and is used in the diet of some people (Sadiq Butt et al, 2008; Koskinen et al., 2009). It is used in food processing products such as flakes, biscuits, breads and extracts of oils used in the pharmaceutical and cosmetic industries (Valentine, 1990, 1995). Oat it is used also in distillation of whiskey (Viggiani & Pezzi, 2002).

In cultivation technologies, it can be cultivated as a simple culture or in combination with other crops. For this reason in the structure of forage crops for fodder or green mass production it occupies an important place especially in the agricultural crop circulation. This is due to the fact that in comparison with other cereals it has a great mass increase in its abilities (Baldoni et al., 2000).

In recent years it is ncreased the demand for the qualitative oat seeds production based on the increase of oat cultivation surfaces. Also the demand from farmers for new cultivars with high grain quality and biomass production has increased extremly. This demand can be achieved through genetic improvement or selection, although recently on genetic improvement programs it is working more to get higher amounts of protein (Peltonen et al., 2004).

In account to the large number of cultivars used today in the world, such as *"Mansholt I", "Nakte Haver", "Overasalt", "Torpan", "Bendo", "Abed Minor"* and *"Argus"* etc. in Italy, *"Argentina", "Alcudia", "Genziana", "Primula Bioda", "Corneil", "Donata", "Fulvia", "Hamel", "Tenebra", "Teo DB40"* (Redaelli et al., 1999; 2013). The oat varietal structure in Albania is poor, since its cultivation are is not large and the *"Këmishtaj"* and *"LxK"* cultivars are the main ones.

In recent years have been introduced hybrid cultivars from overseas which have been studied in advance in the collection trails for different indicators and in particular those of production. Hybrid cultivators produce more, but they do not have the characteristics of traditional cultivars such as nutrient content, resistance to pests and diseases, especially for climatic fluctuations.

Based on some features and indicators, some cultivars have been distinguished from their testing in collection trails because they have interesting indicators for production as well as they are high productive and are resistant to crashes.

These new cultivars for our country, before being recommended for spreading in production, must be subjected to competitive trails (putting them into evidence of zoning comparison). From the preliminary work in the collection trails, the following cultivars have been distinguished: "*Mansholt I"*, "*Nakte Haver",* "*Overasalt"*, "*Torpan"* and *"Bendo"* (Netherlands), "*Abed Minor"* (Denmark) and "*Argus"* (Sweden) which have been put together in comparative trail together with two cultivars made in Albania, "*Kemishtaj"* and "*LxK"*. In the comparative trail, two other cultivars have been added, one produced in the country ("*MVS"* 2008), which is spreaded and is cultivated in the southern area of Albania

and the other from Macedonia ("*MMS"*, 2008). These two cultivars are not studied before for the conditions of the Albanian coastal area.
The main purpose of this research has been the testing of cultivars genetically differencies to look also at the genotype response and on the evaluation of the best cultivars for the production and sustainability indicators to be recommended for the improvement of the varietal structure that is actualy in cultivation in Albania acording to different pedoclimatic zones.

Material and Methods

The experiment was set up in the Experimental Base of Center for Agriculture Technology Transfer of Fushe Kruje. The soil type is aluvional light ash brown. The experiment was set up in 8 variants and 4 replicas, according to the randomized block scheme. Each variant represents a cultivar and the size of each variant was 10 m^2 as follows:

V1 – "Këmishtaj" Cultivar (Local)
V2 – "Lushnja x Kamza" Cultivar (Local)
V3 – "Mansholt I" Cultivar (Holland)
V4 – "Makte Haver Oversalt" Cultivar (Holland)
V5 – "Torpan" Cultivar (Holland)
V6 – "Bendo" Cultivar (Holland)
V7 – "Abed Minor" Cultivar (Denmark)
V8 – "Argus" Cultivar (Sweden)
V9 – "MVS – 2008" Local cultivar
V10 – "MMS – 2008" Foreing cultivar

The applied technology is roughly the same as that is used in the common production. The previous crop has been alfalfa. Land plowing was carried out in October at a depth of 21 - 35 cm. For the preparation of the planting bed were passed twice with discs. In the base fertilizer is used 400 kg/ha^{-1} granulated superphosphate which is distributed in two layers. About one-third of the amount is thrown before the plowing and 2/3 before the discs. Planting was done at the beginning of November, on 05/11/2008 with seed rate of 160 - 180 kg/ha^{-1}. Planting is done by hand, with lines at a distance of 30 cm between rows and the seed was dispersed in chains way in the row. Drainage: Immediately after oat planting was carried out the drainage proceedings of the experimental field and the drainage system has been kept functional during all the oat vegetation period. Additional fertilization: 300 kg/ha^{-1} nitrogen fertilizer (ammonium nitrate with 32% a.m.) was used three times. The first time was thrown into the phase of two - three leaves of the fraternal start with the dose of 80 kg/ha^{-1}, the second time at the fraternal full - fledged start phase with 100 kg/ha^{-1} and the third time at the intensive lifting stage with the dose of 120 kg/ha^{-1}. Attention is also paid to fighting weeds. Combating bad weeds is done through mechanical methods. The harvest is carried out in the full baking phase and with each variant is performed separately. After tiring and cleaning is done the weighing individually of each variant of the experiment.

Results and discussion

During the vegetative period, based on the methodology approach the datas were recorded for the passage of phenological phases (germination, fraternallity, raising, earing, flowering and maturity). From recording of these data it represent that all cultivars compared do not present significant changes in the transition of these phases, so all cultivars have the same vegetative cycle ranging from 196 to 200 days, with small differences of 3 - 4 days.

Observations and notess have also kept for the sustainability of low temperatures, falling and resistence from diseases and pests.
For low temperature stability is done the counting of plants on the trai area before and after wintering. For the year under study no crops are distinguished for this indicator, as the minimum temperatures of the year were not in those limits causing damages during their vegetation.
As for their resistance to plant falling, which is one of the most important indicators in this trend, (assessment is done from 1st up to 5th classes) there are evidenced differences between the cultivars. Thus, the "*Këmishtaj*" cultivar is the most sustainable one against this phenomenon. The cultivars sustainability representes also the *"Torpan"* (Netherlands), *"Argues"* (Denmark) and *"Abed Minor"* (Sweden) cultivars, while the *"LxK"* cultivar is the least-resistent from this phenomenon, where the signs of falling were evidenced from the beginning of the rise and falling arrives till at 4th class.

Figure no. 1. "*Këmishtaj"Cultuvar (fall "0" class)*

Figure no. 2. "*LxK" Cultivar (fall "3" Class)*

In comparative trail are evaluated and other phenotypic indicators such as plant color, presence of anthocyanin, presence of thistles, panicula and grain color as well as biometric indicators.

Table no. 1 Biometric Plant Indicators

No. Variants	Cultivars	Plant length in cm (average)	Length of the main spike in cm	Seed no. in main spike	Weight of main spike (g)	Weight of 1000 seeds (g)
1	*Këmishtaj*	155	27	210	4.4	22
2	*Lushnja x Kamza*	170	35	120	2.4	20
3	*Mansholt I*	165	27	160	3.2	20
4	*Nakte Haver Oversalt*	180	38	180	3.5	19.5
5	*Torpan*	155	30	180	3.9	22
6	*Bendo*	160	30	140	2.8	20
7	*Abed Minor*	170	32	180	4.1	23
8	*Argus*	165	32	170	3.9	23
	Average	**165**	**31.37**	**167.5**	**3.52**	**21.18**

Referring to the data we conclude that all the tested cultivars, based on the absolute weight, are part of the oats group with small seeds and that compared with cultivation oat cultivars studies conducted in other nearby countries (Italy) where the absolute weight is 33.0 g (Redaelli et al., dal 2002 al 2013). Among the tested cultivars there are differences in their absolute weight. Referring to the data of the table above, we conclude that all the tested cultivars, starting from their absolute weight are part of the oats group with small seeds. However, among the tested cultivars there are differences in their absolute weight. In general, there is a correlation linkage in terms of the number grains in the paniculas, the weight of the main spike and their absolute weight, with the yield for each cultivar. Thus, cultivars with a greater number of grains in the main spike and the greater absolute weight result with higher yield per surface unit.

The length of the main plant it is an important indicator for the oat crop, which also affects into its fall resistance. Thus generally short-to-moderate legth cultivars are more resistant to fall than the tall cultivars. Thus the cultivars of the "*Këmishtaj*", *"Torpan"* and *"Argues"*, which are shorter than the other tested cultivars are more resistant to the falling phenomenon. However, they are longer compared to some cultivars in nearby countries as Italy where the average length goes to 107 cm (Redaelli et al., dal 2002 al 2013), in Slovenia 147.8 cm and Morocco 144.1 cm (Toshinobu Morikawa, et al. 2016), from 165 cm that are the tested cultivars. Regarding qualitative indicators such as plant color at different stages of vegetation, the presence of anthocyanin, the presence of flips, the paniculate shape and the grain color, the cultivars tested are peresented with significant variation. Thus, for cultivars, "*Këmishtaj*", *"Torpan"*, *"Bendo"* and *"Mineral Abt"* the color of the plant is dark green, for *"LxK"*, *"Nakte Haver"* cultivars the plant color is common green into light one, while for the cultivars *"Mansholt I"* and *"Argus"* it is common green. The presence of flips is found in *"LxK"* and *"Nakte Haver"* cultivars. In terms of grain color it is evidenced the yellowish-white such as *"Mansholt I"* and *"Bendo"* cultivars, yellow as "*Këmishtaj*", *"Torpan"* and *"Argus"*, yellow in brown into *"Abed Minor"* and *"LxK"* cultivars.

The variation is also visible in the form of panculas. The *"LxK"* cultivar (photo no.3) has an open panicula with almost straight edges. The "*Këmishtaj*" cultivar (photo no. 4) has flagged panicula, *"Argus"* and *"Abed Minor"* cultivars (photo no. 5) have right panicula with a bushy twist.

Figure no. 3. Type of panicula on "*Kemishtaj", "LxK"* and "*Argus"* Cultivars

The most important test indicator that is evaluated during the zonal test is the realized production per surface unit (yield).

Table no 2. Realizyed yield for each cultivar (t/ha).

Cultivars/Variants	Repetitions				Variants amount	Average Yield (q/ha)
	I	II	III	IV		
Këmishtaj (V_1)	2.45	2.6	2.5	2.65	10.2	***2.55***
Lushnja x Kamza (V_2)	1.3	1.1	1.15	1.25	4.8	*1.2*
Mansholt I (V_3)	1.75	1.8	1.6	1.65	6.8	*1.7*
Nakte Haver Oversalt (V_4)	1.9	1.85	1.95	1.9	7.6	*1.9*
Torpan (V_5)	2.6	2.5	245	2.45	10.0	*2.5*
Bendo (V_6)	1.75	1.9	1.9	1.85	7.4	*1.85*
Abed Minor (V_7)	2.15	2.2	2.3	2.15	8.8	***2.2***
Argus (V_8)	2.4	2.25	2.3	2.25	9.2	*2.3*
"MVS – 2008" (V_9)	1.85	1.8	1.75	1.8	7.2	*1.8*
"MMS – 2008" (V_{10})	2.05	1.95	2.2	2.2	8.4	*2.1*
Sum/R	2.02	19.95	20.1	20.15	80.4	2.01

DMV for 0.05=1.518; 0.01=2.06

From statistical data processing, the factual F value for repetitions is smaller than the theoretical F values, for both levels of propiability. This shows that soil fertility has not affected the variability of the experiment. The factual F value for variants is greater than the theoretical F values for both propiability levels. This indicates that the changes in the experiment is the effect of the variants, ie the test cultivars or the factor being put into study. Comparison of the data in Table no. 2 shows that the highest production indicators have the cultivars of "*Këmishtaj"* with 2,55 t/ha^{-1}, *"Torpani"* with 2,5 t/ha^{-1} and *"Argus"* and *"Abed Minor"*, which have respectively 2,3 t/ha^{-1} and 2,2 t/ha^{-1}; even though with lower production than the data coming from studies conducted in other countries (3,97 t/ha^{-1}) from the conventional systems in Latvia (Ingver A. et al.,2008); or (4,50 t/ha^{-1}) in close countries as Italy (Redaelli et al., dal 2002 al 2013), but with significative different ecological conditions. We point out that the climate conditions of the test year were not adequate according to the oat biological requirements. The period of panicula until full ripening is accompanied by numerous rainfall and strong winds. These factors have negatively affected for the achieved yields by the cultivars and their falling rate. This is also argumented by comparing with of the oat biological production capacity.

Conclusions

Testing of new cultivars to spread them in the production under the conditions of poor oat strucure cultivars in Albania is a question of imediate necessity.

From cultivar testing it result that the "*Këmishtaj" cultivar* represents higher productivity indicators with average yield of 2,55 t/ha^{-1}, followed by *"Torpan"* cultivar with a yield of 2,5 t/ha^{-1} and then followed by *"Argus"* and *"Abed Minor"* cultivars with yields of 2,3 t/ha^{-1} and 2,1 t/ha^{-1}.

The production levels of these cultivars are good for the conditions of Albania but are below the average of other countries.

In cultivation testing, the pedoclimatic conditions should be carefully evaluated as they affect production indicators, sustainability and other quality indicators of cultivars.

The above-mentioned cultivars with good quantitative and qualitative indicators should be recomended for the Mediterranean field area of Albania, since they enrich the varietal structure of oat cultivars.

The introduction into cultivation practice of these cultivars will enable the expansion of the cultivated area and will increase its production potential as one of the most important cereals for human and animal feed.

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QUALITY OF NECTARINE FRUIT IN HERZEGOVINA REGION

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Abstract

The aim of our work was to research the influence of agro-ecological factors on quality of nectarine during 2015 and 2016. We conducted research on three different locations in Herzegovinian area. On the locations Stolac, Mostar and Čapljina, we researched plantations of two nectarine cultivars *Big Top* and *Caldesi 2000.* The content of ash, dry matter, total acidity and the content of total sugar in nectarine fruit showed statistically important differences compared with years and locations of studied cultivars. The cultivar *Big Top* had the highest value of dry matter and total acidity on the location of Stolac (15,14 and 0,72%, respectively) The highest content of ash was on the location of Mostar (1,61%), and the highest value of total sugar was registered on the location of Čapljina (11,99%). The values of all the tested parameters were higher in 2015 compared to 2016. The sort *Caldesi 2000* had the highest value of dry matter on the location of Stolac (12,92%). The highest value of ash and the total sugars were registered on the location of Mostar (0,66 and 10,6%, respectively). The highest value of total acidity was registered on the location of Čapljina (1,2%) and the highest value of the total sugar was registered on the location of Mostar (10,6%). As far as the location is concerned, the Caldesi 2000 cultivar is more suitable for the Mostar area. At locations of Stolac and Čapljina both cultivars are suitable for breeding.

Key words: *nectarine, fruit quality, Big Top, Caldesi 2000.*

Introduction

Nectarine is subsort of peach, it has got smooth skin and that is actually difference between peach and nectarine. By crossing and mixing different sorts, we can get nectarines that have the same size as usual peaches, they are also red and they are very sweet as well. Their taste is stronger than the taste of usual peaches. They are important part of daily diet, they have low energy values, a variety of natural sugars, as the source of antioxidant. The Herzegovinian area is very good for growing nectarines, and they are often consumed during summer time. We chose two sorts *Big Top* and *Caldesi 2000*, in order to define quality based on chemical parameters of their fruit. We can evaluate the nectarine fruit using different methods. The methods used in the research are the following: chemical analysis (chromatography values of nectarines fruit), taste or sensory attributes, including fruit analysis (color, texture, juiciness) and organoleptic values of nectarines fruit, and physical values as well (Robertson and coworkers, 1989).

Material and Methods

The field experiment was carried out in the plantation of nectarines in the area of Čapljina, Stolac and Mostar. All experimental researches related to physical and chemical content of nectarines fruit have been done on the sort *Caldesi 2000* and *Big Top.* We did this research during two vegetations: in 2015 and in 2016. Chemical analysis of nectarines fruit were done in Federal Institute for Agriculture Sarajevo. Following chemical parameters were researched: ash content, dry matter, content of total acid, and content of total sugar. Total sugar content was determined by volumetric method by "Luff - Schrool".
Total acidity was determined by potentiometric titration with sodium hydroxide solution

In this paper, the drying method was used at the temperature of 105 ° C. This procedure determines the remainder of the sample after drying at 105 ° C to constant mass. The statistical significance of the quantitative values is determined by Fisher's model of the variance analysis (ANOVA) of the two factorial experiment using a F test for P <0.05 and P <0.01

Fertilization and protection on researched areas

For meliorative fertilization (before planting) as the following fertilizers were used: *NPK 8-26-26, NPK 10-30-20, NPK (SO_3) 5-20-30 S (26) (Florin 1), NPK 7-20-30, NPK 6-18-36 i NPK (MgO) 8-16-24.* In basic fertilization, we apply fertilizer with less nitrogen and more phosphor and potasium, *The programs of protection and fertilization after planating are shown in the tables* 1, 2 and 3.

Table 1. Fertilization and protection in the Mostar area

2015 and 2016	Fertilization	Plant protection
January	NPK (MgO) 8-16-24, 800 kg/ha	/
February	/	Nordox 3x 2kg/ha
March	/	Chorus (200g/ha)+Karate zeon (150ml/ha) and Karate (150ml/ha)+Daconil (2l/ha)
April	NPK 20-20-20	Chorus (200g/ha)+Delan i Dithan+Calypso

During 2015, in the area of Čapljina, there was no need for fertilization, because the soil had enough required elements.

Table 2. Fertilization and protection in the area of Čapljina

2015 and 2016	Fertilization	Plant protection
January	NPK (MgO) 8-16-24, 800 kg/ha	Bordovska čorba
February	/	Chromodin+Confidor+Luna Experience
March	/	Captan 50+Mospilan 20 SP
April		
May	/	Captan 50+Mospilan 20 SP

Table 3. Fertilization and protection in the area of Stolac

2015 and 2016	Fertilization	Plant protection
January	NPK (MgO) 8-16-24 (700 kg/ha)	3% solution of bluo gal and lime
March		Merpan+Kubik Plus+Karate zeon and Mospilan 20 SP+Cadillac 80WP+Akord
April	NPK 30-10-10	Captan 50+Tonus+Dional 500 SC+Dali
May		Affirm+Indar 5 EW+Dithan

Analysis of nectarine fruit

We picked up the fruits from marked threes in all three locations during June and July in 2015 and in 2016. After picking up, fruits were prepared for analysis. We took 50 fruits and we chopped the fruits up and homogenized them, in order to get three average samples of one cultivar. In order to show climate conditions, we used weather measurement units from the Federal hydro meteorological institute in Sarajevo, during both 2015 and 2016. Average monthly temperature was $15,6^0$ C during 2015, in the area of Stolac. In the area of Mostar it

was 16,23^0 C. Average monthly temperature was 15,38^0 C during 2016 (January-July) in the area of Stolac. Monthly average of rainfall was 77,31 l/m^{2,} during 2015 in the area of Stolac. In the area of Mostar, monthly average of rain fall was 110,54 l/m^2. During 2016 (January-July)monthly average of rainfall were 87,74 l/m^2 in the area of Stolac, and 140,72 l/m^2 in the area of Mostar.

Results and discussion

Based on mechanical content of the soil, we defined texture according to USDA classification. The type of soil was mostly sandy loam (table 4.).

Table 4. The soil texture according USDA classification

Location/depth(cm)	sort	Sand %	Loam %	Ash %	Texture
Stolac / 0-30	BT i C 2000*	75	15	10	Sandy loam
Stolac / 30-60	BT i C 2000	73	15	12	Sandy loam
Mostar / 0-30	BT i C 2000	80	10	10	Sandy loam
Mostar / 30-60	BT i C 2000	85	8	7	Sandy loam
Čapljina / 0-30	BT	65	18	17	Loam
Čapljina / 30-60	BT	61	17	21	Loam
Čapljina / 0-30	C 2000	76	13	11	Sandy loam
Čapljina / 30-60	C 2000	76	12	12	Sandy loam
BT= Big Top **C 2000= Caldesi 2000**					

Chemical content of nectarine fruit

The Sort *Big Top* had the highest content of soluble solids and total acids in the area of Stolac (15,14% and 0,72%, respectively). The highest content of ash was in the area of Mostar (1,61%), and the highest content of total sugar was in the area of Čapljina (11,99 %). The highest values were noticed in 2015 (Tables 5 and 6.).

Table 5. Chemical composition of nectarine cultivar Big Top in 2015 and 2016 at locations

Parametar	Soluble solid (%)	ash (%)	total acid (%)	total sugar (%)
2015. Stolac	10,52±13,99	0,01±0,61	0,68±0,72	6,20±7,20
Mostar	12,95±15,14	1,00±1,16	0,43±0,57	4,81±6,74
Čapljina	13,95±14,95	0,58±0,93	0,65±0,68	5,80±6,80
F test Lsd test	F=3,58;P=0,09	F=9,02;P=0,015	F=17,06;P=0,003	F=1,018;P=0,41
2016. Stolac	11,58±12,11	0,40±0,86	0,83±0,96	6,67±10,0
Mostar	12,93±13,46	0,38±0,48	0,47±0,48	7,18±9,43
Čapljina	12,98±13,33	0,40±0,72	0,40±0,43	9,97±11,99
F test Lsd test	F=29,73;P=0,0008	F=1,03;P=0,41	F=127,86;P<0,0001	F=2,97;P=0,12

Table 6. Chemical composition of nectarine cultivar Big Top at the same locations in two years

Parametar	Soluble solid (%)	ash (%)	total acid (%)	total sugar (%)
2015. Stolac	10,52±13,99	0,01±0,61	0,68±0,72	6,20±7,20
2016. Stolac	11,58±12,11	0,40±0,86	0,83±0,96	6,67±10,0
F test Lsd test	t=0,05; p=0,96	t=1,43; p=0,22	t=4,69;p=0,009	t=1,53; p=0,2
2015. Mostar	12,95±15,14	1,00±1,16	0,43±0,57	4,81±6,74
2016. Mostar	12,93±13,46	0,38±0,48	0,47±0,48	7,18±9,43
F test Lsd test	t=1,49; p=0,21	t=4,34;p=0,012	t=0,72; p=0,5	t=2,85;p=0,046
2015. Čapljina	13,95±14,95	0,58±0,93	0,65±0,68	5,80±6,80
2016. Čapljina	12,98±13,33	0,40±0,72	0,40±0,43	9,97±11,99
F test Lsd test	t=3,5; p=0,025	t=0,84; p=0,44	t=18,24;p=<0,0001	t=6,09;p=0,0037

In 2015 we noticed that there was not important difference in the content soluble solid, when we compared all three researched locations (F=3,58; p=0,09). In 2016, the soluble solid in the sort *Big Top* was bigger in the areas of Mostar and Čapljina when we compared it with the area of Stolac (F=29,73; p=0,0008). There was not statistically important difference in the total content of soluble solid in the sort *Big Top* in the area of Stolac, when we compared both years of our research (t=0,05; p=0,96). There was no statistically important difference in total content of dry matter in the sort *Big Top* in the area of Mostar, in both years of our research (t=1,49; p=0,21). Total content of dry matter in the sort *Big Top*, in the area of Čapljina, was statistically higher in 2015 (t=3,5; p=0,025).

Total content of ash in the sort *Big Top* in 2015 was statistically higher in the area of Mostar, when we compared it with areas of Stolac and Čapljina (F=9,02; p=0,015). There was not statistically important difference in total content of ash in the sort *Big Top* in 2016, when we compared all areas of our research (F=1,03; p=0,41). There was not statistically important differences in total content of ash in the sort *Big Top* in the area of Stolac, when we compared both years of our research (t=1,43; p=0,22). Total content of ash in the sort *Big Top*, in the area of Mostar, was statistically higher in 2015 (t=4,34; p=0,012). There was not statistically important difference in total content of ash in the sort *Big Top* in the area of Čapljina, when we compared both years of our research (t=0,84; p=0,44).

The content of total acid in the sort *Big Top* was statistically higher in the area of Stolac and Čapljina, when we compared with the area of Mostar in 2015 (F=17,06; p=0,003). The content of total acid in the sort *Big Top* was statistically higher in the area of Stolac, when we compared it with the area of Mostar and Čapljina in 2015 (F=127,86; p<0,0001). The content of total acid in the sort *Big Top* in the area of Stolac was statistically higher in 2016 (t=4,69; p=0,009). There was not statistically important difference in the content of total acid in the sort *Big Top* in the area of Mostar when we compared with second year of our research (t=0,72; p=0,5). The content of total acid in the sort *Big Top* in the area of Čapljina was statistically higher in 2015 (t=18,24; p=<0,0001).

There was not statistically important difference in the content of total sugars in the sort *Big Top* in 2015, when we compared areas of our research (F=1,018; p=0,41). There was not statistically important difference in the content of total sugar in the sort *Big Top*, in 2016 when we compared the areas of our research (F=2,97; p=0,12). There was not statistically important

difference in the content of total sugar in the sort *Big Top* in the area of Stolac when we compared both years of our research (t=1,53; p=0,2). The content of total sugar in the sort *Big Top* in the area of Mostar was statistically higher in 2016 (t=2,85; p=0,046). The content of total sugar in the sort *Big Top* in the area of Čapljina was statistically higher in 2016 (t=6,09; p=0,0037).

Chemical content of nectarine fruit of the sort *Caldesi 2000*

The sort *Caldesi 2000* had the highest value of dry matter in the area of Stolac (12,92%), the highest value of ash in the area of Mostar (0,66%), the highest content of total acid in the area of Čapljina (1,2%) and the highest content of total sugars in the area of Mostar (10,6%). The highest value of ash was in 2016, and all other values were the highest in 2015. The values of our parameters in both years of our research are shown in the tables number 7 and 8, bellow the text.

Table 7.Chemical composition of nectarine cultivar Caldesi 2000 in 2015 and 2016 at locations

Parametar	Soluble solid (%)	ash (%)	total acid (%)	total sugar (%)
2015. Stolac	11,50±12,92	0,43±0,53	0,58±0,67	5,80±8,60
Mostar	11,70±11,97	0,37±0,66	0,54±0,60	5,80±10,60
Čapljina	10,32±12,37	0,40±0,61	0,70±0,72	6,80±8,60
F test Lsd test	F=1,47; p=0,3	F=0,51; p=0,62	F=12,88;p=0,007	F=0,34; p=0,72
2016. Stolac	9,31±9,74	0,31±0,39	0,93±1,03	4,78±5,27
Mostar	10,35±10,56	0,39±0,52	0,62±0,93	6,68±6,71
Čapljina	8,69±8,91	0,27±0,32	0,88±1,20	8,69±8,93
F test Lsd test	F=77,16;p<0,0001	F=5,57;p=0,043	F=5,89; p=0,038	F=378,28;p=0,0001

Table 8. Chemical composition of nectarine cultivar Caldesi 2000 at the same locations in two years

Parametar	Soluble solid (%)	ash (%)	total acid (%)	total sugar (%)
2015. Stolac	11,50±12,92	0,43±0,53	0,58±0,67	5,80±8,60
2016. Stolac	9,31±9,74	0,31±0,39	0,93±1,03	4,78±5,27
F test Lsd test	t=5,97;p=0,004	t=1,7; p=0,16	t=8,44;p=0,001	t=2,39; p=0,07
2015. Mostar	11,70±11,97	0,37±0,66	0,54±0,60	5,80±10,60
2016. Mostar	10,35±10,56	0,39±0,52	0,62±0,93	6,68±6,71
F test Lsd test	t=12,65;p=0,0002	t=0,83; p=0,45	t=2,24; p=0,08	t=1,08; p=0,34
2015. Čapljina	10,32±12,37	0,40±0,61	0,70±0,72	6,80±8,60
2016. Čapljina	8,69±8,91	0,27±0,32	0,88±1,20	8,69±8,93
F test Lsd test	t=3,41; p=0,027	t=0,2,31;p=0,08	t=2,83;p=0,047	t=2,03; p=0,11

In 2015, there was not important difference in the content of soluble solids, when we compared researched areas (F=1,47; p=0,3). Total content of dry matter in the sort *Caldesi 2000* was statistically higher in the areas of Mostar and Stolac, when we compared it with the area of Čapljina (F=77,16; p<0,0001). Total content of dry matter in the sort *Caldesi 2000* in the area of Stolac was statistically higher in 2015 (t=5,97; p=0,004). Total content of dry matter in the sort *Caldesi 2000* in the area of Mostar was statistically higher in 2015 (t=12,65; p=0,0002). Total amount of dry matter in the sort *Caldesi 2000* in the area of Čapljina was statistically higher in 2015 (t=3,41; p=0,027).

There was no statistical difference in total content of ash in the sort *Caldesi 2000* in 2015, when we compared three researched locations (F=0,51; p=0,62). Total content of ash in the sort Caldesi 2000 in 2016 was statistically higher in the area of Mostar, when we compared it with two other researched locations (F=5,57; p=0,043). There was not statistically difference in total content of ash in the sort *Caldesi 2000* in the area of Stolac, when we compared both years of our research (t=1,7; p=0,16). There was not statistically important difference in total content of ash in the sort *Caldesi 2000* in the area of Mostar when we compared years of our research (t=0,83; p=0,45). There was not statistically important difference in total content of ash in the sort *Caldesi 2000* in the area of Čapljina, when we compared both years of our research (t=2,31; p=0,08).

Content of total acid in the sort *Caldesi 2000*, in 2015, was statistically higher in the area of Čapljina, when we compared it with researched area of Mostar (F=12,88; p=0,007). Content of total acid in the sort *Caldesi 2000* in 2016 was statistically different in the area of Čapljina, when we compared it with the researched area of Mostar (F=5,89; p=0,038). The content of total acid in the sort *Caldesi 2000* in the area of Stolac was statistically higher in 2016 (t=8,44; p=0,001). The content of total acid in the sort *Caldesi 2000* in the area of Mostar was not statistically different when we compared both years of our research (t=2,24; p=0,08). The content of total acid in the sort *Caldesi 2000* in the area of Čapljina was statistically higher in 2016 (t=2,83; p=0,047).

The content of total sugars in the sort *Caldesi 2000* in 2015 was not statistically different when we compared researched areas (F=0,34; p=0,72). The content of total sugars in the sort *Caldesi 2000* in 2016 was statistically higher in the area of Čapljina, when we compared two other researched locations (F=378,28; p=0,0001). There was no difference in the content of total sugars in the sort *Caldesi 2000* in the area of Stolac, when we compared both years of our research (t=2,39; p=0,07). There was not statistically difference in the content of total sugars in the sort *Caldesi 2000* in the area of Mostar, when we compared both years of our research (t=1,08; p=0,34). There was not statistically important difference in the content of total sugars in the sort *Caldesi 2000* in the area of Čapljina, when we compared both years of our research (t=2,03; p=0,11).

According to Bulatović (1992**)** nectarine fruit contains 4-13% sugar, 0,2-1,5% acid, 0,2-0,8% pectin matter, 0,5% mineral matter (especially K, Ca, and P), 3-20 mg% ascorbic acid (vitamin C), while the content of other vitamins is at about 1 mg%. Mišić P.D. (1994) highlights that nectarine fruit contains water (87%) and dry matter (13%), and that is organic matter (carbohydrates, organic acid, proteins, lipids and many others), and inorganic matter and biogenic elements. According to Ninkovski (1984) structure of sugars in nectarine fruits is following: the highest content of saccharine is at about 7,34% from the whole content of fruit, in the 100 g, there is 2,15 g of glucose, and there is the lowest content of fructose (fruit sugar), there is at about 0,44 g in the whole content of 100 g of nectarines mesocarp. Wills et al. (1983) highlight that in nectarine fruit, the most important is saccharine (54-75%), fructose (3-25%), glucose (9-21%) and sorbitol (4-11%) in the whole content of sugars. According to Byrne et al.(1991) the most common organic acid in fruits are malic acid, citric acid and quinolic acid. According to Wills et al.(1983) the content of organic acid is eight times lower

than the content of sugars. Even if the content of acids is not that high, they are important for the taste of fruit. Ninkovski (1984) highlights that citric acid and malic acid are dominant in nectarines fruit, and after them there is ant acid and valeric acid. According to Colarič et al. 2004) there is content of total sugar in the nectarine sort *Spring red* from 67,50 g/kg, and content of total acid from 12,55 g/kg. Content of total sugar in the sort *Venus* was 91,96 g/kg, and content of total acid was 11,50 g/kg. In his research of peach (*Prunus persica L.*), Bakić (2016) told that total content of acid was 0,32- 1,07% in 2012 and from 0,40% to 1,02%, and content of total sugars from 11,10-18,84%.

Conclusion

At the location of Mostar, the cultivar Caldesi 2000 has been proven to be better in terms of the higher content of total acids and total sugars. Cultivar Big Top at Stolac had a higher soluble solids and higher total sugars content.

At the location of Čapljina, the Caldesi 2000 has a higher content of total acids, and the Big Top variety has a higher content of soluble solids.

It can be concluded that both cultivars have a good chemical composition and no major deviations from similar investigations. As far as the location is concerned, the Caldesi 2000 cultivar is more suitable for the Mostar area. At locations Stolac and Čapljina both cultivars are suitable for breeding.

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INFLUENCE OF PRODUCTION SYSTEM ON THE CONTENT OF LYCOPENE IN TOMATO FRUIT AT VARIOUS AGRO-ECOLOGICAL CONDITION

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Abstract

Tomato is the second most consumed vegetable in the EU and a major dietary source of many nutrients, vitamins and antioxidants.Tomato is a rich source of lycopene, β-carotene, α-tocopherol, phenolic components, the necessary minerals, primarily potassium and carboxylic acids including ascorbic, citric, maleic, fumaric and oxalic in human diet. Demand for organic tomatoes is increasing due to its nutritious value.Lycopene concentration of in tomato samples was determined by spectrophotometric method. The results showed that the lycopene content of in the Matias hybrid ranged between 5.42 mg/100g (conventional production) to 5.55 mg/100g (integrated production).The lycopene content in the Sakura hybrid ranged in between 6.30 mg/100g (conventional production) to 6.56 mg/100g (integrated production).The results obtained showed significant statistical differences in lycopene content of Matias varieties which was higher on the ones grown at the location Pocitelj brdo(6.17mg/100g) compared to Butmir(5.48mg/100g). Variety Sakura also had significantly higher values determinetaed the location Pocitelj brdo(6.80 mg/100g) compared to the same variety at the location Butmir(6.43mg/100g).

Keywords: *Tomato,Lycopene, Production systems, Agro-ecological conditions.*

Introduction

In recent years there has been a dramatic increase in the availability of scientific publications about the role of fruits and vegetables in human health. Tomato is the second most consumed vegetable in the EU and a major dietary source of many nutrients, vitamins and antioxidants. Tomato is recommended for strengthening the body defense system but also for good diet because it is nutritious and low in calories. Over the last decade lycopene has been singled out as a significant factor in the prevention of chronic diseases.

Much attention has been focused on oxidative stress-induced cellular damage which is recognized as leading factor for of chronic diseases. Antioxidants play an important role in reducing the harmful effects of oxidative stress. Free radicals can bee removed from the body by using high amounts of lycopene. Tomatoeisrich in this important antioxidant due to its luxurious red colour. Our body cannot produce lycopene. Except in tomato, lycopen is found in the watermelon, pink grapefruit and papaya.

Numerous epidemiological studies have shown that dietary intake of food rich in lycopene can reduce the incidence of some types of cancer, including prostate, breast, lung, and colon cancer, coronary artery disease, hyperglycemia, cataracts and macular degeneration. (Lopez *et al*., 2007;Böhm, 2011).Beside this red coloured carotenoid, other ingredients of tomatoes (e.g. ascorbic acid, folic acid, phenolic compounds) may also be responsible for beneficial effects (Böhm, 2011).

Tomato is a rich source of lycopene, β-carotene, α-tocopherol, phenolic components, the necessary minerals, primarily potassium and carboxylic acids including ascorbic, citric,

maleic, fumaric and oxalic in human diet. Some of these tomatoes compounds have antioxidant activity (Gastélum-Barrios *et al*., 2011.)

Some of these elements such as sodium, potassium, magnesium, copper, zinc and iodine in tomato can reduce the risk of cardiovascular disease, and the organic acid contributes to the securing of the base of ballast acid in the body. Tomato has an effectiveinfluence on the human health. Consumption of tomatoesand its products (ketchup, pasta) prevents development of tumors in the digestive tract and prostate cancer (Caris-Veyrat *et al*., 2004).

Today, tomato is the part of prevention as well as help in treating the most common diseases of modern humanity. This has caused increased interest of consumers for organically cultivated tomatoes, including those produced in greenhouses. The task of producers and manufactures is to provide sufficient quantities of healthy and safe tomatoeproduct with complete information for the consumers about when, where and how it is produced. According to Periago(2011) the results indicate that lycopene content depends on the maturation stage of tomatoes, showing the highest amounts can be obtained in full red stage.

After a 10-year research of the chemical composition of tomatoes from the organic production compared with the ones grown of the integral and conventional production systems Mitchell (2007) came to the conclusion that organic tomatoes contained twice as many antioxidants-flavonoid and significantly more lycopene (quercetin, kamferol and naringenin) that help in the prevention of high blood pressure and also reduce the likelihood of heart disease and stroke. These researchers claim that flavonoids may help in curingsome forms of cancer and dementia. If in the 3-weekly diet 30 g of ketchup or 400 ml of tomato juice a day are included, total cholesterol levels reduces by 5.9%, while the level of LDL decreases by 12.9%.

Tomato is an excellent product in the improvement of health because, in addition to the abovementioned facts, it also contains a balanced mix of elements (K, Fe) and antioxidants including vitamins C and E, lycopene, β-carotene, lutein and flavonoids. Demand for organic tomatoes is increasing, mainly because it contains less nitrate and nitrite than the ones grown under integrated and conventional production systems. Additionally, consumers receive a product free of pesticides, potentially better taste, not genetically modified and are often produced in an area that is very close to the consumers.

Material and methods

In order to evaluate the influence of different production systems (organic, integrated and conventional) and the different agro-ecological conditions on the lycopene content a two years trial was carried out . The trial was conducted under conditions of moderate continental climate Butmir-Sarajevo (altitude of 550m) and the terms of sub-Mediterranean climate site Pocitelj - Čapljina (altitude 75m).

The experiments were separately carried out for organic, integrated and conventional production. All production systems include a common method for the production of tomatoes in the open (block system). In the conventional production system,we used mineral fertilizer NPK (100 kg N, 80kg P_2O_5,100 kg K_2O per hectare. In this system we used chemical for plant protection. For organic production we used organic fertilizer (30 tons per hectare). Diseases and insects were controlled by biological methods. For integral production, we used combined fertilizer and protection measures.

The experiment was set by random order in five repetitions. The size of the experiments parcel was 4.2 m^2.The distance between the plant was 80x50cm.

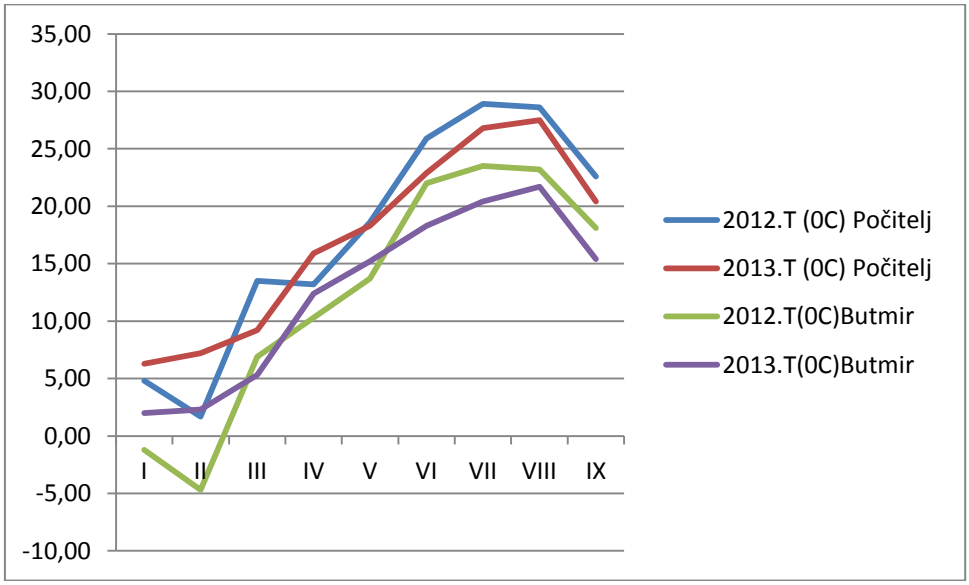
The concentration of lycopene on a sample of 5 plants of each variant experiment was determined by spectrophotometric method in the laboratory of the Federal Institute for Agriculture Sarajevo.In order to obtain information on the concentration of lycopene in a sample, provided is a spectrophotometric analysis of a sample of pure lycopene (standards)

(Shi *at all*.,1999., Shi and Le Maguer, 2000., Lovrić, 1970., Mencarelli and Saltveit, 1988., Tan and Soderstrom, 1988.).

Statistical analysis of the results was determined by analysis of variance. Rating particular significance studied factors and their relationship tested based on the LSD test for significance level of 1% and 5%. Processing of the data obtained in the research is presented in tables and graphs.

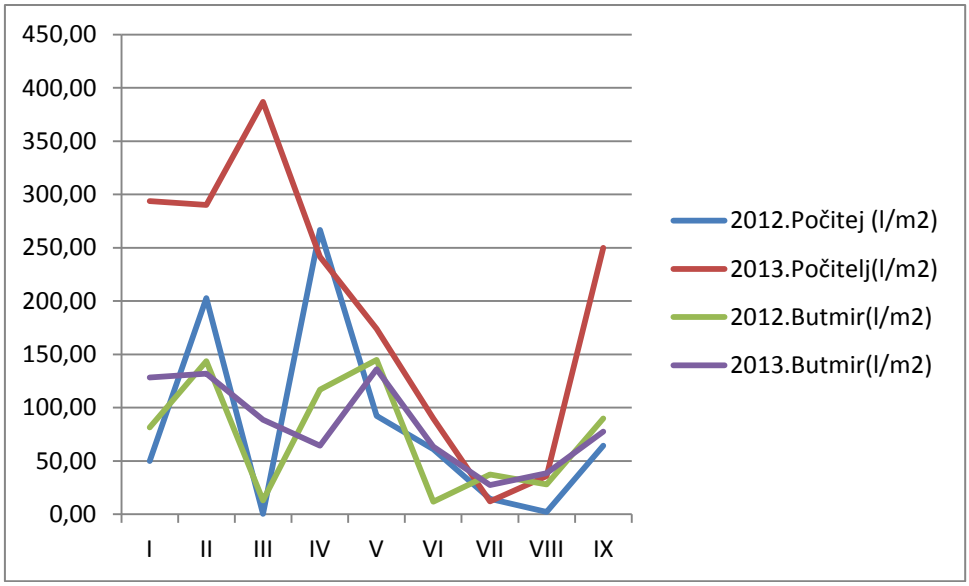
Climatic characteristics

The average monthly temperatures at Butmir in 2012 were the highest in July 23.5(^{0}C), and the lowest in February was -4.7(^{0}C) . During 2013, the average monthly temperature at the Butmira site was highest in August, 21.7(^{0}C), and the lowest in January 2.0(^{0}C). At the locality of Počitelj, the average monthly temperature in 2012 was the highest in July 28.9(^{0}C) and the lowest in February 1.7(^{0}C). During 2013, the average monthly temperature at the Počitelj site was the highest in August, 27.5(^{0}C) and the lowest in January 3.6(^{0}C). (graph 1)



Graph1.Average monthly air temperatures(^{0}C) during 2012-2013

The highest amount of rainfall in the Butmir site in 2012 was in May (144.9 l/m^2) and the lowest in March (13.0 l/m^2). At the site of Počitelj, the highest rainfall sum in 2012 was in April (266.6 l/m^2), the lowest in March (0.3 l/m^2). In 2013, the largest amount of rainfall in Butmir was in May (136.0 l/m^2), while the lowest was in July (27.3 l/m^2). At the site of Počitelj, the highest precipitation peak in 2013 was in March (386.8 l/m^2) and the lowest in August (35.7 l/m^2). (graph 2)



Graph 2.Monthly precipitations (l/m^2) during 2012-2013

Results and Discussion

The content of lycopene in 2012 in different production systems (organic, integrated and conventional) in a variety Matias had different values.The highest content of lycopene in varieties Matias in Butmir was in integral(5.41 mg/100g), then the organic(5.40mg/100g) and conventional production(5.06mg/100g). So,in an integral and organic system of production is expressed statistically significant difference, while the concept inrelation to conventional production showed significant statistical differences.

The highest contentof lycopene in variety Sakura was in the conventional system(6.32 mg/100 g), then the integral(6.30 mg/100g) and organic production(6.28 mg/100g). Statistically significant differences were revealed in production systems at variety Sakura. However, a significant difference between the varieties Matias and Sakura were significantly manifested. Significantly higher yields of lycopene were found in the variety Sakura in all three production systems. At the location of Pocitelj lycopene contentin 2012 in different production systems (organic,integrated and conventional) was also different.The content of lycopene in varieties Matias was the highest in the organic(6.15mg/100g), then the integral(6.06 mg/100g) and conventional production(5.98 mg/100g). Matias varieties in different production systems, no statistically significant differences. The highest content of lycopene in variety Sakura was in the organic system (6.41 mg/100 g), followed by conventional (6.32 mg/100g) and integrated production (6.32 mg/100g). Statistically significant differences were revealed in production systems at variety Sakura, nor have demonstrated a statistically significant difference compared to the sites of production.

Table 1. The content of lycopene in 2012 from different cultivation systems and different localities (mg/100g)

Variant	Location Butmir		Location Počitelj	
	Matias F_1	Sakura F_1	Matias F_1	Sakura F_1
Organic prod.	5.40	6.28**	6.15	6.41
Integrated prod.	5.41	6.30**	6.06	6.31
Convent. prod.	5.06	6.32**	5.98	6.32
LSD 5%	0.09		3.3	
LSD 1%	0.13		3.6	

The content of lycopene in 2013 in different production systems (organic, integrated and conventional production) at variety Matiashad different values.The highest content of lycopene in varieties Matias in Butmir was conventional (5.78mg/100g), then the integral(5.70mg/100g) and organic production(5.57 mg/100g). The demonstrated statistically significant differences in conventional and integratedin relation to organic production.No statistically significant differences between conventional and integrated production.
Variety Sakura had the highest content of lycopene in the integral(6.82 mg/100g), then the organic (6.54 mg/100g) and conventional production (6.29mg/100g). Statistically significan tdifferences have been revealed in the integral over conventional production and statistically significant differences in relation to organic production. Variety Sakura exhibited a significantly statistical difference in all production systems in Butmir in relation to the other studied cultivars. Content of lycopene(2013) on the location Pocitelj in different production systems (organic, integrated and conventional production) was different.Matias variety in different production systems showed no statistically significant differences.The highest content of lycopene in variety Sakura was in the integral(7.28 mg/100 g), followed by conventional(7.26 mg/100g) and organic
production(7.22 mg/100g).Recently,differences and variations in the lycopene content in tomato grown in organic systems versus the conventional way of farming was studied (Riahi *et al*. ,2009.,Barrios – Masias *et al*.,2011., Riahi and Hdider 2013)

Table 2. The content of lycopenein 2013 from different cultivation systems and different localities (mg/100g)

Variant	Location Butmir		Location Počitelj	
	Matias F_1	Sakura F_1	Matias F_1	Sakura F_1
Organic prod.	5.57	6.54*	6.33	7.22*
Integrated prod.	5.70	6.82*	6.23	7.28*
Convent. prod.	5.78	6.37	6.29	7.26*
LSD 5%	0.71		0.89	
LSD 1%	1.01		1.26	

Table 3 shows the influence of locality on lycopene content in tomato fruits Significant statistical differences in the content of lycopene of varieties Matias are higher at the location Pocitelj(6.17mg/100g) compared to Butmir(5.48mg/100g).
Variety Sakura also significantly higher values determined at the location Pocitelj(6.80 mg/100g) compared to the same variety at the location Butmir(6.43mg/100g). Results of the previous researches proved that the concentration of lycopene depends on the vegetation season, location, variety and degree of ripeness. Besides genetic factors, the content varies depending on crop management, especially depending on the input of nutrients into the soil (nitrogen, phosphorus, potassium and calcium) and temperature variations (Garcia and Barrett, 2006., Dumas *et al*., 2003., Adeniyi and Ademoyegun, 2012). The content of lycopene is genetically determined, but is strongly influenced by environmental conditions.

Table3.The effect of locationon the content of lycopene in tomato fruits (mg/100g)

Content of lycopene	Location Butmir		Location Počitelj	
	Matias F_1	Sakura F_1	Matias F_1	Sakura F_1
	5.48	6.43	6.17**	6.80**
LSD 5%	0.23			
LSD 1%	0.29			

Conclusions

The analysis of the two-year results determined the significant influence of the site, ie the agroecological conditions on the value of lycopene:

At the location Butmir in 2012, the highest content of lycopene in the Matias F_1 was in integral production (5.41 mg / 100g), and in the Sakura F_1 in conventional production (6.32 mg / 100g). At the location Počitelj in the same year, the highest content of lycopene was recorded in organic production in both varieties examined.

In the second study year (2013) at the location Butmir, the highest content of lycopene in the Matias F_1 was in conventional production (5.78 mg / 100g), and in the Sakura F_1 in integral production (6.82 mg / 100g). In the same year, at the location Počitelj, the highest content of lycopene in the Matias F_1 was in organic production (6.33 mg / 100g), and in the Sakura F_1 in integral production (7.28 mg / 100g).

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GROWTH PARAMETERS OF TOMATO TRANSPLANTS CULTIVATED BY THE FLOATING CONTAINERS TECHNOLOGY

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Abstract

Commercial production of tomato is based on the use of transplants with a substrate lump around the root, which is also the most expensive transplant production method. In order to explore the possibility of growing transplants using a more cost-effective, innovative hydroponic technology applicable on family farms, a two-factor trial was set according to the method of completely randomized design (CRD). The research factors, term of putting the containers on nutrient solution, and substrate volume of a seeding place, were analysed through three levels. The transplants were grown on substrates of individual seeding place volumes of 27, 37 and 47 ml in containers placed on nutrient solution immediately after sowing, after previous germination and when the plants were in the stage of development of cotyledon leaves. For each type of container, transplants were also grown using the classic method, which was the control variant. The percentage of well-developed tomato transplants was greater for the cultivation on nutrient solution in the containers with previously germinated seeds in comparison with conventionally grown transplants. Also, the transplants grown on nutrient solution had higher values of analysed growth parameters in comparison with classic growing. Each of the tested variants had a highly significant effect on stalk length, diameter and the number of leaves of tomato transplants, while a highly significant interaction of individual variants was also established for stalk length. The research results indicated that high-quality tomato transplants could be produced using the growing technology in floating containers on nutrient solution, which would certainly help in reduction of transplant production costs for smaller commercial vegetable producers.

Keywords: *transplants, tomato, floating containers, nutrient solution*

Introduction

Modern production of transplants is carried out in greenhouses; however, more than 70% of rural producers still produce transplants in hot plots (Parađiković and Kraljičak, 2008). Limitations in the possibilities of high-quality soil disinfection are a big problem in this growing method, which is why a significant part of transplants is ruined by diseases, weediness and manual weeding increase production costs. Commercial production of tomato is based on the use of transplants with a substrate lump around the root, which represent a significant cost item. It is important to choose an appropriate size of individual cell in the container because there is a direct relationship between it and development of transplants (Leskovar, 2001). The size of the container is more important than the type of container (Splittstoesser, 2004) where the volume of substrate is a key factor influencing not only growth but also overall yield and quality (Jankauskienė and Brazaitytė, 2009).

Most producers on small family farms do not have adequate equipment to control temperature and application of irrigation, which puts the production of transplants at risk. Considering these disadvantages, a floating system can be applied as a simpler method for production of transplants. The new method of growing vegetable transplants has been discovered by using hydroponic floating system in the tobacco industry. Interpreting the achievements in

production of tobacco transplants, Carrasco and Izquierdo (2005) gave recommendations for production of vegetable transplants in the floating system. The conventional production of transplants is labour intensive, while cultivation in a floating system can be a less labour-intensive alternative method for the production of transplants of melons (Bilalis *et al.*, 2008), or tomatoes and lettuce, where watering is not necessary (Bilalis *et al.*, 2009). The growth of transplants on nutrient solution is faster than in classic growing technology, and therefore for the same planned planting period, growing of transplants can begin a week later than in classic growing technology (Matotan *et al.*, 2007). Wyatt (1998) was among the first to write about the advantages of growing tomato transplants in the floating system as a sustainable alternative production method. In the floating system, it was possible to produce high-quality tomato transplants (Rideout, 2004), that had higher values of the analysed properties in comparison with those that were grown conventionally, according to Enciso Garay *et al.* (2015).

The objective of the research was to determine the optimum term for putting containers on the nutrient solution after sowing and optimum volume of substrate for growing tomato transplants in the floating system.

Material and Methods

In order to establish the possibility of producing tomato transplants in floating containers on nutrient solution using a technology adapted to family farms in unheated greenhouses on cv. Belle F1, a two-factor trial was set according to the method of completely randomized design (CRD). The main factor, term of putting the containers on nutrient solution (A) was observed through three steps: A1-directly placing the containers on nutrient solution immediately after sowing, A2-placing the containers on nutrient solution after previous germination of seeds, and A3-placing the containers on nutrient solution in the stage of developed cotyledon leaves. The second factor, seeding place substrate volume, or the most favourable number of seeding places in a container (B) also had three steps: B1-23 ml (160 seeding places), B2-37 ml (104 seeding places) and B3-47 ml (84 seeding places). Polystyrene containers (50 x 33 cm) were filled with substrate for production of vegetable transplants Potgrond H (Klasmann). Sixteen containers of each variant were sown, which is a total of 48 containers. One container of each variant was left in the greenhouse where it was further grown in the usual conventional way and was used for comparison of growth as the control variant. Fifteen containers of each variant were put on nutrient solution, occupying an area of 8 m^2 in a reservoir with total area of 22.5 m^2 and water volume 3 m^3. The nutrient solution was prepared using the water-soluble fertilizer Kristalon green special (NPK 18:18:18) in the amount of 1 kg/m^3. Because of evaporation of the solution from the reservoir, fresh water was periodically added trying to keep the EC value constant at a level of 1.5 mS/cm and pH value 5.8-6.3. For the amount of water in the reservoir, 300 ml of Previcur 607 SL fungicide was preventively added. In the fifth week after sowing, on a sample of ten plants of each variant from one container intended for analysis, transplant development parameters were measured: transplant stalk length (cm), transplant stalk diameter immediately below the cotyledon leaves (mm) and counting of developed leaves. The percentage of well-developed transplants suitable for replanting was also established. The research data obtained for growth parameters of the transplants grown on nutrient solution were statistically treated by variance analysis (ANOVA), while the LSD test examined the differences between mean values with the significance level p 0.01. The data were processed using the statistical program SP SS 13 (*Statistical Package for the Social Sciences*).

Results and Discussion

The variants grown on nutrient solution had higher values for the analysed properties compared to conventionally grown transplants (Table 1).

High percentages of well-developed tomato transplants were determined, 96.4%-99.1%, thus achieving one of the preconditions for successful production. The highest percentage of well-developed transplants suitable for replanting was obtained in the containers placed on nutrient solution when transplants had developed cotyledon leaves, while the lowest percentage was in the containers placed immediately after sowing, the value of which was at the level of the control variant. Also, high percentages of tomato seed germination, 89.0%-93.0% in a floating system were reported by Bilalis *et al.* (2009).

In cultivation on nutrient solution, taller transplants were measured, which also had greater stalk diameter and number of leaves in comparison with conventionally grown transplants. Similarly, Wyatt (1998); Verdial *et al.* (1999); Rideout (2004); Enciso Garay *et al.* (2015) showed that tomato transplants grown in floating system were higher and had greater stalk diameter than conventionally grown transplants (Rideout and Overstreet, 2003; Enciso Garay *et al.*, 2015).

Table 1. Development of transplants grown on nutrient solution and with classic technology

Seeding place substrate volume, B (ml)	Term of putting the containers on nutrient solution, A			Control
	After sowing	After germination	In cotyledon stage	
The percentage of well-developed transplants (%)				
23	97.5	98.6	99.4	98.1
37	95.9	98.3	98.6	97.1
47	95.8	97.3	99.4	95.2
Average	96.4	98.1	99.1	96.8
Transplant height (cm)				
23	24.84	26.27	34.80	10.62
37	24.68	18.63	35.34	13.24
47	22.21	19.81	26.65	13.49
Average	23.91	21.57	32.26	12.45
Diameter (mm)				
23	4.27	3.72	4.30	2.50
37	4.34	3.98	4.29	3.21
47	4.08	3.93	4.89	3.27
Average	4.23	3.88	4.49	2.99
Number of leaves				
23	4.10	4.10	4.50	3.00
37	4.20	4.10	4.50	3.10
47	4.10	4.20	5.20	3.00
Average	4.13	4.13	4.73	3.03

Each of the tested variants had a highly significant effect on tomato transplant height, while only the factor term of putting the containers on nutrient solution showed a highly significant effect on stalk diameter and the number of leaves (Table 2).

Transplants in the containers placed on nutrient solution when plants had developed cotyledon leaves were highly significantly highest, while those in the containers placed on nutrient

solution immediately after sowing were lowest. Stalk elongation occurred in containers with larger numbers of seeding places, and significantly higher transplants were on substrate with smallest seeding place volume. Excessive elongation of stalks is a potential problem in both conventional and floating tomato transplant production system (Rideout and Overstreet, 2003). Tomato transplants in the containers placed on nutrient solution in cotyledon stage had the largest diameter, which is at the same significance level with the variant where containers were placed immediately after sowing. Also the significantly largest number of leaves was in the containers placed on nutrient solution when the transplants were in the cotyledon stage. Although the differences in stalk diameter and number of leaves conditioned by substrate volume were not statistically significant, the highest average values were on the largest substrate volume of individual seeding place and decreased with decreasing substrate volume. Similar reports were made by Weston and Zandstra (1986), where tomato transplants grown in the containers with smaller individual seeding place volume developed a smaller number of leaves, and Dumičić *et al.* (2016), who explained that the number of leaves was linearly decreasing under the influence of smaller pot volume, while stalk diameter was not affected by pot volume.

Table 2. Influence of individual factors on measured properties of tomato transplants

Term of putting the containers on nutrient solution, A	Transplant height (cm)	Stalk diameter (mm)	Number of leaves
A1	23.91 b	4.23 ab	4.13 b
A2	21.57 c	3.88 b	4.13 b
A3	**32.26 a**	**4.49 a**	**4.73 a**
Average	25.91	4.20	4.33
$LSD_{p=5\%}$	1.13	0.32	0.29
$LSD_{p=1\%}$	1.50	0.43	0.38
Seeding place substrate volume, B (ml)	Transplant height (cm)	Stalk diameter (mm)	Number of leaves
B1	**28.64 a**	4.10 a	4.23 a
B2	26.22 b	4.20 a	4.27 a
B3	22.89 c	4.30 a	4.50 a
Average	25.91	4.20	4.33
$LSD_{p=5\%}$	1.13	n.s.	n.s.
$LSD_{p=1\%}$	1.50	n.s.	n.s.

Interaction of individual factors had a highly significant effect only on the property transplant height (Table 3).

The maximum transplant height, 35.34 cm, was obtained in the containers placed on nutrient solution when transplants had developed cotyledon leaves on substrate of medium seeding place volume and highly significantly differed from average values of other combinations, with the exception of the combination of the same term of putting the containers on nutrient solution and the smallest seeding place substrate volume, 34.80 cm, from which it did not significantly differ. In tomato transplants, with application of the sub-fertirrigation technique in the stage of fully developed cotyledons, Dumičić *et al.* (2016) explained from presented interactions that transplant height was more influenced by pot volume, as the height was mainly increasing with its decrease, which is consistent with the results of this research.

As a result of interaction of individual factors, the highest values for the properties stalk diameter, 4.89 mm, and number of leaves, 5.20, were not significantly different from average values of other combinations. Thus, with interaction of individual factors, although the differences were not significant, the largest diameter and number of leaves were established in the containers placed on nutrient solution when transplants had developed cotyledon leaves on substrate of the largest seeding place volume.

Table 3. Influence of interaction of individual factors on measured properties of tomato transplants

Term of putting the containers on nutrient solution (A)	Transplant height (cm)			Stalk diameter (mm)			Number of leaves		
	Seeding place substrate volume (B)								
	B1	B2	B3	B1	B2	B3	B1	B2	B3
A1	24.84	24.68	22.21	4.27	4.34	4.08	4.10	4.20	4.10
A2	26.27	18.63	19.81	3.72	3.98	3.93	4.10	4.10	4.20
A3	34.80	**35.34**	26.65	4.30	4.29	4.89	4.50	4.50	5.20
LSD $_{p=5\%}$	1.96			n.s.			n.s.		
LSD $_{p=1\%}$	2.59			n.s.			n.s.		

Conclusions

Using the growing technology in floating containers on nutrient solution, it was possible to cultivate high-quality tomato transplants that had higher values of development parameters in comparison with conventionally grown transplants. The percentage of well-developed transplants was higher for cultivation on nutrient solution in containers placed on nutrient solution with previously germinated seeds and with plants in cotyledon stage in comparison with conventionally grown transplants. Height, stalk diameter and the number of leaves of transplants were significantly greater when containers with plants in the cotyledon stage were placed on nutrient solution. When growing transplants on substrate with smaller volume of individual seeding place, transplants were less developed and stalks were elongated.

In addition to reduction of costs on account of easier system installation and maintenance of water and nutrition regimen, these results indicate the applicability of tomato transplant growing technology on nutrient solution for smaller commercial producers.

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INFLUENCE OF THE SELECTED VARIETIES OF LETTUCE *(Lactuca sativa L.)* ON YIELD AND NITRATE CONCENTRATIONS

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Abstract

Lettuce (*Lactuca sativa L.*) is a highly valued vegetable in human nutrition not only for its richness in minerals and vitamins but also for the fact that nowadays it is produced all year round, and consumed fresh so that all the ingredients stay intact. Taking into consideration the fact that lettuce is the most commonly grown vegetable in the world, there is a large selection of varieties. The aim of the research was to distinguish and to measure the differences in productive characteristics and contents of nitrates in the there the most planted lettuce varieties in greenhouses of Bosnia and Herzegovina. The research was implemented during the growing season 2016/2017. in greenhouse sized 200 m^2. Lettuce was planted 7.12.2017. The research was conducted on lettuce samples of the types butterhead (Shangore and Nantes) and batavia (Funly). At technological maturity lettuce samples were collected for analyses.
The productive parameters which showed significant differences between varieties are average mass of the formed head (0,261-0,372 kg/head) and yield (3,56-5,948 kg/m^2). On the other side, a significant influence of variety was not proved statistically in the case of nitrates content in lettuce heads. The contents of nitrates were ranged (1200 to 2500 mg/kg).

Key words: *Lettuce, Variety, Mass of the formed head, Yield, Nitrates content*

Introduction

Lettuce is an important dietary leafy vegetable that is primarily consumed fresh or in salad mixes. It is one of the most important vegetables grown in greenhouse especially in winter period with or without heating system. Green leafy vegetables contain the highest nitrate levels, and lettuce is classified as having very high nitrate concentrations accumulate in edible parts. The acumulation of nitrates is a consequence excessive adoption of the nitrates from soil or insufficient reduction in the plant. Nitrates themselves have relatively low toxicity to the human body, but their metabolites and reaction products nitrits and nitrosamines in the digestive tract can pose health concerns. The first international risk assessment related to the introduction of nitrates and nitrites was made by JECFA in 1961 (FAO / WHO, 1962). They examined the toxicological impact of nitrates and nitrits, and they established in 1990. Acceptable daily intake, ADI from 0 to 3,7 mg/kg body weight (EC, 1992). Nitrates are mainly created and introduced endogenously (Lundberg et all., 2004 and 2008). The content of nitrates is dependent on growing conditions: temperature, intensity of light, seasons, nutrition and means of fertilization, cultivation methods and, last but not least, variety (Dich et al., 1996). Control of nitrate content in plant products is systematically carried out in developed countries of the world, and there are established guidelines for monitoring the quality of agricultural products in the EU. The maximum allowable quantity of nitrates in the lettuce was determined by the Rule book of the maximum allowed quantities for certain food contaminants (Official Gazette of BiH, No. 68, page 39). If the harvest of lettuce grown in open air is performed in the period from 01.10. - 31.03. it amounts to 4000 mg kg^{-1} of fresh weight, while for a lettuce grown in a protected area it amounts to 5000 mg

kg^{-1}. For harvest carried out in the period 01.04. - 30.09. in a protected area, the maximum permitted nitrate content is 4000 mg kg^{-1} of fresh weight. If in the same period the lettuce is grown on the open field, the value of the maximum allowed quantity of nitrate is 3000 mg kg^{-1} of fresh weight. Therefore, the objective of this paper is to determine the effect of the variety on the nitrates content and on the yield performance of lettuce.

Material and Methods

The research was conducted during the period of October 2016 to May 2018 in greenhouse without heating system, in region of Srebrenik. The experiments were set in a randomized block design with three replications with varieties of Butterhead (Shangore F1 and Nansen) and Iceberg / Crisphead (Funly F1). The lettuce was produced from seedlings planted on black PE foil, at a spacing of 25x25 cm. Each block contained 16 lettuce plants. During the production cycle, all standard measures for the cultivation of lettuce on foil were employed including covering of plants by agrotextile.

The analyses of nutrients contents were performed after harvest. A mixed sample was done from randomly selected thirty plants of each repetition.

- Lettuce plant diameter was measured only from the part of the plant that we can eat.
- Lettuce heads were weighed on digital scales. Average weight of heads and yield per square meter were calculated from the measured values.
- The content of nitraes was determined using the by colometric quick test procedure HACH. Fresh leaves were chopped and pressed in hydraulic press to express plant sap. For the HACH kit, diluted sap was placed in two viewing tubes (suppled with kit). One tube was placed in its slot in a hand-held comparator. The contents of one reagent powder container were emptied into the second viewing tube and the solution was mixed by shoking for 1 min. After mixing, the second viewing tube was placed in its slot in the comparator. The color disk on the computer was rolated until the color on the wheel matched the color in the tube with reagent. The resulting nitrate concetracion was read from comparator.
- Average values of the parameters in the study were statistically processed with computer, using software Excel. Data analysis was performed using the Analysis of Variance (ANOVA) and Least Significant Differences (LSD).

Results and Discussion

Table 1 shows effect of varieties on productive characteristics of lettuce. The productive parameters which showed significant differences between sorts are average mass of the formed head and yield.

Table 1. Sorts effect on leaf yield and yield contributing characteristics of the lettuce.

Parameters / Varieties	Leaf yield plant (kg)	Waste leaves (%)	Yield kg/m2
Shangore	0.371 ± 0.123^{a}	8.337 ± 3.60^{b}	5.95 ± 0.123^{a}
Nansen	0.241 ± 0.781^{b}	9.047 ± 6.50^{b}	3.86 ± 0.781^{b}
Funly	0.261 ± 0.087^{b}	9.192 ± 3.70^{b}	4.18 ± 0.087^{b}

* Values marked by different small letters are statistically significantly different n <0.05.

The highest leaf yield per plant was obtained from Shangore (0,37 kg) and lowest was found in Nansen (0,24 kg). According research Castoldi et al. (2014) the average mass of educated heads was greater than 200 g, which coincides with the results of this research. In contrast, there are studies where the average mass of the head was significantly lower (Valšíková and Viteková, 2006; Todorović et al., 2012).

The highest leaf yield per square meter had Shangore (5,95 kg), which was statistically different from other varieties. The lowest yield (3,86 kg/ m^2) was obtained in the Nansen variety, which also had the smallest head. The factors such as cultivation season, weather conditions and cultivar influenced the formation of phytomass of leaf rosette, which affected its total weight. Koudela and Petříková (2008) also reported that the spread diameter, average weight of heads and yield are affected by variety. These results correspond to the results listed by (Custic et al., 1994; Todorović et al., 2012).

In order to determine whether there was a statistically significant difference in the mass weight between the examined varieties, a variance analysis was performed and a significant F value was established. The Lsd-test was used to test the significance of the differences in the environment.

The nitrates content ranged from 1030 to 2500 mg per kg (Figure 1), it shows that the lettuce produced in this research had a significantly lower level of nitrate than the upper limit allowed by the Ordinance of BiH (Official Gazette of BiH, No. 68, page 39).

Figure 1. Nitrate concentrations in fresh lettuce mass (mg NO_3 kg^{-1} of a fresh mass)

The highest content of nitrates (2500 mg NO_3 kg^{-1} of a fresh mass) was found in Nansen and the lowest in Shangore (1030 mg NO_3 kg^{-1} of a fresh mass). The differences among varieties were not statistically significant. The content of nitrate reported by Prodanović et al., 2001; Toth, 2006; Čustić et al.,1994 is similar to the results of our experiments

Conclusion

According to the study of the influence of sort on the average mass, yiel and nitrate content in a fresh lettuce mass, we can conclude following:

Average mass of lettuce head and hence yield of lettuce varied significantly between different varieties. The best values of all observed pruducitve parameters were determined in Shangore. Leaf yield per plant and yiled depended of sortsand ranged from 0,24 to 0,37 kg.

On the other side, there were no significant differences in nitrates contents. The lowest nitrate concentration in a technological ripeness has been recorded from Shangore (1030 mg NO_3 kg^{-1} of a fresh mass) and the highest has been recored from Nansen (2500 mg NO_3 kg^{-1} of a fresh mass).The tested varieties in protected areas can be successfully grown during the winter period.

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NITROGEN DYNAMICS IN THE SOIL - PLANT SYSTEM UNDER DEFICIT IRRIGATION STRATEGIES IN POTATOES

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Abstract

Experiments were conducted in the open field with silty-clay soil to study the effects of subsurface drip irrigation treatments, full irrigation (FI), regulated deficit irrigation (RDI) and partial root zone drying (PRD), on nitrogen (N) dynamics in the soil – plant system of potatoes. FI plants received 100% of evaporative demands, while RDI and PRD plants, in static approach in 2007, received 70% of water of FI and dynamic approach in 2008 where the reduction of water for PRD irrigation from 70 to 50% was done in the stage of tuber ripening. By four or five harvests during the season we measured N content of leaves, stems and tubers were followed. Results showed, in both years, that N concentration in leaves and stems was progressively reduced during the growing season until final harvest when the lowest values were found in all irrigation treatments. In both years the PRD treatments resulted in the increase N in potato tubers.At final harvest N content was by 16% significantly higher in PRD than in FI plants in 2008. Results for N plant content indicated that PRD treatment could improve allocation of N from shoot to tuber at final harvest, although soil N data pointed out that PRD treatment could be beneficial from the point of increasing N - use efficiency.

Keywords: *Nitrogen content, Solanum tuberosum* L., *Partial root drying (PRD), Regulated deficit irrigation (RDI).*

Introduction

Potato (*Solanum tuberosum* L.) rate fourth in production volume among the world various agricultural products after wheat, rice and corn (FAO, 1995). Potato is grown in different agroecological regions but it is the best adapted to temperate climate and frost – free seasons. It is also sensitive to heat and drought stresses and predictions are that future climate change will significantly affect potato production worldwide Haverkort (2008). As a shallow –rooted crop irrigation is crucial for growing potato plants even in humid areas. A high level of nitrogen (N) is recommended for potato cropping (Darwish et al., 2003) to ensure acceptable yield.

As fresh water resources become scarce, it is difficult to irrigate crops to meet their full demand.

To reduce the irrigation volume, irrigation techniques such as deficit irrigation (DI) have been developed meeting the minimum crop water requirement without any significant reduction in crop yield quality (Davies et al., 2002). In the last decades, one of the management options to overcome the agricultural drought is the use of partial root-zone drying (PRD) and regulated deficit irrigation (RDI) and they are called deficit irrigation methods whose application started on many crops in many countries in the world (Kang and Zhang, 2004). Partial root-zone drying is a deficit irrigation strategy designed to maintain in half of the root system in a dry or drying state, while the other half is irrigated, in order to keep the leaves hydrated. The treatment is then reversed, allowing the previously well-watered side of the root system to dry down while fully irrigating the previously dry side. RDI is a method where the entire root zone irrigated with the amount of water less than the maximum crop evapotranspiration. PRD technique developed at the knowledge of chemical signaling at the level of root-shoot in terms

of lack of water. In most cases, PRD irrigation has shown a great potential to increase irrigation water use efficiency (WUE) and to maintain yield (Davies and Hartung,2004). It has been shown that PRD can greatly induce the initiation and growth of secondary roots, which improve the ability of the plant to absorb both water (Liang et al., 1996) and nutrients from the soil matrix, which may increase the nutrient use efficiency (Han and Kang, 2002). Although several studies on N uptake by potato have been done (Mackand Schjoerring, 2002; Darwish et al., 2003, 2006), a thorough assessment of how different irrigation management strategies affect the N uptake in the plant-soil system under controlled environmental conditions is still lacking. The purpose of the present work was to study how PRD, RDI, and FI irrigation strategies affected nitrogen content in the soil-plant system during the growing season.

Materials and methods

Potato (*Solanum tuberosum* L.) cultivar Liseta was used for investigation. The experiment was carried out during the growing season of 2007 and 2008 in a vegetable commercial farm (Salate Centre) located 10 km north of Serbian capital, Belgrade. The irrigation method used was a drip subsurface method. The subsurface irrigation system was supplied by Netafim (A.C.S. Ltd. Netafim, Israel). For the FI and RDI treatments, one drip line was placed 0.1 m below the top of the ridge although in PRD treatment two drip lines were operated separately and were placed in parallel. The distance between emitters in FI and RDI treatments was 0.3 m and these were place exactly in the middle between two plants. PRD treatments the drip line consisted of two bundled lines each with 0.6 m distance. In partial root drying sealing one side takes until the amount of water in the second half is reduced by 20-30% of the total irrigation and the shifting time interval varied between 3 and 7 days depending on evaporative demand and soil water content. With the RDI and the PRD treatment is started after the phase of tuber initiation (when 80% of tubers more than 20 mm long). In the last 3 weeks of the irrigation period, 70% PRD and RDI was replaced by 50% (PRD and RDI plants received 50% of FI treatment). Because of longer irrigation period, during 2008 season, the number of harvest was increased to five (H1 –H5). Measurement of soil water content was done with a time domain reflectometer (TDR, TRASE, Soil Moisture Equipment Corp., USA). Plant nitrogen content in different plant organs (leaves, stems and tubers) was estimated by Kjeldahl method (Jones, 1990).

The measured traits have been analyzed for statistically significant differences by Students unpaired t - tests (Sigma Plot 6.0 for Windows - SPW 6.0, Jandel Scientific, Erckhart, Germany.

Results and discussion

Results of nitrogen content (Figures 1 and 2) in leaves, stem and tubers (Table 1) were carried out in order to determine whether PRD and RDI irrigation regimes can increase the efficiency of mineral nitrogen absorption from the soil and increase nitrogen translocation from leaves and the stem in the tuber. The positive effect of PRD and RDI irrigation regime on nitrogen absorption and transfer was at the end of the 2007 and 2008 vegetation period where there was a higher accumulation of nitrogen in the tubers. At the end of the experimental period, the PRD irrigation regimes increased nitrogen content in tubers by 11% in 2007. In 2008 at final harvest N content was by 16% ($P<0.05$) significantly higher in PRD than in FI plants. The maximum nitrogen values in both examined seasons, in the leaf and the stem were at the beginning of the vegetation, and then there was a decrease in value by the end of the examined period. These results in 2008 are also the result of the transition from static to dynamic irrigation. These results are in accordance with the Jovanović et al. (2008, 2010), which concluded that the PRD irrigation regime affects the increased translocation of N from the above-ground parts into the tubers and also increase the N – use efficiency. Our results show that the PRD irrigation regime has improved the accessibility of N from the soil, which

has influenced the development of the tuber at later stages of development and has positively affected the yield and size of the tuber. Similar results in potatoes were obtained by Shahnazari et al., (2008), where they also confirmed that PRD treatment may improve soil nitrogen availability during the late phases of potato growing season indicating a higher N mineralization and a greater accumulation of N in plant species. This all indicates that the PRD irrigation regime can improve the transport of nitrogen from leaf and stem to tuber at the end of the vegetation and increase the absorption of mineral nitrogen from the soil. Similar to our results, Shahnazari et al., (2008) found less residual nitrogen in soil at PRD compared to FI irrigation regime, which indicates higher accessibility of nitrogen from the soil to PRD plants, especially in later stages of development.

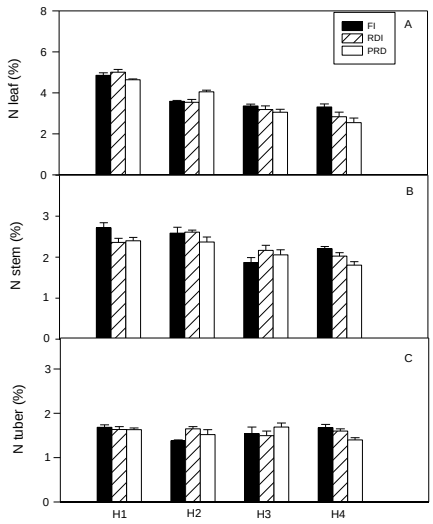

Fig.1.*Nitrogen content (in%) in the leaves (a), stems (b) and tuber (c) of potato plants under PRD,RDI and FI irrigation measured after different harvesting periods (H1,H2, H3 and final harvest) in 2007 experimental season. Bars are means ± S.E.*

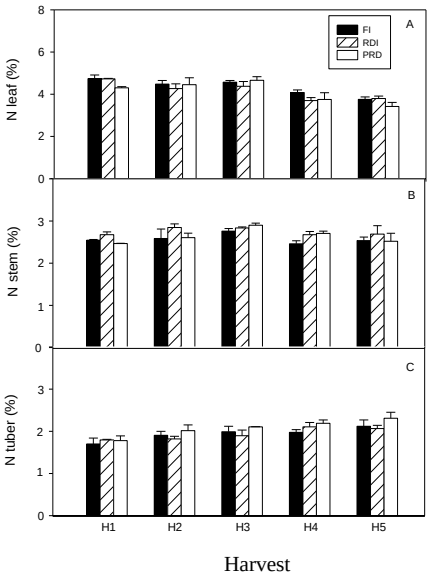

Fig.2.*Nitrogen content (in%) in the leaves (a), stems (b) and tuber (c) of potato plants under PRD,RDI and FI irrigation measured after different harvesting periods (H1,H2, H3,H4 and final harvest) in 2008 experimental season. Bars are means ± S.E.*

Table 1. Influence of FI, RDI and PRD regime of irrigation on content of N in tubers of potato in final harvest 2007 and 2008 years

	2007 year	2008year
Regime		
FI	2.18 ± 0.00	2.25 ± 0.15
RDI	2.30 ± 0.08	2.19 ±0.15
PRD	2.45 ± 0.08	2.68 ± 0.10

Conclusion

Experimental results for both seasons showed that PRD irrigated plants produced tubers with improved quality in terms of higher N content. Results showed, in both years, that N concentration in leaves and stems was progressively reduced during the growing season until final harvest when the lowest values were found in all irrigation treatments. In both years the

PRD treatments resulted in the increase in N in potato tubers. Results for N plant content indicated that PRD treatment could improve allocation of N from shoot to tuber at final harvest. N results might suggest a more beneficial effect of dynamic PRD approach than static PRD on plant growth.

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COMPARATIVE VALUE OF GRASS AND LEGUMES PROTEIN YIELD AT DIFFERENT CUTTING REGIMES IN TEMPORARY GRASSLANDS

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Abstract

The production of bulk feed, with high protein contents, is one of the most important tasks of livestock production. Apart from pure legume crops, high yields of good quality bulk feed can also be achieved by grass-legume mixtures. In practice, temporary grasslands commonly known as grass-clover mixtures, usually comprise various types of grasses and legumes. Given that the quality of forage primarily depends on the botanical composition and stage of the development of plants at the time of grazing or cutting, the aim of this investigation was to determine the influence of the cutting regime on raw protein yield in different species of grasses and legumes in the plant community. A two-year study on protein yield showed that the cutting regime was a very important factor for obtaining high protein yields per unit of surface, since cutting at the flowering stage of legumes in all mixture variants resulted in a statistically significantly higher yield compared with those obtained at other cutting regimes. The maximum two-year protein yields at the cutting regime involving the flowering and budding phases of legumes were obtained by the S2 variant (3084 kg ha^{-1} and 2579 kg ha^{-1}, respectively), with high percentage of bird's-foot trefoil, while at the cutting regime involving the grazing imitation phase, the highest protein yield was obtained by the S3 mixture (2238 kg ha^{-1}) with equal representation of red clover and bird's-foot trefoil (20% each at sowing).

Key words: *temporary grassland, cutting regime, protein yield*

Introduction

In Bosnia and Herzegovina, livestock production is proclaimed as the most important part of agricultural especially in mountain regions into account the fact that food makes 60 to 70% of the total production cost, the production of good quality and cheap bulk feed is particularly important for the success of cattle production.

Perennial legumes, which represent the most important group of fodder plants, are characterized by high content of nutrients (proteins, minerals and vitamins). Perennial grasses are the basis of sustainable livestock farming as they make the basic food for ruminants in moderate climate areas, being components of both sown and natural pastures (Stošić, *et al.,* 2005; Lazarević, *et al.,* 1999), natural and sown meadows (Lazarević *et al.,* 2001) that can be used through cutting (Stošić and Radojević, 1980), silage and haylage (Dinić *et al.,* 2003). In addition to pure leguminous crops, high yields of high quality bulk feed can also be achieved by legume-grass mixtures since legumes contribute to soil fertility and improved nitrogen nutrition, thanks to their ability to fix highlight nitrogen from the soil, in symbiosis with *Rhizobia* bacteria. Therefore, proper choice and selection of types and species of grasses and legumes for grass-clover mixtures has a direct impact on the quantity and quality of the fodder as well as on the length of crop utilization. Namely, there are differences in the chemical composition between different types (Fairey, 2004) and varieties of grasses (Hussein *et al.,* 2002) and clover (Butler and Bailey, 1973), even at the same phytophenological stages of development. It should be particularly noted that the chemical composition of a lawn changes during the growing season, depending on the agro-ecological conditions, the applied agro-

technology, the manner and the intensity of exploitation. In addition to fertilization, the quality and yield of plant mass of sown grassland are largely dependent on the time (regime) of cutting, as well as on the way of cutting in terms whether or not it was mowed or grazed by different types of domestic animals (Marcela Andreata–Koren *et al.* (2009). Direct influence of the frequency of cutting on fodder quality was also pointed out by Minson (1990), who found that the content of crude proteins was higher by 90 g kg^{-1} SM and the content of fibers lower by 197 g kg^{-1} SM on the grassland that was mowed 4 times a year compared to the one mowed two times only.

The aim of the undertaken research was to determine the impact of cutting regimes on crude protein yields of different species of grasses and legumes in the plant community.

Material and Methods

A two-year research was conducted at the Butmir near of Sarajevo, experimental field (2012-2013). The experiment was set up using the random block method in four replications, with elementary plots of 5 m². The sowing was done manually, in rows with 12.5 cm spacing between the rows. The sowing rate was 15 kg ha^{-1} for red clover, 12 kg ha^{-1} for bird's-foot trefoil, 25 kg ha^{-1} for Italian ryegrass, 12 kg ha^{-1} for timothy grass and 30 kg ha^{-1} for orchard grass. Mineral fertilizer NPK (15:15:15) was used in pre-sowing preparation of soil in the amount of 250 kg ha^{-1}. Soil properties of the experimental field: pH-5.0 (in KCl), pH-5.7 (in H_2O); according to humus content of 2.6% it is medium humus soil; low to medium content of phosphorus amounting 9.8 mg/100 g of soil; according to the potassium content, the soil is medium provided with this element (20.8 mg/100 g of soil).

The experiment investigated:

(A) Two species of legumes and three species of grasses, in four replications of the composition of temporary grassland, as follows:

1. Red clover (*Trifolium pratense* L.) 40%, Italian ryegrass (*Lolium italicum* L.) 20%, timothy grass (*Phleum pratense* L.) 20%, orchard grass (*Dactylis glomerata* L.) 20% (Mixture S1).
2. Bird's foot trefoil (*Lotus corniculatus* L.) 40%, Italian ryegrass (*Lolium italicum* L.) 20%, timothy grass (*Phleum pratense* L.) 20%, orchard grass (*Dactylis glomerata* L.) 20% (Mixture S2). 3. Red clover (*Trifolium pratense* L.) 20%, bird's foot trefoil (*Lotus corniculatus* L.) 20%, Italian ryegrass (*Lolium italicum* L.) 20%, timothy grass (*Phleum pratense* L.) 20%, orchard grass (*Dactylis glomerata* L.) 20% (Mixture S3). 4. Italian ryegrass (*Lolium italicum* L.) 33.3%, timothy grass (*Phleum pratense* L.) 33.3% orchard grass (*Dactylis glomerata* L.) 33.3% (Mixture S4).

(B) Three cutting variants a) Cutting at a height of 20 cm (grazing imitation), b) Cutting in the budding phase of the leguminous component, c) Cutting in the flowering phase of the leguminous component. The content of total nitrogen in the plant mass was determined by the Kjeldahl method. Crude protein content was determined based on the level of total nitrogen in dry matter multiplied by factor 6.25. Crude protein yield (CP) was determined on the basis of dry mass yield and the content of crude proteins in it. The test results were processed using mathematical and statistical methods in SPSS and Excel programs.

Results and Discussion

Comparative value of protein yields at different cutting regimes in 2012

In addition to the botanical composition of the mixtures, the cutting regime also had a significant effect on the yield of crude protein (Table 1.), as statistically significantly higher yield of proteins was obtained at cutting in the flowering phase of legumes compared to protein yields obtained at the previous two cutting regimes. There was also a significant difference in the yield of proteins obtained at cutting in the budding phase of legumes compared to the yield obtained in the phase of grazing imitation (except in mixture S4).

The highest protein yield at cutting in the flowering phase of legumes was achieved by mixture S1 (1764 kg ha^{-1}), while the highest yield of crude protein in the phase of grazing imitation and budding of legumes was achieved by mixture S3 (1115 kg ha^{-1} and 1342 kg ha^{-1}).

Table 1. Comparative value of proteins (kg ha^{-1}) in 2012 at different cutting regimes

Mixture variant (Temporary grasslands)	**Cutting regime**		
	Grazing imitation	**Budding**	**Flowering**
Mixture (S1)	1022c	1220b	1764a
Mixture (S2)	652c	1131b	1393a
Mixture (S3)	1115c	1342b	1573a
Mixture (S4)	363b	340b	403a
Average	**788**	**1008**	**1283**

[abc] Values marked with different letters are showing significant differences among cutting regime (P<0.05)

The lowest yield of crude protein, in all phases of development at the time of cutting was found in mixture S4, and its values amounted to 340 kg ha^{-1} (the budding phase of legumes), 363 kg ha^{-1} (the phase of grazing imitation) and 403 kg ha^{-1} (the flowering phase of legumes).

Comparative value of protein yields at different cutting regimes in 2013

The highest average protein yield (1281 kg ha^{-1}) in 2013 was achieved by cutting at the flowering phase of legumes (Table 2.), followed by the budding phase of legumes (1112 kg ha^{-1}), and the lowest at the phase of grazing imitation (1076 kg ha^{-1}). The statistically significantly higher protein yield, regardless of the type of mixture, was obtained by cutting at the flowering phase of legumes (with the exception of mixture S4) compared to the yield obtained by cutting at the budding phase of legumes or the grazing imitation phase. The S2 mixture achieved the maximum yield of crude protein in all cutting variants, ranging from 1435 kg ha^{-1} (grazing imitation phase) to 1690 kg ha^{-1} (legumes flowering phase). The S4 variant, consisting only of grasses, had the lowest crude protein yield at all cutting regimes.

Table 2. Comparative value of proteins (kg ha-1) in 2013 at different cutting regimes

Mixture variant (Temporary grasslands)	**Cutting regime**		
	Grazing imitation	**Budding**	**Flowering**
Mixture (S1)	1017b	1082b	1279a
Mixture (S2)	1435b	1447b	1690a
Mixture (S3)	1122b	1156b	1418a
Mixture (S4)	729a	762a	738a
Average	**1076**	**1112**	**1281**

[ab] Values marked with different letters are showing significant differences among cutting regime (P<0.05)

The minimum protein yield for this mixture was recorded in the cutting at the phase of grazing imitation, only 729 kg ha^{-1}, followed by slightly higher yield of cutting at the flowering phase (738 kg ha^{-1}), and at the budding phase of legumes 762 kg ha^{-1}.

Weather conditions that were slightly more favorable during the vegetation period compared to the year 2012, contributed to the results of this year's research, because although the annual

sum of precipitation was lower relative to the multi-year average, there were no extreme droughts, while temperatures were mostly slightly higher compared to those in the multi-year average.

Comparative two-year protein yield at different cutting regimes

The two-year study on protein yields has shown that the cutting regime had a significant effect on the yield of proteins achieved per unit of surface, (Table 3.) since cutting in the flowering phase of legumes, in all mixture variants, resulted in a statistically significantly higher yield compared to the yields obtained by other cutting regimes.

However, if we compare the protein yield obtained in the other two cutting regimes, it can be concluded that cutting in the budding phase of legumes can obtain a significantly higher protein yield compared to the one obtained at cutting in the phase of grazing imitation.

On the other hand, the influence of the botanical composition, i.e. composition of the mixtures, on protein yields was clearly pronounced. In our research, the highest two-year protein yield at the cutting regime in the flowering phase of legumes, as well as in the budding phase of legumes, was obtained by variant S2 (3084 kg ha^{-1} and 2579 kg ha^{-1}), with a high percentage being bird's foot trefoil, while at the cutting regime in the phase of grazing imitation it was the mixture S3 (2238 kg ha^{-1}) in which red clover and bird's foot trefoil were equally represented (20% each at sowing).

Table 3. Comparative value of the two-year protein yield (kg ha^{-1}) at different cutting regimes

Mixture variant (Temporary grasslands)	**Cutting regime**		
	Grazing imitation	**Budding**	**Flowering**
Mixture (S1)	2040c	2302b	3043a
Mixture (S2)	2087c	2579b	3084a
Mixture (S3)	2238c	2499b	2991a
Mixture (S4)	1092c	1102b	1141a
Average	**1864**	**2120**	**2565**

[abc] Values marked with different letters are showing significant differences among cutting regime ($P<0.05$)

The yield of crude proteins in red clover, bird's foot trefoil, timothy grass, Italian ryegrass, orchard grass and their mixtures indicates that mixtures with a higher content of red clover and bird's foot trefoil produce or can produce higher yields of crude proteins per unit of surface. This is particularly evident in mixtures with a higher proportion of bird's foot trefoil (phase of budding and flowering of legumes), and red clover in the phase of flowering of legumes, where their participation in the mixture was 40%, which quite corresponds to the results of Hannaway *et al.* (1999), Jose *et al.* (2001), Bezdrob *et al.* (2013).

Conclusions

Based on the results obtained in this research, the following can be concluded:

Two-year results of the study on crude protein yields have shown that the cutting regime had a statistically significant effect on the protein yield achieved, where significantly positive differences were obtained at each subsequent cutting regime, irrespective of the botanical composition of the mixtures.

The S2 mixture, consisting of bird's foot trefoil, Italian ryegrass, timothy grass and orchard grass, achieved the highest two-year protein yield in the legumes flowering phase (3084 kg ha^{-1}) and the budding phase (2579 kg ha^{-1}), while the mixture S3 had the highest protein yield in the phase of grazing imitation (2238 kg ha^{-1}).

The mixture of grasses had the lowest total protein yield at any cutting regime - it was even two times lower compared to the average value of all studied mixtures. This shows that leguminous plants ((in our case red clover and bird's foot trefoil), regardless of their participation in the mixtures, had a positive effect on protein yield as their yield was significantly higher compared to the protein yield in grass mixtures.

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DIVERSITY OF *IN SITU* WILD PEAR (*Pyrus communis* L.) POPULATION IN THE LOCALITY OF KOZARA (Bosnia and Herzegovina)

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Abstract

The Balkan Peninsula is considered a secondary centre of genetic diversity of wild pear. To this point, the population of wild pears in Bosnia and Herzegovina (B&H) has not been the subject of comprehensive research. Certain areas are characterized by a large variety of wild pears, one of which is Kozara mountain. The aim of these researches is the morphological characterization of *in situ* populations of wild pear on Kozara mountain, Previja locality (B&H) and the separation of the accessions with certain positive characteristics for selection of varieties and rootstocks. In Bosnia and Herzegovina and in many European countries the wild pear is the main seedling rootstock for grafting cultivated pears. There are nine (9) accessions inventoried in the locality of Previja. The morphological characterization of the vegetative organs of the selected pear trees was performed during two-year period (2012 and 2014) and the following parameters were analyzed: tree trunk characteristics (vigour and habit), characteristics of one-year-old shoots (bark colour, lenticels appearance, length of internodes) and leaf characteristics (leaf blade length and width, petiole length and the appearance of the stipule on the petioles, the shape of the apex and the base of the leaf blade, the shape of the leaf blade). The pomological characterization of the fruits of the selected accessions was carried out during 2012 and 2016 years with the following characteristics: fruit weight, height and width and fruit shape index, number of seeds in fruit, length and thickness of the stalk. The fruit trees had weak to medium vigour with the upright position of branches in most of the analyzed trees. The average weight of fruit of all the accessions in 2012 (15.12 g) was lower than in 2016 (21.01 g). The KP3 accession is distinguished by the number of seeds in the fruit, which is characterized by more than 2 seed germs in one seed case.

Keywords: *Wild pear, Morphological characterization, Leaf, Fruit.*

Introduction

Wild pear (*Pyrus communis* L.) is a valuable and significant forest fruit tree because of its nutritional value (production of distillates, honey), utilisation in fruit production (used as a rootstock for grafting) and wood value. It is a companion of deciduous forests in the Balkan Peninsula and has a wide distribution range (Mratinić and Kojić, 1998). It is also found in both the plains and mountainous areas up to 1,600 m above sea level. Balkan Peninsula is considered a secondary centre of genetic diversity of wild pear. Wild pear is a significant source for genetic improvement and creation of new varieties and rootstocks, but so far it has rarely been used in breeding programs. Most wild fruit tree *in situ* conditions have not been sufficiently explored and processed, as is the case with populations of wild pears in Bosnia and Herzegovina.

Significant studies of wild pear populations in the areas of former Yugoslavia were carried out in the area of Bijelo Polje (Jovančević, 1994), Sarajevo (Kulina, 2001), Western Serbia (Milutinović et al., 2005) and indigenous pear populations in XX area (Paunović et al., 2012). Biodiversity of wild fruit trees in Europe is endangered by the increasing intensification of agriculture and urbanization as well as genetic crossing with cultivated species (Stephan et al.,

2003). Therefore, in areas where it is expected that the existing population of wild fruit trees contains useful properties, it is necessary to carry out the collection of the material and its conservation. Parallel to conservation, description, characterization and evaluation should be performed. The study of phenotypic traits of natural populations is not only relevant for the observation of biological heritage, but also as a potential source of genes for improving cultivated plants (Marino et al., 2013). Mountain areas up to 1.000 m altitude are characterized by the rich and still well-preserved wild pear populations, and one of them is the Kozara mountain, located in the north-western part of Bosnia and Herzegovina. The aim of these researches is the morphological characterization of *in situ* populations of wild pear on the Kozara mountain, the Previja area (B&H) and the collection of accessions with certain positive characteristics for varieties and rootstock selection programs.

Material and Methods

The morphological characterization of wild pear (*Pyrus communis* var. *pyraster* (L.) Ehrh.) included nine (9) *in situ* populations in the Previja area, located on the southeast slopes of the Kozara mountain. Inventory of wild pear trees was carried out on the basis of the vigour, the type of growth and the characteristic of the fruit (size, shape and number of seeds). The accessions are mapped by a hand GPS receiver (GARMIN Oregon 550, USA) and are marked with PCK (PC-*Pyrus communis*, K-Kozara) and numbered from 1 to 9.

The morphological characterization of the vegetative organs of the trees of chosen accessions was carried out during the period 2012 and 2014 years in accordance with the International UPOV (The International Union for the Protection of New Varietes of Plants) descriptors for pear (UPOV, 2000). The trees are characterized by the properties of the vigour and the position of the branches (habit). On the sample of 10 annual shoots we analyzed the following properties: the bark colour on the sunny side, the appearance of lenticels and the length of the internodes. The analysis of the leaf characteristics was conducted on a sample of 25 leaves taken from the central part of the young shoots and the following characteristics were noted: length and width of the leaf blade, the length of the petiole and the occurrence of the stipules on the leaf petiole, the shape of the tip and the base of the leaf blade and the shape of the leaf blade. The analysis of the young shoots and leaves was performed at the end of June, at the end of intensive growth. The pomological characterization of collected fruits was carried out during the season of fruit ripening during 2012 and 2016 years on an average sample of 25 fruits, with the following characteristics: fruit weight, height, width and index of fruit shape, number of seeds in fruit, length and thickness of peduncle.

The data were statistically analyzed by a variance analysis method for a two-factor trial. The significance of the differences was determined by the LSD test for the significance level of $p<0.05$. The absolute variability of the mean values is defined by calculating the standard error (SE). For all studied characteristics, apart from the fruit weight, the coefficients of variation (CV) were calculated.

Results and Discussion

An overview of the characteristics of trees and young shoots of inventoried wild pears is shown in Table 1. In the Balkan researches (Paunović et al., 2012), a vigorous to very vigorous wild pear trees were recorded. In the population of wild pear at the Kozara site, trees with low and medium vigour and upright growth type (habitat) were dominant. Lenticels on one- year- old wild pear shoots from the Kozara site are present in a smaller number, and the bark colour of the accessions are generally various shades of brown, except for the two accessions in which they were gray-green (PCK6 I PCK9).

Table 1. Characteristics of trees and young shoots of wild pear trees *in situ*

Accessions	Tree		One-year-old shoot		
	Vigour	Habit	Colour	Number of lenticels	Length of internodes (cm)
PCK1	weak	spreading	brown red	many	1.96
PCK2	weak	upright	medium brown	few	1.91
PCK3	medium	upright	medium brown	medium	2.14
PCK4	weak	upright	brown red	medium	2.34
PCK5	medium	upright	orange brown	medium	2.16
PCK6	medium	upright	grey green	many	2.30
PCK7	medium	spreading	brown red	medium	1.81
PCK8	medium	semi-upright	brown red	medium	2.19
PCK9	strong	upright	grey green	few	2.37

The qualitative characteristics of the leaves of the selected of wild pear trees are shown in Table 2.

Table 2. Leaf characteristics of *in situ* wild pear accessions

Accessions	Leaf blade			
	Shape of base	Shape of apex	Incisions of margin	Presence of stipules
PCK1	truncate	obtuse	bluntly serrate	absent
PCK2	cordate	obtuse	crenate	absent
PCK3	truncate	obtuse	crenate	absent
PCK4	cordate	right-angled	absent	present
PCK5	truncate	right-angled	bluntly serrate	present
PCK6	obtuse	obtuse	absent	absent
PCK7	truncate	obtuse	crenate	absent
PCK8	truncate	obtuse	crenate	present
PCK9	truncate	acute	bluntly serrate	absent

Most of the selected accessions, i.e. 65% of them, had truncated leaf base form, the obtuse shape of the leaf tip, and the leaf without stipules.

Data on the quantitative characteristics of wild pear leaves are shown in Table 3. Measurements of the leaf characteristics were carried out during two different years regarding the climatic conditions. The 2012 was extremely dry, and 2014 was with a lot of precipitation. The values of leaf dimensions are uniform, with a small variation coefficient of measured properties. A higher variation in the width of the leaf (CV> 15%), with a certain number of accessions, was more pronounced during the dry year (2012). The length of the leaf petiole was between 1.01 cm and 3.76 cm. Accession PCK9 had the longest leaf petiole in average. The leaf dimensions are uniform, and the values of the petiole length are highly variable, and are confirmed by the coefficient of variation (CV), which is, by the length of the petiole, higher than the CV of leaf dimensions characteristics.

Table 3. A quantitative characteristic of leaves of wild pear accessions

Accessions	Year	Leaf blade					
		Length (cm)	CV (%)	Width (cm)	CV (%)	Length of petiole (cm)	CV (%)
PCK1	2012	3.45±0.31	9.03	2.88±0.16	5.56	2.43±0.43	17.75
	2014	3.79±0.29	7.64	3.35±0.27	7.94	1.01±0.20	19.71
PCK2	2012	5.11±0.48	9.31	4.31±0.42	9.80	2.98±0.73	24.38
	2014	4.47±0.43	9.62	3.61±0.28	7.64	1.35±0.24	17.53
PCK3	2012	4.74±0.57	11.94	3.71±0.65	17.42	2.66±0.54	20.20
	2014	4.40±0.51	11.68	3.64±0.33	9.14	1.58±0.31	19.62
PCK4	2012	5.58±0.43	7.74	4.08±0.33	8.16	3.13±0.39	12.44
	2014	6.23±0.38	6.11	3.71±0.39	10.50	2.14±0.37	17.45
PCK5	2012	5.16±0.41	7.88	4.29±0.66	15.27	3.39±0.72	21.31
	2014	5.18±0.49	9.51	3.66±0.32	8.85	1.76±0.24	13.63
PCK6	2012	5.41±0.56	10.39	4.20±0.75	17.75	2.88±0.45	15.64
	2014	6.20±0.57	9.26	4.34±0.38	8.82	2.72±0.40	14.60
PCK7	2012	5.13±0.64	12.39	4.70±0.56	11.96	3.06±0.85	27.87
	2014	4.59±0.52	11.42	3.24±0.46	14.31	1.64±0.50	30.56
PCK8	2012	5.52±0.49	8.93	4.72±0.47	10.04	3.08±0.42	13.51
	2014	5.33±0.44	8.32	4.28±0.64	14.93	2.14±0.45	21.14
PCK9	2012	5.41±0.61	11.31	3.94±0.32	8.18	3.36±0.87	25.97
	2014	6.26±0.42	6.72	4.36±0.54	12.29	3.76±1.44	38.27

The analysis of fruit characteristics of wild pear accessions was carried out over two years, with different climatic factors, in 2012 (dry year) and in 2016 (a year with a favourable precipitation distribution during the growing season). The average values of the weight of the fruit of all tested accessions were higher in 2016 (Table 4), except for PCK7. Four wild pear accessions (PCK1, PCK2, PCK5 and PCK7) had relatively small fruits, weighing up to 10 g. By the size of the fruit, the accession PCK9 is identified with a fruit weight of 42.54 g.

Table 4. Average fruit mass (g) of wild pear accessions

Accessions	Year		Average
	2012	2016	
PCK1	7.40	12.16	9.78
PCK2	6.83	9.89	8.36
PCK3	8.70	19.21	13.95
PCK4	18.10	37.52	27.81
PCK5	7.45	10.68	9.06
PCK6	17.35	19.27	18.31
PCK7	10.32	8.39	9.35
PCK8	19.33	26.84	23.08
PCK9	40.60	44.49	42.54
Average	15.12	20.93	
Level	A	B	AB
F calculated	383.99**	221.68**	27.67**
$LSD_{0.05}$	1.66	0.78	2.35
$LSD_{0.01}$	2.22	1.05	3.15

**statistically highly significant difference; A-accession factor; B-year factor; AB-interaction accessions/year

There are statistically significant differences in the weight of the fruit between analyzed accessions, years of research and the interaction of the accession/year. The results of our research are similar to those of Jovančević (1994) and Paunović et al. (2012). The average mass of wild pear populations of the Bijelo Polje area was 15.84 g (Jovančević, 1994), and in the researches of indigenous populations in Serbia it was 20.05 g, and the highest fruit mass had the genotype K1 (31.17 g) (Paunović et al., 2012). In our research, the accession PCK9 had an average maximum weight of fruit 42.54 grams. The fruits of autochthonous pear varieties "Karamut", "Zobnjača", "Žutica" in the territory of Bosnia and Herzegovina (Đurić et al., 2015) have a lower mass of fruit than the accession PCK9.

The results of measuring the wild pear fruits are shown in Table 4. Wild pear fruit, other than crown, is characterized by a shape that determines the ratio of height and width of the fruit. According to Mišić (1984), wild pear fruits are small, spherical or pear shaped, hard and bitter. The fruits from the Kozara locality are mainly roundly shaped, with the exception of the PCK3 which have a pear shape of the fruit (fruit shape index> 1). The number of normally developed seeds of wild pear fruit varies from 1.2 to 10.1 (Mratinić, 2001). In research in the area of Trebević (Kulina, 2001) the fruit of wild pear had an average of 5.36 seeds and in research of Paunović et al., in Serbia (2012) it contained 3.89 developed seeds. The analysis of wild pear population at the Kozara site showed an average of 5.35 seeds in fruit. By the number of seeds, the accession PCK3 is set apart, and the fruit is distinguished by more than two seeds of embryos in a single seed box, resulting in fruit with more than 10 seeds per fruit. The number of fruit seeds is characterized by high variability. In addition to the PCK3, the accessions PCK4, PCK1 and PCK8 can be separated by a relatively high number of seeds. The aforementioned accessions should be the subject of selection of rootstocks mother trees with further testing of seed germination and seedling characteristics.

Table 5. Fruit quantitative traits of in situ wild pear accessions

Accessions	Year	Fruit					
		Fruit shape index	CV (%)	Peduncle length (cm)	CV (%)	Number of seeds in fruit	CV (%)
PCK1	2012	0.92±0.08	8.37	2.34±0.30	13.02	5.81±2.10	36.21
	2016	0.87±0.05	5.86	1.84±0.20	11.04	5.88±1.36	23.15
PCK2	2012	0.90±0.06	6.55	2.16±0.33	15.24	3.94±1.57	39.85
	2016	0.88±0.03	3.39	3.70±0.28	7.58	5.63±2.39	42.51
PCK3	2012	1.17±0.06	5.35	3.62±0.41	11.25	8.94±1.24	13.84
	2016	1.25±0.08	6.19	2.50±0.32	12.79	9.19±1.91	20.74
PCK4	2012	0.89±0.05	5.56	1.55±0.48	30.95	7.63±1.02	13.44
	2016	0.86±0.07	7.74	1.72±0.26	14.97	6.81±1.94	28.47
PCK5	2012	0.85±0.04	5.11	3.00±0.29	9.77	3.69±1.45	39.26
	2016	0.80±0.03	3.49	3.02±0.32	10.65	4.13±1.78	43.25
PCK6	2012	0.88±0.04	4.54	3.09±0.39	12.46	2.31±2.24	96.98
	2016	0.92±0.08	9.20	3.23±0.32	9.99	1.81±0.98	54.13
PCK7	2012	0.88±0.04	4.18	2.14±0.31	14.39	3.88±2.16	55.65
	2016	0.85±0.03	3.51	2.83±0.25	8.76	6.56±1.59	24.23
PCK8	2012	0.90±0.06	6.23	2.75±0.35	12.64	4.69±1.96	41.75
	2016	0.87±0.05	5.68	2.89±0.42	14.54	6.75±2.72	40.30
PCK9	2012	0.87±0.03	3.25	3.37±0.22	6.64	3.56±1.46	40.96
	2016	0.85±0.03	3.40	3.61±0.41	11.36	4.69±1.89	40.27

The values of fruit peduncle length were between 1.55 cm and 3.70 cm. Four accessions had relatively long fruit peduncle longer than 3 cm in average. The PCK9 had the longest peduncle. Most of the fruit peduncles were longer in 2016.

Conclusions

The Balkan Peninsula is considered a secondary centre of genetic diversity of wild pear, and Kozara locality is one of the more important areas of wild pear genetic diversity. The wild relatives of cultivated varieties represent an important part of plant genetic recourses of a country. The research of wild pear population in Kozara locality shows the great population diversity which can be highly significant in breeding programs of pear varieties and rootstocks. As a significant starting material for breeding work, several accessions can be set apart. Based on the number of seeds in the fruit in the pre-selection of seedling rootstocks for pears accessions PCK3, PCK1, PCK4 and PCK8 can be used. For the breeding of new varieties of pears on the basis of the fruit characteristics (mass and shape) the PCK9, PCK4, PCK8 and PCK3 are suitable.

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POMOMETRIC PROPERTIES OF POMEGRANATE (*PUNICA GRANATUM* L.) IN HERZEGOVINA REGION (BOSNIA AND HERZEGOVINA)

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Abstract

Pomegranate (*Punica granatum* L.) has long been domesticated in our area and it is rightfully considered as one of the autochthonous species of Herzegovinian karst. In lower karst areas, pomegranate occurs in various types of habitats and grows individually or in larger and smaller groups, forming pure populations or populations mixed with other species such as spruce, Jerusalem thorn and others. Wild pomegranate, known under the names *ljutun* and *ljutunac* (*P. granatum* var. *spontanea*) is different from cultivated pomegranate or *sladun*, *sladunac* (*P. granatum* var. *sativa*). A study of the wild-grown population of pomegranate was conducted at several sites in Herzegovina during two vegetation years, 2013 and 2014. In this study, the following pomegranate fruit properties were measured in 11 accessions (phenotypes) from the natural population: fruit weight (g), arils weight (g), weight of 100 arils (g), peel weight (g), peel thickness (mm), fruit height (mm), fruit width (mm), fruit crown height (mm), and fruit crown width (mm). Portions of peel and arils (%) were also determined. The average fruit weight ranged from 73.56 g to 114.82 g. The weight of most samples was higher in 2014 than in 2013. The average arils weight ranged from 32.87 g to 56.76 g. The average portion of arils in 2013 was 41.68% and 50.15% in 2014. The obtained data indicate significant effects of environmental conditions in individual years on properties of the same accessions at some locations, but also significant interaction effects between locations, years and accessions. The year factor had a greater influence on physical parameters of the wild pomegranate fruits than the location factor.

Keywords: *Wild pomegranate (Punica granatum L.), Location, Environmental conditions, Pomometric properties.*

Introduction

Pomegranate (*Punica granatum* L.) has long been domesticated in our area and it is rightfully considered as one of the autochthonous species of Herzegovinian karst, where along with fig and olive represents one of the oldest cultivated fruit species of the Mediterranean and Middle East regions. According to literature data, pomegranate is native to a region of Iran, Afghanistan, the Urals, the southern part of the Caspian Sea and from these areas it spread to the areas around the Mediterranean Sea (Kušan, 1938). Pomegranate was most probably brought to our region and surrounding areas by the Greeks and Romans. Pomegranate is widely distributed along the Adriatic Sea and in river valleys dominated by the Mediterranean influence, the rivers of Neretva in Herzegovina, Krka in Dalmatia and Zeta in Montenegro (Redžić, 1952). In Bosnia and Herzegovina, wild pomegranate is found around Mostar, Blagaj, Stolac, Ljubuški, Čitluk, Grude, Čapljina, part of the Široki Brijeg municipality, Neum and Trebinje. Pomegranate grows from the sea coast up to 580 meters above sea level. The most favorable conditions for growth and development are at altitudes from 20 to 150 meters above sea level, where populations of wild pomegranate are also the most numerous (Ćemalović, 1979).

The fruits of wild pomegranate are smaller than the fruits of cultivated pomegranate. It is a heliophytic plant species that grows alone or in sparse densities in insolated places.

Populations of wild pomegranate predominantly occur on skeleton texture soils of neutral to alkaline pH reaction (Ćemalović, 1979). Pomegranate fruit, root, tree bark, seeds and flower can be used for various purposes. Pomegranate tree bark is rich in tannins and pectins, so it is used for production of pectin, and in leather industry for tanning leather (Džubur, 2007). The beneficial effect on human health is attributed to the content of polyphenolic compounds, particularly anthocyanins, which are characterized by high antioxidant activity and are involved in the neutralization of free radicals responsible for many acute and chronic diseases (Lansky and Newman, 2007, Adhami and Mukhtar, 2006, Reddy *et al*., 2007). Wild pomegranate fruit is not used as table fruit, but only for processing into juices. In the process, it can be blended with cultivated pomegranate juice and grape juice. Pomegranate juice is consumed fresh, in sorbets, it is used for making jellies, cold or warm sauces. Wild pomegranate is morphologically different from cultivated pomegranate varieties (Ćemalović, 1979). The aim of this study was evaluation of the studied wild pomegranate accessions in selected locations of Herzegovina based on pomometric description criteria.

Material and Methods

A study of the wild-grown population of pomegranate was conducted during two vegetation years, 2013 and 2014, in several locations in Herzegovina: Mostar, Počitelj, Međugorje, Stolac and Čapljina. Selection of bushes was carried out on the basis of morphological characteristics of bushes and fruits. A total of 11 wild pomegranate accessions (phenotypes) from the natural population were selected, marked with letters and numbers in the following order: RK1 and RK2 (Mostar), PO1 and PO2 (Počitelj), MK and MNJ (Međugorje), PB1, PB2, PNJ1 and PNJ2 (Stolac) and TR (Čapljina).

Harvesting of wild pomegranate fruit was carried out at the stage of full ripeness in the first half of November. Ten approximately equal wild pomegranate fruits were taken from each of the selected bushes. They were brought to the laboratory, where pomometric measurements of the fruits were conducted. The following wild pomegranate fruit properties were measured in this study: fruit weight (g), arils weight (g), weight of 100 arils (g), peel weight (g), peel thickness (mm), fruit height (mm), fruit width (mm), fruit crown height (mm), fruit crown width (mm). Fruit weight, arils weight and peel weight were measured on technical scale (Kern 440-45). Fruit height, width, crown height and width, peel thickness were measured by digital slide rule (Hm Müllner werkzeuge). Fruit height was measured from fruit top (crown) to fruit peduncle and fruit width was measured transversely to the previous measurement. Portion of peel (%) was calculated from the ratio of peel weight to fruit weight and portion of arils (%) from the ratio of arils weight to fruit weight.

Research data were statistically processed by variance analysis (ANOVA), and then the differences between mean values of the eleven analyzed accessions were examined by LSD test for all significant F tests with the level of significance $p \leq 0.01$. Variance analysis was performed for both study years, and after variance homogeneity tests, a common variance analysis was conducted for both years and for all analyzed properties. The statistical program SP SS 16 (*Statistical Package for the Social Sciences*) and MS Excel 2007 were used for data processing.

Results and Discussion

In both study years, the biggest average fruit height was found in accession PB1 (location Stolac) of 52.26 mm and smallest in accession PO2 (location Počitelj) of 44.42 mm (Table 1). Wani *et al*. (2012) reported the values of fruit height of wild pomegranate genotypes from 59.0 mm to 92.4 mm, while Muradoğlu *et al*. (2006) found average wild pomegranate fruit heights from 60.0 mm to 81.0 mm. A partial agreement can be established by comparing literature data with results of the conducted research and the differences are probably due to

different environmental conditions. Cultivated varieties are characterized by larger fruits, so it stands to reason that they have a greater fruit height. Thus, Gadže (2013) presented average fruit heights of the studied cultivated varieties from 67.18 mm to 85.83 mm.

The biggest average fruit width of 60.05 mm was determined in accession PNJ2 (location Stolac) in both years, while the smallest average fruit width of 50.46 mm was found in accession PO2 (location Počitelj) (Table 1), which coincides with the results of Muradoğlu *et al*. (2006) who reported the average wild pomegranate fruit width values from 30.8 mm to 88.9 mm and Wani *et al*. (2012) from 65.4 mm to 98.4 mm. Different fruit width data were found for cultivated varieties. Thus, Mars and Marrackhi (1999) described average pomegranate fruit widths from 57.6 mm to 111.4 m and Gadže (2013) from 75 mm to 101.12 mm.

The biggest average fruit crown height for both years was found in accession TR (location Čapljina) and it amounted to 14.18 mm, and the smallest average height of 11.91 mm was measured in accession RK2 (location Mostar) (Table 1). Muradoğlu *et al*. (2006) presented crown height values ranging from 11.0 to 26.1 mm, Tehranifar *et al*. (2010) reported the values in the range from 13.45 mm to 24 mm. In cultivated pomegranate varieties, Gadže (2013) presented the values from 17.46 mm to 22.07 mm. It can be concluded that the crown height values of studied wild pomegranate accessions were closer to the lower values of the above-mentioned authors.

The biggest average value of fruit crown width of 16.69 mm was measured in accession PO1 (location Počitelj), and the smallest of 12.73 mm in accession RK2 (location Mostar) (Table 1). Muradoğlu *et al*. (2006) reported the crown width values in wild pomegranate from 11.2 mm to 18.1 mm, which coincides with the results of these studies.

The biggest average peel thickness of 3.44 mm was found in accession TR (location Čapljina), and the smallest of 2.39 mm in accession RK2 (location Mostar) (Table 1). For average wild pomegranate peel thickness, Ćemalović (1979) registered the values from 2 to 4 mm, which means that the peel thickness from this research had similar values to the above-mentioned values. Wani *et al*. (2012) reported the peel thickness in wild pomegranate genotypes between 1.81 mm and 3.64 mm, and Muradoğlu *et al*. (2006) from 1.7 mm to 4.0 mm.

Fruit weight is genetically determined, but also influenced by the climatic factors, as well as substrate (soil) on which the wild pomegranate bush grows. The biggest average fruit weight was found in accession PNJ2 (location Stolac) and it was 114.82 g and the smallest value of 73.56 g was measured in accession PO2 (location Počitelj) (Table 1). In his research, Ćemalović (1979) described the wild pomegranate fruit weight as ranging from 30 to 230 g, which is in agreement with the results of this research. Tabur *et al*. (1987) reported the fruit weight values from 175 g to 259 g, and Muradoğlu *et al*. (2006) reported the wild pomegranate fruit weight values in the range from 131.6 g to 337.1 g, which are higher values in relation to the results of this research.

The biggest average arils weight of 56.76 g was found in accession PB1 (location Stolac), and the fruit of accession PO2 (location Počitelj) had the smallest average arils weight of 32.87 g (Table 1).

Table 1. Average values of pomometric properties of wild pomegranate fruit in two years (2013/14)

Sample	Fruit height (mm)	Fruit width (mm)	Fruit crown height (mm)	Fruit crown width (mm)	Peel thickness (mm)	Fruit weight (g)	Arils weigh t (g)	Weight of 100 arils (g)	Peel weight (g)
RK1	50.14 b	57.60b	12.75 cd	12.80 d	2.48 d	110.34 a	54.29 ab	**19.18 a**	50.77b
RK2	45.83 cde	54.25 c	11.91 d	12.73 d	2.39 d	85.01 c	48.45 b	17.99 a	40.09d
PO1	44.62 de	54.49 c	14.02 ab	**16.69 a**	3.43 a	85.77 c	36.25c	14.35 cd	48.48c
PO2	44.42 e	50.46d	12.93 bcd	13.99 c	2.75 c	73.56 c	32.87c	11.55 f	39.26d
MK	49.43 bc	57.10b	12.89 cd	14.06 c	2.65 cd	103.90 a	49.61b	12.26 ef	51.50b
MNJ	47.85 c	56.80bc	13.16 abc	15.08 b	2.91 b	100.84b	49.42b	15.42 bcd	49.62bc
TR	51.81 a	57.22b	**14.18 a**	16.12 a	**3.44 a**	112.50 a	54.41a b	15.06 bcd	55.71a
PB1	**52.26 a**	58.54 a	12.51 cd	13.93 c	2.83 b	114.67 a	**56.76a**	14.00 de	55.72a
PB2	49.59 bc	58.99 a	13.37 abc	14.40 bc	2.82 bc	112.95 a	52.24a b	13.86 de	55.31b
PNJ1	46.52 cd	54.72 c	13.94 ab	16.33 a	3.39 a	85.15 c	39.32c	15.29 bcd	44.71cd
PNJ2	51.80 a	**60.05 a**	13.34 abc	14.97 b	3.08 b	**114.82 a**	53.48a b	17.37 a	**60.71a**

* Different letters within columns indicate significant differences between the samples based on LSD test (P= 5%; P= 1%)

Ercan *et al*. (1992) reported the arils weight values of studied pomegranate genotypes in the range from 34.0 g to 63.6 g, and Zarei *et al*. (2011) the value of 136.13 g. Gadže (2013) reported that the average arils weight in cultivated pomegranate varieties ranged from 145.01 g to 274.44 g.

The biggest average weight of 100 arils in both years of 19.18 g was in accession RK1 (location Mostar), and the smallest weight of 100 arils of 11.55 g was in accession PO2 (location Počitelj) (Table 1). It is apparent that both the smallest and the biggest average values of weight of 100 arils in these studies were lower in relation to the research of Muradoğlu *et al*. (2006) who described the values for weight of 100 arils of wild pomegranate in the range from 18.5 to 35.0 g. Drogoudi *et al*. (2005) pointed out that aril size is one of the most important factors affecting the quantity of juice and generally pomegranate edibility and reported values of weight of 100 arils from 17.5 g to 66.7 g.

The biggest average peel weight of 60.71 g was measured in accession PNJ2 (location Stolac), and the smallest of 39.26 g in accession PO2 (location Počitelj) (Table 1). Wani *et al*. (2012) reported the values of peel weight in wild pomegranate genotypes from 60 g to 217.75 g, Al-Maiman and Ahmad (2002) reported the value of 69.01 g for peel weight.

The portion of arils in fruits of the studied wild pomegranate accessions ranged from 34.62% to 56.45% with average values of 41.68% in 2013 and 50.15% in 2014 (Table 2).This is consistent with the results of Ercan *et al*. (1992) who presented the values from 43.2% to 62.5%, and Muradoğlu *et al*. (2006) with values from 49.5% to 71.5%.

Table 2. Portions of arils and peel in wild pomegranate fruit

Sample	2013		2014	
	Arils portion (%)	Peel portion (%)	Arils portion (%)	Peel portion (%)
RK1	48.86	51.14	52.27	47.73
RK2	54.53	45.47	56.45	43.55
PO1	34.62	65.38	46.39	53.61
PO2	39.40	60.60	48.59	51.41
MK	45.99	54.01	49.29	50.71
MNJ	43.87	56.14	53.50	46.51
TR	44.36	55.64	50.38	49.62
PB1	45.38	54.62	52.42	47.58
PB2	46.66	53.34	47.80	52.21
PNJ1	46.86	53.14	45.37	54.63
PNJ2	46.84	53.16	49.22	50.78
Average value	**41.68**	**54.79**	**50.15**	**49.85**

The portion of peel in wild pomegranate fruit ranged from 43.55% to 65.38%, and the average values were 54.79% in 2013 and 49.85% in 2014 (Table 2). The results generally correspond to the wild pomegranate peel portion data reported by Wani *et al*. (2012) from 32.6% to 56.05%, while Tabur *et al*. (1987) indicated slightly smaller portion of peel from 28.0% to 39.2%. The ratio of peel and arils or arils yield is a very important property of fruit, and especially in fruits for industrial processing. In this research, the best yields were established in accessions RK1 and RK2 (location Mostar), above 50%, and the lowest in accession PO1 (location Počitelj), less than 50%.

Conclusions

The highest values for fruit weight, peel weight and fruit width were determined in accession PNJ (location Stolac), and fruit height and weight of all arils for accession PB1 (location Stolac), while weight of 100 arils had the highest average value in accession RK1 (location Mostar). The lowest average values for fruit weight, fruit height, fruit width, peel weight, weight of all arils and weight of 100 arils were found in accession PO2 (location Počitelj). The properties of crown height, crown width, and peel thickness had the lowest average values in accession RK2 (location Mostar). For the properties fruit crown height and width, there was no significant difference between the two study years, which indicates that these properties are mostly genetically determined. It should be noted here that the environmental conditions for growth and development of wild pomegranate were favorable in both study years with the fact that the precipitation conditions were more favorable in 2014. It should also be noted that the year factor had a greater influence on pomometric properties of wild pomegranate than the location factor, as well as the fact that the studied locations did not significantly differ in values of climate indicators.

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VEGETATION OF THE CLASS *STELLARIETEA MEDIAE* IN THE "LIJEVČE POLJE" AREA IN NOTHERN BOSNIA AND HERZEGOVINA

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Abstract

This paper shows the results of two-year research (2013-2014) of the weed vegetation of the Lijevče polje area which includes four associations: *Panico-Galinsogetum parviflorae* Tüxen at Becker 1942, *Panico-Portulacetum oleraceae* Lozanovski 1962, *Cynodono-Sorghetum halepense* (Laban 1974) Kojić 1979 and *Erigerono-Setarietum glaucae* Šumatić 1997. Floristic-phytosociology researches were performed by the principles and methods of the Switzerland-French phytosociologic school on 61 localities from the area of municipalities Gradiška, Laktaši, Srbac and Kozarska Dubica in Nothern Bosnia and Herzegovina where 49 relevés were taken. Vegetation research included taking of relevés on test area of 100 m^2: space in row and between rows in orchard and vineyard, in grain crops and stubble from the whole area, root crops, and space between the rows. Relevés in orchards and vineyards on the territory of Lijevče polje were taken in neglected orchards and vineyards and in vineyards which are extensively processed and relevés in thick set crops and stubble were taken from parcels where herbicides were used, as well as from parcels where the application of herbicides was reduced. Also, relevés in root crops were taken in the same manner. Weed flora covered with four plant communities consists of 91 species. Results of correspondence analysis of all association shows similarity of stands association do not stand out subassociations. By projecting the plant communities on the mail components (Principal Component Analysis) and comparting them to the ecological indices (variables), it shows that *Panico-Galinsogetum parviflorae* depends on humidity the (F) and the chemical reaction (R), while *Panico-Portulacetum oleraceae* shows the dependence on the nutrients (N). Association *Cynodono-Sorghetum halepense* shows the dependence on the temperature (T) and the light (L). Association *Erigerono-Setarietum glaucae* is separated because it develops on the stubble.

Keywords*: weed vegetation, ecological indices, Lijevče polje*

Introduction

Weed flora and vegetation mostly depends of ecological conditions of the particular area, the kind of the crop and applied agrotehnical measurments (Shrestha *et al*., 2002, Barać and Garić, 2004). There are several data about weed vegetation of the particular parts of the Balkan Peninsula (Kojić *et al*., 1982; Hadač *et al*., 1997; Dujmović-Purgar and Hulina, 2004; Šilc, 2005; Kropáč, 2006).

The weed vegetation has not been thoroughly researched in the area of Bosnia and Herzegovina and Republika Srpska. In a study of weed vegetation of the Pannonian basin in the Republic of Srpska in row crop, Šumatić (1997) noted association *Panico-Galinsogetum parviglore* Tüxen et Becker 1942, which has distinguished *Panico-Galinsogetum ambrosiosum artemisifoliae* facies, and associations *Galeopsi-Calistegietum sepii* Stepić 1984 and *Erigerono-Setarietum glaucae* (new ass.) in small grains, which is the first serious study of weed vegetation in this area. Weeds in orchards also have been neglected until the research

Mitrić (2004). Kovačević (2008) has been doing research of the vegetation of weeds and vineyards in Bosnia and Herzegovina and found eight associations.
Studying of the weed vegetation in the area of Lijevce Polje is neglected. The last serious research of the vegetation of weed in Lijevce has been done by Kovačević (1956) where two associations are noted. In the grains society we have *Trifolium arvense-Scleranthus annuus* with 62 weed species in grains, and as far as root crops are concerned, there are 64 species under association of *Oxaleto-Chenopodietum polyspermi medioeuropeaneum*. The aim of the paper is to analyze the weed vegetation in the Lijevče polje area from the floristic, ecological and phyto-geographical aspects.

Material and methods

Floristic-phytosociology researches were performed by the principles and methods Braun-Blanquet (1964). The research includes 61 regions from the area of four municipalities (Gradiška, Laktaši, Srbac and Kozarska Dubica) in Bosnia and Herzegovina where 49 relevés were taken. Vegetation research in the field included taking of relevés on test area of 100 m^2: space in row and between rows in orchard and vineyard, in grain crops and stubble from the whole area, root crops, and space between the rows. Relevés in orchards and vineyards on the territory of Lijevče polje were taken in neglected orchards and vineyards and in vineyards which are extensively processed and relevés in thick set crops and stubble were taken from parcels where herbicides were used, as well as from parcels where the application of herbicides was reduced. Also, relevés in root crops were taken in the same manner.
Determination of the plant species was performed according to Josifović (1970), Domac (1994) and Javorka and Csapody (1979). Taxonomy and the nomenclature were given according to Josifović (1970). Ecological optimum for each weed species was determined according to Kojić *et al*. (1997). There are ecological indices for the soil humidity (F), chemical reaction of the soil (R), content of nitrogen in the soil (N), light (L) and temperature (T). Statistical processing of the data was dane by using the program BioDiversity pro (1998) and SPSS 21.0 (2013). Phytosociologic differentation of the associations included the numerical classification which was completed by corrresponence analysis (Sneth and Sokal, 1973).

Results and discussion

In order to have a better control of weeds in the agroecosystem, it is necessary to have knowledge about their floristic composition as well as the characteristics and dynamics of their communities in comparison with ecological conditions which dominate particular area (Kovačević *et al*., 2017). Based on the two-years long floristic-phytosociology research it can be concluded that the weed vegetation in Lijevče polje area consists of four communities.
Weed flora of four plant communities consists of 91 species. Species that have higher degree of presence (II-V) and higher cover value are shown in Table 1.

Table 1. Synoptic table of the plant communities in Lijevče polje area.

Associations*	Pan-Gal	Pan-Port	Cyn-Sorgh	Erig-Seta
Number of species	71	52	34	67
	DP–A**			
Characteristic species of the associations				
Panicum crus-galli L.	V^{+-4}	V^{+-3}	II^{+-3}	II^{+-1}
Setaria glauca (L.) P. B.	III^{+-3}	III^{1-3}	II^{+-3}	V^{+-4}
Sorghum halepense (L.) Pers.	II^{+-2}	II^{+-4}	V^{+-5}	II^{+-5}
Cynodon dactylon (L.) Pers.	I^{2-3}	I^{2-3}	V^{+-4}	I^{4}
Galinsoga parviflora Cav.	IV^{+-2}	III^{+-3}		II^{+-2}
Portulaca oleracea L.	I^{+-2}	III^{+-3}		I^{+}
Erigeron canadensis L.	I^{+}			III^{+}

Other species				
Ambrosia artemisifolia L.	V^{+-3}	V^{+-2}	IV^{+-3}	IV^{1-5}
Convolvulus arvensis L.	V^{+-4}	II^{+-4}	II^{1-4}	IV^{+-4}
Amarantus retroflexus L.	II^{+-2}	III^{+-2}	II^{+-1}	II^{+}
Daucus carota L.	II^{+-2}	I^{+-2}	II^{+}	III^{+-2}
Polygonum lapathifolium L.	II^{+-2}	I^{+-2}	II^{+}	III^{+-1}
Chenopodium album L.	II^{+-1}	I^{+}	II^{+-1}	III^{+}
Plantago mayor L.	I^{+-4}	II^{+-4}	I^{+}	II^{+}
Roripa sylvestris L.	II^{+-1}	I^{+}	II^{+}	I^{+}
Calystegia sepium (L.) R.BR.	II^{1-3}	I^{2}	I^{2}	I^{1}
Ranunculus repens	I^{+-1}	I^{1}	I^{+}	II^{+-2}
Agropyrum repens (L.) Beauv.	I^{1}	I^{1}	I^{1}	II^{+-1}
Anagalis arvensis L.	I^{+}	I^{+}	I^{1}	II^{+}
Sonchus oleraceus L.	I^{+}	I^{+}	I^{+}	II^{+}
Digitaria sanguinalis (L) Scop.	II^{+-2}	I^{+-2}		II^{+-2}
Stenactis annua (L.) Ness.	II^{+-2}	I^{+-2}		II^{+}
Rumex obtusifolius L.		I^{+}	II^{+-2}	I^{1-3}
Polygonum aviculare Agg. L.	I^{1}	I^{1}		II^{+-1}
Verbena officinalis L.	I^{+}		I^{+}	II^{+}
Achillea mellefolium L.	I^{+-2}	II^{1-2}		
Abutilon theophrasti Medic.			II^{+-1}	I^{+}
Cirsium arvense (L) Scop.			II^{+}	I^{+-1}
Oxalis stricta L.	I^{+}			II^{+}
Viola arvensis Murr.	I^{+}			II^{+}
Prunela vulgaris L.	I^{+}			II^{+}
Myosotis arvensis (L.) Hill.	I^{+}			II^{+}

Species that have a degree of presence I are: *Symphytum officinale* L., *Trifolium pratense* L., *Poa annua* L., *Rubus caesius* L., *Papaver rhoeas* L., *Erodium cicutarium* (L.) L'Herit., *Raphanus raphanistrum* L., *Rumex acetosell0a* L., *Crepis setosa* Mall., *Sonchus arvensis* L., *Silene alba* (Mill.) E. Krause, *Verbascum blattaria* L., *Crepis capillaris* (L) Wallr., *Cychorium intybus* L., *Setaria viridis* (L.) P. B., *Medicago lupulina* L., *Epilobium hirsutum* L, *Cirsium eriophorum* (L.) Scop., *Stachys palustris* L., *Artemisia vulgaris* L., *Lysimachia vulgaris* L., *Veronica polita* Fr., *Datura stramonium* L., *Origanum vulgare* L., *Picris hieracioides* L., *Stachys officinalis* (L.) Trev., *Trifolium arvense* L., Rumex crispus L., *Bidens tripartitus* L., *Vicia cracca* L., *Sinapis arvensis* L., *Torilis arvensis* (Huds.) Link., *Matricaria inodora* L., *Galium mollugo* L., *Festuca arundinacea* Schreb., *Stachys annua* L., *Xanthium strumarium* L., *Equisetum arvense* L., *Leucanthemum vulgare* Lam., *Taraxacum officinale* Web., *Mentha arvensis* L., *Potentilla reptans* L., *Lamium purpureum* L., *Trifolium repens* L., *Epilobium palustre* L., *Solanum nigrum* L., *Lythrum salicaria* L., *Mentha longifolia* (L.) Huds, *Stellaria media* (L.) Vill., *Holcus lanatus* L., *Plantago lanceolata* L., *Lactuca serriola* L., *Humulus lupulus* L., *Scrophularia scopoli* Hoppe, *Veronica hederifolia* L., *Sonchus asper* (L) Hill., *Capsella–bursa pastoris* (L) Medic., *Bellis perennis* L. and *Ranunculus arvensis* L.

Legend:

*Pan-Gal-*Panico-Galinsogetum parviflorae*; Pan-Port-*Panico-Portulacetum oleraceae*; Cyn-Sorgh-*Cynodono-Sorghetum halepense*; Erig-Seta-*Erigerono-Setarietum glaucae* **DP-degree of presence (I-V), A (+,1,2,3,4,5)-abundance of the species

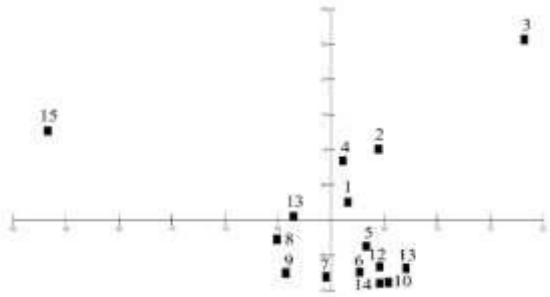
Ass. *Panico-Galinsogetum parvflore* Tüxen et Becker 1942 is widespread in row crops and growing crops (Šumatić 1997; Mitrić 2004; Kovačević *et al*. 2017). In the area subjected to research the association *Panico-Galinsogetum parvflore* Tüxen et Becker 1942 takes an important place in weed vegetation in Lijevče polje area. It can be found in orchards, vineyards, small grains and row crops. Results of correspondence analysis (Figure 1a) show certain similarities of stands association and any subassociations do not stand out. Stands 3 and 15 are singled out from other stands.

Ass. *Potrulacetum oleraceae* Lozanovski 1962 is firstly described in Macedonia. Kovačević *et al*. (2017) described this association in vineyards of Bosnia and Herzegovina. Stands of this association appear in various row crops but in growing crops, too. In weed vegetation of Lijevče polje area this association is row crops such as corn and watermelon and in orchards which are extensively farmed. Correspondent analysis shows a certain similarity of the floristic composition of association stands where stands 9 and 10 are at a distance from the

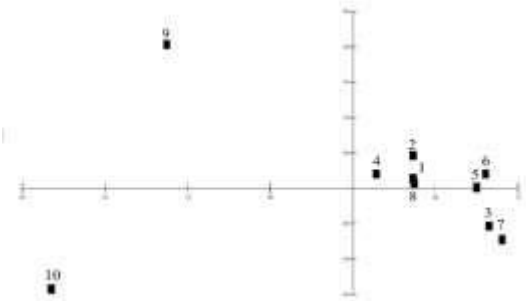
groups of stands in in a coordination diagram. (Figure 1b). This distance of stands is due to the fact that they were registered in the orchard, while others were registered in row crops and small grains.

Ass. *Cynodono-Sorghetum halepense* (Laban 1974) Kojić, 1979 was described in the most details in Kosovo (Kojić and Pejčinović 1982). Stands of association usually occur in row of orchards, as well as in row crops such as corn. In vineyards of Bosnia and Herzegovina this association was described by Kovačević *et al*. (2015). In Lijevče polje area the association is typically present in corn crop, soy crop, beans crop, and in row of orchards. Correspondent analysis showed similarity in terms of floristic features between the first group of stands under the numbers 1-8 and the second group numbered 9-13. Stands 9, 10 and 13 stand out from the rest (Figure 1c).

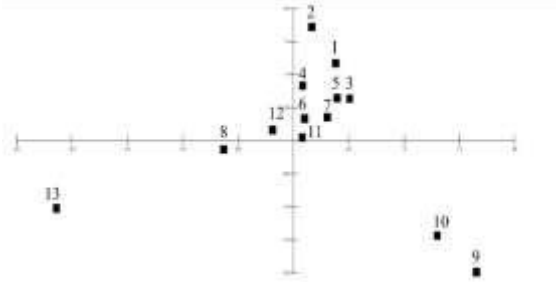
Ass. *Erigerono-Setarietum glauce* Šumatić 1997 was primarily described as the weed community of stubble in Semberija region, all the way to Doboj. Also, this association was described in vineyards of Bosnia and Herzegovina (Kovačević *et al*., 2017). In weed vegetation of Lijevče polje area this association is widespread on stubble, row crops (corn) and in orchards. Results of correspondent analysis confirm the similarity of the floristic composition. Stands 6 and 12 stand out from the other stands (Figure 1d). Stand 12 was registered in an orchard and is floristically richer than the stands registered in row crops.



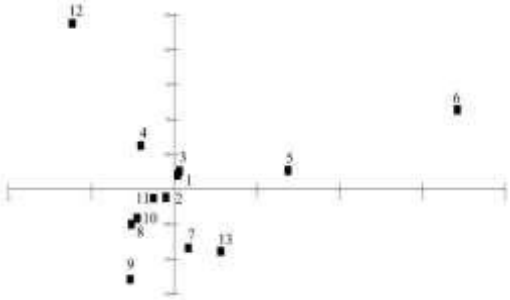
Panico-Galinsogetum parviflore (a)



Panico-Portulaceum oleraceae (b)



Cynodono-Sorghetum halepense (c)



Erigerono-Setarietum glauce (d)

Figure 1. The correspondence analysis of stands of four plant communities.

In terms of ecological relations between described plant communities, according to the ecological indices of the total floristic composition of each community and the established number and coverage of each species in the community, the statistical process of the data was undertaken. Starting from the correlation matrix of the average values of five ecological indices (F, R, N, L, T) for four communities described (Table 4, Table 5), and by applying the method PCA (Principal Component Analysis), the reduction of the beginning group of the changeable (ecological indices) is undertaken onto two main components which explain of the variability of the beginning the changeable.

Table 4. Matrix of Pearson's correlation coefficients of ecological indices.

Correlation	F	R	N	L	T
F	1.000	.906	.756	-.956	-.805
R	.906	1.000	.411	-.890	-.814
N	.756	.411	1.000	-.710	-.429
L	-.956	-.890	-.710	1.000	.619
T	-.805	-.814	-.429	.619	1.000

Table 5. Correlation of principal components with the initial variables.

Variables	Component	
	PC1	PC2
F	.252	.063
R	.232	-.479
N	.184	.923
L	-.239	-.162
T	-.209	.543

Biplot diagram (Figure 2) shows the ecological indices and it is given the projection of the some plant communities onto the main components (IBM SPSS, 2013). The sharp angles between the vectors show the positive correlation while the obtuse angle shows shows the negative correlation of the appropriate changables. So the values of the ecological indices F and R as well as N are positively correlated, and values of the indices L and T are negatively correlated.

Association *Cynodono-Sorghetum halepense* shows the dependence on the temperature (T) and the light (L), while association *Panico-Galinsogetum parviflorae* shows the dependence on the humidity (F) and the chemical reaction (R). Association *Panico-Portulacetum oleraceae* shows the dependence on nutrients (N), while association *Erigerono-Setarietum glaucae* is different because it grows on stubble.

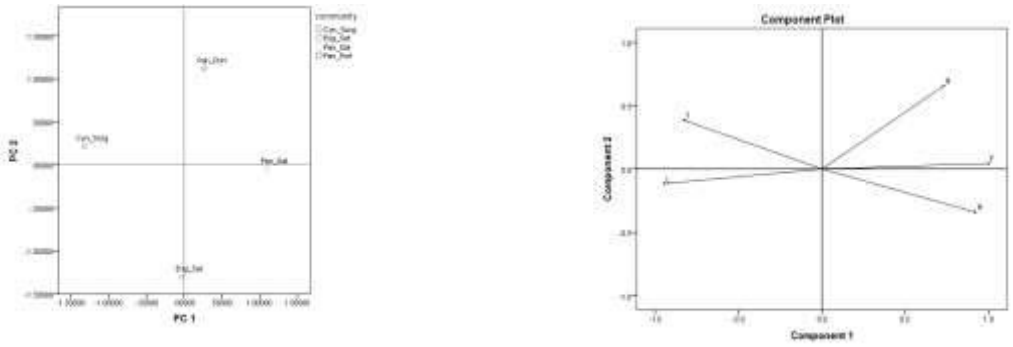


Figure 2. Biplot diagram of plant communities and ecological indices.

Conclusion

On the researched area, it was clarified that four weed plant communities that are characterized and clearly defined which is conditioned by reduced applying of the herbicides and generally weaker agronomic practices which reflected on the floristic difference since it is known that the intensive applying of the herbicides and the other agronomic practices leads to the significant changes of the structure and makes the biodiversity of the weed communities much poorer. This paper shows a comprehensive and fundamental research of weed flora in Lijevče polje area, considering that the latest research was conducted in 1956. Detailed

analysis of the weed vegetation should serve as a basis for successful planning and implementation of measures of weed control.

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EFFECTS OF PLANT DENSITY ON THE YIELD AND TOTAL PHENOLIC CONTENTS OF TARTARY BUCKWHEAT

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Abstract

Tartary buckwheat (*Fagopyrum tataricum* L., Gaertn) is an annual plant from Polygonaceae family. This species is mostly grown in Asia where it is used as a traditional food. In Bosnia and Herzegovina, Tartary buckwheat is grown sporadically and mostly in a mixture with Common buckwheat. In some plant species, increasing the planting density may be used to increase grain yield in crops. The aim of this study was to determine the impact of different planting densities on yield and total phenolic contents in kernel. During three study years (2011-2013), three different planting densities were used: 200, 300 and 400 seed per m^2. Experiment was set up by randomized block method in four repetitions at the village Donje Selo, near Ilijaš. The result showed that plant density did not have a significant effect on plant height, 1000-grain weight, hectoliter mass and phenol contents. Grain yields were significantly dependent on plant density and the year of the study. The lowest grain yield (1666 kg ha^{-1}) was recorded in the sowing variant with 200 seed per m^2 and the largest grain yield (2215 kg ha^{-1}) was recorded in sowing variant with 400 seed per m^2. Phenol contents in kernels depended on years of investigation and ranged from 1.07 to 13.94 mg g^{-1}.

Keywords: *tartary buckwheat, plant density, yield, total phenolic.*

Introduction

Buckwheat is an annual plant and belongs to the family Poligonaceae, which includes two cultivated species: common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*). In general, morphological characteristics and growing technology of tartary buckwheat are similar to common buckwheat, but there are some differences. Tartary buckwheat has many desirable characteristics such as higher seed yield, self-pollination ability, frost resistance, and overall plant vigor. Thanks to the chemical composition, grain of buckwheat is primarily used to obtain high quality flour. Unlike flour of wheat and other cereals, buckwheat's flour contains proteins with good balanced amino acids and have all of essential amino acids (Selimović et al., 2014). In addition to the grain, used to obtain different products, buckwheat recently became more popular due to the use of other plant parts (leaves and flowers) due to their positive impact on human health. Healing properties of buckwheat are derived from the content of the phenolic compounds. The routine has the highest representation of the total phenol. According to Golob et al. (2016) tartary buckwheat contains about a hundred times more rutin than common buckwheat. The ability of buckwheat to synthesize phenolic compounds has emerged as an evolutionary response to adverse conditions of success (Germ, 2004). Content of phenol in buckwheat depends on the species and the variety of buckwheat (Bystrická et al., 2010; Christa and Soral-Śmietana, 2008), altitude (Guo et al., 2001), location and weather conditions (Sakač et al., 2014 and Suzuki et al., 2005). It is believed that the use of buckwheat contributes to the alleviation or prevention of many today's common diseases.Consummation of buckwheat is considered to relieve and heal diseases such as diabetes (Kawa et al., 2003; Li et al., 2016), high blood pressure (Tomotake et al., 2001) and it has anticancer activity (Park and Park, 2004). Buckwheat is mainly produced in moderate climatic zones and in mountain regions. According to FAO data

(FAOStat, 2018), in 2016, it was cultivated on an area of 2.37 million hectares globally. In Bosnia and Herzegovina, Tatarary buckwheat is grown sporadically and mostly in a mixture with common buckwheat. According to 2018 data, B&H cultivation area of buckwheat is 1056 ha and its average yield is 1.07 t ha^{-1} (FAOStat, 2018). Generally, in the world, buckwheats sowing range per area unit ranges from 40 to 100 kg ha^{-1} and it depends on the way of sowing. Literature data is different when it comes to optimal number of plants per area unit. Researchers who worked with the plant density of buckwheat agreed that it significantly changed the yield and may also have an effect on the content of total phenols (Gavrić et al., 2016; Gadžo et al., 2007). In Bosnia and Herzegovina there is only a few scientific papers related to this type of research, thus the focus of this research has been set based on these facts. The aim of this study was to determine how different planting densities reflects on yield and total phenolic contents in kernel.

Material and Methods

Field experiments were conducted in 2011, 2012 and 2013 growing seasons in the village Donje Selo, near Ilijaš. Tartary buckwheat (*Fagopyrum tataricum*) originates from Luxembourg and it was used in this research. Factors of this experiment were three different planting densities: 200 (S1), 300 (S2) and 400 (S2) seed per m^2. Experiment was set up by randomized block method in four repetitions. The size of basic plot was 4,8 m^2. The seeds were sown when the soil temperature was10 ^oC and the sowing was done manually. During the vegetation weeds were removed manually. Plant height and yield were measured in the field. 1000-grain weight, hectoliter mass and phenol contents were determined at Laboratories of the Faculty of Agriculture and Food Science Sarajevo. The total phenolic contents of ethanolic extracts were measured using Folin Ciocalteau reagent as described by Bystrická et al. (2010). Statistical analyses were made using SPSS 22 software programs. The monthly average temperature and precipitation amount was monitored during the period of the research (2011-2013). Data from Meteorological station at Sokolac were used for weather condition analysis (Table 1). During the research period, especially during 2012, above average warm weather was recorded. In 2012, July was the hottest month with the temperature higher than a year-long average for 4.4°C. The temperature increase was accompanied by droughts, with small amounts of rain, especially during the summer when it was most needed for buckwheat. Drought was particularly pronounced during 2011 and 2012, during the period of flowering and grow of grain. The weather conditions in 2013 were more favorable compared to the other two years (2011 and 2012).

Table 1. Average monthly temperature and Amount of rainfall

	Month												
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	
Year	**Average monthly temperature (°C)**												**Average**
1961-1990	-4.8	-2.2	1.6	6.4	11.4	14.3	16.0	15.5	12.0	7.2	2.1	-2.8	6.4
2011.	-2.5	-2.4	1.8	7.7	11.0	15.8	17.5	18.2	15.5	6.4	0.8	-1.0	7.4
2012.	-4.5	-7.2	3.6	7.4	10.9	18.7	20.4	19.7	15.1	9.5	6	-2.8	8.1
2013.	-0.7	0.0	2.3	8.9	12.5	15.6	18.1	18.5	12.3	9.4	4.7	-1.6	8.3
	Amount of rainfall (mm)												**Amount**
1961-1990	53	50	56	62	73	84	73	70	66	66	84	71	805
2011.	30.2	27.6	28.8	42.7	123	62.8	82.2	9.2	36.7	59.6	23.7	100.1	626
2012.	90.8	100.6	23.6	93.7	205.1	11.6	77.6	0.6	59.4	67	50.7	80.7	861
2013.	126.1	138.9	71.7	44	121.5	41.4	28.4	70.6	108	78.5	70.8	5.7	905

Results and Discussion

The plant height is an important morphological characteristic that can effect on lodging crops and lead to loss of yield. The results of the research presented in Table 2 show that plant height has not been significantly dependent on years of research and plant density. During the first year of research (2011), buckwheat was higher for 11.3 cm. Higher plant height was probably caused by bigger amounts of rainfall at the beginning of this year. Similar results were obtained by Glamočlija et al. (2012), who recorded 8.2% lower plants at relatively unfavorable weather conditions (lack of rain) compared to those grown in favorable weather conditions. Similar observations have been also reported by many authors (Ikanović et al.,2013; Maletić and Jevđović, 2003).

Table 2. Plant height, 1000-grain weight and hectoliter mass

Plant density	**Plant height (cm)**				**1000-grain weight (g)**				**Hectoliter mass (kg)**		
	2011.	2012.	2013.	Average	2011.	2012.	2013.	Average	2012.	2013.	Average
S1	99.3	81.3	85.3	88.7^{ns}	17.4	15.8	15.5	16.2^{ns}	59.6	61.5	60.5^{ns}
S2	100.7	94.0	91.9	95.5^{ns}	17.7	15.6	15.2	16.1^{ns}	61.0	62.5	61.7^{ns}
S3	99.0	96.5	88.1	94.5^{ns}	17.6	12.1	15.2	15.0^{ns}	60.1	60.8	60.4^{ns}
Average	99.7^{ns}	90.6^{ns}	88.4^{ns}		17.6^{a}	14.5^{b}	15.3^{b}		60.2^{ns}	61.6^{ns}	

The 1000-grain weight, which is an indicator of grain yield, was significantly dependent on years of research. By observing years of research, the 1000-grain weight value ranged from 14.5 g (2012) to 17.6 g (2011). The lower 1000-grain weight, which has been noted in the second year of research (2012) was the result of lower percipitation levels. These results are similar to Alekseyeva et al. (2001). They found that different agro-ecological conditions in some years have a significant impact on this research feature. Considering sowing variants, the highest value of 1000 grain weight was recorded in S1 variant (16.2 g) and the lowest was in S3 variant (15.0 g). Observed plant density factor over the years of research statistically did not show significant effect on the 1000-grain weight, but it had a negative impact on its value. Hectoliter mass, which is the first indicator of grain quality ranged from 59.6 to 62.5 kg. Difference in hectoliter mass was under the influence of plant density and the years of research were random, which was not statistically significant.

Table 3. Grain yield and Phenol contents

Plant density	**Yield (kg ha^{-1})**				**Phenol contents (mg GAE g^{-1})**			
	2011.	2012.	2013.	Average	2011.	2012.	2013.	*Average*
S1	1308.0	1420.2	2272.5	1666.9^{b}	1.17	13.94	1.46	5.52^{ns}
S2	1722.7	1959.0	2242.7	1974.8^{ab}	1.09	12.52	1.49	5.03^{ns}
S3	2317.7	1862.2	2465.0	2163.6^{a}	1.07	9.24	1.51	3.94^{ns}
Average	1515.4^{b}	1747.1^{b}	2326.7^{a}		1.11^{b}	11.90^{a}	1.49^{b}	

Observing the results from Table 3, it can be concluded that yield significantly depended on the plant density and the year of research. Grain yield was in a positive relation with the sown density. Average grain yield in the S1 variant was 1666.9 kg ha^{-1}, which increased the plant density to 1974.8 (S2) and to 2163.6 kg ha^{1} (S3). With relative indicators, yield increase was 15.6% (S2) and 22.9% (S3). A similar effect of sowing density was observed in all years of

research. Many researchers have also reported higher grain yield at higher planting densities (Gavrić et al., 2017; Thakuria and Gogo, 2000; Singh and Arya, 1996).

This study showed that plant density increase had a positive effect on yield. However, sometimes, an increase in plant density can cause reduction of yield. Gadžo et al. (2007) and Vilcāns et al. (2013) found that by increasing the sowing density reduced the yield. They concluded that the higher planting density leads to lodging and then reduces yield.

In this work, significant yield variations also have been recorded for the years of research. The highest average yield was recorded in 2013 (2326.7 kg ha^{1}) and it was significantly higher than the yield in 2011 (1515.4 kg ha^{1}) and in 2012 (1747.1 kg ha^{1}). Reason why buckwheat achieved the highest yield in 2013 is due to relatively favorable weather conditions. Many studies have shown similar effects years of research on grain yield during the years of research (Glazova, 1998; Gavrić and Gadžo, 2011; Popović et al., 2014).

The results of this research shown in Table 3 show that the content of phenol depended on the years of research. During 2012 (11.9 mg GAE g^{-1} SM), all plant density variants had a high content of phenol and it was about ten times higher than in 2011 (1.11 mg GAE g^{-1} SM). High content was recorded in the year with higher average monthly temperature, and based on this, it can be concluded that the weather conditions are the cause of those differences. Lumingkewas et al. (2015) and Kreft et al. (2013) also concluded that temperature and humidity influenced phenol contents. The three-year average shows (Table 2) that total phenol contents did not significantly depend on plant density, but there are some differences. The lowest content of phenols (3,94 mg GAE g^{-1}) was recorded in S3 variant and the largest content (5.52 mg GAE g^{-1}) was recorded in S1 variant.

Conclusions

According to the presented results of the research, effects of plant density on yield and total phenolic contents of tartary buckwheat during the seasons of 2011, 2012 and 2014, the following conclusions can be reached:

- plant height, 1000-grain weight, hectoliter mass and phenol contents demonstrated no significant changes under the influence of plant density, but they depended on the year of research;
- the grain yield significantly depended on plant density and the year of research;
- the largest grain yield (2215 kg ha^{-1}) was recorded in sowing variant with 400 seed per m^{2};
- phenol contents in kernels depended on years of research;
- high content of phenols was recorded in the year with relatively unfavorable weather conditions.

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THE ACCUMULATION OF BIOMASS IN TRITICALE VARIETIES DEPENDING ON THE TREATMENT WITH PGRs AND DIFFERENT FERTILIZATION LEVELS

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Abstract

A field experiment was conducted during 2016-2018 growing season on the experimental field of the Crop Science Department at the Agricultural University of Plovdiv (Bulgaria). The experiment was arranged using the block method as split plot in three replications and plot size of 10 m^2. Comparative assessment of the biological productivity of the tested Trismart, Musala and Kolorit (standard) varieties was made between the variants with application of PGRs under the action of lower and higher fertilizer rate. The foliar application of the PGRs followed in BBCH 31. During the vegetation of triticale, fresh plants were taken at stages: tillering-leaves, spike emergence-leaves, stem, spikes, maturity- straw, spikes, grains, glumes. The results from the study showed, that the effect of the mineral fertilizer at stage steam elongation on the biomass accumulation of triticale was better expressed than the differences between the varieties. The lowest biological productivity at this growth stage during the years of the study was detected in all varieties treated with the lower fertilizer levels. The higher fertilizer level itself led to 7-8% increase of the dry biomass accumulation during the years of the study. Similar percentage increase was observed in the variants treated with higher fertilizer level in combination with the plant growth regulators. In the analysis of the varieties regarding the different fertilizer level, there were no differences in the organ distribution compared to the total biological yield and by all treatments the straw occupied the largest share, followed by the grain and glumes. The biological yield of triticale at maturity during the period 2016-2018 was mainly formed by the straw, whose share by the different treatments and varieties varied from 47.15 to 50.28 %.

Keywords: *Biomass accumulation, Fertilization, PGRs, Triticale, Varieties.*

Introduction

Cereals are worldwide the most important cultivated crops and account for the main sources of energy and protein in human and domesticated animal diets (Rajaram, 1995). The rising demand for food in developing countries due to the high population growth rates continues to be a significant problem worldwide. Many of the developing counties rely on the import of large quantities of cereal crops such as mainly wheat, rice, maize and barley to meet their food and feed grain needs (Curtis, 2002). In these countries an increase of the national cereal production can be achieved through increasing average yield per unit area or expanding the production area into more marginal lands (Mergoum et al., 2004). Under these environmental conditions triticale can be a viable alternative due to its ability to produce higher biomass and grain yield compared with other cereal over a wide range of soil and climatic conditions (Gulmezoglu et al., 2010; Hein and Waschl, 2015; Ion et al., 2015; Kurowski et al., 2013; Madic et al., 2015). Produced by crossing two different species - wheat and rye with the main goal to combine the superior agronomic performance and the end-use qualities of wheat with the stress tolerance and adaptability of rye, today triticale is cultivated on over than 4 million ha (FAO, 2016). With the aim to evaluate the influence of PRGs and different fertilization levels on the biomass accumulation of triticale varieties, a field experiment was conducted.

Material and Methods

In the period 2016-2018 a field experiment was set on the experimental field of the Crop Science Department at the Agricultural University of Plovdiv, Bulgaria. Three triticale varieties were used - the standard Kolorit, Musala and Trismart. The experiment was arranged using the block method as split plot in three replications and plot size of 10 m^2 planted in the sowing rate of 550 germinated seeds after predecessor sunflower. The action of the PGRs Vitafer Algi (VA) and Vitafer Green (VG) and the untreated (NT) control variants was traced under two different fertilizer levels $N_6P_5K_2$ and $N_{12}P_{10}K_4$. The PGRs were applied in the beginning of stem elongation (BBCH 31). To achieve the aim of the study, during the vegetation of triticale, fresh plants were taken by stages as follows: tillering – leaves; spike emergence-leaves, stems, spikes; maturity – straw (leaves + stems), spikes, grains, glumes. The plants were taken from ¼ m^{-2}, divided into organs, dried to an absolutely dry mass and weighed. The soil on the experimental field of the Agricultural university - Plovdiv has been determined as alluvium, which based on the international classification of FAO belongs to the category of Mollic Fluvisols (Popova et al., 2012). The soil in this region has been developed on sandy-clay and sandy-gravel quaternary deposits, with power of the humus horizon of 20-40 cm. The results were analyzed using the software package IBM SPSS Statistics 22. In order to establish statistically significant differences between the variants, a dispersion analysis (ANOVA) was applied.

Results and discussion

The biomass accumulation at growth stage stem elongation was reported at the end of the period (Fig. 1). In this phase were not observed significant differences between the examined varieties, as by the untreated with growth regulators variants the lowest biomass accumulation of 242.84 kg da^{-1} in 2016 was determined in Musala variety and the highest in the standard – 260.84 kg da^{-1}, while in 2017 the lowest biomass was recorded in the standard (350.64 kg da^{-1}) and the highest in Trismart variety (421.36 kg da^{-1}). In all of the studied varieties the application of the regulators led to rise in the biomass accumulation with circa 8 % compared to the untreated variants. The growth stage spike emergence was characterized by the intensive linear growth of the vegetative organs, which significant increased the biomass accumulation of the varieties. In Kolorit and Trismart varieties the biomass accumulation increase compared to the untreated with growth regulators variants varied between 6-7%, while in Musala variety the increase was 8 %, from 684.90 kg da^{-1} for the untreated to 788.09 kg da^{-1} for the treated with VA. In the second year as a result of the better climatic conditions the biomass accumulation of the untreated variants varied from 1065.25 kg da^{-1} in Trismart variety to 1137.68 kg da^{-1} in Musala variety, which is 6 % higher than the previous year. As a result of the application with VA in phase maturity the biomass accumulation rose with 192.56 kg da^{-1} in Kolorit variety, 145.30 kg da^{-1} in Musala variety and 136.56 kg da^{-1} in Trismart variety during the first year. Similar increase was observed as a result of the application of VG. In the second year Trismart variety reached the highest biomass accumulation of 1756 kg da^{-1} due to the VA application, while in Kolorit and Musala varieties the highest increase of respectively 1579.08 kg da^{-1} and 1691.73 kg da^{-1} was observed after the VG application.

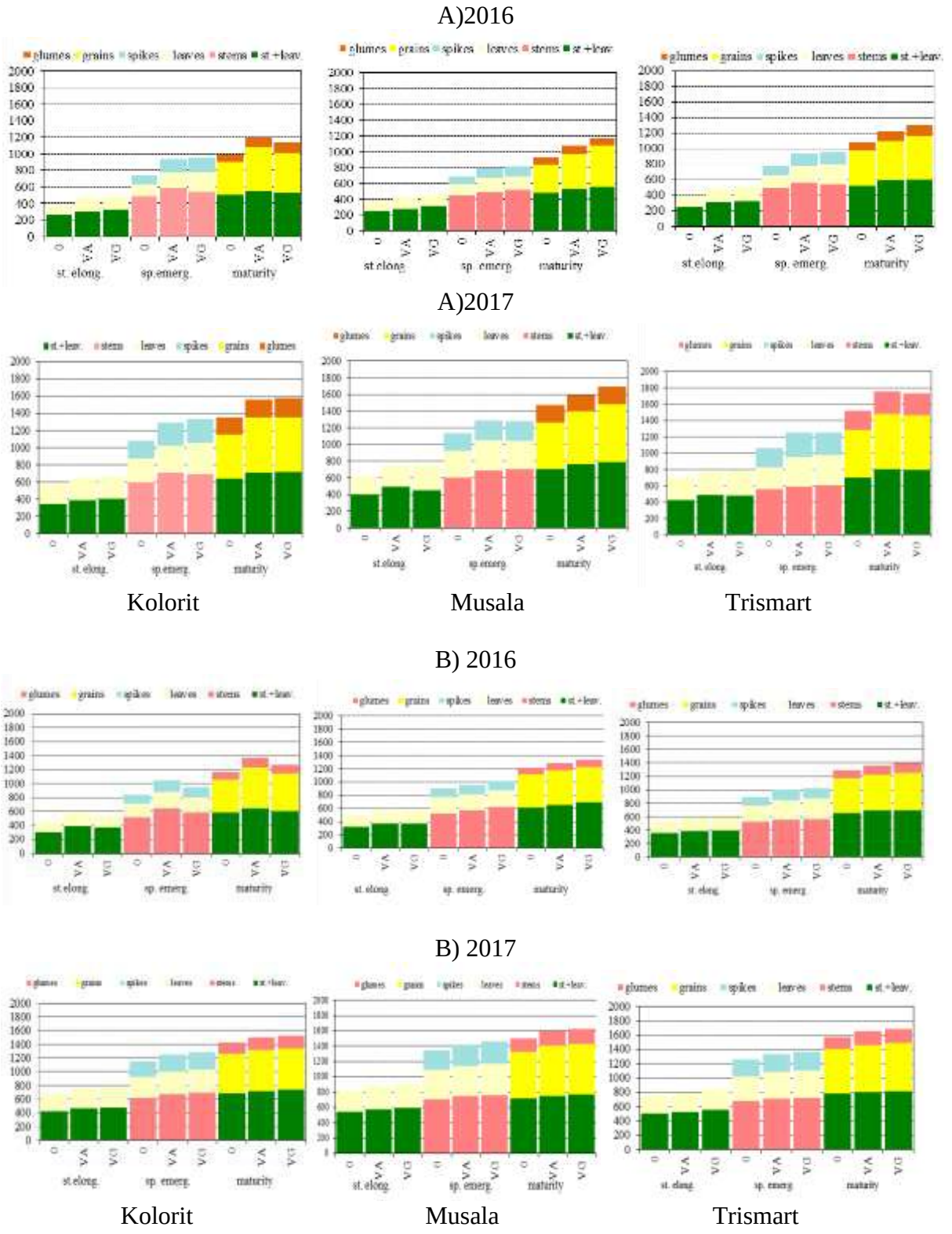

Figure 1. Dry biomass accumulation (A- lower fertilizer level; B-higher fertilizer level)

The results from the study showed, that the effect of the mineral fertilizer at stage steam elongation on the biomass accumulation of triticale was better expressed than the differences between the varieties. The higher fertilizer level itself led to 7-8% increase of the dry biomass accumulation during the years of the study. Similar percentage increase was observed in the variants treated with higher fertilizer level and the plant growth regulators, moreover at stage maturity during the first year of the study Trismart variety with yield of 1396.05 kg da^{-1} was

the most productive, than in Kolorit variety (1370.60 kg da^{-1}) and Musala variety (1334.01 kg da^{-1}). In the second year the tendency was the same and the combined treatment with the higher fertilizer level and VG led to the highest dry biomass accumulation in Trismart variety (1686.87 kg da^{-1}) and the lowest in Kolorit variety (1520.94 kg da^{-1}).

Table 1.Biomass yield in maturity average for the period 2016-2018 under higher fertilizer level, kg da^{-1}

Variety	Treatment	Biomass yield				% of the total yield		
		straw	grain	glumes	total	straw	grain	glumes
Kolorit	NT	637.56^{a}	481.41^{a}	165.45^{a}	1284.42^{a}	49.78^{b}	37.46^{a}	12.76^{b}
	VA	720.16^{b}	618.35^{b}	189.84^{b}	1528.35^{b}	47.15^{a}	40.45^{b}	12.40^{b}
	VG	713.59^{b}	597.26^{b}	187.13^{b}	1497.98^{b}	47.75^{a}	39.88^{b}	12.37^{b}
Musala	NT	696.21^{a}	520.62^{a}	172.12^{a}	1388.97^{b}	50.28^{b}	37.44^{a}	12.28^{b}
	VA	765.88^{b}	609.95^{b}	172.95^{a}	1548.78^{b}	49.50^{b}	39.37^{b}	11.13^{a}
	VG	765.29^{b}	613.31^{b}	168.29^{a}	1546.91^{b}	49.76^{b}	39.53^{b}	10.70^{a}
Trismart	NT	651.61^{a}	530.51^{a}	183.16^{b}	1365.30^{a}	47.90^{a}	38.93^{a}	13.16^{b}
	VA	725.96^{b}	619.17^{b}	215.68^{c}	1560.81^{b}	46.63^{a}	39.80^{b}	13.57^{c}
	VG	715.48^{b}	623.13^{b}	219.51^{c}	1558.13^{b}	45.93^{a}	40.17^{b}	13.90^{c}

*Values with the same letters do not differ significantly

In the analysis of the varieties regarding the different fertilizer level, there were no differences in the organ distribution compared to the total biological yield and by all treatments the straw occupied the largest share, followed by the grain and glumes (Tabl. 1 and 2). The biological yield of triticale at maturity during the period 2016-2018 was mainly formed of the straw, whose share by the different treatments and varieties varied from 47.15 to 50.28 %.

Table 2. Biomass yield in maturity average for the period 2016-2018 under lower fertilizer level, kg da^{-1}

Variety	Treatment	Biomass yield				% of the total yield		
		straw	grain	glumes	total	straw	grain	glumes
Kolorit	NT	635.00^{a}	526.75^{a}	135.57^{a}	1297.31^{a}	49.09^{c}	40.58^{a}	10.33^{a}
	VA	685.65^{b}	589.87^{b}	158.71^{b}	1434.23^{b}	47.79^{a}	41.19^{c}	11.02^{b}
	VG	670.36^{b}	570.83^{b}	154.02^{b}	1395.21^{b}	48.03^{b}	41.05^{b}	10.92^{b}
Musala	NT	665.04^{b}	552.96^{a}	137.67^{a}	1355.67^{a}	49.17^{c}	40.86^{b}	9.97^{a}
	VA	700.73^{c}	590.31^{b}	151.23^{b}	1442.27^{b}	48.77^{b}	40.94^{b}	10.29^{a}
	VG	729.89^{c}	596.74^{b}	154.87^{b}	1481.49^{b}	49.50^{c}	40.24^{a}	10.27^{a}
Trismart	NT	717.04^{c}	572.18^{b}	145.11^{a}	1434.33^{b}	50.04^{c}	39.93^{a}	10.03^{a}
	VA	745.66^{c}	592.61^{b}	167.40^{c}	1505.67^{c}	49.62^{c}	39.35^{a}	11.03^{b}
	VG	754.72^{c}	615.09^{c}	171.65^{c}	1541.46^{c}	49.01^{c}	39.94^{a}	11.05^{b}

*Values with the same letters do not differ significantly

The positive correlative interaction between the PGRs and fertilizer level, which generates yield increase was reported from many researches (Mac'kowiak et al., 2001; Starczewski, Bombik and Dopka, 2002; Wierzbowska, Sienkiewicz and Bowszys, 2010). The results indicated that the biomass formation of triticale as response of PGRs treatment would vary under the different fertilization levels.

Conclusion

At maturity the straw were with the largest share of the plant, followed by the grain and the spikes. However by the two fertilizer levels Trismart variety had the largest total biomass yield and the combined treatment with fertilizer and VG led to the highest percentage share of the grain (40.17%; 49.94%) compared to the Musala and the standard Kolorit varieties.

The foliar treatment with the both plant growth regulators can play an important role in triticale biomass yield formation. Plant treated with the PGRs under different fertilizer rates showed higher biomass accumulation compared to the control plants.

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ENHANCING THE ADAPTATION OF SUGAR APPLE AND CHERIMOYA TO SOIL CONDITIONS OF SOUTH LEBANON BY GRAFTING AND IRON FERTILIZATION

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Abstract

Annona is a subtropical crop of an increasing popularity in Lebanon. Its cultivation is concentrated along the southern coast where climate conditions are the most suitable. Soil analysis at this location revealed a dominance of calcareous-clay soils poor in iron. The paper describes the results of an experiment which evaluated the effect of iron fertilization on the vegetative growth of three annonacultivars obtained by self- or cross-grafting of sugar apple (*Annona squamosa*L.: Sq) and Cherimoya (*Annona cherimoya* Mill.:Ch): Ch/Ch,Sq/Sqand Sq/Ch and cultivated in South Lebanon. Annona scions were grafted on rootstocks of 12 months age and data collection covered various vegetative indicators during on 1 year, 2 years and 3 years old plants. Iron (Fe) was provided to plants with irrigation water once per month starting from April till October in year 1, 2 and 3 with of 30g/plant. Results showed an improvement of vegetative growth in all cultivars compared to control plants during the three years with superiority for the cross-grafted cultivar (Sq/Ch). Iron-fertilized Sq/Ch and Ch/Chcultivars had the highestplant height (around 175cm), number of primary roots (around 42), length of primary roots (27cm), while the cultivar Sq/Ch developed the highest trunk diameter (4.1cm), diameter of primary roots (3cm), leaf biomass (142g) and freshand dry weights of roots (85g and149g respectively).Finally, iron fertilization improved the tolerance of annona plants to the present soil conditions of the southern coast allowing a better performance of plants during their juvenile stage.

Keywords: *South Lebanon, annona, grafting, iron fertilization.*

Introduction

Annonas is a genus of tropical fruit trees belonging the family Annonacea (Chatrou, 1999). Territorial expansion of the family Annonaceae is limited due to specific environmental and soil requirements (Encina *et al*., 2014). In fact, soil characters are extremely important for annona cultivation (Nakasone and Paull, 1998). In some regions of the world, Annonas are grown in calcareous soils. A common problem in these soils is an inadequate supply of soluble Fe which results in Fe deficiency in many crops (Korcak, 1987; Tagliavini *et al.,* 1995) resulting in leaf chlorosis and growth and yield suppression (Zouari *et al.,* 2001). Consequently, a fertilization program based on preliminary soil analysis (Pinto *et al*., 2005) is a prerequisite for excellent seedling growth and the production of good quality fruit (Pinto, 1975). An adequate supply of nutrients should be established targeting cultivated species (Fuehring, 1973). On annona, the amount of fertilizer applied varies with age and stage of growth. Thus, iron fertilization done in the second year should be done on soil analysis while the one of the third year on leaf analysis basis (Silva and Silva, 1997).

Vegetative propagation is the usual way of propagating annona (Heenkenda *et al.,* 2009). Annonas are easily propagated by grafting although the success of grafting varies among species and is influenced by the scion and rootstock compatibility. For instance, *A. cherimoya* roostock is compatible with its own scion as well as with the one of *A. squamosa*, however *A.*

squamosa rootstock is compatible with its own scion but has a low compatibility with the one of *A. cherimoya* (Pinto *et al.,* 2005). Cherimoya (*Annona cherimola* Mill.) is erect with an upright fast growth (Gonzalez *et al.,* 2012) reaching about 5-9 m and can tolerate a wide range of soil types with moderate fertility. Sugar apple (*Annona squamosa* L.) is normally smaller than cherimoya attaining heights of 3-7 m and grows best on fertile soils (Nakasone and Paull, 1998; Folorunso and Olorode, 2006; SCUC, 2006; Orwa *et al.,* 2009).

Lebanon is a coastal Mediterranean country characterized by various types of soils namely red and brown soils, sandy soils, heavily weathered fersialitic soils of volcanic origin (Chalak and Sabra, 2007). In Lebanon, Annonas are mainly cultivated in South Lebanon at an altitude of 0-200 meters above sea level (Yassine and Sassine, 2014) where calcareous soils are dominant (Louay, 1997). These soils are characterized by low content in organic matter and deficiency in nitrogen, zinc and iron (Fuehring, 1973).

Therefore, the current study was conducted in order to investigate the effect of iron application among various annona cultivars formed by self- or cross- grafting of sugar apple and cherimoya and cultivated in the calcareous soil of Southern Lebanese coast.

Materials and methods

Experimental site

The experimental orchard was situated in South Lebanon, at an altitude of 245 m.a.s.l (meters above sea level). Based on Brown (2003) soil at the orchard was identified as calcareous clay rich in phosphorus, nitrogen, sodium and calcium and poor in iron.

Treatments

Three annona cultivars were formed by self- or cross-grafting combinations of sugar apple (Sq)and cherimoya (Ch); self-grafted sugar apple (Sq-Sq), self-grafted cherimoya (Ch-Ch) and cross-grafted cultivar formed by sugar apple scion and cherimoya rootstock (Sq-Ch). Splice grafting was adopted and rootstocks were of 12 months old. Seedlings of each cultivar were transplanted with a distance of 4.5m between rows and 4m between plants into the experimental field. Iron (Fe) was applied monthly through fertigation starting from April with a dose of 30 g/plant. Vegetative growth was compared among plants grown in iron-fertilized soil (S1) and those grown with no iron fertilization (S2) over three consecutive years (year 1: 2012, year 2: 2013 and year 3: 2014).The experimental design was a Randomized Complete Block Design with 6 treatments and 30 replicates per treatment. Control consisted on 30 plants of each cultivar grown in S2.

Measurements

A destructive sampling was done at the end of vegetative growth of each experimental year on 10 plants per treatment including control. Randomly selected plants were subjected to various measurements like plant height (from the soil level till the top of the plant), trunk diameter (using sliding caliper), fresh weight of roots, number and length of primary roots (first ramification from the main root), diameter of main roots and dry weight of roots (obtained after oven drying till constant weight).

Data analysis

The effects of different factors: cultivar (Sq-Sq, Ch-Ch, Sq-Ch), iron fertilization (S1 and S2) and time (year 1, 2, 3) and their interactions on the averages of the measurements were analyzed using Factorial ANOVA. Data analysis consisted on means ± SE compared by Fisher's least-significant differences test (LSD) with a $P_{value}<0.05$ and was performed using STATISTICA10 Program.

Results and discussion

From the probabilities associated with Fisher statistics (Table 1) it was found that non-interactive effects of the factors time (year 1, 2, 3), Fe (iron fertilization) and cultivar (self- or

cross-grafting) were statistically (Pvalue<0.05) significant on all studied growth indicators. Moreover, interactive effects of factors "Time*Fe", "Fe*Cultivar" and "Time*Fe*Cultivar" were significant on averages of all indicators except plant height, length and diameter of primary roots. In addition, interactive effects of the factors "Time*Cultivar" was statistically significant on averages of all indicators except diameter of primary roots.

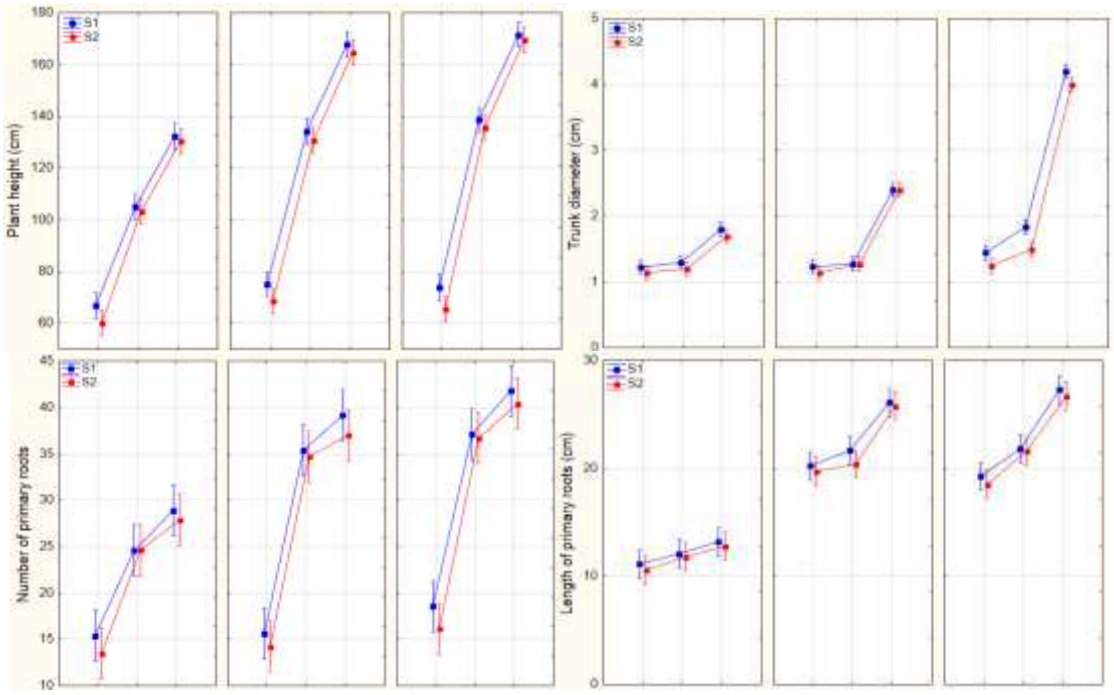
Table 1: ANOVA null hypothesis rejection probability for the effects of the experimental factors and their interactions on the different measurements averages

	P.H	T.D	N.P.R	L.P.R	D.P.R	F.W.L	F.W.R	D.W.R
Intercept	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Time	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Fe	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cultivar	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Time*Fe	0.1312	0.0053	0.0286	0.1507	0.1079	0.0000	0.0000	0.0000
Time*Cultivar	0.0000	0.0000	0.0000	0.0000	0.2457	0.0000	0.0000	0.0000
Fe*Cultivar	0.3701	0.0008	0.0165	0.4490	0.8478	0.0000	0.0000	0.0000
Time*Fe*Cultivar	0.1824	0.0048	0.0000	0.6421	0.3664	0.0000	0.0000	0.0000

P.H: plant height, T.D: trunk diameter, N.P.R: number of primary roots, D.P.R: diameter of primary roots, F.W.L: fresh weight of leaves, F.W.R: fresh weight of roots, D.W.R: dry weight of roots

During the three experimental years, average plant height (Figure 1a) did not significantly differ among the cultivars Sq-Ch and Ch-Ch, while it was significantly higher in both cultivars compared to Sq-Sq in fertilized and non-fertilized plants (Sq-Ch/S1: 171 cm, Ch-Ch/S1: 167 cm, Sq-Sq/S1: 132 cm, Sq-Ch/S2: 169 cm, Ch-Ch/S2: 164 cm and Sq-Sq/S2: 130 cm). Differences in average trunk diameter (Figure 1b) as affected by iron fertilization were only observed during the third experimental year on the cultivar Sq-Ch where iron-fertilized plants of this cultivar had a significantly higher trunk diameter compared to non-fertilized ones (4.2 cm in Sq-Ch/S1 compared to 4 cm in Sq-Ch/S2). In year 1 and year 2, average trunk diameter did not differ among fertilized and non-fertilized plants of self-grafted cultivars (Sq-Sq and Ch-Ch). In addition, in year 3, superiority of this indicator was observed for iron-fertilized plants of Sq-Ch compared to all other treatments as well as to non-fertilized plants of the same cultivar. Root development was increased between year 1 and year 2 and did not significantly differ in year 3 in all plants. In year 1, average number of primary roots (Figure 1c) did not significantly differ among cultivars despite iron application, while in year 2 and year 3 average values of this trait were significantly higher in Ch-Ch and Sq-Ch compared to Sq-Sq. Iron application has only slightly improved number of primary roots in all cultivars (by 3.4 %, 5.6 % and 3.3 % for Sq-Sq, Ch-Ch and Sq-Ch respectively in year 3). Primary roots of self-grafted sugar apple (Sq-Sq) did not significantly grow in length with time with or without iron fertilization (11 cm in year 1, 12 cm in year 2, and 13 cm in year 3). In addition, average length of primary roots (Figure 1d) was higher in Ch-Ch and Sq-Ch compared to Sq-Sq despite the effects of time and iron application. Slight improvements in

length of primary roots were observed in treated plants of all cultivars following iron application. Best improvement was for self-grafted cherimoya in year 2 (21.6 cm and 20.4 cm in S1 and S2 respectively). Observations on the average diameter of the main root (Figure 1e) showed that cultivar (Sq-Ch) has developed the thickest main root among all cultivars (respectively of 3cm compared to 2cm and 1.7cm in Ch-Ch and Sq-Sq cultivars in year 3). Iron fertilization has positively affected this indicator in self-grafted cultivars (Sq-Sq/S1: 1.6 cm and Ch-Ch/S1: 1.8 cm compared to Sq-Sq/S2: 1.5 cm and Ch-Ch/S2: 1.7 cm) while it has negatively affected it in the cross-grafted cultivar (Sq-Ch/S1: 2.7 cm compared to Sq-Ch/S2: 2.9 in year 2). Moreover, iron application has significantly increased the average fresh weight of leaves in year 3 compared to control mainly in Sq-Ch and Ch-Ch (142g and 36g respectively in S1 compared to 90g and 31g in S2). Finally, the cross-grafted cultivar (Sq-Ch) had significantly the highest fresh (Figure 1f) and dry weights (Figure 1g) of roots despite iron application among all cultivars in year 1, 2 and 3. For instance, in year 3 the average fresh and dry weight of roots in S2 were respectively of 130g and 87g in Sq-Ch cultivar compared to 70g and 47g in Ch-Ch and 65g and 43g in Sq-Sq. However, the application of iron was significantly beneficial on both traits only in year 2 on the cross-grafted cultivar (Sq-Ch) (improvement by 15.7 % and 10.5 % in fresh and dry weight of roots respectively).



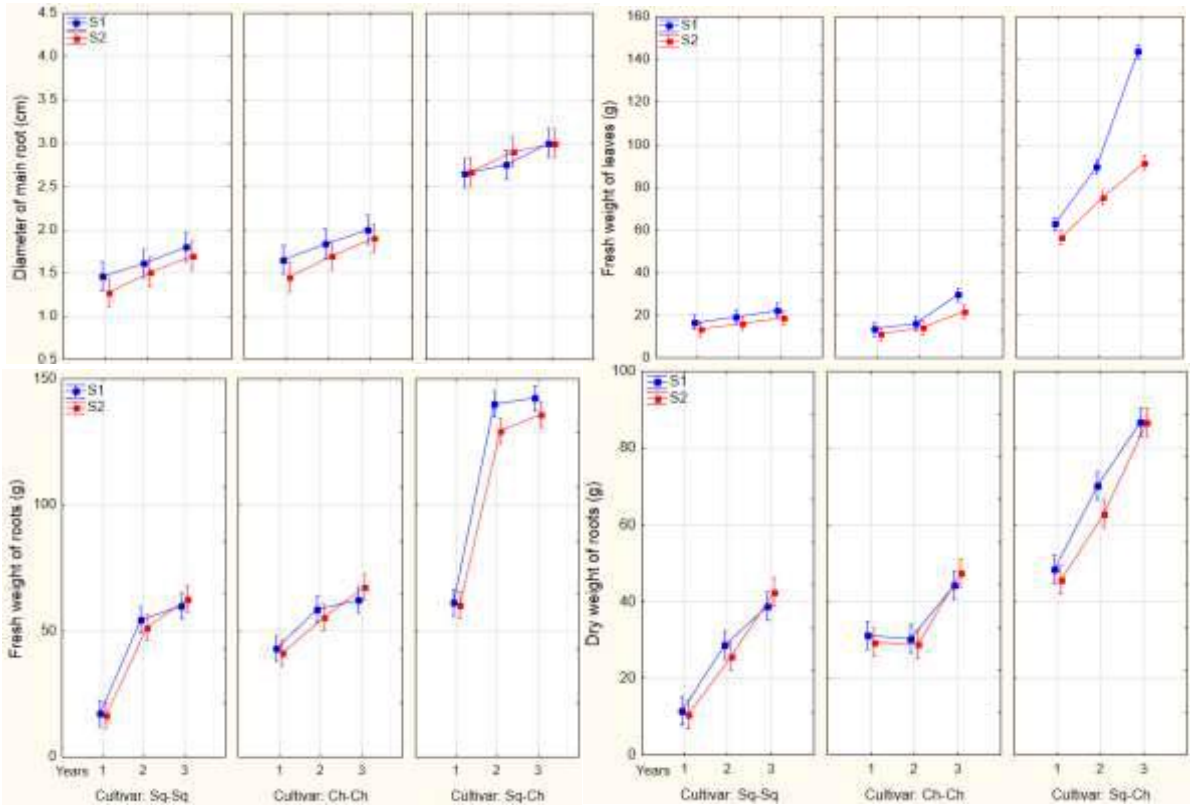


Figure 1: Averages (middle markers) and the 95% limits of confidence (±2 x standard Error: SE) (vertical bars) of various indicators

It is a well-known fact that there is a positive interaction between tree's canopy and its roots. According to Heenkenda *et al*. (2009) the growth and production characteristics of a grafted cultivar are influenced by both the rootstock and the scion; In fact cherimoya is a vigorous and prolific plant (Pinto *et al*., 2005) confirming the current results concerning self-grafted cultivar Ch-Ch plants height. Since the interaction between scion and rootstock is significant (Heenkenda *et al*., 2009), the maximum plant height was recorded for cherimoya grafted on to sugar apple rootstock. Besides, the highest trunk diameter was observed with the cross-grafted cultivar due to the positive scion-rootstock interaction which amplified vegetative growth of both cultivars. Indriyani and Karsinah (2011) have previously reported a better effect of cross-grafting compared to self-grafting on trunk diameter of *A. montana* Macf. x *A. muricata* L. cultivars. The well-developed and vigorous root system of Cherimoya was reflected by the optimal length and number of primary roots of self-grafted Ch-Ch and cross-grafted Sq-Ch cultivars. In fact, the grafting effect boosted the natural capacity of this rootstock leading to the formation of a more ramified root system with optimal root biomass. Consequently, this could have led to a better translocation of nutrients including iron leading to the formation of greater leaf biomass in cross-grafted cultivar. Similar findings were reported by Sebaaly *et al.* (2015) regarding cross-grafted cultivar Sq-Ch who found a positive interaction between sugar apple scion and cherimoya rootstock which improved the vegetative growth of plants grown in other type of soil.

On the other hand, iron application caused a slight improvement in the majority of indicators uniformly on all cultivars except the diameter of main root indicating a positive response of all plants to the newly available micro-nutrient. The most significant effect of iron fertilization was on leaf biomass and trunk diameter. This positive effect was similarly observed by Ojeda (2003) on annona plants grown in hydroponic system and by Bhanukar *et al.* (2018) and Abay and Pirlak (2016).

Conclusions

Combining cross-grafting and iron fertilization is an efficient method to optimize the vegetative growth of annonas in Lebanon. Since sugar apple and cherimoya are the most cultivated species in Lebanon, consequently the current work proposes a strategy to ameliorate the adaptation behavior of species to the dominant soil characteristics in South Lebanon.

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PEA (*PISUM SATIVUM* L.) DIVERSITY IN BULGARIA AND A STRATEGY FOR ITS UTILIZATION

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Abstract

This paper presents the diversity of pea plant genetic resources (PGR) and carries out an analysis of the accumulated database for the National collection, supported by the Institute of Plant Genetic Resources (IPGR) in Sadovo, Bulgaria. Numerous varieties, populations, forms and breeding pea lines, diverse in phenotype, maturity, directions for use and productivity represent the plant materials that are studied and preserved in the IPGR. Special attention is given to the accessions of Bulgarian origin, as they take a central point of the studies conducted in the Institute. The share of the indigenous pea forms is about 12.4% (out of total number of accessions) as the oldest ones were collected during expeditions prior to 1950. Most of the preserved varieties are a result of long-standing selection and people's breeding with a goal for green mass. They are characterized by high winter resistance and rapid early spring growing (*M-9, P-1, №155, 35 [a], 37[a], Podobren Plovdivski, Pleven 10, Mir*). Other varieties from these accessions are bred for dry seed (*Pleven 4, Pikardi, Vessela*). The third ones are bred as a priority for lodging-resistance (*Tedi, Amitie*) and are adapted for direct harvesting (affilate forms). The typical green pea varieties for canning and freezing (*Uspeh, Plovdivska perla*) are stored at the Institute. A large number of old varieties and populations are actively grown in organic farms with a closed production cycle, in home gardens and in smaller farms. It should be mentioned that accessions with alien background are used as gene donors of valuable features for the breeding process or for evaluation of their potential and opportunities for utilization.

Keywords: *Plant Genetic Resources, Pisum sativum L., Organic farms, Breeding process, Utilization*

Introduction

The pea collection is the biggest of all grain legumes preserved in the National Gene Bank. The total number of accessions is 2560. About 25 % of pea collection is in long term conservation, 50 % in middle term conservation and 25 % are in working collection.

The pea collection contains samples from 30 countries. It provides great opportunities for using diverse plant material for scientific purposes, breeding improvement and for direct implementation and reonization (*Koeva et al, 1990; Angelova et al, 2001).* The accessions are grouped according to their important traits for the breeding program and for the farmers– earliness, winter hardiness, productivity, and protein content. (*IBPGR descriptors*)

The aim of this paper is to analyze pea collection, studied by stages for a long period of time.

Material and Methods

Field trials

The article represents the agrobiological characteristics of pea collection since 1986 *(Angelova,1986; Angelova, Ganeva, 1987*). The accessions have a set of valuable qualities (early maturing, lodging resistance etc.) and an economic assessment is made (*Angelova,1986; Angelova & Ganeva, 1987; Angelova, 2001; Angelova & Yancheva, 1996; Angelova & Sabeva, 2013).* According to the methodologies for preliminary study each variety accession is sown in an experimental field with size of two m^2 two times, while for

variety trials conducted in block method, the sowing is repeated four times as the size of the plot is ten m^2 with a relevant standard for comparison. The sowing of spring varieties is carried out as early as possible in the month of February or March. For the winter ones the sowing is in the period from 20^{th} to 30^{th} October.

Soil and Climatic Conditions

The trials are conducted on cinnamon forest soil on the experimental field at the Institute of Plant and Genetic Resources (IPGR) in Sadovo, Bulgaria. The town of Sadovo is situated in the Upper Thracian Lowland with an altitude of 141 meters and is characterized by transient continental climate. The winter is milder and the summer is hotter compared to Northern Bulgaria. The average annual temperature is 12,4°C the lowest one is in January and the highest in July. Sometimes the winter temperatures for two or three consecutive days drop down to minus 18°C and some days even below minus 20°C to be important for survival of winter pea varieties (*Boyadjieva & Stankova, 1990*). The amount of precipitation has its highest values in May with 47,5 mm and the lowest ones in September with 14,9 mm.

Results and Discussion

The National pea collection contains plant genetic resources of different origin, breeding level and botanical possessing. These resources provide an opportunity for improvement of breeding programs in different directions: for food, canning and fodder. The pea accessions are presented from the species of *Pisum sativum ssp. arvense, ssp. Sativum, ssp. hortense*, *ssp. transcaucasicum, ssp. asiaticum.*

The accessions of foreign origin include mainly varieties and breeding lines with a specific quality – gene donors for breeding material.

The plant material of Bulgarian origin occupies about 25%: indigenous landrace populations, old and new breeding varieties, breeding lines and mutant forms. The oldest ones were collected during expeditions prior to 1950. Most of the varieties are a result of long-standing selection and people's breeding with goal for green mass. They are highly winter resistant and early rapid growing in the spring.

The soil and climatic conditions in Bulgaria are favorable for pea growing. The biological characteristics allow to be grown as a spring and a winter crop. The first winter forage pea variety *Plovdivski podobren* is zoned in 1961. Later were bred the varieties *№ 30, Pleven 2, Pleven 5* that are similar in phenotype *to Plovdivski podobreno*. Since 1979 *Pleven10, Lozen 38, Lozen 517* are zoned. During the 1980s and 1990s, *Yubileyni*, *Mir and Vesela* are included on the variety list and still growing in Bulgaria (*Ganeva et al, 1985*; *Angelova & Ganeva, 1987; Angelova & Kalaphieva 2015*).

The accession number 37 from Knezha and accession number 63 from Kalofer could be considered as typical indigenous pea forms. The accession number E-548 from Silistra is added to the collection as a sample, brought by the international expedition in 1959. All of the materials from the plant genetic resources of Bulgarian origin are characterized by high winter resistability as the percentage of survived plants in winter conditions is from 80% up to 97% for a period of 15-20 years. Large parts of them are late varieties and in unfavorable climate conditions could not fulfill their biological potential. They could be used as gene donors for improving winter resistibility of plant (*Figure* 1) (*Ganeva et al, 1985; Angelova & Ganeva, 1987*). The winter varieties *Mir* and *Vessela* are widely used in organic farming in Bulgaria. They are plastic, rapidly growing and develop early in the spring, high productivity for green mass and seeds. These varieties early give yields and allow the next crop sowing to be done on the same plot. When damages from severe temperatures are observed on the central stem, the plants from all elaborated winter varieties react as form basal branches. Their pods very often completely compensate the productive opportunities of the central stem and give good yields *(Bastianelli et al., 1995; Decaux B, 1998).*

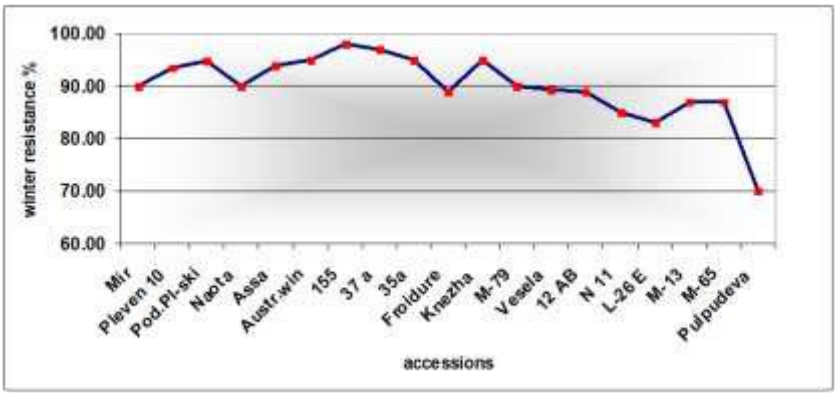


***fig. 1** Winter resistance accessions*

As a result of winter hardiness, the pea accessions are distributed in three groups: winter hardiness – 80% - 100%; low sensitivity – 70% - 80%; high sensitivity – 60% - 70% (*Angelova & Ganeva, 1987*). The main tendencies in the work with spring pea are focused on expanding and enriching the collection of early and middle early forms. They make the most of their biological potential in Bulgaria. The collection spring forms have a wide range of early ripeness.

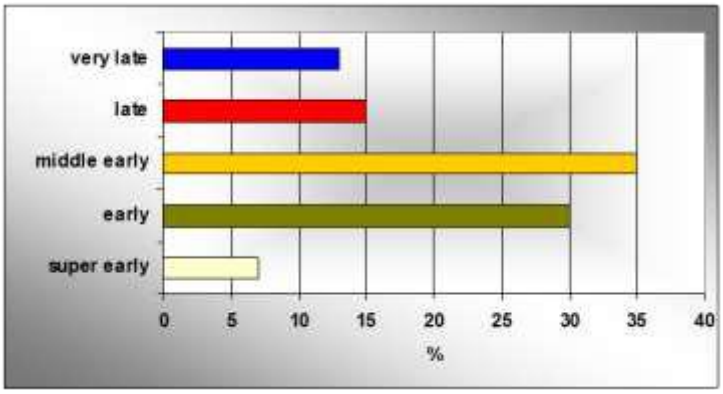


***fig 2.** Maturity of spring pea accessions*

Bulgarian weather conditions are characterized by early summer droughts and dry wind, typical for the Thracian lowland area; the duration of the vegetation period is of great importance. As for the supported collection of spring forms it is from 61 days up to 109 days. (*Angelova & Ganeva, 1987; Angelova S & Ch. Yancheva, 1996; Angelova & Kouzmova, 2001*). The spring varieties bred in Bulgaria are early and mid-early with relatively short vegetation period, good resistance to cold early spring temperatures. Their main requirement is the quality characteristic of the seed. In collaboration with the French National Institute for Agriculture Research (INRA) and in an application of mathematical methods and structural analysis, it has been found that the seed yield of winter pea is formed mainly from the first four productive floors, but more often after the third floor, while for the spring pea, the priority is from first and second floor. The recent breeding achievements in Bulgaria are the lodging resistant varieties from affilate type – *Amitie* and *Tedi, Pikardi* bred at the IPGR in a cooperation with INRA – France; and the highly productive varieties *Mishel* and *Sredez* bred at Dobrudzha Agricultural Institute in General Toshevo, Bulgaria are suitable for growing only in the Northern area of the country. Depending on the growing conditions, the genetic potential for high productivity is shown in different ways. Table 1 presents the statistical data for seed yields for winter and spring pea varieties grown in Bulgaria. During the entire fifteen-year research period, results show that the seed yield for winter pea varieties is from 270,5 kg/da up to 484,4 kg/da while for the spring ones it is from 268,5 kg/da up to 560,5 kg/da.

Table 1. Statistic data for yield in kg/da

	MIN	**MAX**	**MEAN**	**Std. Deviat.**	**V.C %**
Winter varieties					
№ 11	270,5	453,4	328,3	48,194	14,68
Mir	290,4	455,1	329,2	47,063	14,30
Vessela	298,4	484,4	352,3	55,104	15,64
L 12 AB	297,5	447,1	327,4	40,027	12,23
Spring varieties					
Amitie	268,5	560,5	407,1	81,213	19,95
Pikardy	279,5	531	401,1	74,636	18,61
Tedy	271,5	545	410,6	75,808	18,46

Although the yields vary by years, the results show that the varieties make their produce opportunities. All this is confirmed by the studies of (*Koeva et al. (1990)*; *Angelova & Kuouzmova, (2001)* that the temperature and the amount of precipitation affect to a great extent influence the quality characteristics of the accessions. For winter accessions, the variety influence is strongly manifested over the seed productivity while for the spring peas is better manifested the influence of the year (see *Figure* 5). In relation to crude protein (percentage absolute dry content) in the seed, the accessions are divided into four groups (see *Figur*e 6a).

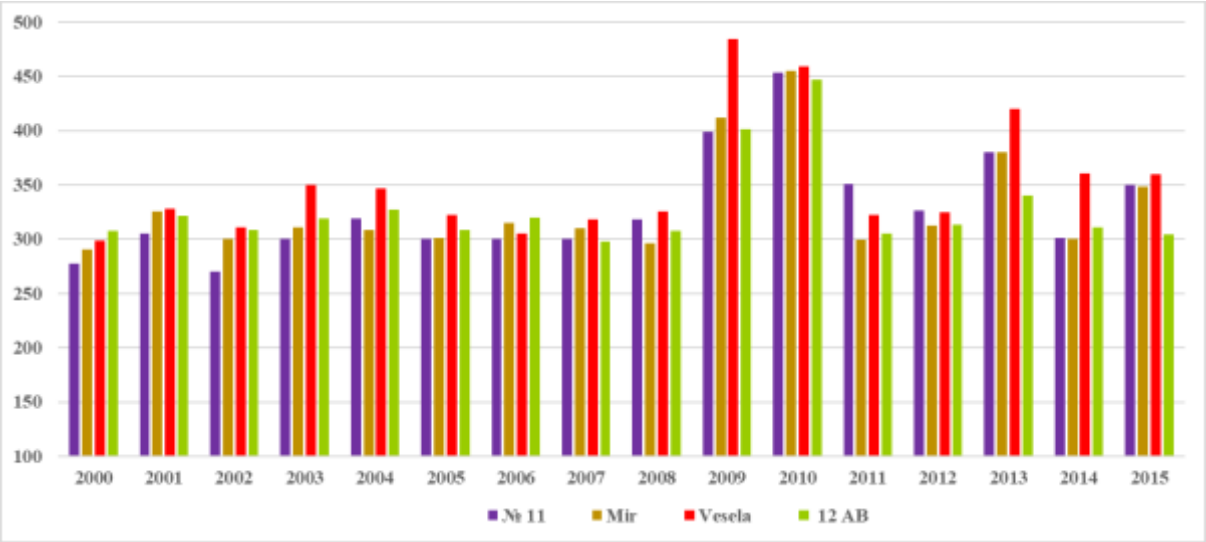


fig 3. *Seed yield from winter pea varieties*

The greater part of Bulgarian varieties fall into the second and third group from 23,1% to 26,0 % (*Angelova & Sabeva, 2008; Angelova & Sabeva, 2013*). The utilization of peas as a fodder plays an important role as alternative to make up for lack of protein during early the spring. The green mass content of crude protein (*see Fig 6b*) in the maturity phase (forming of lower 2-3 beans) is from 15% up to 22% (absolute dry substance) for the period from 10th until 30th of May depending on the variety's early ripening. There are no significant differences between the accessions from the same group. The varieties bred at the IPGR are widely used, and fall into the third group from 19% up to 22% *(Angelova & Sabeva, 2008; Angelova & Sabeva, 2013).*

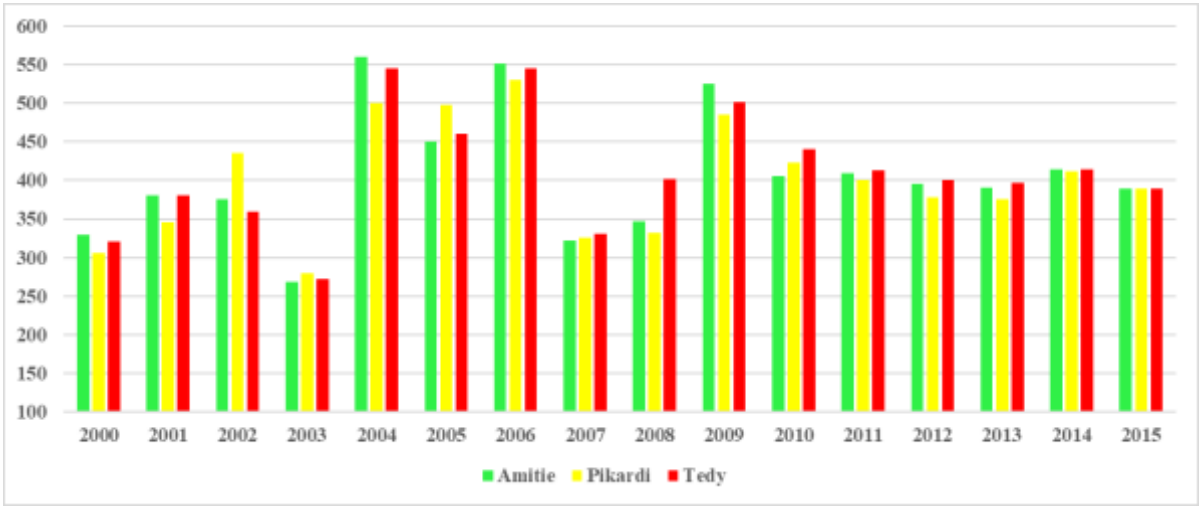


fig 4. *Seed yield from spring pea varieties*

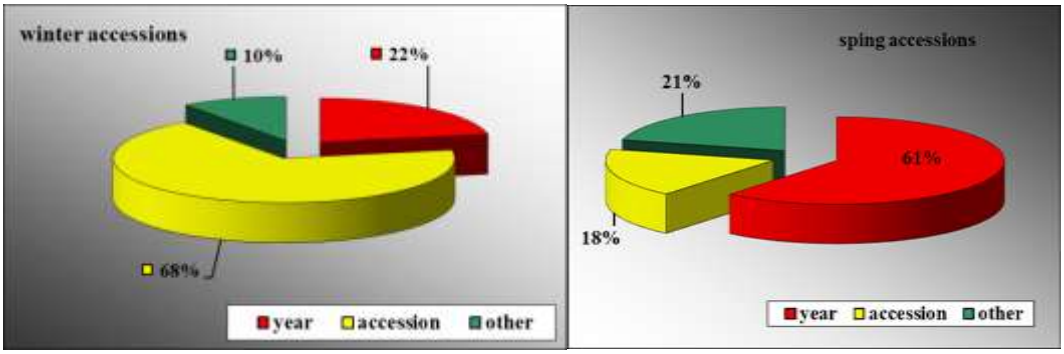
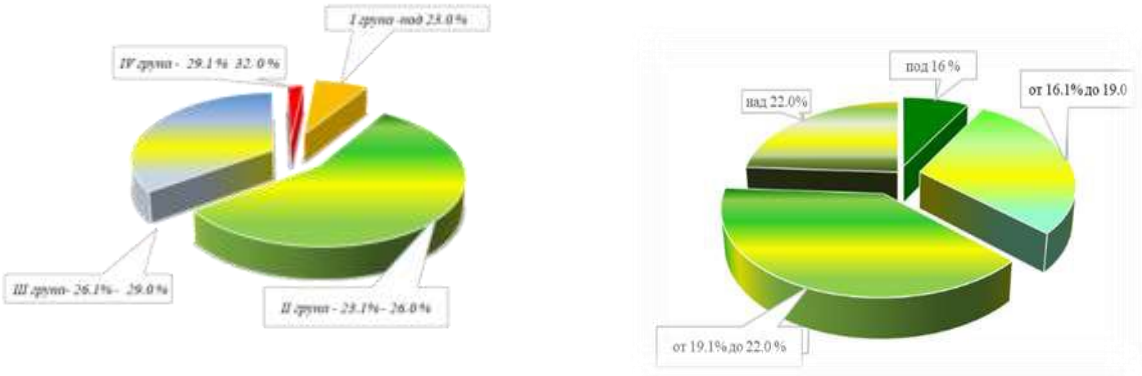


fig5. *Variety influence and year over the productivity*



a b

fig.6 *Crude protein content in the dry seed and in the green mass*

The pea is used as a fodder and as a vegetable in Bulgaria. Besides for fresh consumption, it is a main source for the canning industry, mainly for preservation and freezing. There is a breeding program focused on satisfying the needs of customers at the Institute for Vegetable Crops Maritsa in Plovdiv, Bulgaria. The Bulgarian varieties *Ran I* and *Plovdivska perla* are the most preferred ones for growing in home gardens. From newly bred varieties *Musala* and *Iskar* are mostly used and are suitable for canning and preservation (*Angelova & Kalapchieva, 2015*). A large number from the old varieties and populations are used at organic farms with closed production cycle, in home gardens and in smaller farms. Natural habitats of *Pisum elatius* were identified during expeditions in the last years. Wider populations from the same genus are marked on the Northern and Southern Black sea coast, Kaliakra cape and Kamen Bryag. A seed material included in *ex situ* collection is also collected from the same habitats.

Conclusion

The proper and sustainable use of global and national collections from all crops leads to more efficient breeding activity. The modern collection of *Pisum sativum L*. needs a wide range of starting gene material to include more often indigenous germ plasma, primitive forms and wild species. A constant collection update is required to deepen and improve evaluation methods, necessary for successful and purposeful use, exchange and storage. By using the pea collection we created six pea varieties characterized by good earliness and high productivity. They are namely the following: *Mir*– winter resistant, forage type; *№ 11*- winter resistant; *Vessela*– winter resistant, dry pea; *Pikardi*– spring, dry pea; *Amitie* /af/- spring , dry pea; *Tedy* / af /– spring, dry pea. Winter varieties *Mir* and *Vessela* are used very often for green manure crops in organic farming.

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POST EFFECT OF ORGANIC AND MINERAL NUTRITION ON GROWTH, YIELD AND QUALITY OF SPINACH (*SPINACIA OLERACEA* L.)

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Abstract

Cultivating spinach during autumn, as a sealing crop, has important economic and agro-ecological significance both through the yields achieved and by the more complete and rational utilization of the residual nutrients in the soil after harvesting the previous main crop. A field experiment with spinach variety Matador is conducted on the alluvial-meadow soil. The soil reaction (pH) is slightly acidic, humus content is low (1.33 %). The availability of mobile forms of N, P and K variants of an experiment in post-harvest predecessor are low mineral nitrogen and high P_2O_5 and K_2O. The study aimed to assess the post-effect of organic, mineral and mixed manure and mineral nutrition in the predecessor previous crop early potato with many treatments. Control variant without nutrition (T1), with organic (manure) (T2), with mineral (T3), and with mixed 50% manure-50% mineral nutrition (T4). The effect of treatments on growth, yield, quality of production and quality parameters in autumn growing spinach has studied. The experiment is a completely randomized design. The results indicate that the highest yield of spinach is obtained from the plants with post effect of the mineral nutrition. Plants which were grown after mineral nutrition has higher nitrogen, chlorophyll and nitrates contents. The dry matter content and total sugars are highest in the production of spinach after the previous crop fertilized with manure.

Key words: *organic nutrition, mineral nutrition, spinach, quality parameters.*

Introduction

A major challenge for modern horticulture is to reconcile productivity and sustainability of production systems and nutritional quality of food products. Nutritional quality is a highly complex trait due to both (i) the large number of individual properties which determine crop quality (i.e., concentration and bioavailability of essential and potentially toxic minerals, organic nutrients and accessory health factors) and (ii) the various factors which control them (i.e., genetic and exogenous factors, the latter either natural or man-made) (Wiesler, 2015). Provision of a sustainable environment in the soil by amending with organic inputs can improve the quality and acceptability of crop (Sharma and Agarwal, 2014). Autumn-winter crops can be an important reserve for compensation and increase the income of farmers. For optimum crop rotation schemes according to the requirements of good agricultural practice (GAP), using of leafy vegetable crops with short duration is recommended. Adequate crop for that is spinach, which can used as an indicator who expressed nutritional status of the soil. Leafy vegetables consist from huge diversity, spinach (*Spinacea oleracea* L.) a member of the *Chenopodiaceae* family. Usually spinach plant used as test crop in many agrochemical and ecological studies. (Botev, 2006; Dinev and Mitova, 2011; Mitova, *et al*., 2005; Mitova and Stancheva, 2003). Vegetative organs react quickly and appropriately to various biotic and abiotic effects. In previous studies, the performance of spinach crop was observed following the application of organic and inorganic fertilizers; It was found that the combination of both fertilizers was effective on increasing the mineralization rate of the organic matter (especially nitrate) (Anga,2001) as a result nitrate concentration of the spinach leaves increased.

Therefore, the objectives of this research are to determine post-effect of organic manures and chemical fertilizer on the growth, yield and quality of spinach.

Material and methods

Experiment is laid in experimental open field of IPAZR "N. Poushkarov " located in – Tsalapitsa village on alluvial meadow soil. Initial values of soil reaction (pH) (pH H_2O- 6.8; pH kcl- 6.0) and humus content (1.33%) characterized as slightly acidic soil with low humus content. It was done by the use of spinach cvs."Matador" sowing date in mid-September, after harvesting of early potato production. The production was finished on 10 November. An experiment was laid in Completely Randomized Design the options with 4 replications with the size of the experimental plot- 30 m^2. For the main crop (potatoes) in variants with 100% mineral fertilizer was added 20 kgN.da^{-1} as ammonium nitrate, 12 kg P_2O_5 da^{-1-} as triple superphosphate and 18 kg K_2O.da^{-1} as potassium sulphate.

By using manure which contain of 1.2% total nitrogen. Manure norm 20 kgN.da^{-1} was add with 1667 kg.da^{-1} manure.

In variant, four were added ½ of mineral fertilizer norms and manure. For experiment with spinach did not used any fertilizers. To understand the post effect of fertilization of the previous crop.

Variants of using fertilizers with soil reaction, and the content of nutrients after harvest the potatoes are present in Table 1.

Table 1: Soil agrochemical analysis in depth 0- 30 cm after harvesting of potatoes

Treatments	pH_{kcl}	NH_4-N	NO_3-N	P_2O_5	K_2O
(T1) without nutrition	6.5	10.63	5.70	17.63	24.32
(T2)100% manure	6.9	12.19	0.52	77.50	38.30
(T3)100% mineral fertilizer	6.7	7.52	13.48	21.91	33.35
(T4)with mixed 50% manure-50% mineral fertilization	6.9	4.41	3.63	30.72	28.50

The fresh weight of plants is determined by weight in kilograms per hectare. From the plant samples, after drying at 65˚C with a preliminary fixation, the dry substance (absolute dry matter%) was determined by weight. The total sugar content is determined refractometrically - (%) (Digital refractometer - 32 145). The nitrate content is determined on Merck's RQ flex plus 10 instrument. The content of plaster pigments in the fresh mass (mg.g-1) is determined by the Vernon method, 1960. The content of macroelements in the soil is determined by standard methodologies (Arinushkins, 1970), ammonium and nitrate nitrogen - colorimetric, movable forms of phosphorus and potassium by the method of P. Ivanov (1984).

In plants, total nitrogen was determined by the Keldahl method by decomposition with concentrated H_2SO_4 and 30% H_2O_2. The remaining macroelements were determined by "dry" burning in muffle furnaces and subsequent dissolution in 20% HCl, taking into account the atomic absorption spectrophotometer. The yield data is processed using the Statgraphics / Anova / Multifactor dispersion statistic package.

Results and discussion

The optimum growth and development of plants depend not only on the balanced absorption of nutrients. Their presence in the soil is readily available in readily accessible and digestible forms. External factors also influence the vegetation, which in certain situations can compromise production. Spinach is particularly sensitive to high acidity. The optimum soil pH should be 6.2 to 6.9 (Genkova, 2009). It can be seen (Table 1) that soil acidity is appropriate for the development of spinach. In all embodiments, the mineral nitrogen content

is low, and in the moveable form of phosphorus and potassium-rich forms. The particularly high content of P_2O_5 and K_2O in the soil is the manure variant with manure.
The yield data shows a significantly lower yield in the control variant compared to the plants suffering from the effects of using different fertilizers. It can be assumed that the yield from the non-treated plants should be comparable to the other yields. The reason for the low yield of the untreated variant, despite the fact that the nutrient content is close to that of the other variants, could be the highest density of weeds and the attack of diseases that occurred in the cultivation of the previous potato crop. Spinach from the untreated variant develops slowly, the formed leaves are small, and the plants prematurely shoot (Fig.1)

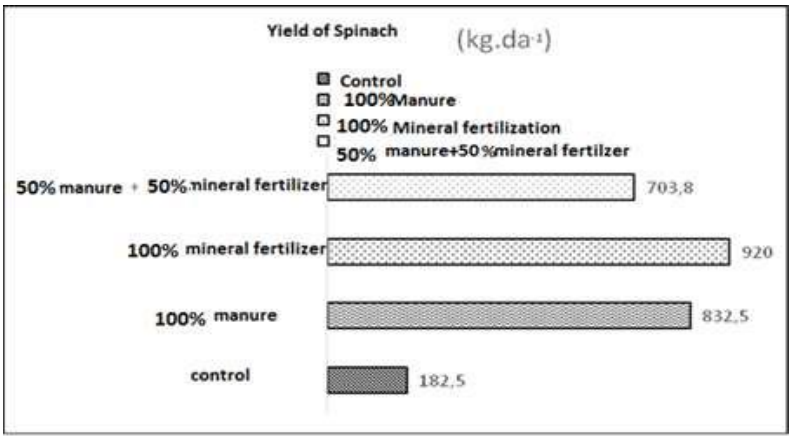

Fig.1: Yield of spinach after treatments Kg/da

The yields obtained in the study are significantly lower than those reported in the literature (Boteva Hr .. 2006, Genkova, 2009, Mitova et al., 2005; Rankov et al., 1994) in fertilization with organic and mineral fertilizers. In this experiment its important to take into account the fact that the obtained yields are the result of the effect of the fertilization of the base crop. The highest yield of spinach in the experiment was obtained in the variant with the effect of mineral fertilization - 920 kg / da(35%), followed by manure variants - 832,5 kg / da(31%) and mixed fertilization - 703,8 kg / da(27%). Differences in yields are statistically satisfied.(Fig.2)

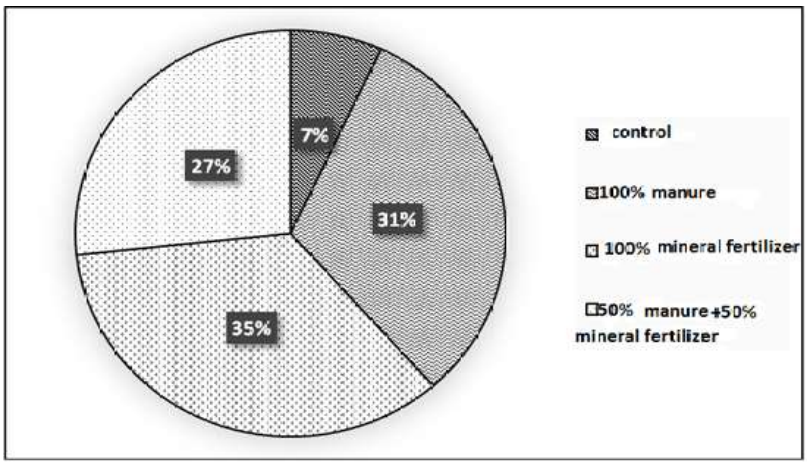

Fig.2: Yield of spinach in percentage %

The differences in yields at both statistical levels between control and fertilizer variants as well as between organic fertilizer plants and mixed and mineral fertilizers are statistically satisfied (Table 2).

Table 2: Statistical analyses of yield

Variants	***1. control***	***2. 100% manure***	***3. 100% mineral fertilizer***	***4. 50%manure+ 50%mineral fertilizer***
Average	703.8	920.0	832.5	182.5
Median	704.4	915.7	831.9	12.5
St Dev	18.11	15.80	17.71	42.5
F-Ratio- 485.37; P- Value- 0.000; (P≥95%)- 48.892; (P≥99%)- 71.142				

The total nitrogen content in the leaf mass varied between (2.0-3.9%) of the plants (Table 3) follows the yields obtained and is comparable to the cited values in the literature (Rankov et al., 2004; Mitova et al., 2015). High concentrations of phosphorus - 1.13%, potassium - 8.9% and calcium - 1.0% in plants with the effect of manure correspond to the high residual contents – these results showed reverse tendency in the movable forms of these elements the soil. The magnesium content in the leaves (0.11 - 0.63%) of all variants is lower than in other publications (Mitova et al., 2015).

Table 3: Content of nutrients (%) in leaves of spinach at end of the study.

Treatments	total N	P	K	Ca	Mg
(T1) without nutrition	2.0	0.9	6.1	0.67	0.11
(T2)100% manure	3.3	1.13	8.9	1.00	0.63
(T3)100% mineral fertilizer	3.9	0.95	7.0	0.70	0.12
(T4)with mixed 50% manure-50% mineral fertilization	2.6	0.96	7.5	0.77	0.34

Plastid pigments are involved in photosynthesis and play a role in plant growth and growth processes. The study of the composition and amount of pigments in plants under different conditions of development is of scientific and practical interest. Despite the unfavorable climatic conditions in the autumn-winter period, the applied fertilization (organic, mineral and organo-mineral) has a positive influence on the synthesis of plastid pigments (Table 4) and the content of chlorophylls in treated variants significantly higher values of Ch "a" + Ch "b" compared to the results obtained in other experiments (Mitova *et al*., 2015, Ratterman, 2006). The highest chlorophyll content in the experiment has plants grown after mineral fertilization - 15.8 mg%. According to some authors (Chinok, 1976), the normal ratio of Ch "a" / Ch "b" should be 3: 1. Bero *et al*., 2007 consider that the ratio of chlorophylls to 2- 3: 1 is constant and depends on a number of factors. In the present study, the nearest ratio of Ch "a" / Ch "b" to the optimal ratio is the 2.25: 1 ratio obtained after mineral fertilization.

Table. 4. Contents of plastid pigments in spinach leaves.

Treatments	Ch"a"	Ch"b"	Carotenoids	Ch"a"+Ch"b"	Ch"a"+Ch"b"
(T1) without nutrition	4.28	2.57	1.83	6.85	1.68
(T2)100% manure	9.04	5.90	2.88	14.94	1.53
(T3)100%mineral fertilizer	10.94	4.86	3.59	15.80	2.25
(T4)with mixed 50% manure- 50% mineral fertilization	7.89	3.57	3.31	11.46	2.21

Leafy vegetables contain relatively low amounts of dry matter, but they are characterized by a high content of vitamins and nitrates (Mitova *et al*., 2005, Mihov *et al*., 1980, Shaban *et al*., 2014). The dry substance is a relatively stable and genetically determined substance. While in fruity vegetable crops its content is lower, in leaf vegetables, in the case of spinach, the dry

matter levels are between 11.4 and 12.15% and are comparable to those of other spinach studies (Mitova *et al*., 2015). The highest levels of absolute dry matter are in the plants grown after treated with manure (Table 5), and for the other variants the differences in dry matter values are insignificant. In the Peev research, 1985, it proves that the sugar content correlates well with that of the dry matter. In this experiment, there is a similar positive relationship between the cited indicators. The foliar fresh weight of plants grown after fertilization with manure has the highest content not only of dry matter, but also of total sugars - 9.3%.

Table 5: Quality parameters of spinach production.

Variants	Absolute dry matter (%)	Total sugars (%)	Nitrate ($mg.кg^{-1}$)
1.control	11.40	5.8	76.23
2.100% manure	12.15	9.3	95.68
3.100% mineral fertilizer	11.95	7.4	176.49
4.50%manure+50%mineral fertilizer	11.85	5.2	79.21
F-Ratio	*0,95*	*149,74*	*92,38*
P- Value-	*0,450*	*0,000*	*0,000*
(P≥95%)	*1,060*	*0,489*	*16,006*
(P≥99%)	*1,543*	*0,712*	*23,289*

The accumulation of nitrates in the production of foliar vegetable crops is genetically predetermined (Genkova, 2009; Stoyanov, 1997; Afaf Ghaleb Hafiz Abu-Dayeh, 2006; Mitova and Stancheva, 2003; Rankov *et al*., 1994). The control of maximum permissible quantities of contaminants in the food is implemented by Ordinance of MAF-31 of 29.07.2004. For spinach, the limit value for nitrate in production harvested in the autumn-winter period is 3000 mg / kg. Experimental nitrate content ranges between 76.2 and 176.5 mg / kg of fresh weight, the highest concentration of nitrates being registered after mineral fertilization. The agrochemical analysis of soil samples from the variants (Table 6) with spinach after harvesting show a significant depletion of nutrients mainly of mineral nitrogen. In accordance with the low yields in the non-preferred embodiment, the residual ammonium and nitrate nitrogen contents are larger than in the other variants. The content of P_2O_5 and K_2O (Table 5) in the soil after the application of manure is still high at the end of the study.

Table 6: Agrochemical soil analyses 0- 30 cm at end of the experiment with spinach.

Treatments	$pH_{кcl}$	NH_4-N	NO_3-N	P_2O_5	K_2O
(T1) without nutrition	6.7	7.42	5.13	14.07	20.66
(T2)100% manure	7.1	6.74	2.00	64.92	34.13
(T3)100% mineral fertilizer	6.8	4.67	4.33	12.28	29.58
(T4)with mixed 50% manure-50% mineral fertilization	6.9	2.56	0.84	27.45	26.50

Conclusion

Cultivating spinach in autumn as a sub crop has important economic and agro-ecological significance both through the yields achieved and by the more sufficient and rational utilization of the residual nutrients in the soil after harvesting the main crop.

-The highest yield of spinach is obtained in the variant with the effect of the mineral fertilization.

-Plants grown after mineral fertilization have higher nitrogen, chlorophyll and nitrate content.

-The dry matter content and total sugars are highest in the production of spinach after a previous crop fertilized with manure.

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TEMPERATURE CONDITIONS FOR GROWING CHERRY *(PRUNUS AVIUM* L.) AND PEACH TREES (*PERSICA VULGARIS* MILL.) IN BULGARIA

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Abstract

In all European regions, variations of temperatures have a cyclic character with a relatively regular change for the periods of active vegetation and dormancy. This regularity is valid and also observed on the large areas in the country. The unfavorable combination of high temperatures and low rainfall, as well as temperature changes at the end of winter and the beginning of spring season are factors which may have critical effects upon the development of orchards. Also important factor often responsible for establishing the warm or cold winter margin for many fruit trees and strongly affecting production near those margins is the amount of chilling available during the plants rest phase of its growth cycle. Chilling is generally considered to be that amount of time during the period the plant is in rest when the temperature is below 0^oC. Cherry (*Prunus avium* L.) and peach (*Persica vulgaris* Mill.) plants are the most widely spread orchards in Bulgaria. They can grow in all over the country, but the optimum results are got only in separate regions, where the meteorological conditions are most suitable for their growth and development. The aim of this paper is to establishing the empirical between the sums of negative temperatures measured in the period from of durable transitions of temperatures below 0^oC (chilling degree days) and the number of days with temperature below 0^oC. The other aim is to find empirical relation between date of durable transitions of temperatures above 10^oC to fruits ripening and the number of days with temperature above 10^oC.

Keywords: *cherry, peach, sum of temperatures, chilling*

Introduction

Since the middle of the last century numerous studies related to the assessment of climate and agro-climatic conditions and their changes during this period has been carried out. They show changing of hydro-thermal conditions connected with observing of extreme meteorological events as droughts, spring frosts, hails, floods etc. observed on the large areas in Europe and particularly in our country. Analysis of data for the last 30 years show that the phenophases of trees occurred earlier in spring. The duration of vegetation period increased with 8 - 13 days on average, over the 20^{th} century (Chmielewski, 2004; Menzel, 2003; Kazandjiev, 2008). High interest is assessment of change of terms for dates of stable transition of average daily temperatures across the different biological threshold as 0, 5, and 10°C during spring and autumn seasons, because they have important scientific and practical application and they show the limit between cold and warm part of the year and trace out duration of the vegetative season and winter dormancy period for different crops (Kazandjiev et al., 2011; Slavov and Moteva., 2007).The period with temperature below 0°C determined duration of dormancy period. The date of steady transition of the air temperature in spring from 5°C was accepted conditionally for the beginning of spring vegetation in orchard (Vandova, 1985; Herschkovich, 1984). In Bulgaria dates of steady transition of air temperatures across 5°C in spring and autumn determined duration of potential vegetative period (RVP). The duration of this period is in turn off 250-260 days, on average. Another index for thermal condition characterizing duration is the period with average daily temperature above 10°C is in turn denoted as the real vegetative period (RVP). This is the period for active vegetation, growth

and development of orchards and agricultural crops. The average duration of this period in Bulgaria is 200-220 days (Kazandjiev, 2008; Kazandjiev, 2011). Detail investigations on the occurrence of phenological stages and sum of temperatures for cherry and peach in Bulgaria was made also by Ganeva et al., (1974) and peach by Draganov et al., (1965) and Vandova et al., (1995, 2002). As regards on temperature conditions the investigations show that the average annual temperature during the period 1971-2000 compare with the period to the contemporary climate has risen by 1.5^{o}C, this increase is greater in Northern Bulgaria, but in South Bulgaria this trend was not observed. As a result the duration of potential vegetative period (PVP) is extended by 6 - 10 days (Kazandjiev and Slavov, 2006; Kazandjiev, 2011).

Material and Methods

Cherry (*Prunus avium* L.) and peach (*Persica vulgaris* Mill.) plants are the traditionally orchards in Bulgaria. The optimum results for the growing are got only in separate regions, where the meteorological conditions are most suitable for their growth and development. Data for this investigation was collected from agrometeorological and climatic archive of National Institute of Hydrology and Meteorology (NIMH). Long-term 1981-2010 daily and monthly meteorological data from 18 agrometeorological stations have been processed. Dates on the phenological development and more precisely the dates of fruit ripening for the cherry and peach trees during the period of 1980-2010 are presented for 18 meteorological stations. Dates of fruit ripening for the cherry and peach during the period of 1980-2010 are presented for 18 meteorological stations. Used stations were grouped by the similarity of agrometeorological conditions. Central North Bulgaria -Ruse; Northeast-Razgrad and Targovishte; Southeast-Yambol and Suvorovo; Central South-Plovdiv, Chirpan, Sadovo and Southwest- Kustendil and Blagoevgrad and eighth stations for peach Central North-Ruse; Northeast: Targovishte and Razgrad; Southeast-Yambol, Sliven and Suvorovo; Southwest-Petrich and Sandanski. All-time series was tested for homogeneity and was controlled statistical parameters - average, standard deviation and variation coefficient. The dates of steady transitions of temperatures and average dates occurrence of phenological stages ripening of fruits are present as time series of days which starting date 1th January, i.e. Julian days. For all 18 agrometeorological station are received the empirical relation between the sums of negative temperatures measured in the period from of transitions of temperatures below 0^{o}C (chilling degree days) and the number of days with temperature below 0^{o}C. We find to empirical relation between chilly degree days and date of durable transitions of temperatures above 10^{o}C to fruits ripening and the number of days with temperature above 10^{o}C.

Results and Discussion

At the beginning of investigation, we develop two types empirical models, describing the dependencies between the sums of negative temperatures measured in the period from of durable transition of temperatures below 0^{o}C (chilling degree days) and the number of days with temperature below 0^{o}C, fig. 1, and sum of active temperatures of date on durable transitions of temperatures above 10^{o}C to fruits ripening and the number of days with temperature above 10^{o}C for cherry trees (*Prunus avium* L.). The linear regressions models describes the relationship between chilling degree days and number of day with T<0^{o}C and sum of active temperatures of date on durable transitions of temperatures above 10^{o}C to fruits ripening and the number of days with temperature above 10^{o}C. Table 1and 2 show the average values of statistical parameters, as well as numeral values of the calculated equations, as average arithmetic values, standard deviation, mean absolute error (MAE) and variation coefficient.

Table 1. Dependencies between chilling degree days and the number of days with temperature below 0°C for cherry tree (*Prunus avium* L.)

Station	Regression model	R-Squared	r-Coef. Corel.	Standard error	MAE
Ruse	y= 0.79 - 6.54*ax	0.56	0.75	67.29	53.97
Razgrad	y=45.88 - 7.16*ax	0.57	0.75	88.31	68.81
Tyrgovishte	y=67.65 - 8.35*ax	0.50	0.70	67.66	64.56
Yambol	y= 9.88 - 5.10*ax	0.50	0.70	53.38	46.34
Suvorovo	y=56.7 - 3.52*ax	0.51	0.71	42.54	34.58
Plovdiv	y=21.50 - 7.72*ax	0.54	0.73	64.16	53.54
Sadovo	y=47.27 - 7.24*ax	0.51	0.71	63.46	49.79
Chirpan	y=50.43 - 10.00*ax	0.55	0.71	20.37	55.74
Kustendil	y=80.24 - 9.06*ax	0.52	0.72	78.44	69.83
Blagoevgrad	y=42.25 - 7.03*ax	0.51	071	52.44	40.12

Table 2. Dependences between sum of active temperatures from transitions above 10°C to fruits ripening and number of days with temperature above 10°C for Cherry trees

Station	Regression model	R-Squared	r-Coef. Corel..	Standard error	MAE
Ruse	y=138.01+16.84*ax	0.56	0.74	138.61	120.95
Razgrad	y=156.09+38.05*ax	0.51	071	234.91	193.28
Tyrgovishte	y=176.7+14.06*ax	0.77	0.87	63.63	53.00
Yambol	y=75.00+16.26*ax	0.77	0.87	71.00	52.07
Suvorovo	y=229.54+13.32*ax	0.66	0.81	75.53	57.03
Plovdiv	y=273.00+12.33*ax	0.73	0.85	80.72	63.61
Sadovo	y=234.15+13.21*ax	0.68	0.82	86.54	67.71
Chirpan	y=479.12+9.56*ax	0.53	0.72	88.32	69.53
Kustendil	y=124.21+15.29*ax	0.82	0.90	59.99	67.11
Blagoevgrad	y=220.96+16.42*ax	0.76	0.87	64.59	55.56

Figure 1. Dependence between chilling degree days and the number of days with temperature below 0°C for cherry trees for representative stations

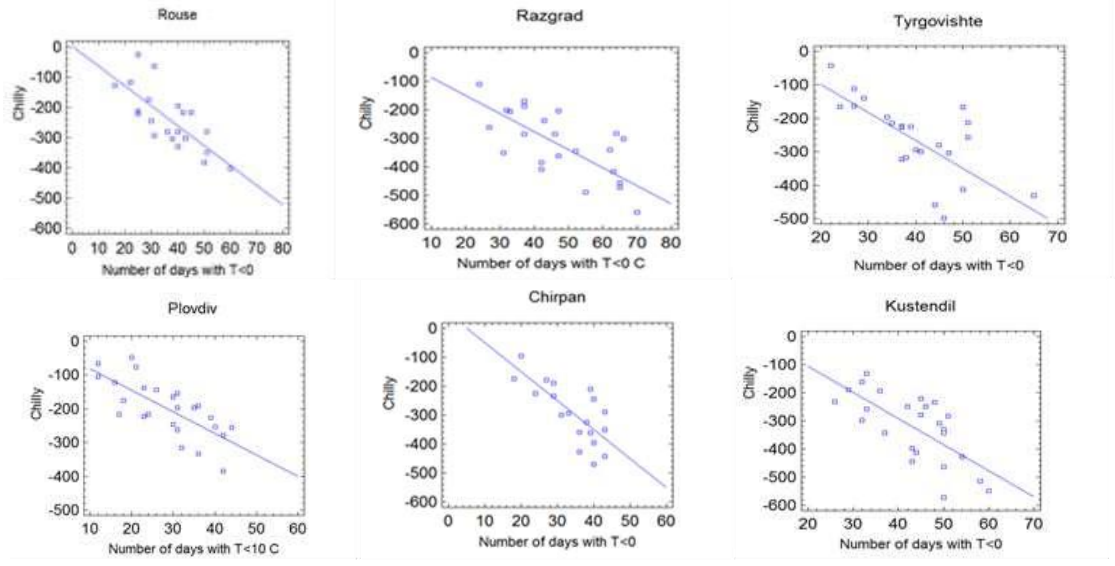
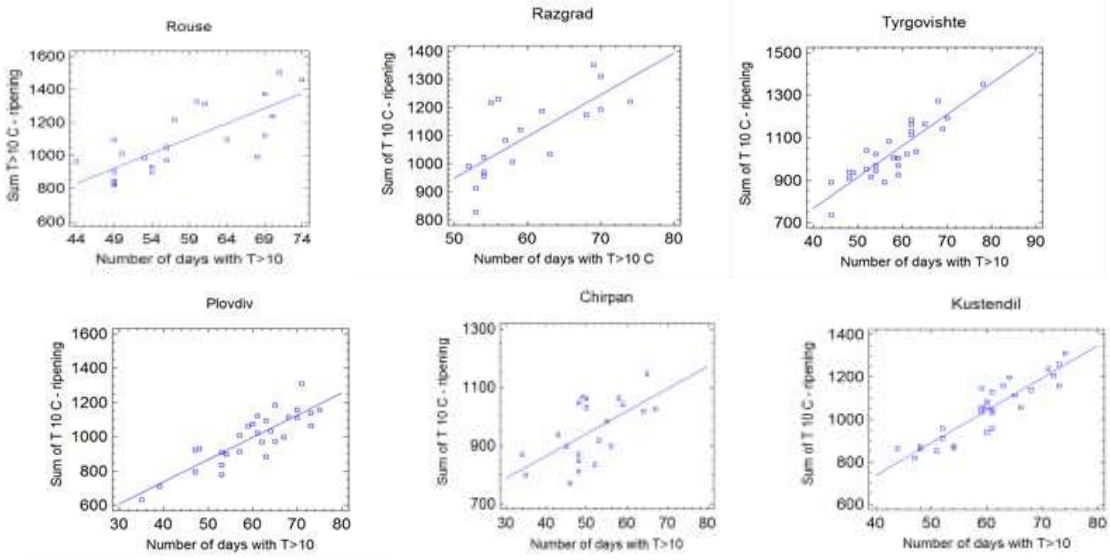


Fig.2. Dependence between sum of active temperatures of date on durable transitions of temperatures above 10°C to fruits ripening and the number of days with temperature above 10°C for cherry trees



The second model describes the relationship between sum of active temperatures of date on durable transitions of temperatures above 10°C to fruits ripening and the number of days with temperature above 10°C for cherry trees, fig. 2.

Also, we develop the empirical models, describing the dependencies between chilling degree days and the number of days with temperature below 0°C and sum of active temperatures of date on durable transitions of temperatures above 10°C to fruits ripening and the number of days with temperature above 10°C for Peach trees The equations of the fitted models and the average values of statistical parameters, as well as numeral values of the calculated equations, as average arithmetic values, standard deviation, mean absolute error (MAE) and variation coefficient are show in table 3 and 4.

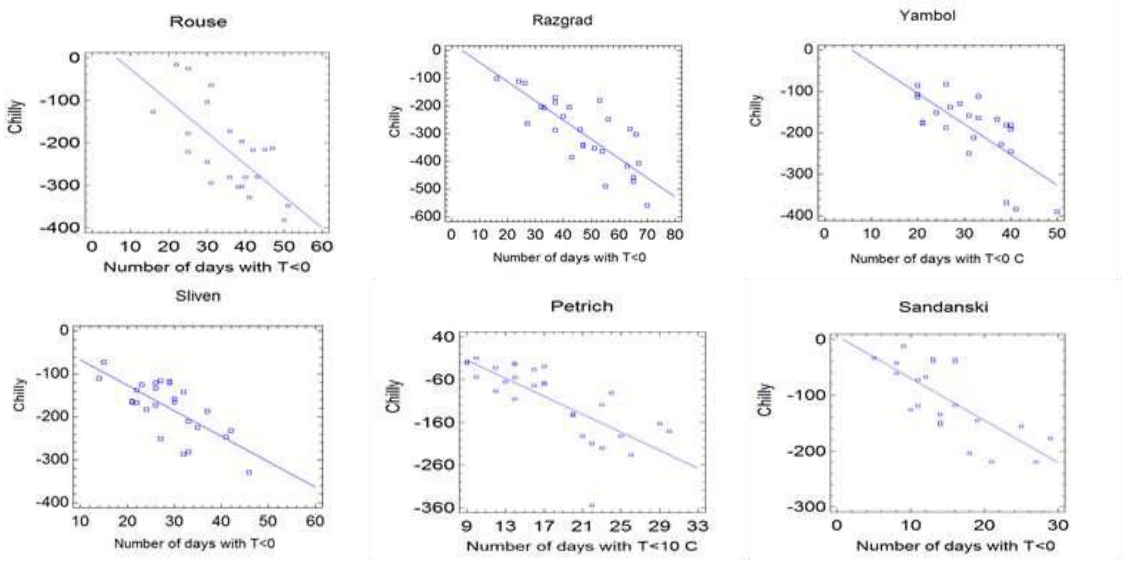
Table 3. Dependence between chilling degree days and number of days with temperature below 0°C for peach trees

Station	Regression model	R-Squared	r-Coef. Corel	Standard error	Mean ab. error
Ruse	y=25.86-6.94*ax	0.66	0.81	78.84	59.59
Razgrad	y=61.08-7.61*ax	0.59	0.76	69.96	60.58
Tyrgovishte	y=2.40-6.35*ax	0.50	0.71	60.05	45.50
Yambol	y=42.57-7.38*ax	0.51	0.71	61.30	48.34
Sliven	y=2.40-6.38*ax	0.52	0.72	44.69	35.86
Suvorovo	y=13.41-5.19*ax	0.57	0.75	44.15	35.08
Petrich	y=71.71-9.67*ax	0.68	0.82	40.65	33.63
Sandanski	y=12.57-5.74*ax -	0.54	0.73	31.93	28.63

Table 4. Dependence between sum of active temperatures from date of temperatures transitions above 10°C to fruits ripening and number of days with temperatures above 10°C for peach trees

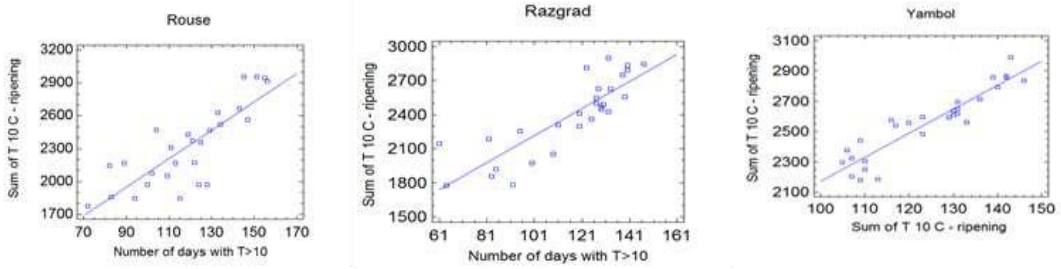
Station	Regression model	R-Squared	r-Coef. Corel	Standard error	Mean ab. err.
Ruse	y=785.47+12.94*ax	0.66	0.81	208.29	157.78
Razgrad	y=779.18+13.76*ax	0.78	0.88	159.44	123.18
Tyrgovishte	y=896.76+16.28*ax	0.76	0.87	198.155	140.04
Yambol	y=588.05+15.84*ax	0.85	0.92	87.20	69.31
Sliven	y=131+29.58*ax	0.54	0.73	244	194.21
Suvorovo	y=594.92+15.09*ax	0.77	0.87	112.52	89.71
Petrich	y=207.46+5.51*ax	0.50	0.70	222.06	186.80
Sandanski	y==19.86+21.19*ax	0.80	0.89	201.62	147.32

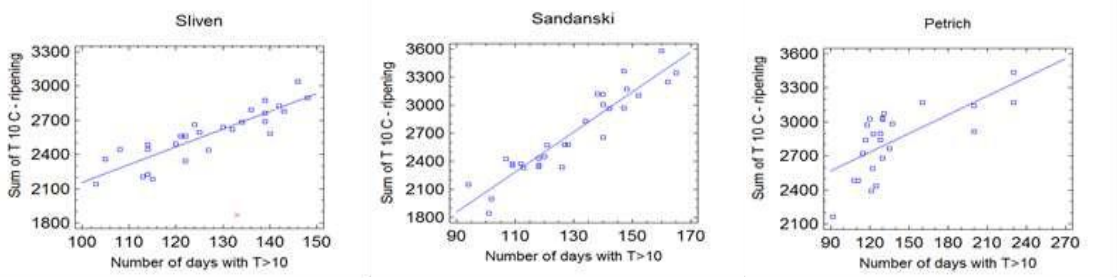
Figure 3. Dependence between chilling degree days and the number of days with temperature below 0°C for Peach trees



The last empirical model describes the relationship between sum of active temperatures of date on durable transitions of temperatures above 10°C to fruits ripening and the number of days with temperature above 10°C for peach trees, fig. 4.

Figure 4. Dependence between date of transitions of temperatures above 10°C to fruits ripening and the number of days with temperature above 10°C for peach trees.





Conclusion

We presented the empirical relations between the sums of negative temperatures measured in the period from of durable transitions of temperatures below 0°C (chilling degree days) and the number of days with temperature below 0°C during the winter dormancy period. Also we find empirical relation between date of durable transitions of temperatures above 10°C to fruits ripening and the number of days with temperature above 10°C during the active vegetation period. The sufficient number of events entailing regression equations, the relatively high values of the correlation index and immaterial equation errors give to recommend that dependencies are useful in agrometeorological practice.

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THE EFFECT OF PGRs AND DIFFERENT FERTILIZATION LEVELS ON THE DRY MATTER FORMATION AND PHENOLOGICAL DEVELOPMENT OF TRITICALE VARIETIES

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Abstract

The biologically active substances known as plant growth regulators (PGRs) are steadily gaining popularity, because they are harmless and ecologically friendly. Their application is not only easy and economically profitable, but also improves the action of the fertilizers, the mineral balance of the plant and leads to yield increase. In order to establish whether the PGRs would influence the phenological development of triticale, a biennial field experiment (2016-2018) was carried out on the experimental field of the Crop Science Department at the Agricultural University of Plovdiv using the block method in three replications, with plot size of 10 m^2. The action of the PGRs Vitafer Algi and Vitafer Green on three triticale varieties (Trismart, Musala and Kolorit (standard)) was conducted under two different fertilizer levels $N_6P_5K_2$ and $N_{12}P_{10}K_4$. The PGRs were applied in the beginning of stem elongation (BBCH 31). The results showed that the both PGRs treatments combined with higher fertilizer levels increased the specific growth rate and dry matter accumulation of the tested plants compared to the untreated variants and the variants treated with lower fertilization rates in combination with PGRs. The beneficial interactive effects of PGRs and fertilization levels on the phenological development of triticale are worthy to further exploration.

Keywords: *Dry matter, Fertilization, PGRs, Phenological development, Triticale*

Introduction

The importance of PGRs was first recognized in 1930s (Harms and Oplinger, 1988). Nowadays the plant growth regulators (PGRs) are widely used in the modern, high input cereal management, because of their large action spectrum. They are furthermore ecologically friendly, harmless, easy to apply and economically profitable. In their nature PGRs comprise a large group of endogenous and exogenous natural chemical compounds that can regulate plant growth in numerous ways (Rajala, 2003). When applicated PGRs can improve yields and their structural compounds (Kolev et al., 2011, Czapla et al., 2000), the mineral balance of the crop (Wierzbowska et al., 2010), the resistance to diseases (Osokina et al., 2015; Novik 1999) and serve as a prevention against lodging (Maciorowski, Stankowski and Piech 2000; Ilumae 2002). The effect of using such substances depends on many factors, e.g. a crop species, environmental conditions or fertilization (Czapla et al., 2007; Wierzbowska et al., 2010). The aim of this study was to evaluate the influence of PGRs and fertilization on the dry matter formation and phenological development in triticale.

Material and Methods

Field trial:

In order to achieve the aims of the investigation, a field experiment was set in the period 2016-2018 on the experimental field of the Crop Science Department at the Agricultural University of Plovdiv, Bulgaria. Three triticale varieties were used- the standard Kolorit, Musala and Trismart. The experiment consisted of a randomized complete block design after predecessor sunflower with three replications and plots of 10 m^2 planted in the sowing rate of 550 germinated seeds m^{-2}. The action of the PGRs Vitafer Algi (VA), Vitafer Green (VG) and

the untreated control variants (NT) was conducted under two different fertilizer levels $N_6P_5K_2$ and $N_{12}P_{10}K_4$. The PGRs were applied in the beginning of stem elongation (BBCH 31).
Estimation methods:
The beginning of the major phenological stages was identified according to Zadoks scale (1974): sowing (00- dry seed); sprung (10- first leaf through coleoptile); third leaf (13- 3 leaves unfolded); tillering (21- main shoot and 1 tiller); stem elongation (31- first node detectable); spike emergence (59- emergence of inflorescence completed); maturity (95- seed dormant). To establish the effect of the temperature and the precipitation conditions of the year, the sum of the active temperatures (above 5^0 C), Σ T act ^{0} C, the average temperature of the period (T aver. ^{0} C) and the sum of precipitation (mm) were calculated for each growth stage period. The dry matter formation and accumulation was determined according to Tretyakova' s (1982) formula for:

- dynamic of growth (g m^{-2} day^{-1}) = (B2-B1)/(D2-D1), where B2 is the dry matter determined at the end of the period; B1- the dry matter in the beginning of the period, D2- the date at the end of the period and D1- the date, when the period starts.

Soil and climate conditions:
According to Popova et al. (2012) the soil on the experimental field of the Agricultural university-Plovdiv has been determined as alluvium, which based on the international classification of FAO belongs to the category of Mollic Fluvisols. The soil in this region has been developed on sandy-clay and sandy-gravel quaternary deposits; with power of the humus horizon of 20-40 cm. Meteorological conditions are different during the years of the study.

Results and discussion

During the years of the study all varieties showed the same duration of the growth stage periods from sowing to tillering stage, due to the same temperature and precipitation requierments (Tabl. 1). The sprung of triticale in 2016 occured 27 days after sowing and in 2017 after 10 days. The duration of this growth stage period depended on the amount of the precipitation, but was limited especially by the average daily air temperature.Although in 2016 the sum of the active temperatures and the amount of rainfall were higher than in 2017, the average daily temperature (6.6 ^{0}C) was lower, which determined the longer growth stage period in the first year.

Table 1. Characteristics of the growth stage period to tillering stage.

Years	Growth stage period	Nr. of days with active t˚C	ΣTact. ˚C	Taver. ˚C	Sum of precipitation (mm)
2016/17	sowing-sprung	27	180.6	6.6	32.9
	sprung-3th leaf	15	75.3	2.2	2.4
	3th leaf-tillering	17	153.5	9.7	124.8
2017/18	sowing-sprung	10	109.1	9.9	28.0
	sprung-3th leaf	12	193.6	9.2	17
	3th leaf-tillering	13	108.1	9.8	42.5

The growth stage period sprung-third leaf lasted between 15 and 12 days during the years of the study. In the first year the growth stage period third leaf-tillering was 17 days and in the second 12 days. Meteorological conditions were most favorable in 2017, where the average daily temperatures of the growth stage periods were stable and without variation and determined the shorter growth stage periods. From tillering to maturity were recorded a different interphase periods of the varieties (Tabl. 2).

Table 2. Characteristics of the growth stage periods tillering-maturity.

A. Kolorit

Years	Growth stage period	Nr. of days with active t˚C	ΣTact. ˚C	Taver. ˚C	Sum of precipitation (mm)
2016/17	tillering-stem elongation	13	147.3	9.7	47.9
	st.elongation-sp.emergence	26	365.6	14.2	35.7
	sp.emergence-maturity	37	746.3	23.7	15.4
2017/18	tillering-stem elongation	29	300.1	10.3	173.7
	st.elongation-sp.emergence	30	488.3	14.8	33.5
	sp.emergence-maturity	56	1115.8	20.8	162.1

B. Musala

Years	Growth stage period	Nr. of days with active t˚C	ΣTact. ˚C	Taver. ˚C	Sum of precipitation (mm)
2016/17	tillering-stem elongation	13	147.3	9.7	47.9
	st.elongation-sp.emergence	21	277.0	12.7	26.1
	sp.emergence-maturity	46	882.8	23.7	15.4
2017/18	tillering-stem elongation	34	266.6	7.8	139.8
	st.elongation-sp.emergence	35	451.8	12.9	58
	sp.emergence-maturity	53	1032.4	20.8	153.8

C. Trismart

Years	Growth stage period	Nr. of days with active t˚C	ΣTact. ˚C	Taver. ˚C	Sum of precipitation (mm)
2016/17	tillering-stem elongation	13	147.3	9.7	47.9
	st.elongation-sp.emergence	32	459.0	17.6	57.8
	sp.emergence-maturity	36	776.0	23.7	15.4
2017/18	tillering-stem elongation	48	398.8	8.3	180.5
	st.elongation-sp.emergence	31	496.3	16.0	24.9
	sp.emergence-maturity	58	1138.3	20.8	162.1

During the first year the growth stage tillering-stem elongation of all varieties was 13 days, due to the same climatic conditions and the coverness of their temperature and precipitation requierments. In 2017 the period was the shortest in the standard (29 days), where the average daily air temperature was the highest (10.3^0 C). In 2016 the growth stage period steam elongation-spike emergence was the shortest in Musala variety (21 days) and although the amount of rainfall (26.1 mm) and the average daily air temperature (12.7 ˚C) were lower compared to the other two varieties it managed to cover its temperature requirements. The average daily air temperatures (17.6 ˚C) and the amount of rainfall (57.8 mm) were the highest in Trismart variety, but the duration of the growth period was the longest (34 days). During the next year the same period was the longest in Musala variety (35 days), due to the lowest sum of the active temperatures. The growth stage period spike emergence-maturity was with duration 36- 46 days in the first year of the study. Musala entered earlier in the growth stage spike emergence, but the next stage lasted longer compared to Kolorit and Trismart varieties, due to the need of accumulating the sum of the active temperatures. In 2016 the growth stage period spike emergence- maturity was the shortest in Trismart variety

(36 days). The maturity period in the second year lasted longer compared to the first year, because of the lower average of daily temperatures. The shortest maturity period was in Musala variety (53 days) and the longest in Trismart variety (58 days).

Table 3. Dynamic of growth, g m^{-2} day^{-1}

Variety	Fertilizer level	Treatment	3th leaf-tillering		tillering – stem elongation		st.elongation – sp.emergence		spike emergence-maturity	
			2017	2018	2017	2018	2017	2018	2017	2018
Kolorit	$N_6P_5K_2$	NT	15,47	12,82	13,51	16,17	165,58	172,89	15,21	21,32
		VA	15,11	15,71	18,89	23,90	224,10	246,16	14,76	19,83
		VG	14,14	15,33	20,70	20,15	216,81	232,65	11,09	22,54
	$N_{12}P_{10}K_4$	NT	13,09	17,10	18,90	18,03	185,58	162,83	18,89	21,14
		VA	17,04	21,54	23,87	26,77	220,18	190,21	19,21	23,71
		VG	13,82	23,07	23,32	25,43	196,98	201,23	18,64	25,42
Musala	$N_6P_5K_2$	NT	13,85	14,86	13,51	19,02	41,11	71,65	27,98	32,11
		VA	15,91	14,62	16,42	22,76	49,36	82,97	32,50	37,87
		VG	14,15	16,97	19,26	19,01	54,30	88,20	40,70	33,92
	$N_{12}P_{10}K_4$	NT	14,87	15,12	20,90	24,89	56,31	77,83	35,23	30,81
		VA	18,17	19,31	22,61	28,90	60,88	85,13	37,40	35,26
		VG	16,04	18,98	24,76	31,43	62,80	82,31	35,93	34,61
Trismart	$N_6P_5K_2$	NT	17,45	14,98	13,47	15,42	46,50	66,48	20,96	27,23
		VA	15,77	17,05	20,03	21,06	54,93	71,04	19,05	25,19
		VG	16,01	16,98	21,33	20,87	54,06	75,12	22,90	28,21
	$N_{12}P_{10}K_4$	NT	14,75	15,22	22,34	25,90	46,06	68,04	26,39	30,75
		VA	15,78	17,74	24,98	28,65	52,43	71,73	23,46	32,22
		VG	15,29	17,12	26,05	26,23	52,90	70,56	24,86	36,80

The dry matter accumulation and its strenght are main physiological parameters for determining the plant productive potential as a source of biomass, which also simultaneously affect the plant reaction against the environmental factors. The dynamic of growth shows the speed of the exchange processes and the growth intensity during the different growth stages of the vegetation. During the vegetation this index changes dynamically, as the speed of the dry matter formation is determined by the specific plant activity. Until tillering the average daily growth varied from 13.09 g m^{-2} day^{-1} to 18.17 g m^{-2} day^{-1} in the first year and from 12.82 g m^{-2} day^{-1} to 23.07 g m^{-2} day^{-1} in the second year and there were no differences between the two fertilizer levels. At tillering it could be observed a positive correlation between the increased fertilizer levels and the average daily growth, as the rise for the untreated variants was in the amount of 1.39 % from Kolorit variety to 1.65 % in Trismart variety in 2016 and between 1.11 % - 1.67 % in 2017. The growth maximum was observed after stem elongation, which can be explained with the stimulating effect of the nitrogen on the synthesis processes of the cereal crops. Another reason for the increased development can be more effective utilize of the soil moisture as a result of the precipitation maximum occurance. The most intensive growth during the years of the investigation was considered by the standard, where the average daily growth of the variant treated with VA in combination with the lowest level of the fertilizer reached a maximum of 224.10 g m^{-2} day^{-1} in 2016 and 246.16 g m^{-2} day^{-1} in 2017. With the vegetation progression followed natural decrease in the dynamic of the growth.

Conclusion

During the growth stage period sowing- germination between the varieties no differences have been established and all varieties entered at the same time due to the same temperature and precipitation requierments. In conditions of insufficient rainfall the duration of germination was determined by the average daily temperature. Different interphase periods were recorded from tillering to maturity for all varieties, what serves for better understanding of the biological requierments of the studied varieties. In the study, it was shown that at tillering a positive correlation between the increased fertilizer levels and the average daily growth could be observed, as the rise for the untreated variants was in the amount of 1.39 % from Kolorit variety to 1.65 % in Trismart variety in 2016 and between 1.11 % - 1.67 % in 2017. The growth maximum was indicated after stem elongation, which can be explained with the stimulating effect of the nitrogen on the synthesis processes of the cereal crops. Hence, further investigation is required to understand better the beneficial interactive effects of PGRs and fertilization levels on the phenological development of triticale.

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COMPARATIVE STUDY OF GRAIN MAIZE HYBRIDS IN THE REGION OF NORTH – EAST BULGARIA

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Abstract

The field experiment was held in Stozher village (north-east Bulgaria) in the period 2014 - 2016. The test was performed by using blocking with four repetitions; experimental field area - 25 m^2 with winter wheat predecessor. The following hybrids were tested; PR38D89, PR37D25, PR36R10, PR37H24 and PR35F38. The total studied area was 500 m². The aim of the investigation was to establish the productivity of five maize hybrids cultivated for grain as well as recommend for growing the most suitable one under non-irrigation in region of north-east Bulgaria. All the stages of the established technology for maize growing were followed. For the purpose of determining the quantity dependence between the studied indicators, the experimental data was processed according to the Anova Method of dispersion analysis, and the differences between the variants were determined by means of the Dunkan's Multiple Range Test. The analysis of the results showed that the production possibility of hybrids maize is determined to a great degree by the meteorological conditions of the year mostly by the precipitation quantity. The highest values of elements of productivity (length of the cob, cop diameter, number of the row per cob, number of the grains per row, number of the grains per cob and mass of grains per cob) were reported with the hybrid PR35F38 and the lowest – with the PR38D89 hybrid. During the years of study the highest yield was obtained from PR35F38 – 6483 kg/ha, followed by PR36R10 – 6200 kg/ha and the lowest one – from PR38D89 hybrid – 5237 kg/ha. We recommend hybrid PR35F38 for growing being the most suitable, under non-irrigation conditions in region of north-east Bulgaria.

Keywords: *maize, hybrids, productive characteristic, yield of grain*

Introduction

The productive potential of maize is manifested when the optimal combination of a complex of factors is achieved, including the hybrid, the agroecological and climatic conditions, as well as the applied agricultural technology (Delibaltova, 2014; Dimitrova et al., 2013; Niaz et al., 2014; Popova et al., 2012; Zivkov and Matev, 2005). The hybrid type with its specific genetic potential is the most dynamic factor in production. The establishment and implementation of new maize hybrids into agricultural practice, as well as their cultivation under different soil and climatic conditions has been the subject of a number of research experiments (Chen et al., 2013; Dong et acal. l.,2016; Kandil, 2013; Popova et al., 2015; Sevov, et al., 2014; Sevov, et al., 2015; Yankov et al., 2014). Choosing the most suitable hybrids for the given region according to the conditions and the cultivation technology, leads to obtaining the targeted results and ensures stable yields (Delibaltova, 2009; Kirchev, 2016; Liu, et al., 2012) . According to Tsankova et al. (2006) the agro-climatic conditions of the region of North-Eastern Bulgaria are suitable for cultivation of FAO 400 – 600 maize hybrids. 'Pioneer' Company offers Bulgarian farmers high quality maize hybrids, which have been adapted to different conditions and included in the Bulgarian varietal list. That was the reason to focus our efforts on studying the highly productive hybrids of 'Pioneer' Company in years with different climatic conditions in the region of North-Eastern Bulgaria.

The aim of the investigation was to establish the productivity of five maize hybrids cultivated for grain as well as recommend for growing the most suitable one under non-irrigation in region of north-east Bulgaria.

Material and Methods

The field experiment was held in Stozher village (north-east Bulgaria) in the period 2014 - 2016. The test was performed by using blocking with four repetitions; experimental field area - 25 m^2 with winter wheat predecessor. The following hybrids were tested; PR38D89, PR37D25, PR36R10, PR37H24 and PR35F38. The total studied area was 500 m². The experiment was performed on leached chernozem soil type.

Ploughing-in of the stubble was performed in August after the forecrop was gathered, and deep ploughing at 25 - 28 cm was performed in October; pre-sowing cultivation with harrowing was performed twice in March and April. Fertilization was performed in autumn before deep ploughing, with 8 kg active substance phosphorus and 10 kg active substance potassium and before sowing with 18 kg active substance nitrogen. The sowing was performed in the second decade of April as the seeds were pre-processed against diseases and pests (by Vitavax – 250 ml / 100 kg seeds and by Diafuran 2.5 l / 100 kg seeds respectively). Herbicide Gardian3000 ml/ ha was applied before sowing and Merlin duo – 1500 ml/ha was applied during vegetation of maize for weed control. All the stages of the established technology for maize growing under non-irrigation conditions were followed. Harvesting was done at full maturity. The grain yield is determined with standard grain moisture of 13%.

The indices length of the cob (cm), cob diameter (mm), number of the row per cob, number of the grains per row, number of the grains per cob, mass of the grains per cob (g) and grain yield (kg/ha) were determined.

For the purpose of determining the quantity dependence between the studied indicators, the experimental data were processed according to the Anova Method of dispersion analysis, and the differences between the variants were determined by means of the Dunkan's Multiple Range Test (Dunkan, 1995).

The major climatic factors determining the growth, development and productivity of maize are temperature and rainfall, their combination and distribution throughout the vegetation season.

The years of the study (2014-2016) differed significantly in the amount and distribution of rainfall during vegetation (Fig. 1). Its amount in the first experimental year was 317 mm, i.e. 63.1 mm higher than the amount measured for a long period of time. During the critical stages of plant growth and development, the crop was well supplied with moisture and that determined 2014 as a favourable year for maize production. The amount of rainfall between April and August 2016 was 256 mm or 61 mm less than the first year of the study, but those values were very close to the values reported for a long period of time, which determined the third year of the experiment as good for the studied hybrids. The least amount of vegetation rainfall was reported in 2015 – only 125 mm, versus 253.9 mm measured for a long period of time. The insufficient amount of moisture in June and July (3 and 4 mm, respectively) when maize was in the critical stages of development, determined the second experimental year as dry and unfavourable for the crop. Comparing the values of the average monthly temperatures during the years of the study showed that they were very close in all the three years and they were higher than those measured for a long period of time (Fig. 2). Just those temperature values in combination with the reported vegetation rainfall had an effect on the growth and development of the plants.

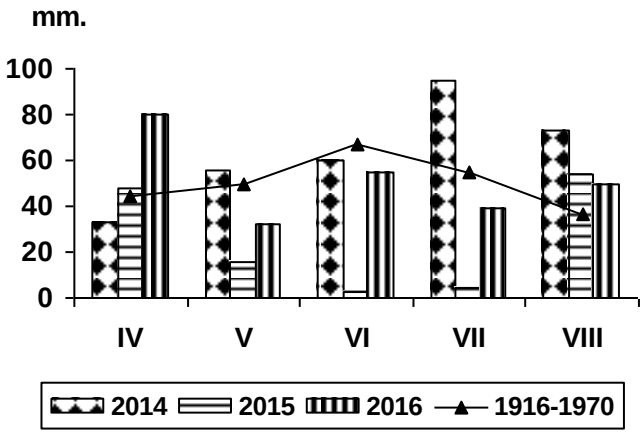

Fig.1. Rainfall, mm

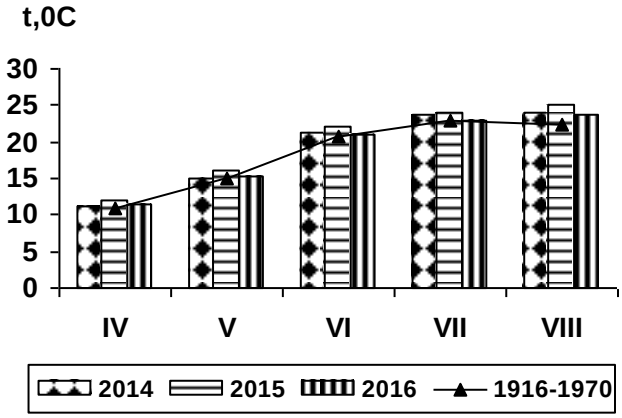

Fig.2. Average monthly air temperature, ^{0}C

The most favourable for maize growth and development was the first experimental year (2014), followed by the third (2016), and unfavourable was the second year (2015), of the experiment, having an effect on the productive potential of the studied maize hybrids.

Results and Discussion

The established structural elements of the yield are presented in Table 1. Data showed that those characteristics change under the influence of the meteorological factors during the years of the study. The highest values of the major structural elements of the yield were recorded during 2014 economic year, followed by those reported in 2016 and the lowest were registered in 2015. Compared to the other studied hybrids, PR35F38 was characterized by longer cobs, a larger diameter of the cob, a larger number of rows and grains per cob, as well as by a higher grain weight per cob.

In the first experimental year, the cob length ranged from 19.5 cm in hybrid PR38D89 to 21.4 cm in PR35F38, whereas in 2015 the values of that characteristic were significantly lower (by 38% on average) and they were from 14.0 to 15.6 cm. In 2016 the cob length ranged from 17.2 to 19.1 cm.

On average for the period of the study, the cob length of hybrid PR35F38 was 18.7 cm and exceeded the hybrids PR36R10 and PR37D25 by 6.2%, the hybrid PR37H24 – by 8.7% and PR38D89 – by 10.6%.

Data about the cob diameter of the studied hybrids showed that during the first experimental year the values ranged from 42 mm in PR38D89 to 55 mm in PR35F38, while in 2015, which was unfavourable for maize production, the values were 7 to 11 mm smaller.

On average for the period 2014-2016, the cob diameter in the studied hybrids ranged from 39.0 to 50.0 mm. The lowest values of that characteristic were reported in hybrid PR38D89 and the highest – in the hybrid PR35F38. Hybrids PR37D25 and PR36R10 had a cob diameter of 43.3 and 46.3 mm, respectively. Hybrid PR35F38 exceeded in cob diameter the hybrids PR38D89, PR37H24, PR37D25 and PR36R10 by 28.2%, 20.0%, 15.5% and 7.8% respectively.

The number of rows per cob is a genetically determined characteristic (Valkova, 2007). The reported values varying in number from 14.0 to 16.0, from 12.6 to 14, and from 13.4 to 14.8 in 2014, 2015 and 2016, respectively, showed that this characteristic was less affected by the conditions during the years of the study than the cob length. However, the number of rows per cob in all the studied hybrids in the first year was 11.5% and 5.5% higher on average than in the second and in the third year.

Hybrid PR35F38 had from 2.8% to 14.3% larger number of rows per cob in the separate years, compared to the others hybrids included in the study. The lowest values of that characteristic were recorded in hybrid PR38D89 – 13.3 rows and on the average for the years of the study, the hybrid had 12% less rows per cob compared to PR35F38 and 6.7% less number of rows per cob on average, compared to hybrids PR37H24, PR37D25 and PR36R10. During 2014, which was favourable for plant growth and development, the highest values of the number of grains in a row were reported. Their number ranged from 34.0 in hybrid PR38D89 to 42.0 in PR35F38. Hybrid PR36R10 surpassed the hybrids PR37D25 and PR37H24 by 4.5 and 5.7%, respectively, and hybrid PR38D89 – by 9.4%, but fell behind PR35F38 by 11.9%.

Table1. Structural elements of the yield

Indices	Years of study	Hybrids				
		PR38D89	PR37D25	PR37H24	PR36R10	PR35F38
Length of the cob, cm	2014	19.5	20.1	19.7	20.2	21.4
	2015	14.0	14.3	14.6	14.7	15.6
	2016	17.2	18.4	17.4	18.0	19.1
	Average	16.9	17.6	17.2	17.6	18.7
Cob diameter, mm	2014	42.0	46.0	44.0	52.0	55.0
	2015	35.0	41.0	38.0	41.0	44.0
	2016	40.0	43.0	43.0	46.0	51.0
	Average	39.0	43.3	41.7	46.3	50.0
Number of the row per cob	2014	14.0	15.0	14.3	15.2	16.0
	2015	12.6	13.2	13.6	13.4	14.0
	2016	13.4	14.0	14.0	14.4	14.8
	Average	13.3	14.1	14.0	14.3	14.9
Number of the grains per row	2014	34.0	35.6	35.2	37.2	42.0
	2015	27.5	29.0	29.5	28.4	31.0
	2016	30.0	34.0	35.0	36.0	38.0
	Average	30.5	32.9	33.2	33.9	37.0
Number of the grains per cob	2014	415.6	530.2	505.1	524.0	583.2
	2015	235.4	278.0	265.0	260.8	311.0
	2016	392.8	470.1	420.0	486.5	507.0
	Average	374.9	426.1	396.7	429.8	467.1
Mass of the grains per cob, g	2014	157.2	161.0	160.7	167.3	174.8
	2015	84.3	97.0	96.2	96.0	97.8
	2016	120.7	140.0	132.3	149.5	160.0
	Average	120.7	132.7	129.7	137.6	144.2

In the dry and unfavourable for maize 2015, a smaller number of grains in a row was established compared to the previous one. In the studied hybrids that characteristic was from 27.5 to 31.0, the differences between the hybrids ranging from 0.9 to 3.5.

In the third year of the study, the maize hybrids yielded from 30.0 to 38.0 grains per row, exceeding those produced in 2015 by 9.1% to 22.6%, but falling behind those produced in 2014 by 10.5% to 13.3%.

The largest number of grains in a row, on average for the study period, was obtained in hybrid PR35F38 – 37.0 grains, followed by PR36R10 – 33.9, PR37H24 – 33.2 and PR37D25 – 32.9. The lowest value was recorded for hybrid PR38D89 – 30.5 grains.

The number of grains per cobs in the studied hybrids varied throughout the years of the experiment and ranged from 415.6 to 583.2 in 2014, from 235.4 to 311.0 in 2015 and from 322.8 to 507.0 in 2016, respectively. In the first year of the study, hybrid PR35F38 produced 167.6, 78.1, 59.2 and 53 more grains per cob, in the second one – 75.6, 46, 50.2 and 33 more grains and in the third one – 114.2, 87, 20.5 and 36.5 more grains, compared to hybrids PR38D89, PR37H24, PR36R10 and PR37D25, respectively.

On average for the period 2014-2016, hybrid PR35F38 yielded 467.1 grains per cob and exceeded the other studied hybrids by 15.2% on average.

The grain weight per cob is one of the most important structural elements of the yield and to a certain extent it determines the productivity of maize hybrids. The values of that characteristic in all the studied hybrids were the highest in 2014, ranging from 157.2 to 174.8 g, while in 2015 and 2016 the values were from 72.9 to 77 g lower and from14.8 to 36.5 g lower, respectively.

The highest grain weight per cob (on average for the experimental period) was established in hybrid PR35F38 – 144.2 g, surpassing hybrid PR36R10 by 4.8%, hybrid PR37D25 by 8.7%, hybrid PR37H24 by 11.2% and hybrid PR38D89 by 19.5%, respectively.

The results obtained for the grain yield of the studied maize hybrids showed that both the productivity elements and the values of that characteristic varied depending on the meteorological conditions during the years of the experiment (Table 2). The amount of rainfall during vegetation, its good distribution and the combination with the average monthly temperatures favoured the higher yields obtained in the first year compared to the second and the third year of the study.

In 2014, the highest statistically significant yield was obtained from hybrid PR35F38 (9100 kg/ha), followed by PR36R10 (8800 kg/ha), PR37D25 (8520 kg/ha) and the lowest – from hybrid PR38D89 (7000 kg/ha).

The insufficient rainfall during the vegetation season in 2015, especially during the stages of maize tasseling and silking, was the reason for the low grain yields. They varied from 2010 to 2650 kg/ha for the studied hybrids. In that year, hybrid PR36R10 surpassed in yield hybrids PR37D25 and PR37H24 by 9.1 and 14.3%, respectively, but fall behind hybrid PR35F38 by 10.4%, the values being significant.

Table 2. Grain yield, kg/ha

Hybrids	Years of study			Average for the period, kg/ha
	2014 kg/ha	2015 kg/ha	2016 kg/ha	
PR38D89	7000 [a]	2010 [a]	6700 [a]	5237
PR37D25	8520 [c]	2200 [c]	7250 [c]	5990
PR36R10	8800 [d]	2400 [d]	7400 [d]	6200
PR37H24	7600 [b]	2100 [b]	7050 [b]	5583
PR35F38	9100 [e]	2650 [e]	7700 [e]	6483
LSD $_{5\%}$	*225*	*86*	*143*	

It was statistically proven that the average yield obtained from hybrid PR35F38 was 27.2% higher compared to the other studied hybrids.

In the third year of the experiment (2016), the grain yield ranged from 6700 to 7700 kg/ha, i.e. it was significantly higher (from 4690 to 5050 kg/ha) compared to the previous 2015. The highest yield was obtained from hybrid PR35F38 and it exceeded PR36R10, PR37D25 and PR37H24 by 300, 450 and 650 kg/ha, respectively, the differences being statistically significant. The lowest yield was obtained from hybrid PR38D89, the values being 14.9% less than the yield from PR35F38, 9.2% less than PR37H24, 6.2% less than PR37D25 and 4.0% less than PR36R10, which was statistically proven.

The highest yield, on average for the period of the study, was obtained from hybrid PR35F38 (6483 kg/ha) surpassing by 4.6%, 8.2% and 16.1% hybrids PR36R10, PR37D25 and PR37H24, and the lowest yield was obtained from hybrid PR38D89 – 5237 kg/ha.

The analysis of variance of the grain yield (Table 3), showed a strong statistically proven effect of the year with its specific climatic conditions – 52%. The genetic potential of the hybrids also had a significant influence on the values of that characteristic (30%).

Table 3. Analysis of variance.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*η 2*
Hybrids	7590904,05	4	1897726,0	13,62	0,000	30,0
Years	271574836,8	2	135787418,0	974,86	0,000	52,0
Interaction	3221296,67	8	402662,08	2,89	0,010	18,0
Residual	6268039,29	45	139289			

A well-proven interaction between the two factors was established (18%).

Conclusions

The productive potential of the studied maize hybrids in North-Eastern Bulgaria is largely determined by the meteorological conditions of the year and, above all, by the amount and distribution of vegetative rainfall.

The highest values of the structural elements of the yield were established in hybrid PR35F38 and the lowest – in PR38D89.

On average during the period of the study (2014-2016), the highest grain yield was obtained from PR35F38 – 6483 kg/ha, followed by PR36R10 – 6200 kg/ha and the lowest – from hybrid PR38D89 – 5237 kg/ha.

Out of the studied maize hybrids grown in the region of North-Eastern Bulgaria, it is recommended to cultivate hybrid PR35F38, as it proved to be more productive than hybrids PR36R10, PR37D25, PR37H24 and PR38D89 in climatically different years.

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EFFECT OF INTERCROPPING MAIZE WITH COWPEA ON FORAGE YIELD AND QUALITY

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Abstract

Low cost and high dry matter content are the reasons why cereals play an important role in feeding ruminant animals. Nevertheless, maize forage is poor in protein content which leads to low quality and nutritive value. Regarding the high feed costs of protein supplementations, legumes can be used in livestock nutrition for their high protein content, and thus, provide cost savings. In this study, maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* L.) were intercropped in different sowing densities and their monocropping equivalents were tested to determine the best intercropping system on forage yield and quality. Maize was cultivated alone (75 000 plants ha^{-1}) and intercropped with cowpea as follows: 75 000 plants ha^{-1} of maize and 37 500 plants ha^{-1} of cowpea (M_1), 75 000 plants ha^{-1} of maize and 50 000 plants ha^{-1} of cowpea (M_2) and 75 000 plants ha^{-1} of maize and 75 000 plants ha^{-1} of cowpea (M_3), in rows alternating with maize. The highest dry matter yield was produced by M_3 (20.4 t ha^{-1}), and the lowest by M_1 (17.3 t ha^{-1}). All intercropped systems had higher dry matter crude protein contents, M_1 (101 g kg^{-1}), M_2 (114 g kg^{-1}) and M_3 (125 g kg^{-1}), than the monocrop maize (79 g kg-1). Intercropping of maize with cowpea reduced neutral detergent fibre and acid detergent fibre contents, resulting in increased forage digestibility. Therefore, maize intercropping with cowpea could substantially increase forage quantity and quality, and decrease requirements for protein supplements as compared with maize monocrop.

Keywords: *Intercropping, Maize, Cowpea, Yield, Quality*

Introduction

In many regions of Europe, whole-plant maize silage is the basic feed used in the feeding of dairy cows and fattening cattle. As a cultivation system, intercropping involves the planting of two or more crop species simultaneously at the same field during a growing season (Kipkemoi et al., 2010, Costa et al., 2012, Ofori and Stern, 1987). Proper spatial arrangements, planting proportions and the maturity dates of components in maize-grain legume intercropping improve biodiversity and have many advantages over monocrop maize cropping. Although maize provides high yield in terms of dry matter, it produces forage with low protein content. However, protein is needed by livestock for growth and milk production. Protein is also needed by rumen bacteria which digest much of the feed for ruminant animals (Ghanbari-Bonjor, 2000). Therefore, it is necessary to provide livestock with protein supplements when forage quality is low. The purchase of protein supplements is expensive and results in high feed costs. Cowpea, an annual legume with a high level of protein (about twice that of maize), can be mixed with maize to improve forage protein content of diets and, thus, the costs of high quality forage production can be lowered. Intercropping has many advantages over sole cropping. It provides an efficient utilization of environmental resources, reduces risk to the cost of production, provides greater financial stability for farmers, decreases pest damages, suppresses weed growth more than monocrop, improves soil fertility through increasing nitrogen to the system and improves forage yield and quality (Francis et al., 1976; Willey, 1979). Many research studies have explored the use of intercropping for

forage production. Toniolo et al. (1987) reported significantly higher crude protein (CP) content of maize-soybean intercropping than that of monocropped maize. Javanmard et al. (2009), worked on intercropping of maize with different legumes, and showed that dry matter yield and crude protein yield of forage were increased by all intercropping compositions compared with the maize monocrop. Dahmardeh et al. (2009) concluded that intercropping of maize and cowpea resulted in more digestible dry matter and also crude protein content than maize mono-cropping. The present study was designed to determine the effect of different patterns of maize-cowpea intercropping in resource consumption on fodder yield and quality.

Material and Methods

A field experiment was carried out during the 2016 growing season at experimental fields in Bjelovar, Croatia. Meteorological data of the experimental site are presented in Table 1.

Table 1. Mean monthly air temperature and rainfall during the 2016 growing season

Weather data	Month					
	April	May	June	July	August	September
Air temperature (°C)	13.0	16.2	21.3	23.3	20.5	18.3
Rainfall (mm)	43.2	85.2	78.4	57.4	114.9	46.7

The experiment was set up as a randomized complete block design with three replicates. Maize was sown as monocrop (SM) and intercropped with cowpea as provided in Table 2. Maize hybrid seed (KWS Kolumbaris) was obtained from Seed Company "KWS". Seed of the cowpea cultivar "Dolga vigna" was obtained from Company "Sjemenarna". The treatment comprising the individual plot size was 12 m × 5 m.

Table 2. Planting densities of solo maize and maize–cowpea intercrops in various treatments

Treatment	Planting density
SM	75 000 plants ha^{-1} of maize
M_1	75 000 plants ha^{-1} of maize and 37 500 plants ha^{-1} of cowpea
M_2	75 000 plants ha^{-1} of maize and 50 000 plants ha^{-1} of cowpea
M_3	75 000 plants ha^{-1} of maize and 75 000 plants ha^{-1} of cowpea

The maize population (75 000 plants ha^{-1}) were spaced at 70 cm × 19 cm and cowpea population (37 500, 50 000 and 75 000 plants ha^{-1}) were spaced at 70 cm × 38.1 cm, 70 cm x 28.6 cm and 70 x 19 cm, respectively, in rows alternating with maize. Basic tillage was carried out by ploughing to 30 cm depth. Presowing preparation was done using a tractor-mounted rototiller. All plots were fertilized with the same amount of fertilizer before sowing, containing 200 kg of N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 200 kg of K_2O ha^{-1}. Maize and cowpea were sown to a depth of approximately 5 cm by hand in April 30, 2016. Herbicides or pesticides were not applied. Hand weeding by hoe was done once when the maize was approximately 30 cm in height. The soil of the research area has an acid pH 5.0 reaction (M-KCl), poor humus (2.1%), poorly supplied with physiologically active phosphorous (8.8 mg P_2O_5/100 g soil), medium supplied with physiologically active potassium (13.4 mg K_2O/100 g soil) and richly supplied with total nitrogen amounting to 0.24%. The fresh fodders were manually harvested when the maize reached soft dough stage and cowpea at R8 stage and then chopped into 20 mm size pieces with a chaff cutter. The dry matter (DM) content was determined by drying in an oven at a temperature of 105°C to a constant mass. Crude protein (CP) was measured according to Kjeldahl (AOAC, 2000), neutral detergent fibre (NDF) and

acid detergent fibre (ADF) were measured according to the Van Soest method. Significance of the differences between the means was determined according to the least significant difference (LSD) at 0.05 probability level.

Results and Discussion

Fresh forage yields differed significantly (P<0.05) among treatments, varying between 45.8 t ha^{-1} (SM) and 61.2 t ha^{-1} (M_3). Dry matter yield of the plots showed trend similar to that for fresh forage yield (Table 3). Monocrop maize had the lowest dry matter yield (16.8 t ha^{-1}).

Table 3. Fresh forage, dry matter and crude protein yield of the studied treatments.

Treatments	Fresh forage yield t ha^{-1}	Dry matter yield t ha^{-1}	Crude protein yield t ha^{-1}
SM	45.8c	16.8c	1.33d
M_1	48.5c	17.3c	1.97c
M_2	54.0b	18.8b	2.14b
M_3	61.2a	20.4a	2.55a

Different letters in the column mean a significant difference (P < 0.05)

Cowpea can be intercropped with maize (Dahmardeh et al., 2009) and sorghum (Ahmad et al., 2007) for a higher yield and quality compared with sole cropping. One possible explanation for the higher yields of intercrops is the ability of the crops to exploit different soil layers without competing with each other. Further, higher consumption of environmental resources, agronomic practices, crop genotypes, photosynthetically active radiation and soil moisture during rainy periods by intercropping can affect the yield and potential use of the intercropping system (Ofori and Stern, 1987; Anil et al., 1998; Lithourgidis et al., 2006). Geren et al., (2008) and Htet et al., (2016) showed that legume contribution to maize in mixtures was significant and increased the total biomass yield of mixtures. In this experiment M_3 treatment had the highest CP yield of 2.55 t ha^{-1} (Table 3) of the treatments. Maize alone had the highest content dry matter (366 g kg^{-1}), while M_3 treatment had the lowest (333 g kg^{-1}). One of the main reasons for intercropping maize and cowpea is to increase silage crude protein level. As crude protein is very important in livestock feeding, silage that contains more crude protein is desirable. In this study, treatment M_3 had the highest crude protein level (125 g kg^{-1} DM), followed by M_2 (114 g kg^{-1} DM), M1 (101 g kg^{-1}), and monocrop maize (79 g kg^{-1} DM), respectively (Table 4). Results in the present study were in agreement with other studies where legumes also increased crude protein concentration when in a mixture with maize (Dawo et al., 2007; Baghdadi et al., 2016; Erdal et al., 2016; Htet et al., 2016). This could be due to higher nitrogen availability for maize in intercropping compared with the monoculture crop (Eskandari et al., 2009; Eskandari, 2012).

Table 4. Nutrient composition of maize and maize-cowpea intercropped fresh forage (g kg^{-1} DM).

Nutrient composition	Treatments			
	SM	M_1	M_2	M_3
Dry matter	366a	356b	348c	333d
Crude protein	79d	101c	114b	125a
Neutral detergent fibre	411a	391b	371c	342d
Acid detergent fibre	231a	217b	204c	192c

Different letters in the row mean significant difference (P < 0.05).

Cowpea fodder is a rich source of crude protein, giving up to 184 g kg^{-1} (Khan et al., 2010) Furthermore, protein content of cowpea forage (220 g kg^{-1}) was higher compared to some legumes such as lablab (Lablab purpureus), mucuna (Mucuna pruriens) and grass species (Sorghum sudanense), though it was the species least consumed by goats (Gwanzura et al., 2011). Dahmardeh et al. (2009) concluded that maximum crude protein percentage of forage was obtained at the milky stage and minimum crude protein was achieved at the dough stage of maize growth in maize-cowpea intercropping. According to the results obtained from this study, neutral detergent fibre contents changed between 411 g kg^{-1} DM (monocrop maize) and 342 g kg^{-1} DM (treatments M_3). The neutral detergent fibre content is important in ration formulation because it reflects the amount of forage that can be consumed by animals (Lithourgidis et al., 2006). High quality forages have low concentrations of both neutral detergent fibre and acid detergent fibre and high digestibility (Peterson et al., 1994). As lower amounts of fiber components help better digestion, it stems that soybean intercropped plots were superior to monocrop maize in terms of neutral detergent fibre and acid detergent fibre. Generally, neutral detergent fibre concentration is greater for grasses than for legumes (Dahmardeh et al., 2009). Acid detergent fibre concentrations, consisting of cellulose and lignin, are important because they determine the ability of an animal to digest the forage. As the acid detergent fibre increases, the digestibility of the forage usually decreases. The content of acid detergent fibre in treatment M_3 (192 g kg^{-1}) was lower than that of other treatments. Similar results have been reported by Dahmardeh et al. (2009) and Htet et al. (2016). Dahmardeh et al. (2009) reported that maximum ADF (31.85%) was recorded by sowing maize alone while increasing the proportion of cowpea seeds to 50% in intercropping with maize, resulted in the lowest ADF (25-89%).

Conclusion

The conclusion of the present study is that intercropping of maize with cowpea at various planting densities was shown to be an effective way to influence fresh biomass production, dry matter and crude protein content to maintain or enhance nutrient quality of fresh forage. Intercropping of maize with cowpea increased crude protein, decreased neutral detergent fibre and acid detergent fibre concentrations in fresh forage. Finally, intercropping with 75 000 plants ha^{-1} of maize and 75 000 plants ha^{-1} of cowpea was most suitable according to the nutrient composition in fresh forage.

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EFFECT OF INTERCROPPING MAIZE WITH SOYBEAN ON FORAGE YIELD AND QUALITY

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Abstract

Cereals are high important in feeding ruminant animals for their high dry matter production and low cost. However, maize forage is poor in protein content which shows its low quality and nutritive value. Regarding to high feed costs of protein supplementations, legumes can be used in livestock nutrition for their high protein content and, thus, providing cost savings. Since legumes have low dry matter yield, acceptable forage yield and quality can obtained from intercropping cereals and legumes compared with their sole crops. In this study, maize (*Zea mays* L.) and soybean (*Glycine max* L. Merril) intercropped in different numbers of rows and their monocropping equivalents were tested to determine the best intercropping system on forage yield and quality. Maize was cultivated alone and intercropped with soybean as follows: 1 row maize to 1 row soybean (1M1S), 1 row maize to 2 rows soybean (1M2S) and 1 row maize to 3 rows soybean (1M3S). The highest dry matter yield was produced by intercropping 1M3S (19.2 t ha^{-1}) and the lowest by 1M1S (16.9 t ha^{-1}). All intercropping had higher crude protein values in dry matter 1M1S (113 g kg^{-1}), 1M2S (122 g kg^{-1}) and 1M3S (130 g kg^{-1}) than the monocrop maize (77 g kg^{-1}). Intercropping of maize with soybean reduced neutral detergent fiber and acid detergent fiber content, resulting in increased forage digestibility. The study showed that among all intercropped forages the 1M3S (1 row maize to 3 rows soybean) was preferable according to forage yield and quality than other intercropping.

Keywords: *Intercropping, Maize, Soybean, Yield, Quality*

Introduction

In many regions of Europe, whole-plant maize silage is the basic feed used in the feeding of dairy cows and fattening cattle. Although maize provides high yield in terms of dry matter, it produces forage with low protein content. Toniolo et al., (1987) observed significantly higher crude protein content of maize-soybean intercropping than that of monocropped maize. Javanmard et al., (2009) worked on intercropping of maize with different legumes. Authors pointed out that dry matter yield and crude protein yield of forage were increased by all intercropping compositions as compared with the maize monoculture. The use of maize grown for ensilaging and the seeding of soybean with maize in alternate-rows as 1 maize + 1 soybean or 1 maize + 2 soybeans highly increased the silage quality and crude protein content (Altinok et al., 2005). The intercropping yields are often higher than in sole cropping systems (Lithourgidis et al., 2007). The reasons are mainly that resources such as water, light and nutrients can be utilized more efficiently than in the respective sole cropping systems (Liu et al., 2006). The underlying principle of better resource use in intercropping is that, if crops differ in the way they utilize environmental resources when grown together, they can complement each other and make better combined use of resources than when they are grown separately (Ghanbari-Bonjor, 2000). Yield advantages occur when intercrop components compete only partly for the same plant growth resources, and inter specific competition is less than intra-specific competition (Vandermeer, 1989). Light, water and nutrients are more

completely absorbed and converted to crop biomass by intercropping. This is a result of differences in competitive ability for growth factors between intercrop components (Davis and Woolley, 1993). Efficient utilization of available growth resources is fundamental in achieving sustainable systems of agricultural production. Intercropping can conserve soil water by providing shade, reducing wind speed and increasing infiltration with mulch layers and improved soil structure (Young, 1997). Physiological and morphological differences between intercrop components affect their ability to use resources; especially cereals with legumes, have several advantages such as higher total yield, better land use efficiency (Dhima et al., 2007), yield stability of the cropping system (Lithourgidis *et al*., 2006), better utilization of light, water, and nutrients (Javanmard *et al*., 2009), improved soil conservation (Anil *et al*., 1998), soil fertility through biological nitrogen fixation, increases soil conservation through greater ground cover compared to sole cropping, and provides better lodging resistance for crops that are susceptible to lodging when grown in monoculture (Lithourgidis *et al.*, 2006) and better control of pests and weeds (Banik *et al*., 2006; Vasilakoglou *et al*., 2008). Atmospheric nitrogen fixation by legumes can reduce the competition for nitrogen in the legume-cereal intercropping system, allowing the cereals to use more soil nitrogen (Eskandari *et al*., 2009). This can affect forage quality of intercrop components because protein content is directly related to the nitrogen content of the forage (Putnam *et al*., 1986). The present study was designed to determine the effect of different patterns of maize-soybean intercropping in resource consumption on fodder yield and quality.

Materials and Methods

A field experiment was carried out during the 2016 growing season at experimental fields in Bjelovar, Croatia. Meteorological data of the experimental site are presented in Table 1.

Table 1. Mean monthly air temperature and rainfall during the 2016 growing season

Weather data	Month					
	April	May	June	July	August	September
Air temperature (°C)	13.0	16.2	21.3	23.3	20.5	18.3
Rainfall (mm)	43.2	85.2	78.4	57.4	114.9	46.7

The experimental design is set up as randomized complete block design with three replicates. Maize was seeded as monocrop (SM) and intercropped with soybean as follows: 1 row corn to 1 row soybean (1M1S), 1 row corn to 2 row soybean (1M2S) and 1 row corn to 3 row soybean (1M3S). Maize hybrid seed "KWS Kolumbaris" was obtained from Seed Company „KWS". The soybean cultivar seed known as „OAC Wallace" was obtained from Company „RWA Agro". The treatment comprising the individual plot size was 12 m × 5 m. The maize and soybean were spaced at 70 cm × 19 cm and 70 cm × 5 cm with population of 75 189 and 285 720 plants per hectare, respectively. Basic tillage was carried out by ploughing to 30 cm depth. Presowing preparation was done using a tractor-mounted rototiller. All plots were fertilized with the same amount of fertilizer before sowing, containing 200 kg of N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 200 kg of K_2O ha^{-1}. Maize and soybean were sown to a depth of approximately 5 cm by hand in April 30, 2016. Herbicides or pesticides were not applied. Hand weeding by hoe was done once when the maize was approximately 30 cm in height. The soil of the research area has an acid pH 5.0 reaction (M-KCl), poor humus (2.1%), poorly supplied with physiologically active phosphorous (8.8 mg P_2O_5/100 g soil), medium supplied with physiologically active potassium (13.4 mg K_2O/100 g soil) and richly supplied with total nitrogen amounting to 0.24%. The fresh fodders were manually harvested when the maize

reached soft dough stage and soybean at R7 stage and then chopped into 20 mm size pieces with a chaff cutter. The dry matter (DM) content was determined by drying in an oven at a temperature of 105°C to a constant mass. Crude protein (CP) was measured according to Kjeldahl (AOAC, 2000), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were measured according to the Van Soest method. Significance of the differences between the means was determined according to the least significant difference (LSD) at 0.05 probability level.

Results and Discussion

Fresh forage yield differences of the treatments were statistically significant (P<0.05). Fresh forage yield varied between 55.6 t ha^{-1} (monoculture maize) and 45.1 t ha^{-1} (1M1S). Erdogdu *et al.* (2013) reported that 50% corn + 100% soybean sown in alternate rows was superior to monoculture maize. Dry matter yield of the plots showed similar trend as for fresh forage yield (Table 2). Monoculture maize had the highest dry matter yield (20.3 t ha^{-1}). Monoculture maize and 1M3S treatment were not statistically different which indicated that 1M3S intercropping system was comparable to monoculture maize in terms of dry matter yield. It was reported that maize-soybean intercrops produced higher dry matter yield than either species sole (Tansı and Sağlamtimur, 1992; Geren *et al*., 2008; Eskandari, 2012; Eslamizadeh, 2015).

Table 2. Fresh forage, dry matter and crude protein yield of the studied treatments

Treatments	Fresh forage yield t ha^{-1}	Dry matter yield t ha^{-1}	Crude protein yield t ha^{-1}
SM	55.6a	20.3a	1.56d
1M1S	45.1c	16.9c	1.91c
1M2S	48.4c	18.7b	2.28b
1M3S	51.7b	19.2a	2.50a

Different letters in the column mean significant difference (P < 0.05)

One possible explanation for the higher yields of intercrops is the ability of the crops to exploit different soil layers without competing with each other. Besides, higher consumption of environmental resources, agronomic practices, crop genotypes, photosynthetically active radiation and soil moisture by intercropping raining period can affect the yield and potential use of the intercropping system (Ofori and Stern, 1987; Anil *et al*., 1998; Lithourgidis *et al*., 2006). Geren *et al*., (2008); Htet *et al*., (2016) indicated that, legume contribution to corn in mixtures was significant and increased the total biomass yield of mixtures. In this study the 1M3S treatmens had the highest crude protein yield value 2.50 t ha^{-1} (Table 2) than other treatments. Sole maize had the lowest content dry matter (345 g kg^{-1}), while 1M3S treatment had the highest value (370 g kg^{-1}). One of the main reasons of intercropping maize and soybean is to increase silage crude protein level. Since crude protein is very important in livestock feeding, silage that contains more crude protein is desirable. In this study in 2016 year, treatments 1M3S had the highest crude protein level (130 g kg^{-1} DM), followed by 1M2S (122 g kg^{-1} DM), 1M1S (113 g kg^{-1}), and monoculture maize (77 g kg^{-1} DM) (Table 3).

Table 3. Nutrient composition of maize and maize-soybean intercropped fresh forage (g kg^{-1} DM).

Nutrient composition	Treatments			
	SM	1M1S	1M2S	1M3S
Dry matter	345d	353c	362b	370a
Crude protein	77d	113c	122b	130a
Neutral detergent fibre	413ns	376ns	381ns	399ns
Acid detergent fibre	228ns	191ns	213ns	218ns

Different letters in the row mean significant difference ($P < 0.05$).

These results showed that an increased proportion of legumes in intercrops increased the crude protein contents in mixture. Results in the present study were in agreement with other studies where legumes also increased crude protein concentration when in a mixture with corn (Dawo *et al*., 2007; Dahmardeh *et al*., 2009; Baghdadi *et al*., 2016; Erdal *et al*., 2016; Htet *et al*., 2016). It could be due to higher nitrogen availability for maize in intercropping compared with the sole crop (Eskandari *et al*., 2009; Eskandari, 2012). According to the results from this study, neutral detergent fibre values changed between 413 g kg^{-1} DM (monoculture maize) and 376 g kg^{-1} DM (treatments 1M1S). The neutral detergent fiber content is important in ration formulation because it reflects the amount of forage that can be consumed by animals (Lithourgidis *et al*., 2006). High quality forages have low concentration of both neutral detergent fiber and acid detergent fiber and high digestibility (Peterson *et al*., 1994). Since lower amount of fiber components help for a better digestion, it stems that soybean intercropped plots were superior to monoculture maize in terms of neutral detergent fiber and acid detergent fiber. Generally, neutral detergent fiber concentration is greater for grasses than for legumes (Dahmardeh *et al*., 2009). The acid detergent fiber concentration values, consisting of cellulose and lignin, are important because they describe the ability of an animal to digest the forage. As the acid detergent fiber increases, the digestibility of the forage usually decreases. The value of acid detergent fiber at tretment 1M1S (191 g kg^{-1}) was lower than that at all tretments. Similar results have been reported by Dahmardeh *et al*. (2009) and Htet *et al.* (2016).

Conclusion

The conclusion of the present study is that intercropping of maize with soybean at various planting densities was shown to be an effective way to influence fresh biomass production, dry matter and crude protein content to maintain or enhance nutrient quality of fresh forage. Intercropping of maize with soybean increased crude protein, decreased neutral detergent fibre and acid detergent fibre concentrations in fresh forage. Finally, intercropping with 1M3S treatment (1 row maize to 3 rows soybean) was most suitable according to the nutrient composition in fresh forage.

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TECHNIQUE OF THE ACCELERATED RECEIVING OF BASIC (ELITE) SEEDS OF NEW AND DEFICIT VARIETIES

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Abstract

The intensification of all processes of cultivation and production of agricultural crops is a basis of the modern agriculture, which means sowing of thoroughbred seeds. Therefore, in the leading countries of the world, special attention is paid on industrial seed farming. The right management of seed farming enables complete realization of productivity of the variety and maintaining its economic and biological properties.

In the modern conditions in seed farming great attention is paid to variety replacement and variety renewal. Fast replacement of a variety provides the complete revealing of potential opportunities of new variety, which ensures the fastrefund. Renewal of the varieties provides the maintaining of high-quality traits of varieties. Proceeding from the methods of releasing of varieties, the duration of their use in production varies from one to five years. Proceeding from international experience, those countries which provide fast replacement of varieties and their renewal, receive big yield per hectare.

Nowadays, in Georgia there is no system of seed farming, the farms as usual use sub-standard seeds therefore productivity of crops is catastrophically low (wheat –1,5 t/hectare, corn - 2,5 t/hectare, potatoes - 15,0 t/hectare).

The system of seed purification of varieties is quite a long process and it takes 6-7 years. Therefore for the reduction of duration of seed purificationwe set a goal to develop the accelerated method of receiving seeds. On the basis of the researches conducted in 2013-2017 was developed the technique of the accelerated receiving of basic seeds of new and deficit varieties in 3-4 years instead of 6-7 years.

Key words: *seed farming, grade, wheat, elite, standard seeds.*

Introduction

Seed farming is a branch of agriculture, which plays an important role in receiving of a big and qualitative yield [1,2,3,4,5].In the modern conditions in seed farming great attention is paid to variety replacement and variety renewal. Fast replacement of a variety provides the complete revealing of potential opportunities of a new variety, which ensures the fast refund. At distribution of new varieties isimportant the development of separate elements of technology of their cultivation, which is differentiated depending on features of a variety. An important part of seed farming is the variety renewal, which provides maintaining of its high-quality traits [4. Proceeding from the methods of release of varieties, the duration of their use in production varies from one to five years. It is possible to sow hybrid seeds only for one year, in case of correct conducting of seed farming, hybrid varieties can be sown for 4-5 years.

The correct conducting of seed farming requires knowledge of biology of the variety [Naskidashvili Petre et al.(2013), Kevkhishvili Vladimer.(1998), Kevkhishvili Vladimer. (2001).]. Variety is a spontaneous, stable and discreet biological system. Degree of resistance of variety depends: on the consistence of the pollination way, on the level of a modification variation, on the level of cross-pollination by cultures, as in the self-pollinated varieties, as well as in the cross- pollinated ones. For example,in the cross-pollinated cultures,self-pollination decreases the productivity, also the development of plants is slowed down, many

traits get worse, ineach subsequent generation the regression is increased (5-14 generations). The reasons of deterioration in high-quality traits and features are: 1.Mechanical contamination and the pollination by the other varieties; 2. Splitting; 3. Diseases of plants. 4. Identification of mutations [Samadashvili Tsotne., Naskidashvili Petre.(2014), Popolzukhin Petre et al.(2008)];

Mechanical contamination often causes biological contamination of the variety.Biological contamination takes place in case of the absence of isolation of the sown plots. For the evaluation of mechanical contamination on seed sites is carriedout field approbation.

Seed farming ensures maintaining of high-quality traits in the process of production and provides the growth of productivity by 25-40% [1,4]. Seed farming is in close connection with the seeds maintaining,which studies questions of production of seeds and methods of definition of increase in their quality.

Nowadays, in Georgia there is no system of seed farming, the farms as usual use sub-standard seeds therefore productivity of crops is catastrophically low (wheat - 1,5 t on hectare, corn - 2,5 t/hectare, potatoes - 15,0 t/hectare ,at the same time these indices in the Europe are correspondingly : 6,5 t/hectare, 9,0 t/hectare, 60 t/hectare). The Law on seed farming, adopted in Georgia define the international names of categories of seeds:breeder seed, prebasic (superelite) seed, basic (elite) seed and certified (reproductive) seed.

Breeder seeds are the seeds received from agricultural crops and which are produced by the breeder of a variety or the person employed by him. Breeder provides maintenance of all valuable characteristics of the variety. Breederenters the variety at the StateRegister. Production of such seeds takes place at primary stage of seed farming under the name of breeding seeds. The seeds which meet requirements of the standard are called standard seeds. These seeds guarantee a high yield [SamadashviliTsotne., NaskidashviliPetre. (2014)].

World breeding achievements gave us the opportunity to carry out variety replacing once in 6-8 years. The system of seed purificationis quite a long process and it takes 5 years. Therefore, for the reduction of duration of seed purification we set a goal to develop a method of the accelerated receiving of elite seeds.

Material and Methods

For the initial material from the nurseries of selection weretakenwinter wheat varieties Bezostaya-1 and Saul-9. Both varietiesare recommendedfor the production in all wheat producing regions of Georgia. In 2013, on the Sartichalatesting station selected 3000-3000 original ears and sown in the rows on the Mukhraniagricultural experiment station. In 2014-17 studying of the generations and nurseries was carried out in four replications. Studying area of each variety made 50 sq.m.For thedevelopment of the accelerated method of seed purification four options were taken:

I option – the nursery of selection of the first year, nursery of selection of the second year, nursery of multiplication of the first year, nursery of multiplication of the second year,multiplication of the third year, superelite (5 years).

II option – the nursery of selection of the first year, nursery of selection of the second year, nursery of multiplication of the first year, nursery of multiplication of the second year, superelite (4 years).

III option – the nursery of selection of the first year, nursery of selection of the second year, nursery of multiplication of the first year, superelite(3 years).

IV option – the nursery of selection of the first year, nursery of multiplication of the first year, nursery of multiplication of the second year, superelite(3 years).

Phenological and biological studying of wheat varietieswas carried out according to the method developed by UPOV.

During the vegetative period it was studied viability of plants,the survival, thetillering, the blossoming, the maturing .The assessment of wheat variety resistance to basic diseases was carried out: to yellow and stem rust, septoria, head blight and mildew.. Economic signs of each separate varietywere studied: the plant height, productive tillers, ear length andnumber of cones on an ear, number of grains in an ear, the weight of grains of one ear, and the weight of 1000 grains. The productivity was defined in four replications on the area of2sq.m. For the establishment of varietal purity of both varietieson separate sections were studied theindexes of varietal purity, for the assessment of reliability of average sizes were defined an standard deviation, average error and variation factor. (Tables 1 and 2).

Researches were conducted on Sartichala testing territory of Gardabani district and Mukhrani testing station of Mtskheta district. Soil and climatic conditions of both territories satisfied biological requirements of a winter wheat. In the researcheswas applied the modern technology of receiving seeds of wheat (well-timed and high-quality processing of the soil, complex of agricultural measures, wide-row crops, carrying measure of fight against diseases, wreckers and weeds). In 2014-2017 the vegetative period of wheat was characterized by various climatic conditions,which was reflected in a drought and sharp fluctuations of high temperatures.

Results and Discussions

During the vegetative periodphenologicalstudy was conducted: on emergence, tillering, maturing, resistance to lodging, resistance to diseases.

In 2014-17 the economic indicators did not differ significantly and they completely revealed the characteristic traits.

During 2014-17 the height of plants varied: in Bezostaya-1 between 90.4-104.4 cm, in Saul-9 - 95.2-106.8 cm, ear length in Bezostaya-1 up to 7.6-11.1 cm, in Saul-9 –up to 10.4-11.8 cm, productive tillers of variety Bezostaya-1 within 1,7-2,5, of variety Saul-9 respectively-9 - 2,8–3,8. By number of grains in one ear of a control plant of variety Bezostaya-1 - 39.5-45,3, weight 2.2–3.0 g; in an ear of a control plant in Saul-9 respectively 42.1–52.3, weight 2.3-2.6 g; weight of 1000 grains of a variety Bezostaya-1 - 45.1-47,5 grams, of variety Saul-9 the weight of 1000 grains - 43.5-45,6 grams.

The results of studying in nurseries of selection in 2014-17 are presented in the Tables No. 1-2. Apparently in 2014 the number of off-types of variety Bezostaya-1 in the nursery of breeding of the first year was 15 plants, of variety Saul-9 - 7. In the nursery of the second year of breeding - respectively 1 and 0 plants. The received results meetthe standard requiredfor the seeds of the first category.

The number of off-types in the nursery of multiplication of the first year as a result of sowing the seeds received in the nursery of selection of the first year in variety Bezostaya-1 was 18, in variety Saul-9 - 8. By the data received in 2015, variety Bezostaya -1 did not meet the standard. Number of atypical plants in nursery of multiplication of the first year as a result of sowing the seeds received in the nursery of selectionof the second year in variety Bezostaya-1 was 6, in variety Saul-9 - 2. Bothindicators completely met the standard.

In the nursery of multiplication of the second year the number of off-types in varietyBezostaya -1 did not increase -5 plants, and invariety Saul- 9 was sharply reduced and was only 2. In nursery of multiplication of the third year the number of off-types sharply increases and in variety Bezostaya-1 reached 33, and in a variety Saul-9 - 18. In the first case the seeds were sub-standard, and in the second – on the verge.

Table 1. Nursery of reproduction of seed purificationof wheat variety Bezostaya-1 2014-2016

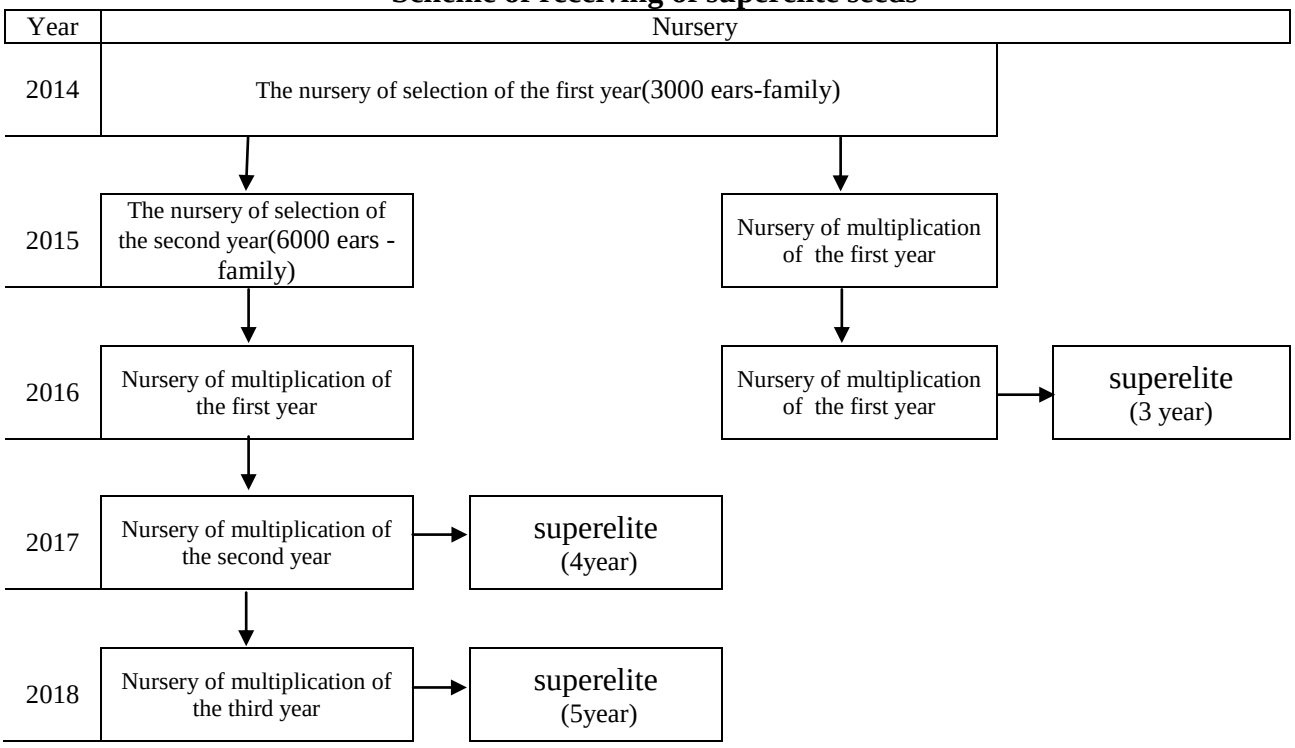
Year / nursery	Number of typical plants 1 m2									Number of off-types1 m2										Statistical analysis, %	
	I	II	III	IV	V	VI	VII	VIII	total	I	II	III	IV	V	VI	VII	VIII	total	varietal purity (%)	Standard Error of means	Variation factor
The nursery of selection of the first year of 2014	418	387	407	398	367	402	478	489	3346	2	3	2	1	2	2	1	2	15	99,54	0.06	0.18
The nursery of selection of the first year of2015	348	367	390	407	415	312	385	382	3006	0	0	0	0	1	0	0	0	1	99,97	0.03	0.08
The nursery of selection of the second year of 2015	378	412	422	413	378	356	413	447	3210	2	4	2	2	2	2	2	2	18	99,44	0.06	0.17
2015 Nursery of multiplication of the first year	516	498	479	490	513	506	459	476	3937	0	1	2	0	1	1	1	0	6	99,85	0.05	0.15
2016 Nursery of multiplication of the first year	453	464	476	450	478	487	453	442	3703	0	2	0	0	1	0	0	0	3	99,92	0.06	0.16
2016 Nursery of multiplication of the second year	417	432	403	387	401	432	412	456	3340	0	1	1	1	0	0	0	2	5	99,85	0.06	0.17
2016 Nursery of multiplication of the third year	367	349	373	385	361	390	402	368	2995	5	3	5	5	4	5	3	3	33	98,9	0.09	0.26

Table 2. Nursery of reproduction of seed purificationof wheat variety Saul 2014-2016

Year / nursery	Number of typical plants 1 m2									Number of off-types1 m2										Statistical analysis, %	
	I	II	III	IV	V	VI	VII	VIII	total	I	II	III	IV	V	VI	VII	VIII	total	varietal purity(%)	Standard Error of means	Variation factor
The nursery of selection of the first year of 2014	478	489	482	523	436	487	405	472	3772	2	0	1	1	0	0	1	2	7	99,81	0.06	0.17
The nursery of selection of the first year of 2015	476	482	503	489	512	545	390	423	3820	0	0	0	0	0	0	0	0	0	0	0	0
Nursery of selection of the second year of 2015	480	455	419	435	502	478	418	422	3602	2	1	1	1	1	0	1	1	8	99,78	0.04	0.11
2015 Nursery of multiplication of the first year	506	506	492	509	517	501	478	507	4016	0	1	0	0	0	0	0	1	2	99,95	0.03	0.09
2016 Nursery of multiplication of the first year	496	437	466	493	516	507	512	504	3931	0	1	0	0	0	0	0	1	2	99,95	0.03	0.09
2016 Nursery of multiplication of the second year	417	468	498	503	518	546	518	576	4044	0	0	0	0	1	0	0	1	2	99,96	0.03	0.08
2016 Nursery of multiplication of the third year	403	433	412	456	456	501	439	465	3565	3	2	2	2	3	0	2	4	18	99,47	0.08	0.25

On the basis of the obtained data it is possible to present the scheme of options of the accelerated technique of receiving elite seeds as following:

Scheme of receiving of superelite seeds



On the basis of the data, obtained after using the technique of the accelerated receiving of elite seeds, confirmed with a statistical analysis, it is possible to receive the elite seeds in 3-4 years (scheme) instead of the classical scheme (7 years) provided by seed purification. The technique of the accelerated receiving of the elite seeds gives to farmers to use elite seeds and by that to provide annually a big and steady crop before the variety replacement.

Conclusion

On the basis of the experiment conducted during 2014-2017 and the analysis of the received data, we can draw the following conclusions:

For the production of elite seeds, it is possible to use the accelerated method, which gives a chance to accelerate this process for 2-3 years.

For the production of elite seeds it is possible to use the following scheme: nursery of selection of the first year, nursery of selection of the second year, nursery of multiplication of the first year, nursery of multiplication of the second year, superelite (4 years) or nursery of selection of the first year, nursery of selection of the second year, nursery of multiplication of the first year, superelite (3 years) or nursery of selection of the first year, nursery of multiplication of the first year, nursery of multiplication of the second year, superelite (3 years).

For the farmers, who own the large areas of land and need a large amount of sowing material it is better to use the following scheme: nursery of selection of the first year, nursery of selection of the second year, nursery of reproduction of the first year, superelite (4 years).

For the farmers, who own the small areas of land for producing of necessary amount of sowing material it is better to use this scheme: nursery of selection of the first year, nursery of selection of the second year, nursery of reproduction of the first year, superelite (3 years).

Four-year researches have convinced us, that the scheme for the production of elite seeds: nursery of selection of the first year, nursery of reproduction of the first year, nursery of reproduction of the second year, superelite (3 years) doesn't guarantee the receiving of standard seed material at the subsequent reproduction, therefore using of this scheme isn't recommended.

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SINGLE-PLANT SELECTION AT ULTRA-LOW DENSITY OF THREE BEAN CULTIVARS AND SALINITY TOLERANCE DURING GERMINATION

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Abstract

Single-plant selection under ultra-low density (interplant distance of 100 cm), between 3 common bean (*Phaseolus vulgaris* L.) cultivars, the Greek cultivars, Iro and Pirgetos and an imported one, was performed in a honeycomb design experiment established during 2017 in the main farm of the Western Macedonia University of Applied Sciences in Florina resulting in first generation sister lines. Divergent selection of individual plants characterized as providing high (H) and low (L) yield led to 3 H and 3 L first generation genotypes, for each cultivar respectively. Salinity tolerance during germination and early seedling growth was evaluated for 18 first generation sister lines and 3 original cultivars at 0, 60, 120 and 180 mM NaCl in a randomized complete block design experiment with four replications. Germination percentage (G%), mean germination time (MGT), coefficient of velocity of germination (CVG%), time to 50% germination T_{50}, sensitivity index (SI) and fresh and dry weight of radicles and hypocotyls were measured. Salinity stress delayed germination and affected early seedling growth significantly, with the biomass of radicles plus hypocotyls decreasing with increase in salinity stress, in all genotypes tested. Significant differences were observed among first generation sister lines and the original cultivars in their response to the highest salinity stresses. Significant differences between H and L yielding lines were also shown on SI in salt stress especially within the cultivar Iro. Further research is needed to confirm the results of the present study so that any existing variation is beneficially exploited.

Keywords: *Ultra-low plant density, Salt tolerance, bean germination, Sensitivity index.*

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most widely cultivated legume crop, representing near 50% of grain legumes for human consumption (McClean *et al*., 2004; Acosta-Gallegos *et al*., 2007). Common beans are essential in Mediterranean diet schemes and they are recognized as a significant source of high quality and low cost protein. Common bean is the most significant among the other pulses in Greece and in the recent years the cultivated areas are increased steadily especially in Northern and Central parts of the country (Vakali *et al.,* 2017). Modern agriculture depends by far on uniform crop varieties in order to meet a growing demand for food by the world's population, and in most cases several landraces have progressively been replaced by elite cultivars which have high agronomic performance to ensure increasing production and income, satisfying the farmers and consumer's needs (Mavromatis *et al.*, 2007). Farmers mainly use foreign certified bean varieties despite the existence of Greek varieties (Vlahostergios, 2001), which however give unstable productive results. The existence of genetic heterogeneity in Greek genotypes is offered for plant selection with methods of classical improvement and main criterion plant yield (Papadopoulos *et al.,* 2007). An intracultivar single-plant selection under ultra-low density has been extensively used to exclude plant-to-plant interference for resources as nil-competition boosts phenotypic expression and erases the masking effects induced by the negative relationship between yielding and competitive ability thus making selection of

desirable genotypes within a narrow gene pool applicable (Fasoula and Tokatlidis, 2012;Tokatlidis, 2015).

Soil salinity is an important constraint affecting agricultural productivity worldwide with over 100 million hectares worldwide affected (Qadir *et al.,* 2008). Salinity stress could be caused by poor irrigation water containing high amounts of salts, accumulation of salts in the top layer of the soil and the capillary rise of salts from underground water into the root zone due to excessive evaporation. Salinity impairs seed germination, reduces the ability of plants to utilize water and causes a reduction in growth rate as well as changes in plant metabolic processes (Munns and Tester, 2008). The probable cause of salinity toxicity is due to osmotic stress associated with lack of cell-wall extension and cell expansion leading to cessation of growth and ion toxicity interfering with nutrient imbalance within the plant thus lowering net photosynthetic rates in the affected plants (Khadri *et al.,* 2007; Cabot *et al.,* 2009). Legumes are considered a relatively salt sensitive family with the common bean germination and growth being affected at low salinity levels less than 2 dSm^{-1}. Effects of salinity during germination and early seedling growth have been reported in even lower concentrations in different bean genotypes (Bayuelo-Jimenez *et al.,* 2002; Gama *et al.,* 2007; Gutierrez *et al.,* 2009). One way reducing the deleterious effects of soil salinity on bean crop production is the development of salt-tolerant cultivars either exploiting intraspecific variability or by transferring genes from closely related wild species adapted to high salinity (Jimenez et al., 2002).

The present study was undertaken to characterize intraspecific variability for NaCl salinity tolerance in first generation sister lines of three different bean varieties during seed germination and early seedling growth. The range of variation that could exist may be utilized for identifying and developing improved genotypes which could perform better under adverse conditions.

Materials and Methods

Three common bean determinate type genotypes, two Greek cultivars Iro and Pirgetos developed by the Hellenic Industrial and Fodder Crops Institute and an imported one Northern Type were cultivated in the experimental farm of the University of Applied Sciences of W. Macedonia in Florina Greece (40°46′ N, 21°22′E, 707 m asl), in a sandy loam soil with pH 6.3, organic matter content 14.0 g kg^{-1}, N-NO_3 100 mg kg^{-1}, P (Olsen) 50.3 mg kg^{-1} and K 308 mg kg^{-1} and water holding capacity 21.8% (0 to 30 cm depth). The ultra-low density (interplant distance of 100 cm), was achieved with individual plants occupying equidistant hills in a zig-zig pattern. This density was used to preclude interplant competition and allow plant yield efficiency to be fully expressed. The trial was composed of 350 individual plants from each genotype evenly and systematically allocated according to a replicated honeycomb design (Fasoula and Tokatlidis, 2012). A total of 400 Kg/ha 0-20-0 and 200 Kg/ha 11-15-15 fertilizers were applied at planting, while additional N (50 g per plant of a 27-0-0 fertilizer) was top-dressed when plants had reached the appropriate developmental stage. Complete weed control was obtained by tilling and hand whereas irrigation was performed by a drip irrigation system. Plants were harvested individually and grain yield was measured at the physiological maturity stage and recorded at a per-plant basis.

Divergent selection of individual plants characterized as providing high (H) and low (L) yield led to 3 H and 3 L first generation genotypes, for each cultivar respectively. The 18 first generation sister lines and the 3 original cultivars were evaluated for salt tolerance during germination and early seedling growth at 0, 60, 120 and 180 mM NaCl concentration. Seeds were surface sterilized with 2% sodium hypochloride solution for 5 minutes, rinsed with sterile distilled water for several times and blotted onto sterile paper. Ten seeds from each

genotype were manually scarified by removing 1 mm of the testa with a scalpel and placed in covered, sterilized disposable Petri dishes containing filter paper moistened once with 10 ml of distilled water or NaCl solution. The Petri dishes were tightly sealed with Parafilm to prevent evaporation of water and changes in the solution concentration. A randomized complete block design with a split plot arrangement of treatments and four replications was used with NaCl treatments as main plots and the genotypes randomized within each main plot. The experiment took place in a dark automatically controlled growth chamber with a mean temperature of 25^{o}C and relative humidity 85-90%.

Seeds were considered germinated when the radicle reached 2 mm in length. Percentage germination was recorded every 24 h for 6 days. Germination percentage (G%), mean germination time (MGT), time to 50% germination T_{50} and sensitivity index (SI), ratio of median response at the three salt concentrations to the median response at the control, were calculated. At the final day fresh weights of radicles and hypocotyls were recorded and subsequently were dried at 50^{o}C for 72 h and dry weights were measured.

Comparison of means was conducted by Least Significance Difference Test (LSD) after analysis of Variance c(ANOVA), for two-factor randomized complete block design.

Results and Discussion

Final germination, germination percentage compared to the original genotypes for all first generation sister lines and mean days to 50% germination T50 are presented in Table 1 for the all NaCl treatments. Germination responses to 60 mM NaCl were not significantly different than the controls and are not shown. In all genotypes tested the germination rate was delayed in response to the highest salt concentrations and also the mean time to germination increased with the addition of NaCl. This is in agreement with other studies where moderate and high salt stress has adversely affected seed germination through osmotic effects (Bayuelo-Jimenez *et al.,* 2002; Gholami *et al.* 2009). The increase in T50 was greater at 180 mM than 120 mM with all sister lines regardless the yielding type performing better under both treatments reaching 50% germination in significantly less time than the original genotypes. No significant differences in mean germination time were shown between high yielding lines compared to the low yielding lines in all three original genotypes.

With regard to final germination percentage many first generation sister lines were relatively salt tolerant, exceeding 80% germination at 120 mM NaCl whereas two sister lines of genotype Pirgetos and one of Northern type exceeded 90% germination (Table 1). The 180 mM NaCl salt stress treatment markedly reduced final germination for all genotypes tested but all first generation sister lines performed either equally or better compared to their original genotype. Our results demonstrated genetic variation in seed germination responses to salinity among first generation sister lines and their original genotypes. Similar observations have been reported in bean (Papadopoulos *et al.,* 2007) and in lentil (Vlahostergios *et al.,* 2018) where compared to the mother populations, higher yields combined with reduced abiotic and biotic stress incidences were observed in their first-generation sister lines. The same genotypes which germinated over 90% in the 120 mM NaCl stress also reached germination percentages between 65-78% at the highest salt treatment of 180 mM which is higher than what is observed in most saline agricultural soils (75-150 mM; Gutierrez *et al.* 2009).

The sensitivity index of each genotype was estimated by the relative increase in mean germination time with the addition of NaCl compared to the control. There were differences on the stability of response to germination between original genotypes and first generation sister lines and also between high and low yielding lines especially within genotype Iro in both high salt treatments.

Table 1. Germination responses and Sensitivity Index (SI) of high (H) and low (L) yield first generation sister lines compared to the three original bean genotypes at 0, 120 and 180 mM NaCl stress.

		Salt concentration										
		0 mM			120 mM				180 mM			
Gen/ sister lines	Yield type	G %	OG* %	T50** d	G %	OG %	T50 D	SI	G %	OG %	T50 d	SI
Pirgetos (A)		93		1.30±0.10	80		2.50±0.05	1.9	50		2.70±0.49	2.1
A1	H	88	95	1.00±0.17	73	91	1.40±0.16	1.4	55	110	1.50±0.28	1.5
A2	H	100	108	1.10±0.21	78	97	1.40±0.18	1.2	55	110	1.70±0.12	1.5
A3	H	95	103	0.90±0.14	93	116	1.30±0.31	1.5	75	150	1.40±0.14	1.5
A4	L	97	105	0.70±0.19	95	119	1.50±0.16	2.1	78	155	1.80±0.36	2.6
A5	L	95	103	1.20±0.18	83	103	1.70±0.35	1.4	50	100	1.90±0.32	1.6
A6	L	97	105	1.10±0.20	85	106	1.70±0.16	1.5	75	150	2.00±0.37	1.8
Iro (B)		93		1.50±0.11	70		2.2±0.05	1.5	45		2.20±0.22	1.5
B1	H	100	108	1.20±0.24	88	125	1.50±0.29	1.3	78	172	2.20±0.20	1.8
B2	H	100	108	1.40±0.17	75	107	1.50±0.19	1.1	60	133	2.10±0.39	1.5
B3	H	95	103	1.20±0.10	68	96	1.90±0.41	1.6	60	133	1.90±0.69	1.6
B4	L	98	105	1.20±0.14	88	125	1.40±0.40	1.2	48	106	1.60±0.59	1.3
B5	L	95	103	1.40±0.12	68	96	1.40±0.10	1.0	53	117	1.70±0.36	1.2
B6	L	98	105	1.30±0.25	83	117	1.60±0.20	1.2	58	128	1.70±0.12	1.3
N. type (C)		98		1.30±0.18	65		2.50±0.50	1.9	45		2.60±0.49	2.0
C1	H	98	100	1.20±0.20	83	127	1.30±0.30	1.1	45	100	1.70±0.07	1.4
C2	H	98	100	1.30±0.14	75	115	1.50±0.29	1.6	63	139	2.50±0.52	1.9
C3	H	98	100	1.30±0.22	78	119	1.80±0.55	1.4	50	111	2.10±0.37	1.6
C4	L	98	100	0.90±0.28	78	119	1.10±0.38	1.2	40	89	1.80±0.29	2.0
C5	L	100	103	1.00±0.22	93	142	1.40±0.20	1.4	65	144	1.80±0.32	1.8
C6	L	95	97	1.00±0.22	83	127	1.70±0.16	1.7	65	144	1.90±0.36	1.9

* % germination of the Original Genotype

** Days to 50% germination ±SE

Table 2. Seedling growth of high (H) and low (L) yield first generation sister lines combined for all three original bean genotypes under NaCl stress.

		Fresh weight			Dry weight		
		g seedling^{-1}			g seedling^{-1}		
NaCl mM	Genotype yielding	Radicle	Hypocotyl	Total	Radicle	Hypocotyl	Total
0	H	1.11	2.25	3.36	0.10	0.27	0.37
	L	1.45	2.78	4.23	0.13	0.31	0.44
	LSD* $_{0.05}$	0.29	0.36	0.61	ns	0.03	0.05
60	H	1.05	1.74	2.79	0.11	0.23	0.33
	L	1.25	2.16	3.41	0.12	0.26	0.38
	LSD $_{0.05}$	0.19	0.27	0.48	ns	0.02	ns
120	H	0.41	1.03	1.43	0.04	0.13	0.17
	L	0.47	1.25	1.72	0.05	0.14	0.19
	LSD $_{0.05}$	0.05	0.21	0.48	ns	ns	ns
180	H	0.20	0.55	0.75	0.02	0.08	0.10
	L	0.18	0.52	0.69	0.02	0.07	0.09
	LSD $_{0.05}$	ns	ns	ns	ns	ns	ns

* LSD: Least Significant Difference at P≤0.05, ns: No significant differences

Salt stress inhibited seedling growth in all genotypes tested with the magnitude of reduction being highly dependent on the salt concentration and hypocotyl growth to be more sensitive than radicle growth (data not shown). Seedling growth of high and low yielding first generation sister lines, averaged over all three original genotypes is shown in Table 2. Fresh weight of radicle, hypocotyl and total fresh seedling weight was significant different between low and high yielding lines with the low yielded genotypes to have performed better in the low and moderate salt concentrations. Similar response was shown even in the control treatment. The low yielding lines had much lower seed size with irregular seed surface which could explain faster water absorption and thereby higher seedling growth at the initial stages (Debouck, 1999). In the highest salt stress concentration there were no significant differences between the two yielding type sister lines. When dry weights were compared significant differences were shown only in hypocotyl dry weight under the low salt treatment.

In conclusion, the results of this study demonstrate that there is intracultivar variation on salt tolerance during germination and early seedling growth within first generation sister lines and these could be used to identify and develop improved genotypes which could perform better under adverse conditions. Further research is underway to confirm the results of the present study and to exploit any existing variation.

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STUDY OF THE PERFORMANCE OF GREEK DURUM WHEAT CULTIVARS IN A COLD ENVIRONMENT

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Abstract

Durum wheat is one of the most important cereal crops in Greece, both because of its nutritional value and its economic importance, and itsgood adaptation to the soil and climate conditions of the country as well. To study how the Greek durum wheat cultivars perform in the cold environment of Florina under low input conditions,eight Hellenic durum wheat varieties (cvs. Anna, Elpida, Selas, Mexikali, Athos, Papadakis, Aiantas and Thraki) were used. The complete randomized block design was applied with four replications and the experiment was established in the farm of the Western Macedonia University of Applied Sciences in Florina. The following morphological traits were measured: height and total height, the blooming, the length of spike, the number of fertile grain per spike, the length of grain as well as the germination capacity, the yield, the weight per thousand grains, the hectoliter weight, the protein content and the ashes. Differences were found between durum wheat varieties regarding germination and yield as well as the agronomic traits. No significant differences were found concerning the protein content and the ashes. More early blooming variety was the variety Thraki and the more late blooming was Athos. Regarding yield, durum wheat cultivars Anna, Mexicali and Athos were ranked first, followed by cultivar Thraki.It was concluded from the results of the present study that most of the examined cultivars performed satisfactory under low temperature and low input conditionsand could be used by wheat breeders to produce new promising varieties under similar conditions.Further research is needed to confirm the results of the present study.

Keywords: *durum wheat, yield potential, cold resistance, low input conditions.*

Introduction

Durum wheat is one of the most important cereal crops in Greece, because of its economic and nutritional value. Durum wheat has a number of traits which make it ideal for pasta-making. It also has high protein content, while its strong gluten matrix retains the starch molecules during cooking (Feillet 1984). One of the most decisive factors in the reliable production of winter cereals, including durum wheat, is resistance to extreme climatic conditions in winter.

The main factors causing winterkill (alone or in combination) are related to low temperature per se (such as extreme air or soil temperatures, below the critical temperature of a particular wheat cultivar), or prolonged periods of low sub-zero temperatures; in particular, mid-winter temperatures below -15ºC result in the rapid loss of winter hardiness (Gusta *et al*., 1997).

Handling a complex trait such as winter hardiness in a breeding program is a difficult task, due to the large number of genes involved and the numerous interactions with the environment (Saulescu and Braun 2001). A factor limiting breeding for increased frost tolerance is the variation in the occurrence of frost stress across years. But the main difficulty in breeding cold tolerant wheat is that high freezing tolerance is generally associated with lower yields and later maturity. However Friedrich *et al.,* (2013) studied the association of frost tolerance with other important traits in durum wheat reported that frost tolerance was not negatively associated with other important agronomic and quality traits.

So in order to discuss the improvement of winter hardiness, it is necessary to evaluate the relationship between yield, quality, and winter hardiness to increase the acceptance of winter durum new cultivars. The association between frost tolerance and quality is controversially discussed by breeders. Lafferty (2010) considers the combination as challenging, but not impossible and suggests a selection of quality on a level of classical quality spring durum varieties, moderate frost tolerance on bread wheat level and excellent yield performance.
The creation and use of high-yielding varieties that are resistant to abiotic stresses (such as drought, salinity, extremely low or high temperatures) and adapted to extreme environments is considered necessary (Acevedo and Fereres 1994, Lazaridou and Xynias 2017).Winters in Florina area, and especially the previous winter (2017-18) are characterized by very low temperatures so the evaluation of the existing genetic material of durum wheat (a crop adapted to dry- heat conditions) characterized by tolerance to cold is the only approach that could contribute to the problem.
The aim of the present study was to investigate the combination of frost tolerance with important agronomic and quality traits and with high grain yield in durum wheat varieties. This study was undertaken to evaluate the behavior of eight Hellenic durum wheat varieties in the cold environment of Florina under low input conditions.

Materials and methods

For the purpose of the study eight durum wheat cultivars (Anna, Elpida, Selas, Mexikali, Athos, Papadakis, Aiantas andThraki) developed at the Cereal Institute of Thessaloniki (Cereal Institute of Thessaloniki 1985) were used. The examined cultivars were sown in early November 2016 in a field at the University of Applied Sciences of W. Macedonia Farm in Florina Greece, in a sandy loam soil. Seedbed preparation included mould board plough, disc harrow and cultivator. Nitrogen and P_20_5 at 80 and 40 kg ha^{-1}, respectively, were incorporated into the soil as diammonium phosphate (20-10-0) before sowing. The crop was kept free of weeds by hand hoeing when necessary. The plots were consisted of five rows of which the three inner were harvested. The randomized complete block design (Snedecor and Cochran 1980) with four replications was used. The following morphological traits were measured: height after tillering and total height, the blooming, the length of spike, the number of fertile grain per spike, the length of grain as well as the germination capacity, the yield, the weight per thousand grains, the hectoliter weight, the protein content and the ashes. The blooming was measured as number of days from the sowing to heading. Finally an observation about thefrost tolerance of the above mentioned durum wheat cultivars was made. Data were statistically analyzed and the means were compared according to HSD test at p=0.05.

Results and Discussion

Significant differences were recorded between the examined cultivars in yield (significant differences at p = 5%, Table 1). Yield ranged from 1103.4 $kgha^{-1}$in cultivar Aiantas to 3824.1 $kgha^{-1}$in cultivar Anna (Table 1). More productive cultivars are Anna and Mexicali followed by Athos. The insufficient yield performance of cultivars Elpida and Aiantas may be due to very low germination capacity of those cultivars. Symptoms of frost stress were observed at the cultivar Athos in the leaves of three out of four replications, in plants of one replication of cultivar Mexicali, Papadakis, and Selas. All other cultivars did not show any symptoms. Nevertheless the cultivars Athos and Mexicali are among the most productive cultivars so the frost didn't cause a serious damage for the cultivars, in other words the cultivars managed to overcome this damage. Anna is the most productive variety and the most tolerant, although it is known from the literature that the traits that are associated with freezing tolerance, such as delayed spring growth or small cells, can have negative effects on yield (Saulescu and Braun

2001). The above results suggest that there must be no effect of the frost tolerance on the durum wheat cultivars performance.
Regarding the final height and the height after tillering, the differences were significant (Table 1). Total height ranged from 0.641 m in cultivar Aiantas to 0.768 m in cultivar Anna. Anna is the tallest cultivar but generally the height (after tillering and final) is low for all cultivars studied because of the extremely low temperatures of the winter. Aiantas and Thraki are the shortest varieties although Aiantas is the tallest variety after tillering.

Table 1. The germination capacity, the yield, the height after tillering and the final height of eight durum wheat cultivars

Cultivar	Germination capacity %	Yield Kg/ha	Height after tillering(m)	Final Height (m)
Anna	74.25ab	3824.1a	0.248b	0.768a
Elpida	10.25d	1561.6bc	0.244b	0.744ab
Mexikali	78.75ab	3565.5ab	0.249ab	0.702bc
Athos	78.25ab	3044.1ab	0.231b	0.711bc
Thraki	71.25ab	2573.5bc	0.236b	0.643d
Papadakis	80.00a	2256.5bc	0.235b	0.668cd
Selas	56.25bc	2250.3bc	0.231b	0.681ab
Aiantas	33.75c	1103.4bc	0.275a	0.641d

Means in columns followed by different letters, are significantly different at $p<0.05$ by Tukey's HSD test.

In all other morphological traits (the length of spike (m), the number of fertile grain/spike the length of grain) statistically significant differences were observed between the examined cultivars. (Table 2).

Table 2. The length of spike, the number of fertile grains/spike the length of grain of eight durum wheat cultivars

Cultivar	Blooming (days to heading)	length of spike (m)	number of fertile grain/spike	length of grain (m)
Anna	156	0.144a	27.90ab	0.0069a
Elpida	160	0.142a	29.50a	0.0069a
Mexikali	155	0.127b	21.22b	0.0066ab
Athos	170	0.121b	24.16ab	0.0062b
Thraki	165	0.129ab	23.22ab	0.0070a
Papadakis	167	0.135ab	25.80ab	0.0066ab
Selas	160	0.137ab	24.74ab	0.0067ab
Aiantas	159	0.144 a	25.57ab	0.0066ab

Means in columns followed by different letters, are significantly different at $p<0.05$ by Tukey's HSD test.

Khan *et al.* (2013) reported significant and positive direct effect of spike number and large indirect effects of days to heading and plant height on grain yield. In the present study it was found that the grain yield is not related to the number of kernels per spike, but to the plant height and the number of days to heading. (Tables1,2). The tallest and earliest varieties (Anna

and Mexikali) were the most productive, except cv. Athos, which is the latest variety and simultaneously among the most productive. The same was reported by Mekhlouf *et al.*,(2006) who found out that the grain yield ability, and the tolerance to low temperatures were unrelated and were represented by separate PC (principal components) components.
In all other quality traits studied, statistically significant differences were observed between the examined cultivars except protein content which is very low in all cultivars studied probably due to extreme conditions of the year (Table 3).

Table 3. The weight /thousand grains, the hectoliter weight, the protein content, and the ashes of eight durum wheat cultivars

Cultivar	hectoliter weight	weight /thousand grains	the protein content %	Ashes %
Anna	57.6	82.50	10.65a	1.65ab
Elpida	56.0	80.10	10.90a	1.53b
Mexikali	50.0	80.90	10.69a	1.63ab
Athos	45.4	85.30	10.39a	1.66a
Thraki	57.2	80.50	10.90a	1.57ab
Papadakis	48.0	83.30	11.10a	1.56ab
Selas	49.0	80.10	10.52a	1.65ab
Aiantas	48.2	78.50	11.25a	1.57ab

Means in columns followed by different letters, are significantly different at $p<0.05$ by Tukey's HSD test.

The results of this study were in agreement with Friedrich *et al.*,(2013) who found that frost tolerance was not negatively associated with other important agronomic and quality traits. In the present study the most tolerant varieties Anna, Elpida, Thraki and Aiantas had good quality features. Szucs et al. (2003), after eight years of experiments, reported that the durum wheat genotypes studied had average state of hardening and winter hardiness equal or better than those of winter *T. aestivum* varieties. From the present study it seemed that actually the durum wheat cultivars used had almost equal cold tolerance than those of *T. aestivum* varieties cultivated in the field in area near this experiment.

Conclusions

It was concluded that there are considerable differences in performance between the genotypes studied in the cold environment of Florina. From the present study it is found that the grain yield is not related to the number of kernels per spike, nor to the genotypic capacity to cold tolerance, but to the number of days to heading. The earliest varieties were the most productive. The above results suggest that there must be no effect of the frost tolerance at the productivity of durum wheat cultivars. Therefore, the breeding objective should not be to maximize winter hardiness, but to develop cultivars with the minimum winter hardiness necessary for a satisfactory performance for that region. A first estimate of these results leads to the conclusion that sufficient performance is not necessarily associated with cold resistance. However further research is needed to confirm the results of the present study.

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PLANT PROTECTION OF GERANIUM (PELARGONIUM) IN HORTICULTURAL OF KECSKEMÉT

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Abstract

In Hungary, the second best-selling potted and balcony plant is geranium (*Pelargonium*) after cyclamen (*Cyclamen* sp.). Of the more than 240 types of *Pelargonium*, the most sensitive and most infected is the garden geranium (*Pelargonium hortorum*). Due to the large crossing of natural species, a large number of hybrid varieties are grown. The research was carried out in Kecskemet at the Gyenes Flower gardening. In horticulture, there are more than 80 different colors of the geranium standing, running, semi-trailer types, and the most recent F1 hybrid types and English gnawing. The geranium plants are grown in 3 greenhouses on Klasmann's peat. The research describing crop protection was carried out between 2013 and 2018. The greatest damage was caused by the Western flower thrips (*Frankliniella occidentalis*). The following insecticides were used against it: Mospillan, Admiral, Cyperkill and Lannate. The leaves and a bud damage caused destroyed the plants to a large extent. Major damage was caused by the greenhouse leaflet (*Trialeurodes vaporariorum*) and to the peat fly (*Bradysia* sp.). The larva of peat fly damaged the geranium cuttings, which could cause the geranium to develop and therefore death of the plant. Of the diseases, botrytis (*Botrytis cinerea*) was the most typical. Typical geranium rust (*Puccinia pelargonii-zonal*) did not occur in horticulture, due to the adequate prevention. Horticulture expands year by year and changes the proportions of the types of geranium according to customer needs.

Keywords: *Geranium (Pelargonium), Gyenes Flower gardening, plant protection, Frankliniella occidentalis, Botrytis cinerea*

Introduction

The geranium (*Pelargonium*) is part of the *Geraniaceae* family. Among the genus of the geranium there are annual or perennial herbaceous species, rarely woody (semi-shrubs or shrubs) (Nagy, 1975; Honfi *et al.*, 2011). The most common species is the garden geranium (*Pelargonium hortorum*) (Szántó *et al.*, 2003). To Europe, the first geranium (*Pelargonium triste*) was imported by the Dutch around 1600 from South Africa.

Today, the cultivated geranium species are of hybrid origin. The bloom depending on the variety and the season lasts 20-40 days. It is an extraneous pollinating plant. The geranium home country (South Africa), pollen mediated by the birds, while in Hungary the need for artificial pollination when the core recovery. Generally, fertility is poor. The germination strength is 71%.

The types of geranium are divided into two major groups according to the propagation method: F1 hybrids can be grown from seed heterosis and vegetative shoots propagated heterozygous varieties (Armitage and Kaczperski, 1992; Hass-Tschirschke, 1994; Burri, 2004). Heterosis breeding of varieties mainly deal with the United States. Horticulture varieties grown in Europe did not extend to plant cultivation. Vegetative propagated varieties can be grouped by growth. Small individuals grow to 20-30 cm (for example: variety of Radio, variety of Friesdorf). Middle-height individuals are 30-40 cm high (e. g. variety of Adonis, variety of Rubin). High individuals are 40 cm high (for example: variety of Hungaria) (Nagy, 1975).

The temperature requirement of the geranium moves within wide limits. Optimum temperature is 16-18 °C day and night of 12-14 °C vegetative periods. During cuttings it is 20 °C, but it can withstand 25-30 °C during the summer planting. It then blooms with the most intense water supply. The optimum temperature of the hybrid varieties is 18-24 °C (Dobay, 1998).

In addition to the optimal light conditions, the geranium develops and blooms favorably. With 25,000 lux illumination both in cultivation and outdoors, there is rapid growth, rich in flowering, rich in branching, stem does not stretch. It does not damage the 50,000 lux light when the required water content is available in the soil (Nagy, 1991).

Most of the geranium species live in their natural habitat in a dry, whimsical precipitation distribution area. Relative humidity should not exceed 70-75% in the glasshouse and foil. Particularly in the cool, dark, winter months, night and morning condensation are dangerous. If the temperature reaches the dew point, the water will precipitate on the glass, foil and drop into the plants, leading to the spread of the Botrytis (Glits and Folk, 2000). Winter irrigate geranium enough for 2-3 weeks.

The geranium species live in their original habitat on sandy humus soil. In cultivation, soil is not particularly demanding, just like other ornamental plants. The soils in the pH range of 6.3-7.2 are ideal. Nitrogen, phosphorous and potassium-rich, but excessive N content causes strong vegetative development, hindering flowering. The N deficiency causes short leaves and small leaves. Lime-rich sensitive, it induces chlorosis in plants. In the soil, the salt concentration cannot rise above 0.5% otherwise the lower leaves will be smothered by salt suffocation. Effective defense is frequent, abundant, relieving irrigation. Rooting medium consists of 70-80% peat, 20-30% clay minerals, 10-20% bark + 2.5-3 kg / m^3 carbonated lime + 0.5 kg / m^3 complex fertilizer (Nagy, 1975).

Land disinfection is inevitable because the source of soil used in propagation and cultivation is very high. In addition to soil-fungi, bacteria and weeds, we find viral infected plant parts in the soil. The soil is disinfected by steaming at high temperatures at 92-95 °C for 4-6 hours (Gerbár, 1992).

Among the most commonly used geranium pests to count the greenhouse whitefly (*Trialeurodes vaporariorum*) during the cutting propagation and growing. In summer, on the leaves of the geranium the Silver Y (*Autographa gamma*), on the geranium flower the cotton bollworm (*Helicoverpa armigera*) causes harm. Of the flower of geranium, it is regularly damaged by Western flower thrips (*Frankliniella occidentalis*) (Glits *et al.*, 1997).

Material and methods

The experiment was set up in Gyenes Flower gardening between November 2013 and March 2018 in Kecskemét (Bács-Kiskun county), Hungary. The gardener was founded in 1978, initially for gerbera (*Gerbera*) and yucca (*Yucca*) were their main crops, but switched to the geranium (*Pelargonium*) cultivation as a result of market. In horticulture, there are about than 80 variety geranium of the standing, running, semi-trailer types and English gnawing. The *Pelargonium* was different sizes and colors. These are the 6-pack, 10 to 14 cm pot made commodity.

The cuttings are taken from parent plants from excellent propagation material and rooted in a computer-controlled greenhouse with soil heating. In this way they can provide high quality cuttings for customers.

The Gyenes Flower gardening distributed by Klasmann-Deilmann GmbH, Germany Company, Klasmann TS 3 types of peat used in the cultivation of geranium. The composition of Klasmann TS 3 is a medium decomposed white filler, 0-25 mm; chemical properties: pH (H2O, v / v 1: 2.5) 6.0; Nutrient content (g / l): 1.0 and added nutrients: Nitrogen (mg N / l) 140; Phosphorus (mg P2O5 / l) 100; Potassium (mg K2O / l) 180; Magnesium (mg Mg / l)

100; Fe 13% EDTA. Physical properties: dry matter content <10%, water capacity 75-80%, air capacity 10-15%.
The following fungicides were used in protection of geranium cultivation: Amistar Top, Teldor, Signum, Topsin-M, Rovral Aquaflow.
The following insecticides were used during the experiment: Admiral, Mospilan, Kohinor, Vertimec, Warrant, Spin Tor, Cyperkill, Actara, Sumi-Alfa, Decis, Danadim, Conserve, Karate Zeon, Naturalis, Chess, Steward, Confidor, Movento, Teppeki, Laser, Dimilin, Lannate.

Results and discussion

In the Gyenes Flower gardening, we examined at plant protection of geranium with a period of nearly five years (November 2013 - March 2018). The dates of protection against pathogens and the applied fungicides are given in Table 1.

Table 1. The following fungicides were used in plant protection of geranium cultivation.

Year	Fungicide	Active ingredient
2013	Amistar Top	azoxystrobin + difenoconazole
	Teldor	fenhexamid
2014	Teldor	fenhexamid
	Amistar Top	azoxystrobin + difenoconazole
	Signum	boscalid + pyraclostrobin
2015	Teldor	fenhexamid
	Signum	boscalid + pyraclostrobin
	Amistar Top	azoxystrobin + difenoconazole
	Topsin-M	tiafanat methyl
2016	Topsin-M	tiafanat methyl
	Signum	boscalid + pyraclostrobin
	Teldor	fenhexamid
	Rovral Aquaflow	iprodion
2017	Signum	boscalid + pyraclostrobin
	Teldor	fenhexamid
	Rovral Aquaflow	iprodion
	Amistar Top	azoxystrobin + difenoconazole
2018	Amistar Top	azoxystrobin + difenoconazole
	Rovral Aquaflow	iprodion
	Teldor	fenhexamid

*Source: Nóra Pap and Viktor József Vojnich

During the examined period (2013-2018), the following pathogenic agents damaged of geranium in the Gyenes Flower gardening:
1. Fungal diseases: *Botrytis cinerea*, *Pythium debaryanum*, but rarely occurred *Alternaria porri*, *Phytophtora cryptogea*. The greatest destruction was caused by botrytis (*Botrytis cinerea*). We used the Signum and Teldor fungicide against botrytis. In addition, we increased the spacing of geraniums, and we also defended the early irrigation and frequent ventilation.
2. In order to prevent bacterial diseases, we used sterile new soil. We also protect against bacterial pathogens by continuous disinfection and ventilation of pots and greenhouses.
3. Viral disease did not occur during the examined period. The dates of protection against pests and the applied insecticides are given in Table 2.

Table 2. The following insecticides were used in plant protection of geranium cultivation.

Year	**Insecticide**	**Active ingredient**
2013	Admiral	pyriproxyfen
	Mospilan	acetamiprid
2014	Kohinor	imidacloprid
	Mospilan	acetamiprid
	Admiral	pyriproxyfen,
	Vertimec	abamectin
	Warrant	imidacloprid
	Spin tor	spinozad
	Cyperkill	cypermethrin
	Actara	thiamethoxam
	Conserve	spinosad
	Sumi-alfa	esfenvalerate
	Decis	deltamethrin
	Danadim	dimethoate
2015	Decis	deltamethrin
	Warrant	imidacloprid
	Danadim	dimethoate
	Cyperkill	cypermethrin
	Actara	thiamethoxam
	Spin tor	spinozad
	Karate Zeon	lambda-cyhalothrin
	Naturalis	Beauveria bassiana
	Comserve	spinosad
	Chess	pymetrozine
	Mospilan	acetamiprid
	Sumi-alfa	esfenvalerate
	Steward	indoxacarb
	Confidor	imidacloprid
2016	Karate Zeon	lambda-cyhalothrin
	Decis	deltamethrin
	Sumi-alfa	esfenvalerate
	Actara	thiamethoxam
	Confidor	imidacloprid
	Spin tor	spinozad
	Vertimec	abamectin
	Movento	spirotetramat
	Cyperkill	cypermethrin
	Chess	pymetrozine
	Teppeki	flonicamid
	Laser	spinozad
	Dimilin	diflubenzuron
	Mospilan	acetamiprid
	Admiral	pyriproxyfen
2017	Dimilin	diflubenzuron
	Laser	spinozad

	Mospilan	acetamiprid
	Admiral	pyriproxyfen
	Spin tor	spinozad
	Steward	indoxacarb
	Vertimec	abamectin
	Cyperkill	cypermethrin
	Actara	thiamethoxam
	Karate Zeon	lambda-cyhalothrin
	Lannate	methomyl
2018	Vertimec	abamectin
	Lannate	methomyl
	Karate Zeon	lambda-cyhalothrin
	Actara	thiamethoxam
	Laser	spinozad
	Cyperkill	cypermethrin

*Source: Nóra Pap and Viktor József Vojnich

During the experiment, the most common damages were caused by pests of greenhouse whitefly (*Trialeurodes vaporariorum*) and the Western flower thrips (*Frankliniella occidentalis*). Silwet adhesion enhancer was used to enhance the effectiveness of the insecticide. The following insecticides were used for the *F. occidentalis*: Admiral, Mospilan, Cyperkill, Vertimec, Actara, Conserve, Decis, Danadim, Spin Tor, Laser.
The following agents were used for the greenhouse whitefly: Admiral, Warrant, Sumi-Alfa, Karate Zeon, Lannate, Actara.
We used the Klasmann TS 3 growing medium of geranium cultivation, weeds did not occur, so we did not have to defend it.

Conclusions

During the experiment period (November 2013 - March 2018) botrytis caused the greatest damage to the geranium plantation. Amistar Top and Signum fungicides were the most effective against of *Botrytis cinerea*. The most common disease of geranium, rust on *Pelargonium* (*Puccinia pelargonii-zonalis*) was not typical of Gyenes Flower gardening.
Viral and bacterial diseases did not occur during the period under review.
Among the pests, the greatest damage was caused by the Western flower thrips (*Frankliniella occidentalis*) and the greenhouse whitefly (*Trialeurodes vaporariorum*).
The Mospilan, Admiral and Cyperkill insecticides were the most effective against of *Frankliniella occidentalis*. In addition to the insecticides, we also used an adhesion enhancer, Silwet. The Admiral insecticide was the most effective against of *Trialeurodes vaporariorum*.
We did not have to defend against weed because the Klasmann peat in which the geranium was raised was weeds free.

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EFFECT OF ETHYLENE ON ANTIOXIDANT ENZYMES ACTIVITY IN ETHYLENE-INSENSITIVE CUT ROSES (*ROSA HYBRIDA* L.)

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Abstract

Cut roses (*Rosa hybrida* L.) have been classified as ethylene-sensitive, but the nature of the ethylene sensitivity changes in these flowers has not been well characterized. Therefore, in this work, ethylene-insensitive cut roses consisting *etr1-1* gene were evaluated. Cut roses prepared from an isolated greenhouse at commercial stage, after transferring to the laboratory, were compared regarding the content of antioxidant enzymes by ethylene treatment (0, 0.5 and 1 $\mu l\ l^{-1}$). Evaluations were carried out in wild type and transgenic line in bud and half-open stages. The research was performed in completely randomized factorial design by four replicates. After measuring and applying the statistical differences at the $P \leq 0.05$ level via SAS software was done. The results showed that ethylene significantly increased superoxide dismutase, catalase and guaiacol peroxidase activities in bud and half-open stages in wild type and transgenic line. Maximum antioxidant enzymes activity was observed in wild type roses in bud and half-open stages treated by 1 $\mu l\ l^{-1}$ ethylene which had a significant difference in comparison with the same stage in transgenic line. According to the increasing trend of these enzymes activities in wild type and transgenic lines with higher concentrations of ethylene, it seems that, ethylene by inducing senescence oxidative damage could accelerate flower senescence. Therefore, genetic manipulation of ethylene receptor genes of ethylene-sensitive flowers as well as roses could decrease oxidatve stress during senescence and considerably improved longevity.

Keywords: *Antioxidant enzyme, Cut roses, Ethylene.*

Introduction

Roses are the most economically important ornamentals and belong to the top ten flowers in the worldwide. Cut roses account for about 21% and 31% of all cut flowers traded in China and in European auctions, respectively (Heinrichs, 2008; Meng *et al.*, 2013). Unfortunately, many consumers consider roses to have a very short vase life. Senescence is the end of the relatively short life of cut flowers that leads to the visible symptoms of various physiological characteristics (Rani and Singh, 2014). Ethylene, as a gaseous plant hormone, plays prominent role in acceleration of senescence phenomena of most plant concomitantly with increasing in endogenous ethylene production (Lei *et al.*, 2009; Rani and Singh, 2014; Rogers, 2012). In general, during the senescence process, ROS[1] content increase and accelerate senescence process by increasing cell membrane permeability. In fact, low concentrations of ROS including singlet oxygen ($^1O_2$), the alkoxy radical ($RO^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), hydroperoxyl radical ($HO_2^{\bullet-}$) and the peroxy radical ($ROO^{\bullet}$) are essential as secondary messengers for cell signaling to initiate biological processes, whereas oxidative stress during senescence denotes higher ROS levels which cause damage a range of

[1] Reactive oxygen species

macromolecules such as lipids, proteins and DNA (Lei *et al*., 2009; Rani and Singh, 2014; Rogers, 2012). When ROS overproduction is triggered with progressive senescence, antioxidant system comprising of enzymatic ascorbate peroxidase, guaiacol peroxidase, glutathione reductase, catalase, superoxide dismutase and nonenzymatic components scavenge these compounds in plants (Chakrabarty *et al.*, 2009; Rani and Singh, 2014).
Plant breeding by genetic engineering technique such as transformations by *ert1-1* mutated gene resulted in plants with considerably higher ethylene tolerance (Wilkinson *et al.*, 1997). In this regard, succeeding studies were reported in *Kalanchoe* (Sanikhani *et al*., 2008), *Campanula* (Sriskandarajah *et al*., 2007; Sriskandarajah *et al*., 2010), and *Dianthus* (Gubrium *et al*., 2000), which provided a better flower longevity. ETR1 is found to be both essential and sufficient for mutations among the ethylene receptors (Chen *et al.*, 2002; Dong *et al*., 2008; Grefen *et al*., 2008).
Senescence of rose flower is still not completely understood. In order to understand petal senescence, it is important to explore the mechanisms of oxidative stress management. In this study, the levels of endogenous antioxidant enzymes during senescence of *etr1-1* transgenic rose flowers pulsed with ethylene were investigated.

Materials and methods

Plant material and ethylene treatment

Cut roses, wild type (Wt) and transgenic line (Tr), at the bud commercial were obtained directly from Iranian Research Organization for Science and Technology (Tehran). Then cut flowers were selected for the uniformity in size, shape, and freedom from malformations or damage related with harvesting and transport handling. Lower leaves were removed and flower stems were trimmed to a uniform length of 35 cm and placed in dH_2O in a growth room equipped with a controlled environment maintained at 25 ± 2 °C, 60% ± 5% relative humidity and 15 μmol m^{-2} s^{-1} light intensity for 16 h day^{-1} by cool-white fluorescent lamps.
To investigate the effects of ethylene, the samples were subjected to 0, 0.5 and 1 μl l^{-1} exogenous ethylene for 24 h in the polyethylene plastic bag. In this regard, each flower was sealed in a 7 L polyethylene plastic bag with 1 ml KOH-type for respiration CO_2 absorbance. Aliquots of pure ethylene gas were injected by syringe into the polyethylene bag. Bag ethylene concentrations were checked by gas chromatography and flowers were held in dH_2O.
Petals were collected in bud (B) and half-opened (H) stages as shown in Fig. 1. In addition, when wild type was in half-open stage, transgenic line was in bud stage at the same time. Therefore, this stage in transgenic line was called TBH (transition bud to half-open stage). Transgenic petals were collected in this stage, too (Fig. 1). In the following, immediately frozen in liquid nitrogen and then stored at -80 ºC.

(1) (2) (3)

Fig. 1. Three studied stages of flower development in transgenic rose: (1) bud = B, (2) transition bud to half-open stage = (TBH) and (3) half-opened = (H).

Antioxidant enzyme assay

Superoxide dismutase activity

Total superoxide dismutase (SOD) activity was determined by measuring its ability to inhibit the photochemical reduction of MTT according to the method of Giannopolitis and Ries (1977) with some modifications (Chowdhury and Cboudburi, 1985; Zhang *et al*., 1995). The 3-ml reaction mixture contained 75 μM MTT, 4 μM riboflavin, 13 mM methionine, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.4). Riboflavin was added last. The test tubes containing the mixture were placed under two fluorescent lamps at 4000 lux. The reaction was started by switching on the light and was allowed to run for 15 min. The reaction was stopped by switching off the light, and the absorbance at 560 nm was recorded. A non-irradiated reaction mixture that did not develop color served as the control, and its absorbance was subtracted from A_{560}. The reaction mixture which lacked enzyme developed maximum color as a result of maximum reduction of MTT. The reduction of MTT was inversely proportional to the enzyme activity. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of MTT reduction at 560 nm.

Catalase activity

Catalase (CAT) activity was determined spectrophotometrically by measuring the rate of H_2O_2 disappearance at 240 nm, taking at 240 nm as 40 mM^{-1} cm^{-1}(Patterson *et al*., 1984). The reaction mixture contained 50 mmol/L potassium phosphate (pH 6.8), 0.1 mmol/L H_2O_2. The reaction was run at 25°C for 2 min, after adding the enzyme extract containing 20 μg of protein, and the initial linear rate of decrease in absorbance at 240 nm was used to calculate the activity.

Guaiacol peroxidase activity

Guaiacol peroxidase (G-POD) activity was measured spectrophotometrically at 25°C by following the method of Tatiana *et al*. (1999). The reaction mixture (3 ml) consisted of 25 mmol/L potassium phosphate (pH 6.8), 40 mmol/L H_2O_2, and 20 mmol/L guaiacol. The reaction was started by the addition of an enzyme extract equivalent to 50 μg protein. The formation of tetraguaiacol was measured at 470 nm (ε = 26.6 mmol/ L^{-1} cm^{-1}).

Statistical Analysis

All the data were analyzed using SAS 9.1 for Windows. The data were initially compared by one way analysis of variance (ANOVA) and difference was detected using the Duncan test. The difference between treatments was considered to be statistically significant when *P* values $\leq$ 0.05. Figures were performed using Excel and means ± standard error (S.E.) were shown. Experiments were repeated two times.

Results and discussion

SOD, CAT and G-POD activities increased during vase life and the highest activity was observed at the final stage after harvest in wild type (Fig. 2). Wild type had significant difference in all enzymes in comparison with transgenic line in the same stages (Fig. 2). It seems that ethylene exposure could be enhanced the activity of these antioxidant enzymes. Total SOD, CAT and G-POD activity enhanced in B and TBH stages in transgenic line by increasing ethylene to 0.5 μl l^{-1}. This up-trend continued in SOD activity by 1 μl l^{-1} ethylene while the activity of CAT and G-POD showed down-trend (Fig. 2b, c). It is proved that ethylene existence is necessary during opening phase (Olsen et al., 2015; Rani and Singh, 2014). SOD activity in TBH stage significantly increased in compare to other stages (Fig. 2a). TBH was considered as a transition stage from bud to half-open in transgenic line. Increasing ROS and ethylene production in TBH stage confirmed these results (data not shown). Probably, higher SOD activity in this stage could be related to ethylene sensitivity and starting the opening phase.

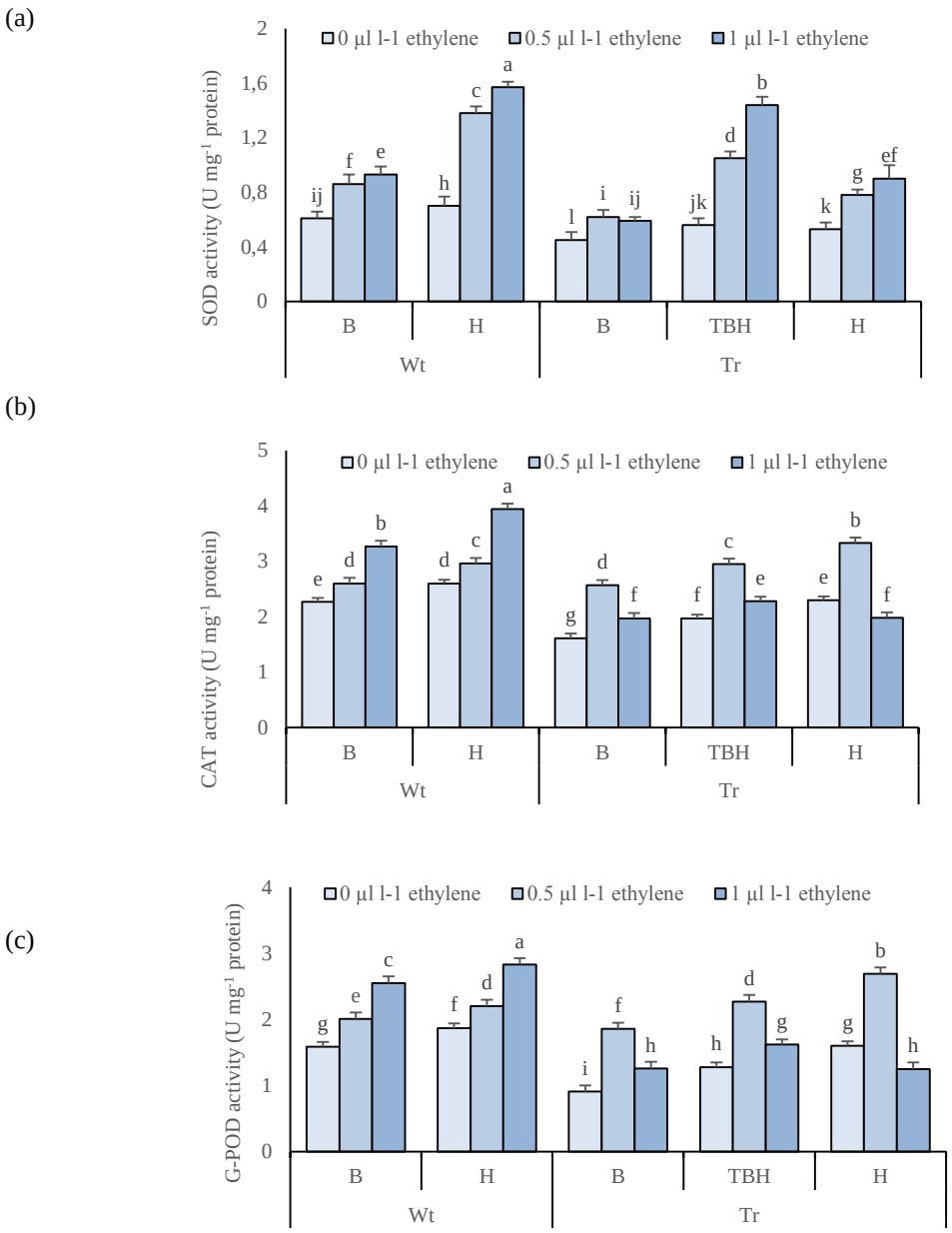

Fig 2. Changes in the level of SOD, CAT and G-POD activity in the petals of wlid type and transgenic rose line during different stages of senescence. Different letters in each bar differ significantly according to Duncan test ($P \leq 0.05$).

It has been reported, an progressive increase in antioxidant enzymes activity and increase in endogenous H_2O_2 in some plant species such as carnation, daylily and rose during senescence and application of exogenous ethylene accelerated this process (Chakrabarty *et al.*, 2009; Rani and Singh, 2014). Moreover, enhanced antioxidant enzymes activity was associated with an increase in the level of peroxides and free radicals which react with cellular constituents (Rani and Singh, 2014). In addition, antioxidant enzymes activities was found to be much higher in senescent leaves than those of in young (Rani and Singh, 2014). In this research, the main emphasis is given on enzymatic components like CAT, SOD, and G-POD which showed antioxidant behaviour with progressive senescence. Catalase is considered as an important biological factor that its major function is in the process of superoxide metabolism and plays an important role in releasing oxygen and hydrogen peroxide free

radicals and preventing creation of hydroxyl radicals (Spanou *et al*., 2012). Superoxide dismutase plays a critical role in inhibition of superoxide and guaiacol peroxidase acts as detoxification of hydrogen peroxide (Miao and St Clair, 2009). It has been reported, CAT, SOD, and G-POD play role in protection of balance of oxygen in plant tissue (Miao and St Clair, 2009; Spanou *et al.*, 2012). Accordingly, the present research indicated antioxidant enzymes activities increased during vase life by increasing exogenous ethylene concentration in wild type and transgenic line (Fig. 2). The higher activity was observed in wild type compared with transgenic line in all developmental stages (Fig. 2). The induction of SOD, CAT and G-POD activities results in decreased sensitivity to free radical-induced cellular damage (Fig. 2). The increased SOD, CAT and G-POD activity that have observed in our study could reflect a similar process of oxidative stress with the implication of these enzyme activities as part of the antioxidant response against H_2O_2. In addition, these data are consistent to previous studies that the dominant ethylene-insensitive *etr1-1* mutant has reduced affinity for ethylene and delayed flower senescence (Müller and Stummann, 2003; Olsen *et al*., 2015).

Conclusions

In conclusion, senescence could be slowed down by retarding peroxidation and high levels of antioxidant enzymes are correlated with delayed senescence. The compromise of oxidative stress in aging is a common feature to cut flowers, and this factor could be a key to designing adequate methods to prevent or delay deterioration and to improve the conservation of cut roses. It seems that transformation by *etr1* in ethylene-sensitive flowers as well as roses could be increased longevity considerably based on the disruption in perceiving ethylene by increasing antioxidant enzymes activity.

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PHYSIOLOGICAL EFFECTS OF GRAFTING IN APRICOT TREES

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Abstract

Grafting of fruit trees is a widely used agronomic practice. Depending on the purpose of its application, grafting may influence many phenological traits of the plant, such as vegetative growth, precocity of fruiting, balance between vegetative growth and fruit production, ripening time and fruit quality. In physiological terms, grafting may modify plant photosynthesis, transpiration and water use capacity. The aim of the present study was to determine the effects of different grafting techniques and of different combinations of rootstock and scion and to characterize the changes induced in the plants. In the experiment, apricot *cv.* San Castrese was studied in combination with two different clonal rootstocks: Isthara and GF677. The effects of interstock grafting were also investigated, using the Japanese apricot species *Prunus mume*. Various tests and physiological measurements were completed to quantify the effect of grafting on vegetative growth and development of the plants. All measurements were taken on leaves located in the middle of the main branches using the photosynthesis-meter Li-Cor in closed systems. Botanical assessments at the grafting point and other physiological data allowed the evaluation of the effects of grafting on plant physiology. Since water equilibrium is controlled by a complex physiological system, dependent on genetic characteristics of the species and the cultivation conditions, this study confirmed significant differences between different plants. The use of stomata conductivity and leaf temperature as indicators for determining the water balance in plants offers an effective and rapid method for characterizing the influence of water stress on plants and allows the determination of its degree of resistance to drought.

Keywords: *Rootstock, scion, disaffinity, intermediate grafting, graft union.*

Introduction

Grafting is a very ancient agronomic practice that is used for different purposes, among which the following: to make the grafted cultivars adapted to different pedo-climatic environments, to achieve a better resistance to phytosanitary adversities (pests and pathogens), to control vegetative growth and canopy habitus (Duval et al., 2012). Three main factors determine the success of the grafting: the affinity, the technique of execution and the environmental conditions. From a physiological point of view, the grafting may modify the photosynthetic efficiency, the transpiration and the hydraulic balance of the plant (Pina and Errea, 2005). During their life together, the two grafted plants influence each other reciprocally, with modifications in their vegetative and productive characteristics. The influence of the scion on the rootstock is less evident because it affects the root system, while the rootstock determines the physiological and vegetative behaviour of the cultivar, with important effects on the vigour and on growth of the scion. The most important effects induced by the rootstock concern the climatic and pedologic adaptability, the control of vigour, the quantity and quality of fruit (Di Vaio et al., 2009). The rootstock also affects the water-plant relations and the gas exchanges of the cultivar (Hernández et al., 2010).

The affinity between the rootstock and the variety is very important for the uptake of water and of the mineral elements that are necessary for an optimal growth of the grafted plant. The vascular discontinuity of the grafting joint negatively influences the continuity of

communication between scion and rootstock, being the cause of physiological disorders having consequences on plant growth. The negative effects of incompatibility become evident starting from the first phase of the grafting union and, afterwards, during the plant growth and yield, when nutrient and water requirements increase (Martinez-Ballesta et al., 2010). To overcome eventual problems related to disaffinity between rootstock and scion cultivar, the use of the intermediate graft is recommended. This technique, in addition to allowing the use of a larger number of rootstocks, also allows to restrict the growth of extremely vigorous varieties and to improve fruit quality, as a consequence of a new physiological equilibrium that is established in the plant (Tombesi et al., 2010).
The complex interactions due to the coexistence of the two plants united by grafting are strictly connected to and influenced by the cultivation environment. To study these interactions, the present experiment was set up for the establishing the capacity of assimilation and the water balance of the apricot cultivar San Castrese, grafted using both the traditional method and by means of the double inter-stem grafting.

Material and Methods

Rootstock 'GF677' is commonly used for apricot because it provides an optimal vegetative growth and an excellent fruit quality (fig. 1). Field trials were conducted at the Tree Fruit Research Centre in Rome (Italy) for the observation of the pomological traits of apricot cultivar San Castrese, grafted onto 'GF677'. Some years after planting (5-7 years) however, this particular scion/rootstock combination appeared to be weak at the point of grafting, which eventually caused the fracture of the plant at the junction point (fig. 2).

Fig. 1: Apricot cultivar San Castrese grafted onto GF677 rootstock, tree row (left) and fruit at maturity (right)

The adoption of the intermediate grafting technique was proposed as a possible solution to avoid this incompatibility. The physiological effect of a specific scion/rootstock combination, also using the Japanese apricot *Prunus mume* Sieb: et Zucc. as the interstock, was evaluated in these trials. The effect of the interstock on the plant's characteristics was determined by observing the assimilation of nutrients and the water balance throughout the productive life cycle of the plant. The Japanese apricot *Prunus mume* was chosen as the interstock, because it has a high affinity with 'GF677', the peach-almond hybrid rootstock. In addition, *P. mume* can recreate tissue continuity at the grafting point very quickly. For a further comparison, the hybrid rootstock 'Isthara' [(*Prunus cerasifera* x *P. salicina*.) x (*P. cerasifera* x *P. persica*)] was used in the tests, because it is commonly used as the rootstock in apricot orchards in Italy.

Fig. 2: The plant suffering grafting incompatibility (left),
which caused the fracture at the junction point (right)

The plant material was prepared in two phases. In June, one-year old plants of both rootstocks, 'Isthara' and 'GF677', were whip grafted with the intermediate scion *Prunus mume*, applying the technique of the green grafting during the growing season (fig. 3). A month later, after having obtained an adequate growth of the intermediate, the cultivar 'San Castrese' was also grafted as a scion over the intermediate, using the same grafting technique.

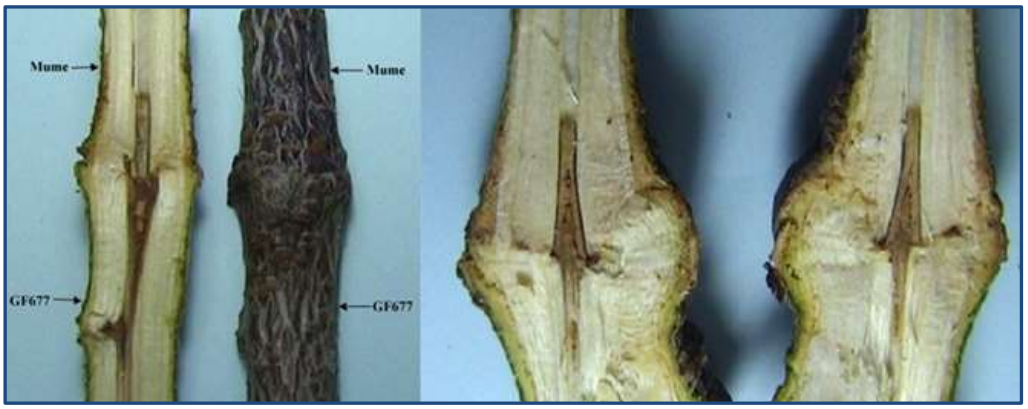

Fig. 3: The grafting of 'GF677' with *Prunus mume* interstock (left),
cross section of the well-established graft junction (right).

The planned tests were prepared applying the same method to graft the following combinations: S. Castrese/Isthara and S. Castrese/GF677. The plants were grown in pots with a controlled irrigation regime, aimed to evaluate the resistance to water drought stress. During the vegetative period, during the hours of the day characterized by the maximum sunlight, many physiological measurements were carried out, among these: photosynthesis, transpiration, conductance and stomatal resistance using Li-6400 (Licor, USA). (Fig. 4), (Horton et al., 2001). The values relating to air temperature 'Delta T' and to 'vapour-pressure deficit' (VPD) were also calculated from the data analysis.

Fig. 4: Portable gas-exchange system, Li-6400 (Licor, USA), with standard

Results and Discussion

The field trials show that both traditional grafting and, even more, intermediate grafting have an effect on the physiological parameters and on the assimilation capacity of the scion trees (Rosati et al., 1997).

The differences in the net photosynthesis values obtained for the two grafting systems (traditional and intermediate), show that the use of *Prunus mume* as an intermediate improves the affinity between the cv. San Castrese and the two rootstocks 'GF677' and 'Isthara', achieving a 10% and 30% increase in assimilation capacity, respectively. The same trend is also observed for transpiration, that increases by 15%-25%, and also for stomatal conductance, that is 25%-30% higher. The increase in these values is attributed to the improved connection between rootstock and scion, due to the optimal reconstruction of the tissues at the grafting point, after the insertion of the scion wood.

Regarding the plant's hydraulic balance, with reference to the ambient temperature trend, the data shows a large variability for transpiration, in particular when drought conditions occur. With the decrease of available soil water, each grafting combination shows its own threshold for resistance to water stress. With the use of the interstock, the rootstock's capacity to use the water decreases as the water content of soil in reduced. For this reason, when the orchard faces low water availability, the transpiration values are lower for both combinations, but in particular when the intermediate is grafted onto both rootstocks ('GF677' and 'Isthara'). However, the rootstock 'Isthara' is capable of maintaining a higher transpiration rate, despite the hydraulic stress, thus showing a higher resistance to drought.

The difference between leaf and ambient temperatures, 'Delta T' values, obtained for the two grafting techniques (traditional and intermediate grafting) in an irrigated field, show that in the double grafted plants, the leaf temperature is lower than the ambient temperature. This is attributed to the improved affinity in the rootstock/intermediate/scion combination, that allows better transpiration, compared to the traditional grafting. Regardless the type of grafting, the leaf temperature depends on water availability and on the transpiration capacity of the plant. The vapour pressure deficit (VPD) is responsible for the movement of water vapour from inside the leaf the atmosphere. The higher the values for VPD, the higher the loss of water due to transpiration. This phenomenon also limits photosynthesis, because in water stress conditions, the gas exchanges are reduced to limit transpiration. The plants with the intermediate grafting treatment have usually lower VDP values, compared to those grafted in the traditional way. Data analysis shows the existence of the correlation between transpiration and VDP and the correlation with the Delta T, confirming the importance of the climatic conditions on the normal physiological activities of the plants in the orchard.

Conclusions

The analysis of the data collected throughout the trials clearly show the influence of the grafting technique, in particular the use of the intermediate has positive effects on the physiological activity of the plant and on the hydraulic balance. The use of the intermediate *Prunus mume* Sieb: et Zucc. has improved the affinity between the apricot cultivar 'San Castrese' and the hybrid rootstock 'GF677'. Instead, the direct grafting of 'San Castrese' onto 'GF677' has been seen to develop physiological problems as a consequence of the weakness of the graft union, after some years from planting. In addition, the double grafting treatment in an irrigated field, has positively influenced the expression of the genetic potential of the cultivars and improved their drought resistance.

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EFFECTS OF NANO-FERTILIZERS AND GREENHOUSE CULTIVATION ON PHENOLOGICAL DEVELOMENT-STAGES AND YIELD OF SEEDLESS TABLE GRAPES VARIETIES

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Abstract

In order to meet the market demand, new seedless varieties of table grapes in Lebanon should be supplied on the market outside harvest season and their cultivation areas must be expanded to new Lebanese regions. Greenhouse cultivation could be a way to achieve this goal and nano-fertilizers would help vines to withstand the warm conditions of the Lebanese coast. In fact, there were not enough studies conducted to investigate the effect of those two agricultural practices on table grapes. The current study investigated the effect of nano-fertilizer (LITHOvit AMINO 25) application and greenhouse cultivation on phenological development-stages and production of three ARRA seedless varieties: ARRA 15, ARRA 18 and ARRA 19. Experimental treatments were: open-field (control), greenhouse, open-field/nano and greenhouse/nano. Greenhouse cultivation induced earliness in budburst and fruiting (one week), flowering (2 weeks) and harvest (3 weeks) compared to open-field despite nano-effect in all tested varieties. LITHOvit treatment did not affect budburst, fruiting, flowering and harvest dates in cultivation systems except on ARRA 18 cultivated in open-field where it caused early fruiting by 1 week. The yield was increased by LITHOvit application outdoor compared to indoor (by 19.1%, 19.1% and 6% in ARRA19, ARRA18 and ARRA 15, respectively). Finally, greenhouse cultivation seems to be an effective method to supply table grapes outside the harvest season, while LITHOvit application did not enhance vines productivity.

Keywords: *Lebanon, LITHOvit, budbreak, productivity.*

Introduction

Nano-fertilizers (NFs) are synthesized or modified form of traditional fertilizers bulk materials or extracted from different vegetative or reproductive parts of the plant by different chemical, physical, mechanical or biological methods using nanotechnology (Singh, 2017). NFs have higher surface area due to very low size of particles which are smaller than root and leaves pores (Sasson *et al.,* 2007) where highly energized LITHOvit and amine particles, sprayed finely onto the leaf surface, are taken up directly through the stomata. This led to increase yields combined with a reduced requirement for water, since with LITHOvit plants are able to keep their stomata closed longer when they are subjected to water stress (GmbH, 2018). In general, NFs are known to prevent plants from different biotic and abiotic stress where it build carbon uptake (Manjunatha *et al.*, 2016), improve chlorophyll formation and photosynthetic activity (Liu *et al.,* 2005), increase dry matter (Quasem *et al.,* 2009), penetration and uptake of nutrients (Nair *et al.,* 2010), result better crop yield and food quality and enhance soil fertility (Qureshi *et al.,* 2018). In particular, LITHOvit increases the photosynthesis rate, improves enzyme activity and brings about a general enhancement of metabolic activities which improve crop yield, quality and storage properties, accelerate growth, intensify green coloration, increase growth, vitality, pest resistance, frost and drought tolerance, enhance the supply of essential trace elements to the plants and reduce the water

requirement (GmbH, 2018). According to GmbH (2018) LITHOvit can be used on arable and pastures land as well as in greenhouses, forestry and horticulture. It is harmless to human and animals and not hazardous to water. In fact, LITHOvit can be applied at the beginning of foliation, at the time of flowering and during fruit growth. It can be also used once or several times, separately or in combination with a plant protection agent. Additionally, it can be applied using any agriculture sprayer with agitation or hand operated sprayer. It is recommended to apply it twice on vine.

From the available sources just one experiment was found as published study on the application of NFs on table grapes where Sabir *et al.* (2014) showed that nano-size Ca-based fertilizer led to remarkable enhancements in foliar development and chlorophyll concentration and improvement of vine growth, yield, berry quality attributes and leaf nutrient content of grapevines (cv. 'Narince') grown under non-favorable alkaline soil conditions. However, Davarpanah *et al.* (2017) found that the foliar application of nitrogen NFs on pomegranate increased fruit yield and other qualitative parameters.

Actually, Lebanese farmers are seeking to meet market demand for new seedless table grapes varieties with high yield and extra-seasonal products. Some previous study conducted on the effect of greenhouse cultivation on table grapes in Lebanese coast shows earliness in harvest (El Masri *et al.,* 2018). Greenhouse did not show any effect on yield and plant productivity while it was not the case in some other studies. In addition, farmers have found that nitrogen uptake is the main reason improving yield (Manjunatha *et al.,* 2016). The study objective was to investigate the effect of nitrogen Nano-fertilizers (particularly LITHOvit amino 25) on the yield and occurrence of several phenological development-stages on three seedless table grapes cultivars grown under greenhouse and open-field conditions in Lebanese coast.

Materials and methods

The experimental site was located in Byblos at 140 m above sea level. The total planted area was 0.6 hectares (ha) divided on 0.3 ha under greenhouse and 0.3 ha in open-field. Four years old seedless varieties were used in this study. The following cultivars were used: ARRA 15 (white grape), ARRA 18 (black grape) and ARRA 19 (red grape). Each cultivar occupied 0.1 ha in both cultivation systems. Vines were grafted on rootstock 1103P, trained by pergolas system and irrigated with drip irrigation. In December, vines were pruned leaving 6 buds on the main shoot (per cane). LITHOvit AMINO 25 was used as a fertilizer. LITHOvit AMINO 25 is a nutritious organic nitrogen fertilizer, a new top quality nanotechnology product created by Tribodynamic activation and micronization. It is a calcium carbonate from selected natural limestone with 25 % water-soluble free L-amino acids. It contains: 56.3 % calcium carbonate (CaCO3), 3.75% magnesium carbonate (MgCO3), 31.7% alkaline constituents (expressed as CaO-calcuim oxide), 0.2 % iron (Fe) , 0.02 % manganese (Mn) (those elements are essential in all LITHOvit product while their concentration differs in each one) and 3.1 % total nitrogen (N) (GmbH, 2018). LITHOvit amino 25 was applied on vines using two different patterns. The first application pattern started before the begging of the season (1st of September) through the 3 applications with a range of 2 weeks between each one. The second application pattern started a week after the bud break, through the 2 applications with a range of 2 weeks between each one. It was applied at a concentration of 1.5 g/L as foliar application. The four experimental treatments were: open-field (control), greenhouse, open-field/nano and greenhouse/nano. Each sub-treatment was consisted on 9 plants of each variety cultivated under the same experimental conditions. Budburst, fruiting, flowering and harvest dates were recorded by daily observation and values were expressed as number of weeks after first bud burst (week 0). At harvest yield was calculated in each treatment. Statistical analysis was done by Spss program.

Results and Discussion

Results of factorial ANOVA showed that interactive and non-interactive effects of the various factors were significant at P value ≤ 0.05 on date of fruit set. The non-interactive effect of the factor variety and the interactive effect between System of cultivation and variety had also a significant difference on date of harvest. In addition, the non-interactive effect of the factor System of cultivation had a significant difference on all parameters (Table 1).

Table 1: LSD table (parameters: yield, date of fruiting and date of harvest)

	Yield	Date of fruit set	Date of harvest
Variety	.021	.000	.000
System	.020	.000	.000
LITHOvit	.952	.001	1.000
Variety*System*LITHOvit	.855	-	-
Variety*System	.253	.000	.000
System*LITHOvit	.952	.001	1.000
Variety*LITHOvit	.954	.000	1.000

LITHovit didn't affect date of any phonological development-stage any in both cultivation systems, except fruit set of ARRA 18 cultivated in open-field, where 1 week earliness was observed after LITHOvit application. On the other hand, greenhouse cultivation caused earliness in all phonological dates despite the effect of LITHOvit. As example, regarding treated and non-treated cultivar ARRA 15, dates of budburst, flowering, fruiting and harvest were earlier 1, 2, 1 and 3 weeks respectively under greenhouse than in open-field. In the control cultivar ARRA 15 was the most delayed cultivar compared to cultivar ARRA 18 and cultivar ARRA 19 where harvest was delayed by 2 and 3 weeks respectively. However, fruit set of cultivar ARRA 19 was also delayed by 1 week compared to the two other cultivars. In fact, all dates were similar regarding all cultivars under greenhouse cultivation (Table 2).

All treatments had almost the same average yield while the lowest one was recorded in treated and non-treated plant of cultivar ARRA 19 grown under-greenhouse. It was recorded that LITHOvit application increase yield by 19.1%, 19.1% and 6% in cultivars ARRA19, ARRA18 and ARRA 15 respectively outdoor compared to indoor but this increase could not considered as significant effect (Figure 1).

Table 2: Budburst, flowering, fruiting and harvest dates recorded in all treatements.

variety	Phenological dates (week)	Open-field	Open-field /nano	Greenhouse	Greenhouse/ nano
ARRA 19	budburst	1	1	0	0
	flowering	5	5	3	3
	fruit set	7	7	6	6
	harvest	19	19	17	17
ARRA 15	budburst	1	1	0	0
	flowering	5	5	3	3
	fruit set	8	8	6	6
	harvest	20	20	17	17
ARRA 18	budburst	1	1	0	0
	flowering	5	5	3	3
	fruit set	8	7	6	6
	harvest	18	18	17	17

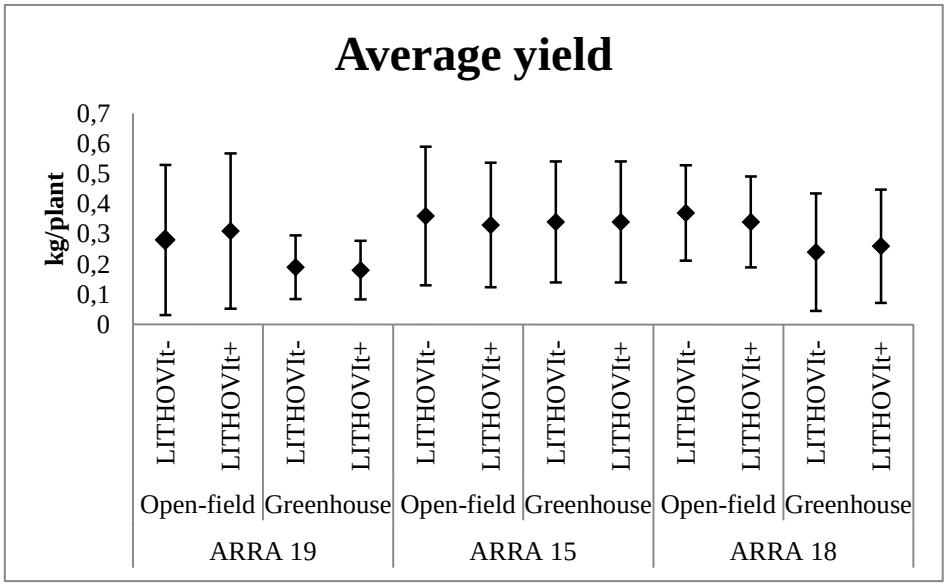

Figure 1: Average yield of plants in each treatment (kg/plant).

Early ripening under greenhouse confirmed the findings of Morettini (1974) on cv. Cardinal in Central and Southern Italy. Also, the earliness obtained by 7 to 30 days in harvest date agreed with findings of Kamiloğlu *et al.* (2011) and Novello *et al.* (2000). Moreover, the precocity in harvest has been occurring after an earlier budbreak indoor compared to outdoor. In fact, greenhouse has induced an earliness of 7 days of budbreak due to the increase of air temperature which in turn induced a faster accumulation of growing degree days (Joly, 2005; Novello and de Palma, 2008). Earlier harvest under protected cultivation was also induced by earlier maturity of fruits on vines (Ying, 2005). Ying (2005), Novello *et al.* (2000), Kamiloğlu *et al.* (2011) and Qin (2013) have observed superiority in yields under protected cultivation which contradicted our results. The effect of LITHovit amino 25 which is considered as nitrogen nano-fertilizers could be due the disturbance of vine development due to the lack of enough chilling hours in Lebanese coast. In fact, no study was published on its effect on vines to compare results.

Conclusion

Greenhouse cultivation has shortened the phenological cycle of plants reaching an earlier maturity of fruit clusters and consequently earlier yields. However, LITHovit did not show any significant results and this could be due to the lack of enough chilling hours which affect negatively budbreak and therefore flowering and fruiting capacities. Consequently, it is recommended to conduct the same study combining with addition hormone treatment which could help vine to function normally in warm winter region and compare those results with results obtained in presented study or to conduct the same study on vine planted in normal conditions in order to know the real reason behind the effect of LITHOvit on vine, since there was a lack of published paper and experiment on the effect of Nano-fertilizers on table grapes.

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MITIGATING SALINITY EFFECTS ON ZUCCHINI PLANTS BY APPLICATION OF GLYCINE BETAINE

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Abstract

Most of irrigated arable lands in Arabic countries are suffering of salinity problems either in soil or irrigation water. This problem has a strong impact on countries such as Egypt with limited natural resources of agricultural land and irrigation water, more precisely in the Northern parts of the Nile River. Therefore, this work was designed to investigate the responses of Zucchini plants (*Cucurbita pepo L.*) to salinity while applying glycine betaine (GB) to improve such responses. The experiment was carried out in an open-field in Beheira governorate, Egypt in order to study the effect of exogenous application of GB on improving salt tolerance of zucchini plants (*var. cylindrica*). Salt stress was induced by daily fertigation using saline water of an EC=6 dS/m. GB was applied through foliar spray with a concentration of 10 mM one week (first application) and four weeks (second application) after transplanting. It was found that GB application induced superiority in all tested indicators except in fruit number. Stem length, fresh weights of leaves and stems, chlorophyll content, fruit yield and weight of individual fruit were improved by 18.7%, 127%, 58%, 7%, 26% and 45% respectively. Moreover, fruits of treated plants were bigger and richer in total proteins, potassium, phosphorus and water compared to control fruits. Results revealed that GB application has helped in mitigating the detrimental effects of salinity on growth and production of zucchini plants under the current experimental conditions.

Keywords: *glycinebetaine, salt stress, zucchini, plant growth, production.*

Introduction

Plants often face the challenge of several suboptimal environmental conditions which limit plant growth and productivity. One of the major constraints to agricultural development is salt stress. Salt accumulation in soils causes high osmotic pressure reducing water potential and inhibiting plant growth (FAO, 2018). A soil is defined as saline when its electrical conductivity (EC) exceeds 4 dS/m at 25◦C (Jamil *et al.*, 2011). In Egypt, seawater intrusion in the Nile Delta is considered as a major problem facing agriculture (Mabrouk *et al.,* 2013). Although most crop plants are not able to grow in high concentrations of salts (Flowers, 2004), they have developed well-organized defense mechanisms to protect themselves from the salinity-induced damages including hormonal regulation, accumulation of compatible solutes (Bohnert and Jensen, 1996), ionic homeostasis (Hasegawa et.al., 2000) and osmoregulation.

Various compatible solutes enable plants to tolerate abiotic stress, and glycinebetaine (GB) is one of the most-studied among such solutes (Chen and Murata, 2011). Glycinebetaine (GB) is an organic osmolyte that acts as an osmoprotectant in response to salt stress in many crop species like barley and sugar beet (Mäkelä, 2004).. Glycinebetaine (N,N,N-trimethylglycine) is a quaternary ammonium (Rhodes and Hanson, 1993) resulting from the dehydrogenation of choline or N-methylation of glycine (Sakamoto and Murata, 2002) and isolated from sugar

beet molasses (Filipčev *et al.*, 2015). On the other hand, other crops are not able to synthesize GB (Sakamoto and Murata, 2000) such as zucchini. Zucchini is one of the most popular vegetable crops in Egypt where it is highly cultivated and consumed due to its beneficial nutritional properties. However, being a moderately salt-tolerant crop, its cultivated area has declined from the year 2011 due to salinity built up in Egyptian soils (Ramadan, 2014).
Positive effects of the exogenous application of glycinebetaine (GB) under salt stress conditions were reported on various crops such as rice (Harinasut *et al.*, 1996), wheat (Diaz-Zorita *et al*., 2001), tomato (Tantawy *et al.*, 2009; Razaei *et al.*, 2012) and maize (Nawaz *et al.*, 2012). However, little literature has discussed the case of zucchini. Consequently, the current work was carried out in order to evaluate the effect of exogenous GB application on vegetative growth, production and fruit composition of salt-stressed zucchini plants.

Materials and methods

The experiment was carried out in a private farm situated in Beheira governorate, Egypt. Soil analysis was performed at two different depths (0-30 cm and 30-60 cm) and soil type was determined based on Mcdonald *et al.* (1998) as sandy clay loam, with a pH of 7.4-7.7 and an electrical conductivity of 4.16-4.77 dS/m at both depth levels.
Seeds of zucchini (*Cucurbita pepo* var. *cylindrica*) were sawn in trays in a greenhouse in mid-June. In mid-July, 60 seedlings (5 to 6 leaves) were transplanted in open-field with a planting distance of 50x75 cm and were irrigated with sweet water (EC>2 dS/m).

Experimental treatments

One week after transplantation, 30 plants were treated by GB solution while the remaining plants were not (control). Second application of GB was carried out three weeks later and GB was provided with a concentration of 10 mM and an application rate of 0.5 L per plant. Salt stress was induced on all zucchini plants through saline irrigation starting at 10 DAT (days after transplantation). Saline solution was prepared by diluting well water of an initial EC=10 dS/m with sweet water to reach an EC=6 dS/m.

Data recording

At 45 DAT (before harvest), 15 plants of each treatment were selected for evaluating their stem length (from cotyledons level to the highest growing point of the stem) and leaf number. Total chlorophyll content was measured on field prior to first fruit formation using a Minolta SPAD chlorophyll-meter (SPAD unit is evaluated by 10 mg chlorophyll/100g fresh weight). This indicator was evaluated on 10 plants per treatment by selecting the most expanded leaves. Fruits were harvested twice a week after reaching a marketable stage. Plant yield was evaluated after determining the fruit number and individual fruit weight per plant. After harvest, 15 plants per treatment were randomly selected and removed from the soil to record the fresh weight of aerial parts (leaves and stems). Moreover, three representative fruits per treatment were sampled and sent to the laboratories of the National Research Center (NRC), Giza, Egypt for measuring fruit length and diameter (using a sliding caliper), moisture, protein, phosphorus and potassium contents. The various components were determined based on AOAC (2005) standard methods.

Statistical analysis

The statistical analysis was made using SPSS. The experimental design was a randomized complete block design (RCBD) with two treatments and 30 replicates (plants) per treatment. T-test was adopted for evaluating differences among both treatments.

Results and discussion

There were detrimental effects of salinity on different growth indicators investigated on zucchini plants while GB treatment induced improvements in such indicators except for leaf number (Table 1). In fact, average stem length was significantly higher in GB-treated plants

compared to control and the increment reached more than 18%. . Similar effect of GB was observed on average fresh weight of leaves (improvement by 127 %) and stems (improvement by 58 %). Moreover, total chlorophyll content was significantly enhanced by GB treatment (improvement by 7 %).

Table 1: Averages of vegetative traits in treated and non-treated zucchini plants

	S.L (cm)	L.N	Chl_T (mg/100g)	S.F.W. (g/plant)	L.F.W (g/plant)
Control	51.67[a]	23[a]	46.1a	150[a]	351.67[a]
GB-treatment	61.33[b]	25.33[a]	49.3b	238.33[b]	798.33[b]

Numbers followed by the same letters are not statistically different at *P<0.05*
S.L: stem length; L.N: leaf number, Chl_T: total chlorophyll content, S.F.W.: stem fresh weight, L.F.W.: leaves fresh weight.

Furthermore, GB application has significantly increased plant yield compared to control as shown in Table (2). GB-caused improvements in average weight of individual fruit rather than average fruit number that did not differ significantly among treatments. Average fruit length and diameter did not significantly differ among treatments although they were slightly higher in fruits of treated plants compared to control (Table 2).

Table 2: Averages of productive traits in treated and non-treated zucchini plants

	F.N.	F.W (g)	Y. (g/plant)	F.L. (cm)	F.D. (cm)
Control	5.6[a]	91.07[a]	510[a]	9.6[a]	3.8[a]
GB-treatment	4.85[b]	132.4[b]	642.41[b]	12.5[a]	4.3[a]

F.N: fruit number, F.W: fruit weight, Y: yield, F.L: fruit length, F.D: fruit diameter

Moisture, protein, potassium and phosphorus contents did not significantly differ among fruits of treated and control plants although all components were slightly higher in treated fruits (Table 3).

Table 3: Composition of zucchini fruits of treated and non-treated plants

	Moisture (%)	Protein (%)	Potassium (ppm)	Phosphorus (ppm)
Control	91.3[a]	1.36[a]	2688.07[a]	504.28[a]
GB-treatment	94.6[a]	1.45[a]	2754.73[a]	523.54[a]

Salinity-caused reductions in stem length, leaf number, chlorophyll content as well as stem and leaves fresh weights confirmed earlier findings on barley (Iyengar *et al.*, 1977), tomato (Al-Rawahy, 1989), radish (Jamil *et al.*, 2007) and squash (Mutowal *et al.*, 2013).
Salinity affects crop growth by creating osmotic imbalance in the cell (Morgan, 1984). Salts accumulated in soil and/or water may inhibit plant growth by reducing plant ability to take up water and by accumulating salts in the transpiration stream causing cell injuries in leaves and further reductions in growth (Greenway and Munns, 1980).
Amelioration in some growth indicators that was obtained following GB application was due to the role of GB in increasing water retention of cells and facilitating nutrient absorption (Ashraf and Foolad, 2007). Moreover, GB could have alleviated the negative effect of salinity

on shoot growth by inducing the production of additional vacuoles in root cells, which resulted in a greater accumulation of Na^+ in the root and a decrease in its transportation to the shoot (Rahman *et al.*, 2002). On the other hand, the non-ameliorative effect of GB on leaf number of treated plants confirmed results of Mohammed and Tarpley (2011) on rice and contradicted those of Razaei *et al.* (2012) on squash plants. In addition, GB application has improved chlorophyll content by protecting the photosynthetic machinery and stabilizing the activity of repair proteins under high concentrations of NaCl (Giri, 2011).

Figure 1: Malformed fruits by control plants (left) and well-formed fruits by GB-treated plants (right) grown under saline conditions.

Salt stress affects flowering and fruit set which reflects on number of fruits per plant, and the latter being the most influencing component of fruit yield (Rezaei *et al.*, 2012). Although Glycine betaine is an effective strategy for increasing yield of crop plants under abiotic stress (Chen and Murata, 2008), GB treatment did not enhance fruit number similar to the findings of Sajyan *et al.* (2018) on tomato. However GB treatment improved yield by 22 % due to higher fruit weight compared to control. Likewise, there was improvement in yield by GB treatments observed by Park *et al.* (2006) and Tantawy *et al.* (2009) on tomato. Moreover, fruits of control plants were malformed while those of treated plants had a better appearance (Figure 1). Fruit quality was influenced by salinity which caused inhibition of water uptake by roots and consequently lowered water transportation to the fruits (Azarmi *et al.*, 2010). The lower moisture content in control fruits was due to the high osmotic pressure induced by salinity which was responsible for the reduction of the cytoplasmic volume and the loss of cell turgor (Cicek and Cakirlar, 2002). However, treating plants with GB allowed the formation of longer and thicker fruits which might be due to better expansion of fruit cells and a better accumulation of assimilates in fruits. The nature of glycine betaine as an amino product could have enhanced the protein content of zucchini fruits confirming the finding of Al-Taweel *et al.* (2007). Glycine betaine has helped to restore the nitrogen fixation activity under salt stress and to antagonize the inhibition of protein biosynthesis, improving plant tolerance to stress (Giri, 2011). Also, saline water irrigation had a negative effect on the potassium uptake due to the competition between K^+ and Na^+ on the absorptive sites of plant roots (Ioneva, 1988). High levels of NaCl alter the export of phosphorus from root vacuoles to other plant organs (Navarro *et al.*, 2001). This caused lower potassium and phosphorus content in zucchini fruits, while on the contrary both elements were higher in fruits of treated plants as was observed in this study.

All above mentioned responses reflected on total plant fresh weight which was negatively affected by salinity and positively by GB application. Although number of leaves was not statistically different, there was a tendency to be negatively lower under salinity stress conditions. According to Alamgir and Ali (2006) salt accumulation has inhibited the formation of leaf primordial causing reduction in leaf number. This means lower leaf area which reflected negatively on a reduction in light interception hence photoassimilate

production. In addition, salt stress may cause a decline in photosynthesis due to lower stomatal conductance, depression in carbon uptake and metabolism, inhibition of photochemical capacity or a combination of these factors (Osuagwu and Udogu, 2014).

Conclusions

The result of osmotic effects of salt stress on the water and nutrient status of stressed plants ended with altering major growth parameters resulting in yield reduction. On the other hand, GB application, as an osmoprotectant, is an efficient and eco-friendly method that counteract those negative effects on zucchini plants by improving their tolerance.

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DETERMINATION OF CHEMICAL COMPOSITION AND AMINO ACIDS IN WHEAT BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

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Abstract

Wheat cereal grains are among the most important staple foods for the world's population. Near-infrared spectroscopy (NIRS) for determining grain chemical constituent contents has been studied over the past few decades around the world. The grain industry is in need of an automated, economical, and rapid means of determining grain quality. The aim of this work was to determine the chemical composition of wheat (*Triticum aestivum* L.) by near infrared reflectance (NIR) spectroscopy. This paper presents the chemical and amino acid composition of twelve spring wheat (n = 6) and winter wheat (n = 6) varieties grown in one location in Lithuania. Wheat grain samples of the 12 varieties were analysed for crude protein, crude fat, crude ash, crude fibre and amino acids using the FOSS NIRS™ DS2500 system according to the manufacturer's instructions in CARAT laboratory, Adisseo, Commentry, France. The results showed that crude protein content was between 11.53–13.97 g 100 g^{-1} and in winter wheat varieties it was 9.68–11.00 g 100 g^{-1} dry matter. Analyses showed that starch content in spring and winter wheat varieties ranged from 53.29–58.67 and 60.51–63.89 g 100 g^{-1} respectively, crude fat in spring and winter wheat were 2.54 and 2.19 g 100 g^{-1} respectively, ash 1.71 and 1.69 g 100 g^{-1} respectively. Comparison of spring and winter wheat varieties grown in Lithuania suggests that varieties of spring wheat outperform winter wheat in protein, crude fat, ash and amino acids contents.

Keywords: *amino acids, wheat, chemical composition, grains, NIRS.*

Introduction

Wheat cereal grains are among the most important staple foods for the world's population. Near-infrared spectroscopy (NIRS) for determining grain chemical constituent contents has been studied over the past few decades around the world. The current visual methods for grain quality estimation are subjective and time consuming. The grain industry is in need of automated, economical, and rapid means of determining grain quality. Near-infrared spectroscopy has been used for moisture and protein contents of cereal grains (Delwiche, 2004), including wheat, in Lithuania over the past decade. However it has been reported that near-infrared spectroscopy may be used for moisture, starch, crude protein, crude fibre, and crude ash determination in cereal grains (Shenk et al., 1992). Application of NIR spectroscopy in qualitative analysis and NIRS is currently becoming more useful in cereal grain applications.

Wheat grading systems are commonly based on the wheat protein content (Williams, 2007; Hulasare et al., 2003). The price of wheat depends on the protein content, often with substantial price differences between grades. Grain chemical composition (including amino acids) of different cereal varieties depends on various factors such as grain size, sowing time (Kong et al., 2014), genotype, climatic conditions (Janušauskaitė et al., 2013), environment (Akçura et al., 2011), fertilisation, and soil conditions (Manès et al., 2012). NIRS is widely used in routine screening as it provides a quick, non-destructive assay requiring small or no sample pretreatment (Ferrio et al., 2000). The application of NIRS in wheat quality control has been characterized by rapid development from prediction of major constituents in wheat

grains (moisture, protein, fat, starch, ash) to prediction of functional properties (Williams, 2007).

It is important to investigate the chemical composition of wheats in a given geographic location because their nutritional value depends on variety, fertilization, environmental conditions. NIRS can be more efficient in the use of resources. Thus, the objective of this study was to determine the chemical and amino acid composition of wheat varieties grown in a location in Lithuania using NIRS methodology. It's not the first time that this methodology has been used in Lithuania, it's already a routine procedure in this country.

Materials and Methods

Grain samples of varieties of winter and spring wheat were collected from the Kaunas Plant Variety Testing Station (PVTS) – Sitkūnai (55°02′31″ N latitude; 23°48′50″ E longitude) grown in a location in Lithuania (Figure 1). Six spring wheat varieties - *'Cornetto', 'Diskett', 'Ethos', 'Rospuda', 'Sonett', 'Tybalt'* and six winter wheat varieties - *'Agil', 'Kovas DS', 'Mariboss', 'Mulan', 'Rigi', 'Zentos'*, were used in this study. Spring wheat fertilization was applied as $N_{16}P_{38}K_{76}$ additionally–N_{75}. Winter wheat fertilization was applied as $N_{11}P_{22}K_{55}$, additionally–$N_{68.8}$+$N_{68.8.}$ Soil parameters were: humus–1.65%, pH–7.6, P_20_5–140 mg kg^{-1}, K_20–327 mg kg^{-1}. During the experimental year (2014), the average temperature ranged from 8.9°C at tillering to 18.5°C at waxy maturity stage of cereals. Rainfall was between 1 and 100.2 mm per year during different development stages of cereal development (Alijošius et al., 2016).

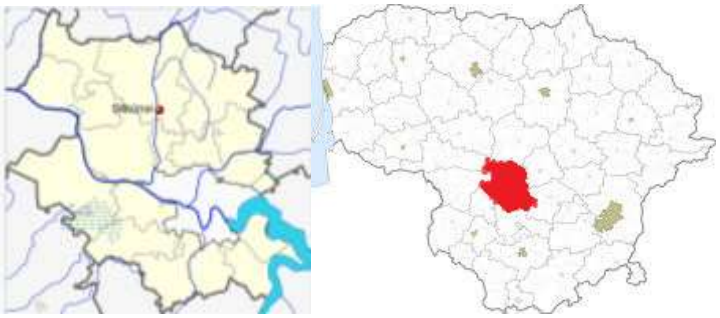

Figure 1. Map of the wheat sample collection region (red colour) in Lithuania

Wheat cereal grain samples (from each varieties 3 samples) were taken and analysed in accordance with the Commission regulation (EU) No 691/2013 of 19 July 2013, amending Regulation (EC) No 152/2009 as regards methods of sampling and analysis. Grain samples with three subsamples for chemical analyses were ground in an Ultra Centrifugal Mill model ZM 100 (Retsch GmbH, Germany) with a 1.0 mm sieve. Chemical compositions (dry matter, crude protein, crude fat, ash, crude fibre, starch, amino acids) were determined in spring and winter wheat by near-infrared spectroscopy (NIRS) without any previous sample pretreatment, except milling (CARAT laboratory, Adisseo, Commentry, France). Chemical composition was analysed using the FOSS NIRS™ DS2500 system according to the manufacturer's instructions.

The significance of differences between samples was established using one–way analysis of variance (ANOVA), and data are reported as means and standard deviations. Means were compared using Duncan's *t*-test ($P<0.05$). ANOVA was conducted using the statistical package SPSS 22.

Results and discussion

The chemical compositions of wheat varieties are shown in Table 1. About 70% of the bioavailable energy comes from carbohydrates, with starch being the most important fraction for animal nutrition (Klein et al., 2001). The majority of the carbohydrate content in cereal grains occurs as starch, which is readily digested (Franco et al., 1995; Hizukuri et al., 2006). Starch contents in analysed grain samples significantly differed between spring and winter wheat varieties ($P<0.05$). In spring wheat, grain starch contents ranged from 53.29 g 100 g^{-1} DM ('Ethos') to 58.67 g 100 g^{-1} DM ('Cornetto'). The highest starch content was determined in winter wheat variety 'Mariboss' (63.89 g 100 g^{-1} DM) and the lowest in 'Mulan' (60.51 g 100 g^{-1} DM).

Table 1. **Chemical compositions** (g 100 g^{-1} DM) **of the 12 spring and winter wheat varieties**

Variety denomination	Dry matter	Crude protein	Crude Fat	Ash	Crude fibre	Starch
			Spring			
'Cornetto'	85.71^{a}	13.28^{b}	2.44^{d}	1.70^{c}	3.10^{d}	58.67^{d}
'Diskett'	86.13^{a}	13.17^{b}	2.99^{a}	1.83^{a}	4.25^{a}	53.83^{e}
'Ethos'	85.74^{a}	13.97^{a}	2.67^{b}	1.75^{b}	3.78^{b}	53.29^{b}
'Rospuda'	85.61^{a}	12.31^{c}	2.25^{f}	1.64^{d}	2.68^{f}	58.34^{a}
'Sonett'	84.85^{a}	11.53^{d}	2.58^{c}	1.62^{d}	3.15^{c}	57.71^{f}
'Tybalt'	85.24^{a}	12.86^{b}	2.31^{e}	1.69^{c}	2.93^{e}	58.25^{c}
Mean	**85.55**	**12.85**	**2.54**	**1.71**	**3.32**	**56.68**
SD	**±1.50**	**±0.82**	**±0.26**	**±0.07**	**±0.55**	**±2.44**
			Winter			
'Agil'	86.51^{a}	10.55^{b}	2.19^{c}	1.73^{a}	2.82^{a}	61.13^{d}
'Kovas DS'	87.06^{a}	9.87^{c}	2.12^{d}	1.71^{ab}	2.65^{d}	60.88^{f}
'Mariboss'	86.03^{a}	9.68^{c}	2.38^{a}	1.70^{ab}	2.81^{ab}	63.89^{b}
'Mulan'	86.86^{a}	10.73^{ab}	2.15^{d}	1.65^{d}	2.78^{c}	60.51^{c}
'Rigi'	86.83^{a}	10.85^{ab}	2.05^{e}	1.66^{cd}	2.67^{d}	60.66^{e}
'Zentos'	86.78^{a}	11.00^{a}	2.24^{b}	1.69^{bc}	2.79^{bc}	61.14^{a}
Mean	**86.68**	**10.45**	**2.19**	**1.69**	**2.75**	**61.37**
SD	**±1.50**	**±0.54**	**±0.11**	**±0.03**	**±0.07**	**±1.26**

Means in the same column with different superscript letters are significantly different, $^{a-f}$ – $P < 0.05$, with spring and winter wheats tested separately.

The highest crude protein concentration was determined in spring variety 'Ethos' (13.97 g 100 g^{-1} DM) and the lowest in winter variety 'Mariboss' (9.68 g 100 g^{-1} DM). The winter wheat varieties accumulated less crude protein than spring varieties, differing overall by over 2%. Crude fat and ash concentrations were also higher on average, being 2.54 and 1.71 g 100 g^{-1} DM, compared with 2.19 and 1.69 g 100 g^{-1} DM overall for winter wheats. Mean crude fibre content present in this study in spring varieties ranged from 2.68 to 4.25 g 100 g^{-1} DM ($P<0.05$), but in winter varieties from 2.65 to only 2.82 g 100 g^{-1} DM.

Profiles of amino acids differed significantly between spring and winter varieties (Table 2). Eleven free amino acids were detected in the spring wheat and winter wheat samples; three from the aspartate family (Lys, Met and Thr), one from the glutamate family (Arg), three from the pyruvate family (Leu, Ile, Val), three from the aromatic amino acid family (His, Phe, Trp) and one from the 3 phosphoglycerate family (Cys). The ranges of amino acids in most wheat varieties were 0.12–1.07 g 100 g^{-1} DM. Wheat proteins are low in some amino acids essential to the human diet. Lys is a limiting amino acid in most cereal products and

during baking it is believed that Maillard reactions may affect the content and composition of related products lowering its availability (Charissou et al., 2007). In this study, comparing spring and winter wheats the lowest amount of lysine was found in the winter variety 'Mariboss' (0.27 g 100 g^{-1}) and highest amount in spring variety 'Ethos' (0.39 g 100 g^{-1}). High contents of phenylalanine (0.51–0.64 g 100 g^{-1}) and arginine (0.52–0.63 g 100 g^{-1}), and low levels of tryptophan (0.14–0.17 g 100 g^{-1}) were determined. Other amino acids, such as aspartic acid and glutamic acid, were not determined in this experiment. However, using RP-HPLC-FLD, Anjum et al. (2005) determined amino acid concentrations in flour to vary as follows: aspartic acid 0.453–0.841, glutamic acid 0.743–1.313g/100 g. Complementary results of Boila et al. (1996) gave leucine 1.04 g/100 g, arginine 0.695 g/100 g, and histidine 0.352 g/100 g.

Table 2. Amino acid contents in different spring and winter wheat varieties (g 100 g^{-1} DM)

Variety denomination	**Lys**	**Met**	**Cys**	**Thr**	**Trp**	**Val**	**Ile**	**Leu**	**Phe**	**His**	**Arg**
						Spring					
'Cornetto'	0.34^{c}	0.22^{b}	0.30^{b}	0.43^{b}	0.17^{b}	0.63^{b}	0.50^{b}	1.01^{b}	0.66^{b}	0.29^{c}	0.63^{c}
'Diskett'	0.38^{b}	0.21^{c}	0.29^{c}	0.42^{c}	0.16^{c}	0.62^{c}	0.48^{c}	0.92^{e}	0.64^{c}	0.30^{a}	0.66^{b}
'Ethos'	0.39^{a}	0.23^{a}	0.31^{a}	0.45^{a}	0.18^{a}	0.68^{a}	0.54^{a}	1.07^{a}	0.72^{a}	0.32^{b}	0.71^{a}
'Rospuda'	0.33^{d}	0.21^{c}	0.28^{d}	0.41^{d}	0.16^{c}	0.60^{e}	0.47^{d}	0.93^{d}	0.62^{d}	0.29^{c}	0.59^{e}
'Sonett'	0.33^{d}	0.20^{d}	0.27^{e}	0.39^{e}	0.15^{d}	0.57^{f}	0.43^{e}	0.85^{f}	0.58^{e}	0.27^{d}	0.58^{f}
'Tybalt'	0.33^{d}	0.21^{c}	0.29^{c}	0.42^{c}	0.17^{b}	0.61^{d}	0.48^{c}	0.97^{c}	0.64^{c}	0.29^{c}	0.61^{d}
Mean	**0.35**	**0.21**	**0.29**	**0.42**	**0.17**	**0.62**	**0.48**	**0.96**	**0.64**	**0.29**	**0.63**
SD	**±0.03**	**±0.01**	**±0.01**	**±0.02**	**±0.01**	**±0.03**	**±0.03**	**±0.07**	**±0.04**	**±0.02**	**±0.05**
						Winter					
'Agil'	0.30^{a}	0.19^{a}	0.25^{b}	0.35^{b}	0.15^{a}	0.51^{c}	0.39^{b}	0.74^{d}	0.52^{c}	0.25^{a}	0.52^{d}
'Kovas DS'	0.29^{b}	0.18^{b}	0.23^{c}	0.33^{c}	0.14^{b}	0.48^{d}	0.36^{c}	0.70^{e}	0.48^{d}	0.24^{b}	0.49^{e}
'Mariboss'	0.27^{c}	0.17^{c}	0.22^{d}	0.31^{d}	0.12^{c}	0.44^{e}	0.34^{d}	0.65^{f}	0.44^{e}	0.22^{c}	0.46^{f}
'Mulan'	0.30^{a}	0.19^{a}	0.25^{b}	0.36^{a}	0.15^{a}	0.51^{c}	0.39^{b}	0.78^{b}	0.53^{b}	0.25^{a}	0.53^{c}
'Rigi'	0.30^{a}	0.19^{a}	0.26^{a}	0.36^{a}	0.15^{a}	0.52^{b}	0.40^{a}	0.77^{c}	0.54^{a}	0.25^{a}	0.54^{b}
'Zentos'	0.30^{a}	0.19^{a}	0.26^{a}	0.36^{a}	0.15^{a}	0.53^{a}	0.40^{a}	0.80^{a}	0.54^{a}	0.25^{a}	0.55^{a}
Mean	**0.29**	**0.19**	**0.25**	**0.35**	**0.14**	**0.50**	**0.38**	**0.74**	**0.51**	**0.24**	**0.52**
SD	**±0.01**	**±0.01**	**±0.02**	**±0.02**	**±0.01**	**±0.03**	**±0.02**	**±0.05**	**±0.04**	**±0.01**	**±0.03**

Means in the same column with different superscript letters are significantly different, $^{a-f}$ – $P < 0.05$, with spring and winter wheats tested separately.

Conclusions

This study demonstrated that the chemical composition and amino acids differed significantly amongst six spring and six winter wheat varieties, determined with NIR spectroscopy. Comparison of spring and winter wheat varieties grown in Lithuania suggests that varieties of spring wheat outperform winter wheats in protein, crude fat, ash and amino acid contents.

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THE IMPACT OF CLIMATE CONDITIONS ON THE LEAF SIZE OF BASMA TOBACCO

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Abstract

Morphological traits are of major importance in determination of the type of tobacco and varieties within the type. These traits differ to a lesser or greater extent and are genetically controlled. Under the influence of inadequate agro-ecological and technological conditions, tobacco types can also show strong variations in bio-morphological properties, especially in dimensions of the leaves. Such variations can occur despite the genetic control and are known as "plasticity" of tobacco. Therefore, before starting the production of some tobacco type or variety, it is necessary to know the basic preconditions for its stable production in order to avoid major variations in morphological and production characteristics. Three-year investigation 2009, 2010 and 2011 was carried out in the experimental field of Tobacco Institute – Prilep Republic of Macedonia. The trial was set up using the method of randomized blocks in 5 replications, with three varieties of Basma tobacco (MK-1, MB-2 and MB–3) and one Yaka variety (YK 7-4/2) as a check. The aim of investigation on morphological traits length, width and angle of the top and bottom leaf was to get a real picture of the varieties and their plasticity under the influence of different climate conditions. Research workers of the Scientific Tobacco Institute – Prilep created several varieties of Basma tobacco, including MK-1, MB-2 and MB-3, approved by the State Commission for variety testing in 2010, which yield and quality guarantee a cost - effective production. The creation of new tobacco varieties is a long-term commitment of the Tobacco Institute - Prilep and continuous process which has not been interrupted up to this day, giving a great contribution to the production. The significance of the trials was computed statistically and with the use of LSD test.

Keywords: *morphological traits, basma, length, width, leaf.*

Introduction

Oriental tobaccos Prilep, Yaka and Dzebel account for almost 95% of the total tobacco production in R. Macedonia. In recent years this stock has been enriched with Basma tobacco, which yield and quality meets the criteria and standards of manufacturers and companies that purchase tobacco from this region.

Of the 25-30 000 tons annual oriental tobacco production in R. Macedonia, over 90% are intended for exports. Republic of Macedonia is recognized as tobacco country small in size and population but one of the biggest in production of good quality oriental tobacco per capita. With the change of smoker's taste preference, the manufacturers requirements for particular raw materials of oriental tobacco used in cigarette blends also change. For these reasons, each year foreign buyers are offered raw materials of various types and varieties to satisfy their requirements in terms of flavor, taste and other properties of tobacco. The Basma tobacco is one of the most highly valued oriental tobaccos in the world. It is mainly grown in Greece and Turkey, but after the dramatic decline in these two countries (especially in Greece), tobacco companies see a chance to relocate a part of the production to the Republic of Macedonia, in areas where soil and climate conditions favor the production of this type. Leaf size in various years of investigation and with various amounts of precipitation show

that top leaves of Basma tobacco are typical representatives of oriental tobacco. According to the Rules for assessment and purchase of oriental tobacco, the top leaves are considered oriental if their size does not exceed 13 cm (Mitreski M., 2012). In order to make its production more attractive to manufacturers, the price of Prilep tobacco is slightly higher compared to other oriental tobaccos. Due to its quality, there are no problems with the export of this type and it gives incentive for production of newly created varieties which would be interesting for farmers, manufacturers, traders etc.

Materials and Methods

Four varieties of oriental tobacco were used as material for the three-year investigation (2009/2010/2011): YK 7-4/2 as a check (ø), and Basma varieties MK-1, MB-2, and MB-3. Seedling was produced in traditional way, in cold beds covered with polyethylene, in the experimental field of the Scientific Tobacco Institute - Prilep.

During the breeding process, all necessary cultural practices were applied: covering - uncovering, watering, fertilization and protection, simultaneously in all tested varieties, in order to obtain a healthy and well-developed seedlings. Fertilization was performed with 300 kg / ha NPK mineral fertilizer (8:22:20). The trials were set up using the method of randomized blocks with 5 replications, at 45 × 12 cm spacing on previously treated soil.

There were five rows in each plot, three of them were used for harvesting and two for protection and for morphological measurements. Leaf length and width were measured on 50 plants of each variety and the obtained data were statistically processed following the analysis of variance (ANOVA), using the LSD test. Meteorological data on the amount and distribution of precipitations in mm during the growing season May - September were obtained from the Meteorological Station at Tobacco Institute-Prilep.

Results and Discussion

Amount of precipitation

The requirements for water and warmth of each plant, especially of tobacco, are changing during the entire biological cycle. According to Atanasov D. (1962), tobacco plant requirements for water are variable and so the amounts of water should be gradually reduced during the vegetation period. The maximum precipitation for successful growth of oriental tobacco is 250 mm and the minimum is 100 mm, but since it is not sufficient for production of a good quality tobacco raw, additional irrigation is needed. The same author reported that the optimum precipitation during the field growth is 120 - 150 mm. According to Pelivanoska V. (2007), if there is only one rainy day in a month, with insignificant amount of rainfall per square meter, it should not be taken into consideration.

Nuneski R. (2008), in his trials with Izmir oriental tobacco in Turkey, reported that even low precipitations measured in July and August had a good impact on ripening and drying of the upper insertions, which are the main carriers of raw tobacco quality. In certain periods of plant growth, however, additional interventions with water were applied. Data in Table 1 show irregular distribution of rainfalls during the three years of investigation. Thus, the mean precipitation in May ranged from 55.0 mm in 2009 to 64 mm in 2010. During the vegetation period June – September, the lowest average precipitation by decades (0 mm) was recorded in July 2009 (third decade), June 2011 (third decade) and July 2011 (second decade), August 2010 (third decade) and September 2009 (first decade), when additional irrigation was applied depending on plant requirements.

The highest precipitation average was recorded in the third decade of June 2009 (68 mm). From June to September, the lowest mean monthly precipitation was recorded in July 2009 (8.0 mm) and the highest in June 2010 (87.0 mm).

According to the data presented in Table 1, the lowest amount of precipitation during the growing season (May-September) was recorded in 2011 - 180.0 mm, and the highest in 2010 - 298.0 mm.

Table 1. Amount and distribution of precipitations in mm (May-September) – location: Tobacco Institute – Prilep

Year	Decade	Months					Total precipitations May-September (mm)
		May	June	July	August	September	
2009	I	14.0	2.0	2.0	16.0	--	196.0
	II	1.0	5.0	6.0	4.0	13.0	
	III	40.0	68.0	--	23.0	2.0	
	Monthly	55.0	75.0	8.0	43.0	15.0	
2010	I	18.0	18.0	25.0	43.0	26.0	298.0
	II	17.0	47.0	8.0	2.0	1.0	
	III	29.0	22.0	23.0	--	20.0	
	Monthly	64.0	87.0	55.0	45.0	47.0	
2011	I	20.0	31.0	8.0	9.0	--	180.0
	II	32.0	20.0	--	1.0	23.0	
	III	11.0	--	9.0	1.0	15.0	
	Monthly	63.0	51.0	17.0	11.0	38.0	

Leaf size (length and width) of the undertop and top leaves of fresh tobacco

Data presented in Table 2 and Fig. 1 show that leaf length by years ranged from 10.3 cm in variety MK-1 in 2009 to 14.0 cm in MB-3 variety in 2010 and leaf width ranged from 5.4 cm in 2009 in MK-1 to 7.7 cm in Basma variety MB-3 in 2010.

According to the average values, the shortest leaf was measured in variety MK-1 (11.5 cm) and the longest in variety MK-3 (12.6 cm), which is 5% more compared to the check. In all three years of investigation, statistically significant differences at 1% were estimated in MB-3 variety in 2011, while other varieties did not show such differences. The smallest average width of the leaf was recorded in variety MK-1 – 6.3 cm and the largest in variety MB-3 – 6.8 cm, which is 6.25% more than the check variety. In the three years of investigation on the character leaf width, the investigated varieties did not show statistically significant differences. The increased amount of rainfalls in 2010 resulted in increased dimensions of leaves, but still within the permitted range.

JK-7-4/2 **MK-1** **MB-2** **MB-3**

Fig. UNDERTOP LEAVES

Figure 1. Undertop leaves of fresh tobacco

Table 2. Leaf size (length/width) of the undertop leaves of fresh tobacco

Variety	Year	Length, cm	Average 2009 / 2011	Differences Absolute	Differences Relative %	Width, cm	Average 2009 / 2011	Differences Absolute	Differences Relative %
YK 7-4/2 ∅	2009	10.8	12.0	/	100.00	5.6	6.4	/	100.00
	2010	13.2				7.1			
	2011	11.9				6.6			
MK-1	2009	10.3	11.5	-0.5	95.83	5.4	6.3	-0.1	98.44
	2010	12.7				7.1			
	2011	11.4				6.5			
MB-2	2009	10,8	12.3	+0.3	102.50	5.6	6.5	+0.1	101.56
	2010	13.8				7.2			
	2011	12.2				6.8			
MB-3	2009	10.5	12.6	+0.6	105.00	5.6	6.8	+0.4	106.25
	2010	14.0				7.7			
	2011	13.2^{++}				7.2			

year 2009, leaf length
LSD 5% 0.98 cm $^{+}$
1 % 1.38 cm^{++}
leaf width
LSD 5% 0.27 cm+
1% 0.38 cm ++

year 2010, leaf length
LSD 5% 0.94 cm $^{+}$
1% 1.32 cm^{++}
leaf width
LSD 5% 0.43 cm +
1% 0.46 cm ++

year 2011, leaf length
LSD 5% 0.84 cm +
1% 1.18 cm ++
leaf width
LSD 5% 0.40 cm+
1% 1.56 cm ++

Top leaves are the smallest leaves of tobacco plant. They are characterized by a sharp apex and the broadest leaf base (Fig. 2). The largest width is measured at the base of the leaves and they account for 6-7% of the total yield of the stalk.

Figure 2. Top leaves of fresh tobacco

According to data in Table 3, the variety MB-3 had the smallest length of the top leaf in 2009 (8.1 cm) and the biggest length in 2010 (12.9 cm).
The shortest top leaf was measured in the variety MK-1 (9.6 cm), and the longest (10.5 cm) in varieties YK 7-4/2 (∅) and MB-3. The tested varieties did not show statistically significant differences during the three years of investigation.
Leaf width ranged from 4.8 cm in MK-1, MB-2 and MB-3 in 2009 to 7.1 cm in MB-3 variety in 2010. The average width of the top leaf ranged from 5.5 cm in MK-1 to 5.9 cm in the check variety YK 7-4/2 and MB-3. No statistically significant differences were recorded in the investigated varieties with regard to this trait.

Table 3. Leaf size (length/width) of the top leaves of fresh tobacco

Variety	Year	Length, cm	Average 2009 / 2011	Differences Absolute	Differences Relative %	Width, cm	Average 2009 / 2011	Differences Absolute	Differences Relative %
YK 7-4/2 ∅	2009	8.5	10.5	/	100.00	4.9	5.9	/	100.00
	2010	12.3				6.6			
	2011	10.7				6.1			
MK-1	2009	8.4	9.6	-0.9	91.43	4.8	5.5	-0.4	93.22
	2010	11.3				6.4			
	2011	9.2				5.3			
MB-2	2009	8.5	10.0	-0.5	95.24	4.8	5.7	0.2	96.61
	2010	12.1				6.8			
	2011	9.5				5.4			
MB-3	2009	8.1	10.5	0	100.00	4.8	5.9	0	100.00
	2010	12.9				7.1			
	2011	10.6				5.7			

year 2009, leaf length
LSD 5% 0.83 cm +
1% 1.17 cm ++
Leaf width
LSD 5% 0.24 cm +
1% 0.35 cm ++

year 2010, leaf length
LSD 5% 0.72 cm +
1% 1.01 cm ++
Leaf width
LSD 5% 0.41 cm +
1% 0.58 cm ++

year 2011, lea f length
LSD 5% 0.45 cm +
1% 0.64 cm ++
Leaf width
LSD 5% 0.34 cm +
1% 0.48 cm ++

Based on the results of the three-year investigation on morphological traits of oriental Basma tobacco in the Republic of Macedonia, the following conclusions can be drawn:

Conclusions

- The trial was set up on colluvial-diluvial soil, with low humus and nitrogen content and good supply of available phosphorus and potassium. The use of mineral fertilizers (300 kg/ha N8:P22:K20) is a guarantee for obtaining higher yield and good quality.
- The amount of precipitation during the growing season May-September ranged from 180.0 l/m^2 in 2011 to 298.0 l/m^2 in 2010. Due to the uneven distribution of rainfalls in this period, it was necessary to apply additional water interventions in the field.
- The smallest length of the undertop leaf was measured in variety MK-1 – 11.5 cm and the largest in variety MK-3 – 12.6 cm, which is 5% more compared to the check variety. During the three-years investigation, statistically significant differences at a level of 1% were recorded in MB-3 variety in 2011 and no such significance was observed in other varieties.
- The smallest leaf width of the undertop leaf of 6.3 cm was measured in MK-1 variety and the largest width of 6.8 cm was recorded in MB-3, which is 6.25% more than the check variety.
- The length of the top leaf ranged from 9.6 cm in variety MK-1 to 10.5 cm in the check variety YK 7-4/2 and in MB-3. The investigated varieties did not show statistical significance in the three years of investigation.
- The average width of the top leaf ranged from 5.5 cm in MK-1 to 5.9 cm in the check variety YK 7-4/2 and in MB-3. With regard to this trait, the investigated varieties did not show statistically significant differences compared to the check variety. According to the results obtained, the MK-1 variety is characterized by the smallest length and width of the top leaf.

- The results of the three-year investigation under variable amounts and distribution of rainfalls indicate that the top leaves of Basma varieties in the region of Prilep are typical of the oriental type of tobacco.

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QUALITY OF THE POMEGRANATES VARIETIES "HICAZ" AND "KARAMUSTAFA" FROM THE REGION OF MACEDONIA

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Abstract

Pomegranates are valuable fruits due to the high level of anthocynanins in juice and phenolic compounds responsible for antimicrobial activity of pomegranate peel. Significant amounts of phenolic acids, vitamin C and other polyphenolics are responsible for health benefits of the consumers. The objects of our study was the impact of variety on the quality of pomegranates grown in the region of Macedonia. More precisely, morphological parameters for fruits and chemical characteristics of pomegranate juice were significantly affected to the variety of pomegranates. Morphological parameters, in particular fruit weight, fruit height, equatorial and calix diameter favorited "Hicaz" variety in comparison to autochthonous "Karamustafa" variety of pomegranates. Furthermore, the pH value of fruit juice, the level of phenolic acid and percentage of glucose and fructose indicate significant differences between varieties. The quantity of anthocyanins (537 mg/L), catechins (50 mg/L) and total phenolic compounds (3367 mg/L) indicated higher quality of pomegranate juice from "Hicaz"pomegranate variety. The effect of pasteurization did not influenced significantly to the quality of pomegranate juices from both varieties.

Key words: *pomegranate juices, morphological parameters, total phenolic compounds, anthocyanins, catechins*

Introduction

Pomegranates are valuable fruits due to the level of anthocynanins in juice and phenolic compounds responsible for antimicrobial activity of pomegranate peel (Dimovska *et al*., 2018). Significant amounts of phenolic acids, vitamin C and other polyphenolics are responsible for health benefits of the consumers (Dimovska *et al*., 2017).

Pomegranates are native from Persia and it is believed that the origin is in Central Asia. The morphological changes might have occurring during domestication and the most indicative changes are usually notice on flower, rind and aril colour, fruit size, percentage of sugar and organic acids as well as quality of the seeds (Teixeria da Silva *et al*., 2013)

The objects of our study were the impact of variety on the quality of pomegranates grown in the region of Macedonia. More precisely, this study examined the effect of variety on morphological parameters for fruits and chemical characteristics (Brix, pH, organic acid, total phenolic compounds, catechins and anthocyanins) of pomegranate juice from the autochthons varieties "Karamustafa" and "Hicaz".

Materials and Methods

Sample preparation

The sampled fruits were selected randomly in order to separate three replicates for analysis, using 20 kg per repetition and cultivar. Pomegranates were weighed, cut in halves, and arils were hand separated from the pith avoiding contamination by components in membranous walls (septum). Juices of each culivar were obtained by pressure of arils and weighed to determine the juice yield. Samples of freshly prepared juice from both varieties ("Hicaz" and "Karamustafa") were stored frozen one year (−20 ◦C) until have been analyzed.

Physical properties

Size of the randomly selected 100 dried arils was determined from the axial dimensions of arils. Length was measured using a digital caliper at the accuracy of 0.01 mm. Weight of the hundred dried arils was determined by randomly selecting five samples and weighing in an electronic balance of 0.001 g sensitivity.

Chemical analyses

For determination of titratable acidity (TA), 2 ml of fresh juice was diluted with 70 mL of distilled water and titrating with 0.1 M NaOH to an end point of pH 8.2 using a Metrohm 862 compact titrosampler (Herisua, Switzerland). The results were expressed as percentage of citric acid (% CA). Total soluble solids (TSS, °Brix) was measured using a digital refractometer (Atago, Tokyo, Japan) calibrated with distilled water. The pH values were determined at room temperature using a calibrated pH meter (Crison, Model 00924, Barcelona, Spain). All measurements were made by three repetitions

The total phenolic content of pomegranate juices was determined with Folin–Ciocalteau reagent. For each sample, 50 μL of diluted (1:5) oil extract were added to 750 μL water and 50 μL of Folin–Ciocalteu reagent. The solution with total volume of 850 μL was incubated in the dark for 5 min. Then, 150 μL of 20% sodium carbonate solution was added and samples were incubated in the dark for 1 h. Reference solution was prepared with distilled water instead pomegranate juice and treated with the Folin–Ciocalteu reagent in the same way as the assayed samples. The samples turned to a blue colour with different degrees, depending on the content of phenolic compounds in the samples. The absorbance at 765 nm was recorded against the absorbance of the reference solution. The color intensity was measured spectrophotometrically by UV/VIS spectrophotometer (Shimadzu 1800, Shimadzu corporation, Kyoto, Japan). Determination of monomeric anthocyanins was performed by the colorimetric method of Singleton and Rossi (1965). The measurements were performed by three repetitions

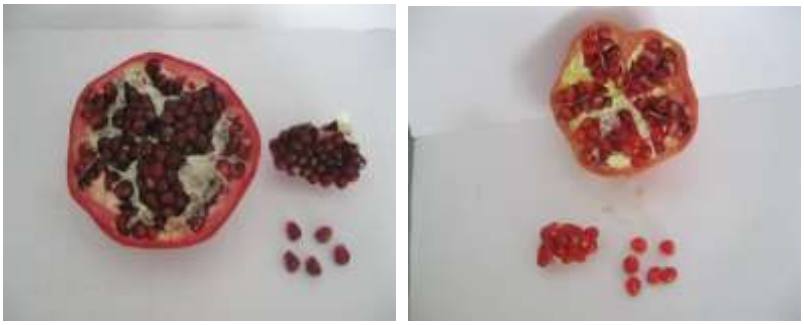
Results and discussion

The chemical parameters of the samples of pomegranate juices from two varieties are presented in Table 1. As we can see, there is no significant difference between results obtained for Brix and pH for both pomegranate varieties. Furthermore, there is no statistical difference between pasteurized and fresh pomegranate juices. On the other hand, there is significant difference between the level of organic acids, monomeric anthocyanins, total phenolic compounds (TPC) and total catechins which were higher for "Hicaz" variety in comparison to "Karamustafa" variety.

Data from physical and morphological characterization of pomegranates from "Hicaz" and "Karamustafa" varieties from 2016 and 2017 vintage years are presented in Tables 2 and 3. The pomegranates from "Hicaz" variety had higher average fruit weight. Percentages of grain and peel were higher for "Karamustafa" variety as well as weight of 100 berries and percentage of skin. The percentage of carpels was more than double for "Hicaz" variety as well as equatorial and calyx diameter and fruit height with and without calyx (Table 3). Pomegranates from "Karamusfata" variety had reddish yellow colour of the fruit and pink colour of the fruit juice. On the other hand, pomegranates from "Hicaz" variety had reddish colour of the fruit and dark pink colour of the fruit juice (Table 1 and Fig.1.). The average fruit weight of pomegranates from "Hicaz" variety was the same as "Chioukhi" variety of pomegranates from Morocco. On the other hand, calix diameter for "Hicaz" variety of pomegranates was similar to Moroccanish "Ounk Hmam" variety and calyx for "Karamusfata" variety was more similar to some Spanish varieties of pomegranates [5].

Table 1. Chemical parameters of pasteurized and fresh pomegranate juice

	Brix	pH	Organic acids	Total phenolic compounds (TPC)	Total catechins	Total antocyanins
Pomegranate juice			g/l	mg/l GAE	mg/l TCE	mg/l
Karamustafa (fresh)	17	3.05	5.4	2038	22.91	117.31
Hicaz (fresh)	16	3.04	29.6	3367	50.1	537.13



a b

Fig.1. Pomegranate seeds from "Hicaz" (a) and "Karamustafa" variety (b)

Table 2. The technological characteristics of pomegranate fruits

Variety	Yeas	Repetition	Average fruit weight (g)	Percentage of grain (%)	Percentage of peel (%)	Weight of 100 seeds g	Weight of 100 berries g	Percentage of skin %	Percentage of carpels %	Percentage of juice (%)	Color of fruit	Color of juice
Hicaz	2016	I	525.00	50.24	25.95	4	30.37	0.49	15.59	45.21	Reddish	Dark pink
		II	368.75	50.85	31.36	3	32.50	0.78	14.91	46.08		
		III	283.75	50.92	31.72	3	28.12	0.70	15.77	45.49		
	2017	I	398.90	50.74	25.41	4.5	28.61	1.61	12.05	45.26	Reddish	Dark pink
		II	340.44	47.42	24.30	4.0	29.32	1.74	14.84	45.58		
		III	240.77	50.35	23.35	3.5	23.41	1.74	13.04	47.21		
Karamustafa	2016	I	285.43	53.85	42.14	3	27.86	1.50	8.10	48.26	Reddish yellow	Pink
		II	235.00	51.06	35.56	2.5	27.86	2.13	6.08	46.48		
		III	198.28	53.31	37.10	2.5	29.28	2.37	7.20	48.76		
	2017	I	370.12	64.19	22.55	2.5	33.12	0.55	5.70	50.70	Reddish yellow	Pink
		II	246.06	61.58	21.19	2.5	35.39	0.90	5.19	51.00		
		III	225.76	55.86	19.56	2.8	37.63	1.00	5.24	49.25		

Table 3. The morphological characteristics of the fruits

Variety	**Year**	**Repetition**	Fruit weight (g)	D1 (mm)	D2 (mm)	L1 (mm)	L2 (mm)	L3 (mm)
Hicaz	2016	I	525.00	101.0	26.8	90.3	101.6	27.1
		II	368.75	90.0	23.3	80.6	87.6	26.1
		III	283.75	84.7	18.2	75.0	85.2	25.3
	2017	I	398.90	93.6	21.8	**82.2**	**98.6**	**33.0**
		II	340.44	87.1	24.5	**78.0**	**93.6**	**26.0**
		III	240.77	77.4	23.0	**67.1**	**79.0**	**24.0**
Karamustafa	2016	I	285.43	84.8	17.7	73.3	87.3	29.3
		II	235.00	80.0	18.9	70.8	82.9	28.1
		III	198.28	75.5	16.5	66.1	80.2	27.0
	2017	I	370.12	91.3	14.2	82.1	94.6	25.7
		II	246.06	79.1	11.9	96.6	79.5	25.0
		III	225.76	79.0	11.0	67.3	74.6	25.1

D1-equatorial diameter, D2 – calyx diameter, L1 – fruit height without calyx , L2 – total fruit height, L3 – calyx height,

Conclusion

The purpose of the present study was to evaluate the morphological, physical and chemical parameters, in particular organic acids, total phenolic content (TIC), catechins and anthocyanins in two varieties of pomegranates juices from the region of Macedonia. The lower level of phenolic compounds and acids as well as catechins and anthocyanins had pomegranate juice from the autochtonous variety "Karamustafa" and the higher level had the pomegranate juice obtained from "Hicaz" variety. Based on the explanation above, we can summarize that pomegranate juice from "Hicaz" variety is enough rich source with polyphenolic compounds. Finally, we recommend the juice from this pomegranate variety for further investigation in order to identify and quantify the level of polymer compound and other valuable bioactive compounds with significant impact on human health.

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FRUIT CHARACTERISTICS OF FEIJOA GROWN IN A TROPICAL HIGHLAND OF MEXICO - PULP COMPOSITION, OIL IN SEEDS, AND ESSENTIAL OILS

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Abstract

The feijoa (*Acca sellowiana*) is a fruit tree recently grown in Mexico, where trees had been established in humid tropical highland conditions of Veracruz, at 1900 m of altitude. Industrial potential of feijoa at the moment are unknown. The objectives of this study were to determine aromatic compounds in the skin of the fruit, oils in the seeds, and some physical and chemical characteristics in the pulp. Commercial-sized fruits were obtained from eight-year-old trees with a single compost application per year and without artificial irrigation. Feijoa seeds had 69.4% unsaturated fatty acids, mainly linoleic (46.2 %) and linolenic (3.7). The major compounds in the essential oil of the feijoa skin were benzoate 2-methylpropane and caryophyllene acids with 18.41% and 10.28 %, respectively. The total dry mater, humidity, ash, proteins, ethereal extract, crude fiber in the pulp differed slightly from those reported in the literature of feijoa cultivated under temperate conditions. The feijoa fruit produced under tropical highland conditions is adequate as a fresh commodity and for making industrial food products.

Keywords: *Acca sellowiana, essential oils, fruit skin, oil in seeds, isobutyl benzoate.*

Introduction

The feijoa is a new fruit tree crop in Mexico, and the few agronomic experiences with this Myrtaceae are found in the humid tropical highlands of Veracruz at 2000 m of altitude where precipitation is around1900 mm per year, with annual average temperature of 17 C. Feijoa center of origin is located in the highlands of Paraguay, southern Brazil and Uruguay, and northeastern Argentina (Keller & Tressens, 2007). In New Zealand and Australia, feijoa cultivation has been very popular and the fruits are exported as fresh fruit or industrialized products. Its cultivation has increased in Italy, China and Turkey due to its high market sales potential and its medicinal anticancer, anti-inflammatory, antiviral, antimicrobial, hepatoprotective, anti-osteoporosis, antihyperthyoid, antioxidant and immunomodulatory properties (Lim, 2012). The feijoa is also used as a preventive treatment for gastritis and ulcers (Monforte *et al*., 2014). However, the information about fruit composition and specifically on oils in the seeds, and essential oils in the skin of feijoa under tropical highlands conditions is limited. The essential oils are volatile substances of organic nature, responsible for the aroma of plants, and the term of oil is not related to fats. Some of the feijoa volatile compounds are the methyl and ethyl benzoates, which represent approximately 90% of the volatile fraction and are responsible for the aroma of the fruit (Binder & Flath R, 1989). Regarding to the oil content of the feijoa seeds, Andrade *et al* (2012), obtained a yield of 1.5% of lipids in 100 g of feijoa seeds. The composition of the oils in the feijoa varies according to its geographical origin (Weston, 2010). The objectives of this study were to determine some physical and chemical properties in the pulp of the fruit, and determine oils in the seeds, and essential oils in the skin of the fruit. This is the first report regarding oils in the seeds of feijoa, and essential oils in the skin of feijoa fruit produced under tropical highland conditions.

Materials and methods

Plant material. All the fruits evaluated were harvested at physiological maturity and harvested in an orchard located at 2000 m of altitude in Veracruz, Mexico. All the trees were seedlings planted at 4 x 4 m that received water from the rain.

Bromatological analysis. Total moisture, total dry matter, ash, crude protein, ether extract, crude fiber and carbohydrate percentages were determined in 10 ripened feijoa fruits harvested in each of four accessions that were propagated by seed. The determinations were carried out in accordance with several Mexican standards for the bromatological analysis of food.

Oil in the seeds. In fruits randomly harvested a total of 30 g of seeds were extracted. The oil of the seeds was obtained using an instrument of destilation Soxhlet (Meyer *et al*., 2008). The fatty acid profile was determined by gas chromatografy (Agilent 6890) with a coumn AT-FAME (López-Yerena *et al*., 2018).

Essential oils. They were isolated from the fruit skin using the Clevenger device. The essential oil was analyzed in a gas chromatograph coupled to an Agilent 7890 B ion trap. A capillary column (30m x 250 µ x 0.25 µ) was used for the separation.

Data analysis. A completely randomized design was used to evaluate the physical and chemical characteristics of the fruits. The variables were evaluated with an analysis of variance and significant differences among means were determined using the Tukey test ($P \leqslant 0.05$). Standard errors were also determined. Data analysis was done with the InfoStat statistical package (Di Rienzo et al., 2016).

Results and discussion

Bromatological analysis

There were significant differences ($P \leq 0.05$) among the four accessions propagated by seed in all the physical and chemical variables evaluated (Table 1). The total moisture, ash, crude protein, ether extract, crude fiber and carbohydrates was slightly different from the values found by González-García *et al.* (2018), using other seedlings of feijoa receiving a different amount of precipitation in Veracruz, Mexico, or with selected feijoas in Colombia (Fischer *et al*., 2003). The total dry matter of the fruit (%) (Table 1) coincide with the 15±1 % reported by Sun-Waterhouse et al (2013) in New Zealand. The dry matter content determine quality in fruits (Famiani *et al*., 2012). Thus, in general these parameters changed little in the feijoa fruit produced under a tropical highland condition. Likewise, fruit size values of feijoa grown in a tropical conditions are adequate for the market (González-García *et al*., 2018).

Oil content of the seeds

The content was of 0.23 %, and this value was two times lower than the reported by Andrade *et al.* (2012) in Brazil with feijoa trees grown under temperate conditions. The linoleic acid achieved the highest value with 46 % (Table 2), and Andrade *et al.*, (2012) also found the linolenic acid (84%) as the main fatty acid in the feijoa seeds. In this work it was confirmed that the linoleic acid is the main fatty acid in the seeds of feijoa. The palmitic acid had 21 %, and the oleic acid 19 % (Table 2). Lower values has been reported for the oleic (Andrade *et al*., 2012) in comparison with those found in the present study. Most of the fatty acids (69 %) in the seeds were polyunsaturated.

Essential oil

The yield of the essential oil obtained from the feijoa skin was of only 0.3%. Low essential oil recoveries have also been obtained in New Zealand (Shaw *et al*., 1983). There were 57 compounds detected. The isobutyl benzoate or benzoic acid 2-methylpropyl ester (numbers 29 and 57 in Table 3) achieved the highest content (18.41%) followed by the caryophyllene oxide (10.28%) (number 22 in Table 3). Two important volatile components of the feijoa fruit are methyl benzoate and ethyl benzoate (Hardy and Michael, 1970), then the isobutyl

benzoate (Table 3) plays an aromatic role because this compound belongs to the family of benzoic esters. The caryophyllene oxide, is a natural bicyclic sesquiterpene present in many essential oils. Saj *et al*. (2008), reported that the feijoa essential oil had a broad spectrum of antibacterial and antifungal activity due mainly to the caryophyllene oxide. Another compound identified was azulene (4.53%), and some derivatives of azulene prevent gastric lesions (Amagase *et al*., 2013). Feijoa fruit produced under tropical highland conditions of Veracruz are less aromatic than the produced in New Zealand. The % of the caryophyllene oxide was lower than the found by Saj *et al*. (2008). It seems that the environment conditions where our fruit grew influenced negatively the production of essential oils in the skin.

Table 1. Physical and chemical characteristics of feijoa fruit

Determination (%)	Accesion 1		Accesion 2		Accesion 3		Accesion 4	
Total moisture	85.09	± 0.08 b	86.80	± 0.06 a	86.70	± 0.00 a	85.78	± 0.00 b
Total dry matter	14.91	± 0.08 a	13.30	± 0.06 c	13.28	± 0.00 c	14.22	± 0.00 b
Ash	0.41	± 0.05 a	0.36	± 0.05 a	0.33	± 0.05 b	0.46	± 0.05 a
Crude protein	1.09	± 0.15 b	1.29	± 0.15 a	0.92	± 0.15 c	1.08	± 0.15 b
Ether extract	0.40	± 0.06 a	0.27	± 0.06 b	0.32	± 0.06 b	0.39	± 0.06 a
Crude fiber	2.09	± 0.06 b	2.01	± 0.06 c	2.14	± 0.06 a	2.16	± 0.06 a
Carbohydrates	10.92	± 0.69 a	9.36	± 0.49 b	9.57	± 0.44 b	10.11	± 0.59 b

Values with similar letters in each line are equal by Tukey, ($p \leq 0.05$) ± standard error.

Table 2. Fatty acids (%) in seeds

Fatty acids		(%)
Palmitic	C16:0	20.86
Palmitoleic	C16:1	0.48
Estearic	C18:0	2.77
Oleic	C18:1ω9	18.57
Linoleic	C18:2ω6	46.23
Linolénic	C18:3ω9,12,15	3.69
Behenic	C20:0	0.91

Table 3. Chemical composition of esencial oils of the skin

No. Compound	Name of the compound	T.ret, (min)	%
1	2-butenoic acid methyl ester, (E)	5.439	0.028
2	benzeneethanamine, 4-fluoro-α-methyl-	8.623	0.056
3	phenethylamine, o-fluoro-α-methyl-	18.180	0.725
4	1-cyclohexene-1-propanol, 2,6,6-trimethyl-	24.228	0.107
5	2-butenoic acid, methyl ester, (E)-	27.301	0.273
6	α-guaiene	28.236	0.155
7	naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	41.434	0.157
8	himachala-2,4-diene	41.901	0.138
9	1,2,4-methenoazulene, decahydro-1,5,5,8a-tetramethyl-, [1S-(1α,2α,3aβ,4α,8aβ,9R*)]-	44.048	0.417
10	(-)-aristolene	46.961	0.306

11	5-iodopryid-2(1H)-thione	48.259	0.82
12	(+)-2-carene, 2-isopropenyl-	49.761	0.196
13	naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	51.896	1.142
14	spiro[4.5]dec-6-en-8-one, 1,7-dimethyl-4-(1-methylethyl)-	54.633	0.49
15	1,1,4,5,6-pentamethyl-2,3-dihydro-1H-indene	56.158	0.236
16	Ledol	56.824	1.241
17	ledene oxide-(II)	58.286	3.562
18	1-naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1α,4aβ,8aα)]-	59.073	0.209
19	spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene-, (-)-	61.144	0.187
20	4aH-cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1aα,4β,4aβ,7α,7aβ,7bα)]-	62.807	2.92
21	aromadendrene, dehydro-	63.499	0.78
22	caryophyllene oxide	66.416	10.28
23	benzoic acid, 3,17-diacetoxy-4,4,10,13-tetramethylhexadecahydrocyclopenta[a]phenanthren-7-yl ester	66.486	6.504
24	Aromandendrene	67.788	6.396
25	propanoic acid, 2,2-dimethyl-, 2-(1,1-dimethylethyl)phenyl ester	68.511	0.085
26	benzoic acid, heptyl ester	69.009	0.841
27	azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1α,3aβ,4α,7β)]-	70.032	4.535
28	5-azulenemethanol, 1,2,3,3a,4,5,6 ,7-octahydro-α,α,3,8-tetramethyl-, [3S-(3α,3aβ,5α)]-	71.151	2.669
29	benzoic acid, 2-methylpropyl ester	72.293	13.56
30	S-benzyl-N2-(4-nitrobenzoyl)cysteine N′-(4-hydroxybenzylidene)hydrazide	73.029	1.127
31	Ledol	73.508	0.279
32	diepicedrene-1-oxide	74.497	3.949
33	humulane-1,6-dien-3-ol	77.595	3.839
34	Alloaromadendrene	78.527	0.049
35	trans-α-bergamotene	81.509	0.765
36	1,3,6,10-Dodecatetraene, 3,7,11-trumethyl-.(Z,E)-	86.239	0.253
37	naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	86.844	0.568
38	spiro[4.5]dec-7-ene, 1,8-dimethyl-4-(1-methylethenyl)-, [1S-(1α,4β,5α)]-	87.699	1.119
39	cis-α-bisabolene	87.746	0.984
40	tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-, (1R,2S,7R,8R)-	88.818	0.918
41	γ-elemene	90.945	2.154
42	6-(1,3-dimethyl-buta-1,3-dienyl)-1,5,5-trimethyl-7-oxa-bicyclo[4.1.0]hept-2-ene	90.963	0.995
43	pregn-5-en-20-one, 3-(acetyloxy)-, cyclic 20-(1,2-ethanediyl acetal), (3β)-	91.16	0.232
44	naphthalene, 1,2-dihydro-1,1,6-trimethyl-	92.227	0.542
45	naphthalene, 1,2-dihydro-1,1,6-trimethyl-	94.944	0.766
46	2,5-dimethyl-4-phenylpyridine	96.995	0.637
47	naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	98.073	0.587
48	2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	98.162	0.506
49	cholan-24-oic acid, 7,12-bis(acetyloxy)-3-ethoxy-, methyl ester, (3α,5β)-	100.09	0.517
50	1-phenanthrenecarboxylic acid, 7-ethyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a,7-trimethyl	104.52	0.121
51	ergost-5-en-3-ol, 22,23-dimethyl-, acetate, (3β)-	114.70	0.43
52	1,2,3,4-butanetetrol, 1,4-dibenzoate, (R*,S*)-	115.90	0.56
53	9-isopropyl-1-methyl-2-methylene-5-oxatricyclo[5.4.0.0(3,8)]undecane	124.71	0.311
54	β-guaiene	129.84	0.034
55	imonen-6-ol, pivalate	132.69	0.169
56	benzyl Benzoate	136.71	1.979
57	benzoic acid 2-methylpropyl ester	152.80	4.856

Conclusions

Some chemical characteristics such as total dry matter, total moisture, protein, carbohydrates, fats, ash and fiber of feijoas harvested in Veracruz, Mexico, were comparable with values reported in other countries where feijoa is cultivated under temperate conditions. The main oil in the seeds of feijoa was the linoleic acid. Then main essential oils were benzoic acid 2-methylpropyl ester and the caryophyllene oxide. The trees used in this study were propagated by seed and varied among them in fruit chemical characteristics then trees well adapted for tropical highlands require be selected. The skin of the feijoa containing essential oils and other chemical properties could be used in the alimentary industry..

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MORPHOLOGICAL CHARACTERISCS OF FRUITS AND PITS OF SOME JUJUBE (*ZIZIPHUS JUJUBE* MILL.) GENOTYPS

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Abstract

During the two consecutive years (2015-2016) the main morphological characteristics of jujube fruits and pits of three cultivated and three 'domestic' genotypes were studied. The research was carried out on three well known cultivars ('Lang', 'Li' and 'Tsao'), and three domestic genotypes with higher fruit weight. The highest weight of fruit (24.32 and 23.97 g) was registered in cultivars 'Lang' and 'Li', statistically significantly higher compared with other cultivars and genotypes. Cultivars with higher weight of fruit ('Lang' and 'Li') also had the highest weight of flesh (23.57 and 23.03 g), and weight of pits, although the smallest weight of pit was registered in cultivar 'Tsao'. The highest flesh to stone (pit) ratio was registered in all cultivated cultivars ('Lang' 96.87%, 'Li' 95.95 % and 'Tsao' 95.15%), mostly due to the higher mass of flesh. Also, 'Tsao' cultivar had the smallest mass of pit (0,40 g), statistically significantly smaller than other cultivated cultivars, and domestic genotype 3. Statistically highly significant correlation between the weight of fruit and the weight of the flesh (r=0,986**), and the pit (r= 0,929**) was found. Morphological characterization can be first step in future breeding program for improvement domestic genotypes aiming to increase fruit size, and keeping the quality of fruit.

Keywords: *morphological characteristic, jujube, Ziziphus jujube Mill.*

Introduction

Jujube, also known as Chinese date, is the deciduous subtropical fruit species from family Rhamnaceae. Jujube's fruit belongs to drupe type with single hard pit (stone). It is considered to be resistant to winter cold (Lyle, 2006) and other unfavorable ecological condition (arid environment, saline soil etc). Jujube grows as a small-medium tree (2-5 m high) with very hard and strong wood, although it sometimes forms a bush.

It originates from the regions of the middle and lower reaches of the Yellow River (China), and is often referred to as Chinese date (Yao, 2012). It is mostly cultivated in China, where this species extends over 200.000 ha and more than 400 cultivars is registered (Lyle, 2006). According to recent data, China has 700-800 cultivars of jujube (Yao,2012). Some of them are used for fresh consumption, but also some cultivars are good for processing (drying, candied) or as an ornamental tree. China is the largest producer with 90% of the total world production (Zheng at al., 2010).

According to some allegations in our region jujube was known 2.500-3.000 years ago, and it is believed that have been brought from Syria (Nikolić and Radulović, 2010). There are no plantations, but in the coastal region and its hinterland it grows in the gardens and yards. It can be considered as a true ecological fruit since no cultural practices are applied. Fruits of this species can be found on the market from the end of August to the half of October. Domestic people prefer jujubes with crisp and sweet taste of flesh, what is the case in period when the fruit was half colored in dark brown. Since the production is very small, virtually everything is sold on the local market.

In Chinese folk medicine jujubes have been used as a very important plants due to its helty-beneficial efects (Gao et al., 2012). Jujube fruits are reach in fibre, minerals, sugars, organic and phenolic acids, and especially in vitamin C (Gao et al., 2012, Li et al, 2007).

The genetic diversity of this species grown in Montenegro is unexplored. The aim of this paper is to compare morphological characteristics of jujube with larger fruits compared to commercial cultivars. This can be the first step in selecting and promoting potentially interesting genotypes for propagation.

Materials and methods

The research comprised 6 jujube genotype during two consecutive years (2015-2016). Three researched genotypes are introduced cultivars 'Li', 'Lang' and 'Tsao', and three genotypes are choosen from domestic population with larger friuts. The research was carried out at the Tomba locality in Bar Municipality, Montenegro. Each genotype is represented by one tree.

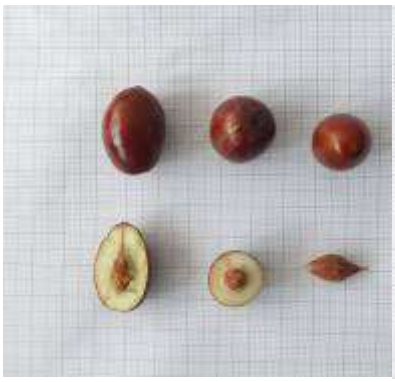

Figure 1. 'Domestic 1' genotype

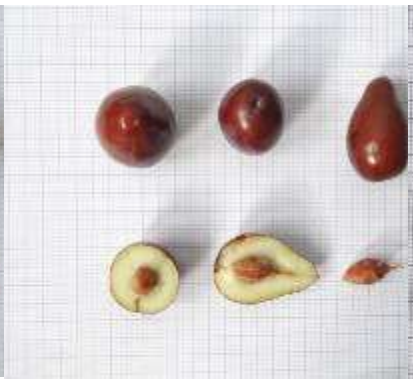

Figure 2. 'Domestic 2' genotype

Figure 3. 'Domestic 3' genotype

Figure 4. 'Li' cultivar

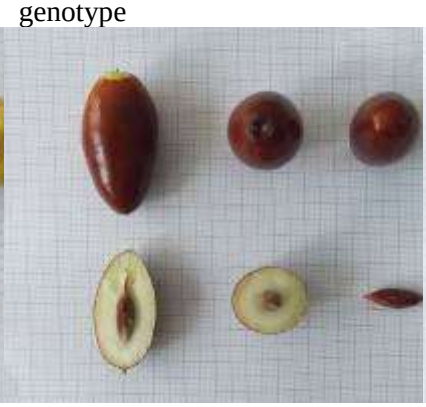

Figure 5. 'Tsao' cultivar

Figure 6. 'Lang' cultivar

The fruits for analysis were harvested when the epidermis of fruits were 50% colored in dark brown and the flesh was crisp and sweet (Stage 85, Hernandez et al., 2015). The following parameters were tested for morphological analysis: fruit weight (FW), flesh weight (WF), fruit length (FL), maximum width (MW), smallest width (SW), pit weight (PW), pit lenght (PL), pit width (WP) and ratio fruit/pit weight (RF). Fruit weight and pit weight were measured by digital balance "E Mettler" with an accuracy of 10^{-2} g. The dimensions of fruit and pit were measured by digital caliper with a precision of 10^{-2} mm. The data were tested by one-way analysis of variance and the significance of differences of means was tested by the LSD test for the levels of significance 0.05 and 0.01. The correlation among variables was calculated according to Pearson's correlation matrix for all studied parameters. Coefficient of determination was calculated as a square of correlation (Hadživuković, 1991).

Results and discusion

Fruit size, color and shape of fruit are the main attributes that attract customers' attention. This is mostly influenced by genetical potential, environmental conditions and yield. Although the weight of fruits contribute to higher yield, this is not the only factor for evaluating cultivars.

Table 1. Comparison of means (LSD) for main characteriscs of fruit of jujube genotypes

Factors Cultivars	Fruit weight FW (g)	Flesh weight WF (g)	Fruit lenght FL (mm)	Max. width MW (mm)	width SW (mm)
'Domestic 1'	4.63 c*	4.19 c	28.65 c	18.80 d	18.05 d
'Domestic 2'	4.04 c	3.63 c	29.16 c	19.66 d	18.65 d
'Domestic 3'	6.52 bc	5.99 bc	29.59 c	21.67 c	21.28 c
'Li'	23.97 a	23.03 a	39.01 b	36.60 a	33.79 a
'Tsao'	8.29 b	7.90 b	40.14 b	21.83 c	20.94 c
'Lang'	24.32 a	23.57 a	46.42 a	34.85 b	31.69 b
Critical values for comparison					
$LSD_{0.05}$	3.8044	2.6556	3.0252	1.5627	1.8948
$LSD_{0.01}$	2.6831	3.7654	4.2895	2.2157	1.3363
* The letters indicated differences at 0.05 level of significance					

The fruits of the highest weight were registered in the cultivars 'Lang' and 'Li', statistically significantly higher than in other studied cultivars. The genotype 'Domestic 3' has moderately large fruits, a little bit smaller than the 'Tsao' cultivar. Variations in the mass of jujube fruit are stated by many authors (Grygorieva et al., 2014, Hernandez et al., 2016, Markovski and Velkoska-Markovska, 2015). According to these authors, the weight of the fruits of some cultivars varies from very low mass (2.8 g,), to very large 29.3 g.

The quality and taste are factors that are more difficult to determine (Crisosto et al., 2010), but it is obvious that most authors from countries of similar ecological conditions (Spain, Macedonia) reported similar consumers demands in terms of fruit quality. Also, smaller fruits can have a higher nutritional value (higher content of vitamin C), compared to cultivars of larger fruits (Markovski and Velkoska-Markovska, 2015). Particularly appreciated is the crispness of the flesh. The cultivars of the largest weight of fruit ('Lang' and 'Li') have the largest mass of flesh This characterstic is determined by a relatively small mass of the pit, so the flesh of the friut has the highest importance for the fruit's size. This can be registered from the results of the correlation dependence analysis (Table 3). The weight of the flesh has a statistically significant effect on the mass of the fruit (r = 0.9984), and according to the coefficient of determination mass of fruit is 98 % determined by the mass of flesh. Length and width of the fruit are characteristics that determine its shape. The fruit shape can be round, oblong, oval, ovate, obovate, oblate or abnormal shapes (Yao, 2012). Our genotypes mostly have an elongated-ellipsoidal shape. The friuts of 'Li' cultivar have an apple-like shape, while the 'Lang' cultivar is pear-shaped. The biggest difference between MW and SW is registered in cultivar 'Lang'. Due to this, the 'Lang' cultivar at the cross-section has an ellipsoidal shape, while other studied cultivars mainly have a round shape.

Table 2. Comparison of means (LSD) for main characteriscs of pit of jujube genotypes

Factors Cultivars	Pit weight PW (g)	Pit lenght PL (mm)	Pit width PW (mm)	Fruit/pit ratio RF (%)
'Domestic 1'	0.44 d	18.92 c	7.54 d	90.31 cd
'Domestic 2'	0.41 d	18.42 c	7.69 cd	89.47 d
'Domestic 3'	0.53 c	18.56 c	8.14 bc	91.73 c
'Li'	0.93 a	22.87 b	9.84 a	95.95 ab
'Tsao'	0.40 d	23.84 b	6.68 e	95.15 b
'Lang'	0.76 b	29.76 a	8.53 b	96.87 a
Critical values for comparison				
$LSD_{0.05}$	0.0551	2.3393	0.5092	1.5704
$LSD_{0.01}$	0.0781	3.3170	0.7220	2.2268
* The letters indicated differences at 0.05 level of significance				

The folk name for Jujube in Montenegro is "iglica" which means needle. The name was given by the narrow and long pit inside the fruit. Cultivars with higher weight of fruit have a higher average weight of pits ('Li' and 'Lang'). The exception is the 'Tsao' cultivar which has the statistically significant smallest mass of pit (0.4 g). This is probably caused by the smallest width of the pit. Due to the higher weight of the fruits cultivars 'Lang' and 'Li' have statistically significantly higher fruit/pit ratio in comparision with other cultivars. This is a consequence of a larger difference in the weight of the fruit, while the variation in the weight of the pit is significantly lower. Similar results were reported by Grygorieva et al. (2015) and Sulusoglu et al, (2014).

Table 3. Correlation matrix beetwen research characteristics

Factor	FW	WF	FL	MW	SW	PW	PL	WP	RF
FW									
WF	**0.9904** **0.0000**								
FL	0.8243 0.0010	0.8289 0.0009							
MW	**0.9333** **0.0000**	**0.9925** **0.0000**	0.7735 0.0032						
SW	**0.9890** **0.0000**	**0.9879** **0.0000**	0.7630 0.0039						
PW	**0.9292** **0.0000**	**0.9259** **0.0000**	0.5735 0.0512	0.9563 0.0000	0.9628 0.0000				
PL	0.7932 0.0021	0.7983 0.0019	**0.9717** **0.0000**	0.7302 0.0070	0.7123 0.0093	0.5255 0.0793			
WP	0.7502 0.0050	0.7444 0.0055	0.2613 0.4120	0.8069 0.0015	0.8163 0.0012	**0.9328** **0.0000**	0.2230 0.4859		
RF	0.8634 0.0003	0.8663 0.0003	**0.9416** **0.0000**	0.8327 0.0008	0.8355 0.0007	0.6827 0.0144	0.8714 0.0002	0.3901 0.2100	

$p < 0.05$ * statistically significant

$p < 0.01$ * statistically highly significant are indicate in bold

The studied characteristics of morphological traits have shown certain statistically significant correlation dependencies. As already mentioned, the most significant correlation dependence is recorded between the weight of the friut and the weight of the flesh. The weight of the friut is statistically significantly correlated in relation to the fruith width (both MW and SW) and the mass of the pit. The length of the pit is in a statically significantly correlation dependency by the length and especially the width of the fruit. Regarding to this characteristic, 'Tsao' cultivar is an exception, because it has a smaller width of the pit which significantly reduced its weight. According to the results given in Table 3, the fruit/pit ratio most dependens on the length of the fruit. Similar results of correlation dependency are stated by Grygorieva et al. (2014). According to these results, the weight of the fruit is determined by the width of the fruit ($r = 0.97$) and the fruit length ($r = 0.77$).

Conclusions

Jujube production in Montenegro is at a low level and does not meet the needs of the local population. Genetic diversity of this species has not been studied, but it can be assumed that there are potentially interesting genotypes. Jujube as a species can be grown on land that is not suitable for cultivation of other fruit species, so it is necessary to research the existence of other jujube genotypes with preferable fruit characteristics. One of them could be genotype 'Domestic 3', which has a satisfactory weight of fruit and taste, and should be recommended for multiplication.

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QUALITY OF CEREAL GENOTYPES AS NUTRITION IMPROVEMENT TOOL EVEN AT INTOLERANT CONSUMER POPULATION

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Abstract

Lately, the number of people suffering from gluten-based foods is increasing. Therefore, dietary foods that contain gluten have become very popular, especially among people who do not have any problem with gluten tolerance. With a properly selected cereal type, knowing their qualitative properties, it is possible to make a balanced diet for the population with diagnosed gluten intolerance. The conducted study covers seven types of commercial cereal genotypes as soft wheat, durum wheat, barley, rye, oats, millet and brown rice. Durum wheat grains are the richest in proteins (13.31 g 100 g^{-1}) and fat poorest (1.84 g 100 g^{-1}). The same species had also the highest wet gluten content 26.33 g 100 g^{-1}, while in the barley it was the lowest (8.19 g 100 g^{-1}). Regarding the dry gluten, the highest value is found in soft wheat grains (14.81 g 100 g^{-1}) and at least in rye (3.03 g 100 g^{-1}). Differences between investigated commercial cereal cultivars regarding quality traits are statistically significant.

Key words: *cereal grains, genotypes, gluten intolerance, nutrition, quality.*

Introduction

One of the most important tasks of modern agriculture is to provide enough food for an ever-growing population. Cereal grains and products derived from them represent an important component in human nutrition both in developed and in developing countries. Cereals constitute basic food for humanity and represent the main constituent of animal feed (Koehler, Wieser, 2013). Almost two thirds of the total daily intake of carbohydrates originates from them. They are grown on nearly 60% of the cultivated land in the world. Today, humanity has a range of grains from various commercial cereals, and also are added grains that have no grain origin. The habits of consumers are changing, so the area under alternative cereals in the world is growing slowly. High content of carbohydrates makes cereals suitable as an energy source because of fast digestion and the energy released for the body. In developed European countries annual consumption of cereals varies and ranges from 60-80 kg per capita (Mickovski-Stefanović et al., 2012). Among them, wheat is the most important. White flours are the most important grain products. Soft wheat is our most important bread cereal. According to the statistical data, wheat bread today represents a big part of the diet for more than 70% of the planet's population. The protein content of cereals is high in the case of durum wheat, soft wheat, oats, barley and rye, where proteins are higher than 10.0% represented. Bearing in mind that the daily recommended protein intake is 0.8 mg g^{-1} thereby mix of cereals (Damgaard et al., 2013) with nuts is an excellent source of essential components for the start of the day (Jablonskytė-Raščė et al., 2013). On the other hand, the amount of fat is low and therefore in the risk groups of populations with hyperlipidaemia, cereal intake, especially durum and barley is recommended. Cereals contain significantly less protein than leguminous grains with 20.00% to 40.00% leading on the list of vegetable protein source (Bartkienė et al., 2016). This fact is valuable not only for the human population but also for animal (Gonzalez, 2015). Even in the preparation of ready-made flour, a bean starch is used for the preparation of gluten-free bread (Krupa et al., 2010). In the grain 95% of the

proteins are represented by gluten, which is a complex composed of gliadins (prolamines) and glutenins (glutelines) (Žilić, 2011). Certain populations of people are intolerant on gluten having digestive problems and allergic reactions particularly on gliadins and many suffer from celiac disease which is a serious illness and requires a special dietary regime. Gluten intolerance does not know age limits (Miyazaki et al., 2006), so it occurs in babies and young children and in adults at different age. It is important to mention that the amount of gluten in the cereals has changed and went through an evolutionary pathway, as evidenced by the research carried out by (Bustamante et al., 2017). Addressing the gluten intolerance and possibility to achieve a balanced diet (Dickey, 2008) is a complex and frequently asked question. This research problem sometimes depends on cultural beliefs and dietary habits (Ferrari, 2014).

With a carefully selected cereal type and making a choice between commercial cultivars, it is possible to make a balanced mix for populations that show any allergic reactions, but also for those with accurately diagnosed gluten intolerance (Newton et al., 2011). It is especially important to keep in mind that some of the cereals, such as oats and rice are gluten-free and they are excellent foods which are rich in fibres. This opens new perspectives on reducing or avoiding an incorrectly balanced meal and ensuring a functional diet, respectively. Therefore, the goal of this research was to determine the quality properties of seven types of genotype commercial cultivars pointing out which cereal contains most proteins that appear as allergenic gluten-based ingredients.

Materials and methods

Plant material. The investigations involved seven types of cereals: soft wheat, durum wheat, barley, rye, oats, millet, brown rice and polished rice. For each type of commercial cereal cultivars were purchased 30 samples in 2017 from different places like supermarkets, green markets, healthy food stores and organic food stores. From healthy food stores were purchased 1.00 kg of bulk package. Regions and cities were carefully selected to cover almost the entire territory of the country as following Skopje, Veles, Shtip, Kochani, Strumica, Prilep and Ohrid in Macedonia. Cleaned and dried up to reaching 11.0% grain moisture samples were put in fridge as short-term stored in glass jar under 4°C. Each sample of commercial cultivars was manually cleaned and put in plastic jar and tempered separately maximum to 14.0% moisture. All samples were kept under ambient temperature for 24 hours. From each of samples were measured ten sub-samples with weight of 100.00 g seeds in order to obtain a well-balanced analytical sample.

Laboratory analysis. All samples were analysed for moisture, protein, fat, fibre, wet and dry gluten content in whole grains g 100 g^{-1} dry mass (DM) according the standard recognizable accredited methods (http://www.iarm.gov.mk/files/Akreditirani-tela/Laboratorii/OB05-25_LT-036.pdf). The content of the protein is determined as nitrogen content according to Kjeldahl method (conversion factor to protein 6.25), fat content by Soxhlet method, crude fibre according the method of determining and calculating by Kürschner-Hanak method and gluten by a grain analyser "Infratec 1241 Foss" with a calibration package IM 9200 ("Foss", Denmark). Moisture content was determined by drying of plant material at 105 ± 2°C to constant weight (ICC 109/01:1976 - Determination of the moisture content of cereals and cereal products).

Statistical analysis. The obtained data were statistically analysed by one-way analysis of variance (*ANOVA*), statistical program *SPSS*. Differences between cereal grains concern quality traits are considered $P < 0.05$ and $P < 0.01$.

Results and discussion

With respect to structures and amount of chemical constituents, very significant differences exist between cereals and even between cultivars within each cereal. These differences in a large extent determine the quality of products made from cereal grains (Koehler, Wieser, 2013). Regarding the ambient conditions under which were kept grain samples the moisture content almost does not show larger variations. Regarding commercial cultivars, the highest average moisture content was found in durum wheat 12.64% DM and the millet is characterized by the lowest 10.73% DM (Table 1).

Table 1. Moisture content (%) and quality of analysed grains (g 100 g^{-1} dry mass) * at commercial cultivars

Parameter	Species							
	soft wheat	durum wheat	barley	oat	rye	brown rice	polished rice	millet
Moisture	11.15b	12.64a	12.44a	11.61b	12.46a	12.47a	11.07b	10.73bc
Protein	12.61b	13.31 a	12.72b	12.86b	12.27c	8.17e	7.07f	10.00d
Fat	1.87e	1.84ef	2.12e	4.91a	3.38b	2.52d	3.48b	3.08c
Crude fibre	2.89d	2.94d	5.64c	9.89a	7.48b	3.08d	0.40e	2.98d
Wet gluten	21.08b	26.33a	8.19d	–	14.58c	–	–	–
Dry gluten	14.81a	8.76b	4.06c	–	3.03d	–	–	–

Statistical analysis						
Species	moisture	protein	fat	fibre	dry gluten	wet gluten
Soft wheat	11.15±1.14	12.61±0.49	1.87±0.38	2.89±0.06	21.08±1.58	14.81±1.17
Durum wheat	12.64±0.93	13.31±0.71	1.84±0.16	2.94±0.27	26.33±0.77	8.76±0.38
Barley	12.44±7.46	12.72±0.46	2.12±0.38	5.64±0.49	8.19±0.85	4.06±0.21
Oat	11.61±1.65	12.86±0.61	4.91±0.42	9.89±0.54	GF	GF**
Rye	12.46±0.83	12.27±0.90	3.38±0.51	7.48±0.60	14.58±0.69	3.03±0.22
Brown rice	12.47±1.03	8.17±0.56	2.52±0.33	3.08±0.27	GF	GF
Polished rice	11.07±1.08	7.07±0.37	3.48±0.16	0.40±0.03	GF	GF
Millet	10.73±1.08	10.00±0.91	3.08±0.75	2.98±4.56	GF	GF

	LSD $p < 0.05$	LSD $p < 0.01$
Moisture	0.562	0.741
Protein	0.332	0.438
Fat	0.217	0.286
Crude fibre	0.203	0.267
Wet gluten	0.529	0.670
Dry gluten	0.335	0.443

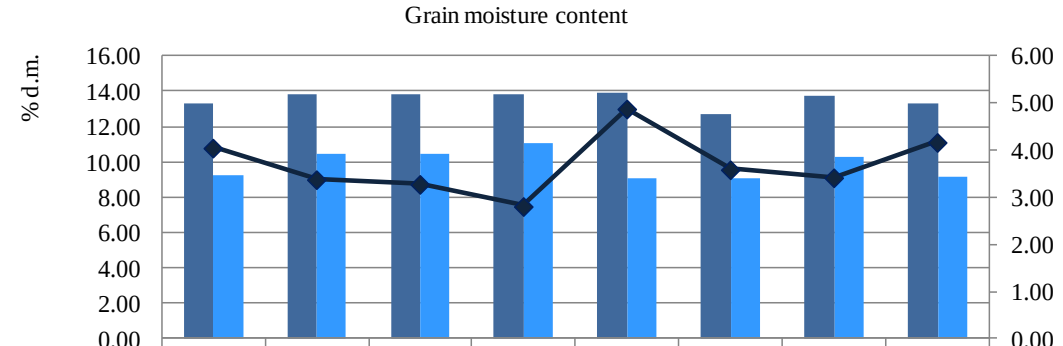
Note. * – data expressed as means (n = 30 ± SD), **GF – gluten-free.

Among the analysed samples for each cereal (n = 30), the biggest differences were recorded in the grains of polished rice 4.18 (13.3–9.12), and most of the moisture was found in an oat sample 13.94% DM, while in millet grains at least 12.68% DM (Fig. 1).

Proteins, after carbohydrates, make second important group of constituents of cereal grains. They occupy an average range of about 8–11% of the grain mass (Table 1). However, there are significant variations in the protein content. For example, soft wheat grains may vary from less than 6% to more than 20% (Souci et al., 2008). The results given in Table 1 show that most proteins contained durum grains 13.31 g 100 g^{-1}, followed by oat, barley and soft wheat in the range of 12.86–12.61 g 100 g^{-1} which is significantly lower compared to other studies. Polished rice is the poorest 7.07 g 100 g^{-1} and cannot be considered as an important source of protein, which is in agreement with the results of other studies (Šramkova et al., 2009).

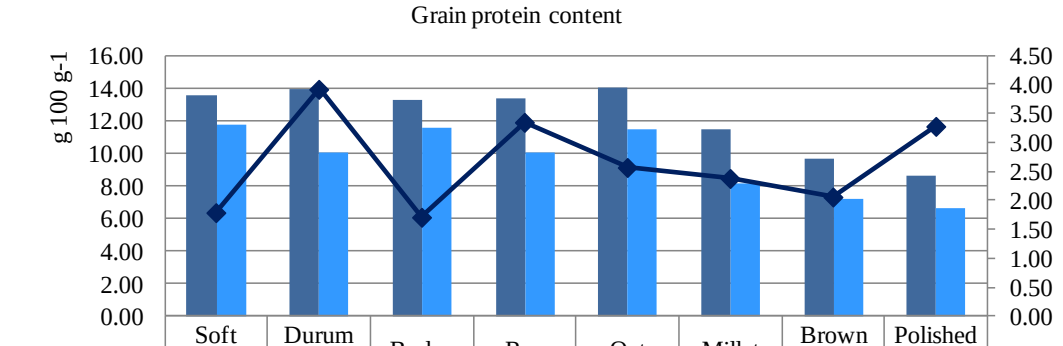
The analysed samples in the same type of cereal show the highest differences among rye 3.35 (13.36–10.01 g 100 g^{-1}), and the smallest in barley 1.71 (13.28–11.57 g 100 g^{-1}), but

corresponded with investigations by Lexhaller et al. (2016) (Fig. 2). These results are somewhat lower compared to the reference values given by Luallen and Eliasson (2004) and Heshe et al. (2016). The content of proteins in these species compared to the content in millet, brown rice, polished rice and rye marked as important.



	Soft wheat	Durum wheat	Barley	Rye	Oat	Millet	Brown rice	Polished rice
max	13.29	13.82	13.78	13.85	13.94	12.68	13.72	13.30
min	9.23	10.43	10.49	11.03	9.05	9.08	10.29	9.12
Difference	4.06	3.39	3.29	2.82	4.89	3.60	3.43	4.18

Figure 1. Differences between minimum and maximum value for grain moisture at commercial cultivars (n = 30)



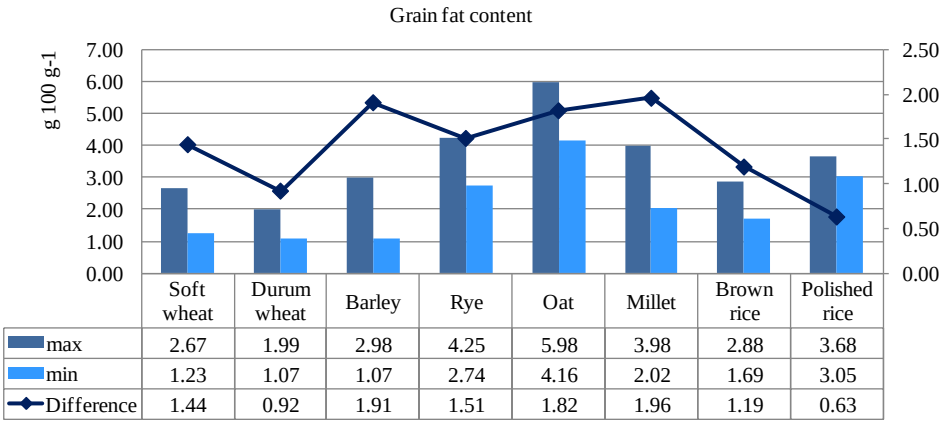
	Soft wheat	Durum wheat	Barley	Rye	Oat	Millet	Brown rice	Polished rice
max	13.56	13.93	13.28	13.36	13.99	11.44	9.62	8.65
min	11.77	10.01	11.57	10.01	11.42	8.16	7.24	6.59
Difference	1.79	3.92	1.71	3.35	2.57	2.38	2.06	3.28

Figure 2. Differences between minimum and maximum value for grain protein content at commercial cultivars (n = 30)

Generally taken over these studies, fats are slightly represented in cereals (Newton et al., 2009), but play a great importance in the formation of inclusion complexes with proteins (Luallen and Eliasson, 2004) and with starch (Horstmann et al., 2017). Cereal lipids originate from membranes, organelles, and spherosomes and their content in the grains depend on cereal species and vary from 1.7–7% of the dry weight (Delcour and Hoseney, 2010). Except oats (~7%), cereal lipids belong to the minor constituents (2–4%) (Belitz et al., 2009). Crude fat is mostly contained in commercial oat grains 4.91 g 100 g^{-1} and this value is significantly higher compared to other cereals (Gujral, 2012). The lowest fat content was found in durum 1.84 g 100 g^{-1}, which coincides with carried out study (Kumar et al., 2011). Significant differences in fat content were also found by comparing polished rice (3.48) and rye (3.38) with all other cereal species (Table 1).

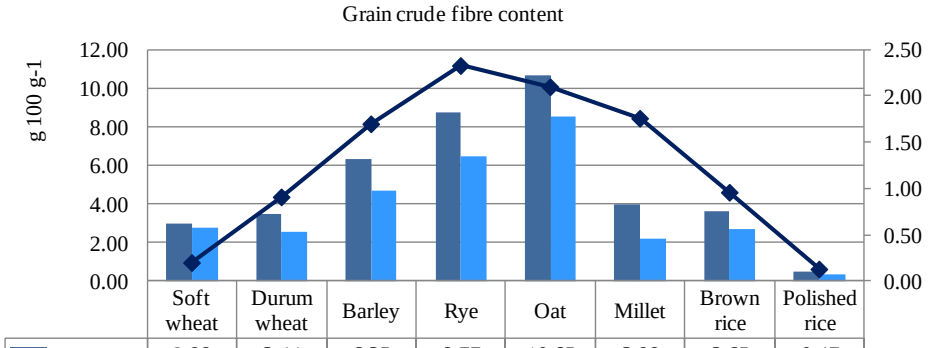
The largest differences between the maximum and the minimum value of fat were found in the millet samples 1.96 (3.98–2.02). The smallest fat content had polished rice 0.63 (3.68–3.05 g 100 g^{-1}) (Fig. 3).

Plant fibre has influenced the digestive micro flora as prebiotics serve for its nutrition and maintenance with the highest average content (Horstmann et al., 2017). The highest content of fibre is measured in oat grains 9.89 g 100 g^{-1} and at least at polished rice 0.40 g 100 g^{-1}, which is quite understandable if it is known that they are present in the grain coat layers (Sarawong et al., 2014) (Table 1). The crude fibre content in oat and rye grains was statistically significantly higher compared to all other species. Obtained results corresponded with literature data (Gebruers et al., 2008) for wheat and durum seeds. Analysis of laboratory samples showed the highest variation at rye 2.33 (10.65–8.55 g 100 g^{-1}), and the lowest in the polished rice 0.13 (0.47–0.34 g 100 g^{-1}) (Fig. 4).



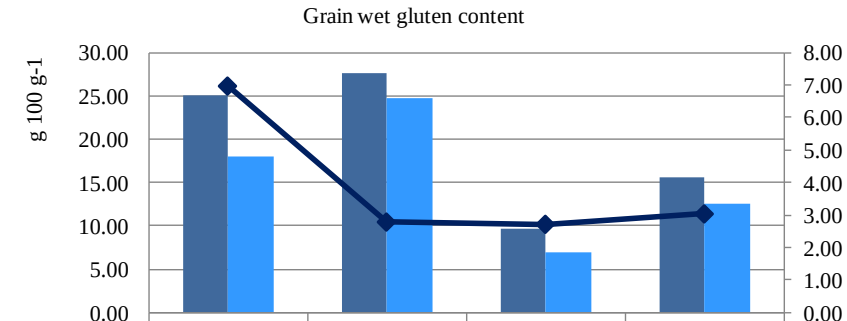
	Soft wheat	Durum wheat	Barley	Rye	Oat	Millet	Brown rice	Polished rice
max	2.67	1.99	2.98	4.25	5.98	3.98	2.88	3.68
min	1.23	1.07	1.07	2.74	4.16	2.02	1.69	3.05
Difference	1.44	0.92	1.91	1.51	1.82	1.96	1.19	0.63

Figure 3. Differences between minimum and maximum value for grain fat content at commercial cultivars (n = 30)



	Soft wheat	Durum wheat	Barley	Rye	Oat	Millet	Brown rice	Polished rice
max	2.98	3.44	6.35	8.77	10.65	3.98	3.65	0.47
min	2.78	2.53	4.65	6.44	8.55	2.22	2.69	0.34
Difference	0.20	0.91	1.70	2.33	2.10	1.76	0.96	0.13

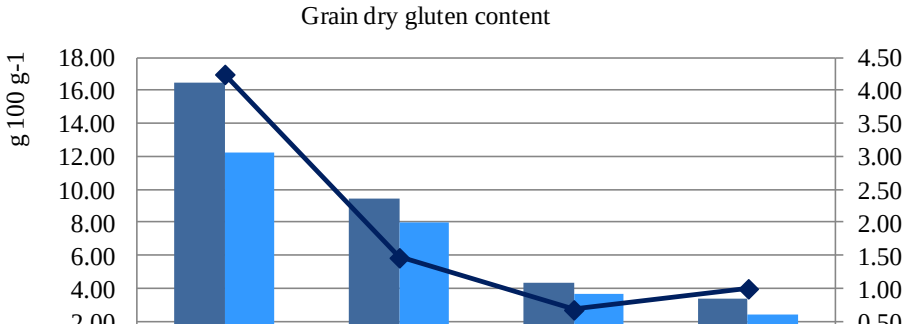
Figure 4. Differences between minimum and maximum value for grain crude fibre content at commercial cultivars (n = 30)



	Soft wheat	Durum wheat	Barley	Rye
max	24.99	27.56	9.67	15.62
min	18.02	24.78	6.96	12.59
Difference	6.97	2.78	2.71	3.03

Figure 5. Differences between minimum and maximum value for grain wet gluten content at commercial cultivars (n = 30)

The gluten content is quite variable within each studied crop (Barada, 2010), and regarding dry gluten it generally corresponds with the results of so far researches (Bustamante et al., 2017), although our results are higher for wet gluten. The highest mean value for the content of the wet gluten at commercial cultivars was confirmed in durum 26.33 g 100 g^{-1}, while the lowest was in barley 8.19 g 100 g^{-1}. It is evident that highest variation was found among wheat samples 6.97 (24.99–18.02), and the lowest in barley 2.71 (9.67–6.96) (Table 1, Fig. 5). The differences in the content of wet gluten among all studied cereals were statistically significant.



	Soft wheat	Durum wheat	Barley	Rye
max	16.51	9.45	4.35	3.39
min	12.26	7.98	3.67	2.39
Difference	4.25	1.47	0.68	1.00

Figure 6. Differences between minimum and maximum value for grain dry gluten content at commercial cultivars (n = 30)

Unlike the oat which is gluten free cereal (Hegazy et al., 2009) wheat grains contain the highest average value of dry gluten 14.81 g 100 g^{-1} where are the largest variations between the individual laboratory samples 6.97 (16.51–12.26). The maximum measured value was found in wheat 16.51 g 100 g^{-1}, while an average the lowest in rye 3.03 g 100 g^{-1}. The rye contained minimum content in general measured 2.39, in contrast to the variations between analytical samples that are the smallest in barley 0.68 (4.35–3.67) (Table 1, Fig. 6).

Conclusions

Based on the results presented, it can be concluded:

The most proteins contain grains of durum commercial cultivars 13.31 g 100 g^{-1}, followed by rye and barley while polished rice is the poorest 7.07 g 100 g^{-1}. Durum protein content compared to the content in wheat, millet, brown rice, polished rice and rye showed significant differences.

Crude fat is mostly presented in oat grains 4.91 g 100 g^{-1} and this value is significantly higher than other cereals and the lowest fat contain durum grains 1.84 g 100 g^{-1}. Studied cereal grains concern fat are statistically significant different.

The highest content of fibre is recorded in oat 9.89 g 100 g^{-1} while at least at polished rice 0.40 g 100 g^{-1}. Fat content in oat and rye grains are significantly higher compared to other cereals. The content of the wet gluten component is the highest in durum 26.33 g 100 g^{-1}, while in the barley is the lowest 8.19 g 100 g^{-1}. Regarding the dry gluten, the highest value is found in wheat grains 14.81 g 100 g^{-1}, while in the rye is the lowest 3.03 g 100 g^{-1}. Differences in the gluten content the both wet and dry among all studied commercial cereal cultivars were statistically significant.

Cereals remain to be food for all the people of the planet although at some of them which are intolerant caused allergic based diseases and this study shown that with properly selection and combined with other non-cereal grains can be food worldwide.

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POTENTIAL ADAPTABILITY OF PIGEON-PEA GENOTYPES UNDER DIFFERENT AGRO-ECOLOGICAL ENVIRONMENTS OF MOROCCO

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Abstract

Pigeon pea (Cajanus cajan (L.) Millsp.) is the fifth most important food legume cropped in rain-fed agriculture in the semi-arid tropics of south Asia, Africa, and America Latin. It is used as source of proteins for food, feed add to the uses of wood in the fire and hedges. It grows in a wide range of edaphic and climatic conditions, making it well-suited to succeed in a variety of agricultural systems. Drought resistance of pigeon pea might be suitable in marginal land of Morocco in current climate change and might offer opportunities to diversifying protein's sources for both human and animals. Thus, seventeen genotypes received from ICRISAT germplasm were evaluated in different agro-ecological conditions of Morocco. The study aimed to analyze genetic variability, yield potential, yield components and grains and pods nutritional value using sixteen quantitative traits. Recorded data were used for descriptive and variance analysis and to build hierarchical cluster based on similarity and dissimilarity among genotypes. The results showed a high significant variability among genotypes for almost all measured quantitative traits. The genotypes AN33, AN34, AN21 and AN32 showed higher values for yield potential and grains nutritional value. Cluster analysis grouped the evaluated genotypes in four groups at 82% of similarity. The first cluster grouped the four previous performed genotypes, while the second and the fourth clusters grouped each one six genotypes. Whereas, the third cluster grouped a single genotype ATZ19 that was distinguished by late blooming, high pods weight, pods width, high number of seeds per plant and small seed size.

Keywords: *Pigeon pea, adaptability potential, yield and yield components, Morocco*.

Introduction

Pigeon pea (Cajanus cajan (L.) Millsp.), is one of the major pulse crops of the tropics and sub tropics (Vanaja et al., 2010). It belongs to Fabaceae (Leguminosae) family that provides sustainable farming system especially for marginal lands through their ability to improve soil texture and fertility. In addition, their grains may contribute to food and feed security thanks to their high nutritive value. Pigeon pea is a perennial crop with more diverse uses way than others grain legumes (Domoguen et al. 2010). Furthermore, Pigeon pea is a harsh crop that is adapted to a wide range of environments and cropping systems add to its high drought tolerance. It is mainly used as de-hulled split peas, as immature green seeds and pods are also consumed fresh as a green vegetable or as fodder. Regarding these specific characteristics, we have been encouraged to introduce and extend this crop to Moroccan farming system to take benefit from its high tolerance to water deficit and the high nutritional value of its seeds and pods. Thus, present research aimed to evaluate the adaptation potential of a collection of pigeon pea genotypes received from ICRISAT germplasm in different agro-ecological Moroccan environments.

Material and Methods

Plant material

Potential adaptation of seventeen genotypes of pigeon pea received from ICRISAT germplasm was evaluated under agro-ecological Moroccan environments (Table 1). Sixteen agro-morphological and nutritional quantitative traits were used on the base of "Descriptors pigeon pea manual CIRP" (1993) (Table 2). The agro-morphological traits were recorded on three plants per genotype, and three simples of pods per plant. The nutritional quality concerns crude fibers content that is determined by the Weende and Prosky method (1988), and crude protein content that is determined according to Kjeldahl method (ISO16634).

Table 1: List of studied pigeon pea genotypes

N°	Genotypes
1	AN21
2	AN32
3	AN33
4	AN34
5	AN90
6	ATZ19
7	ATZ21
8	ATZ28
9	ATZ29
10	ATZ36
11	ATZ38
12	ELK45
13	ELK46
14	ELK48
15	ELK49
16	ELK50
17	ELK51

Table 2: List of measured quantitative traits

Quantitative traits	Abbreviation
1. Days of flowering	DF
2. Days of maturity	DMAT
3. Pod length (cm)	PdL
4. Pod width (cm)	Pdw
5. Weight pod (g)	WPd
6. Number of Pod per plant	NPd/PL
7. Number of seeds per pod	NSd/Pd
8. Number of seeds per plant	NSd/PL
9. Seed length (cm)	SdL
10. Seed width (cm)	Sdw
11. Seed yield per plant (g)	SdYield/PL
12. Weight of 1000 seed (g)	W-1000Sd
13. Seed Protein content (%DM)	SdCP
14. Pods Protein content (%DM)	PdCP
15. Fibers content of seeds (%DM)	SdCF
16. Fibers content of Pods (%DM)	PdCF

Data analysis

The measured quantitative data were subjected to descriptive and variance analysis (ANOVA). A hierarchical cluster was built on the base of similarity and dissimilarity among the studied pigeon pea genotypes.

Results and discussion

Quantitative traits variability

Descriptive analysis revealed a large quantitative traits variability among pigeon pea genotypes mainly for plant yield components characters such as number of seeds per plant (CV=117.6%), seed yield per plant (CV=111.4%), weight of pod per plant (CV=106.3%) and number of pod per plant (CV=103.9%) (Table 3). These results reveal a variable genotype potential adaptation in Moroccan growing season conditions. Indeed, the earliness was ranged from 89-163 days after sowing. Six of evaluated genotypes are earlier (AN21, ATZ29, AN32,

AN33, AN34 and AN90). Flowering date was significantly and negatively correlated with seed yield per plant (-0.517***), number of pod per plant (-0.546***), pods length (-0.314*), seeds per plant (-0.476**) and crude proteins content of pods (-0.650***). While, significant and positive correlation was revealed between earliness and seed size as seed length (0.724***) and seed width (0.630***). Plant production potential was evaluated on the base of number of pod per plant, number of seeds per plant, seed yield per plant and 1000 seeds weight. The mean plant yield potential was 51g varying from 3.0g (ELK48) to 187.5g (AN21). The most productive genotypes were AN21, AN32, AN33, AN34 and ATZ19. Plant seed yield potential except flowering date, was positively and significantly correlated with number of pods per plant (0.920***), weight of pod per plant (0.88***), pods length (0.513**), pods width (0.322*), number of seeds per plant (0.984***) and with pods crude proteins content (0.648***).

The nutritional quality of seeds and pods was evaluated on the base of crude proteins and crude fibers contents. The mean crude proteins content was 16.3%DM and 6.23%DM respectively of seeds and pods. Seeds proteins content varied from 12.6%DM (ATZ38) to 20.8%DM (ELK51). While, pods proteins content varied from 5.02%DM (ATZ19) to 9.06%DM (AN32). Crudes proteins were more concentrated in seeds than in pods. Seeds proteins content was negatively and significantly correlated with 1000-seeds weight (-0.375**) and positively with pods length (0.376**). Whereas, pods proteins content was positively correlated with number of pods per plant (0.636***), weight of pods per plant (0.492**), pods length (0.501**), number of seeds per plant (0.593***) and seeds yield per plant (0.648***). The mean content of crude fibers in seeds (4.50%DM) ranged from 4.09%DM (AN21) to 4.98%DM (AN90) without significant variation among genotypes. The mean content of crude fibers in pods (3.50%DM) ranged from 3.01%DM (AN21) to 4.47%DM (AN33) (Table 3).

Table 3: Agro-morphological quantitative traits variability among seventeen genotypes of pigeon pea

Genotypes	DF	DM	NPd/Pl	WPd	PdL	Pdw	NSd/Pd	SdL	Sdw	Nsd/PL	Sd/Yld/PL	W1000Sd	SdCP	PdCP	SdCF	PdCF
AN21	104,0^{c}	195,0^{ab}	397,6^{bc}	152,7^{bc}	4,7^{c}	0,6^{cd}	3,6^{de}	0,4^{de}	0,3^{bc}	1480,3^{c}	130,0^{bc}	83,0^{b}	17,0^{c}	7,3^{cd}	4,1^{c}	3,3^{de}
AN32	104,0^{c}	195,0^{a}	336,8^{b}	184,1^{cd}	6,0^{de}	0,4^{abc}	3,6^{de}	0,4^{de}	0,3^{ab}	900,0^{ab}	101,5^{cd}	106,5^{cde}	15,9^{cd}	8,8^{e}	4,2^{abc}	5,4^{de}
AN33	104,0^{c}	195,0^{a}	470,3^{cd}	291,0^{e}	6,9^{e}	0,7^{e}	3,3^{de}	0,5^{bc}	0,3^{abc}	1496,0^{c}	152,8^{c}	105,2^{cde}	19,18^{f}	6,9^{b}	4,9^{d}	4,5^{c}
AN34	89,0^{a}	195,0^{a}	584,8^{d}	205,8^{cd}	6,94^{e}	0,5^{cd}	4,3^{fg}	0,4^{de}	0,3^{abc}	1218,0^{c}	118,3^{bc}	113,6^{de}	17,3^{c}	7,7^{cd}	4,5^{bcd}	5,5^{de}
AN90	104,0^{c}	195,0^{a}	22,0^{a}	7,3^{a}	5,5^{b}	0,6^{cd}	3,5^{de}	0,4^{de}	0,3^{ab}	89,0^{a}	14,6^{a}	117,2^{de}	16,7^{bc}	7,9^{d}	4,7^{cd}	5,7^{e}
ATZ19	147,0^{f}	161,0^{a}	153,0^{a}	240,2^{cd}	4,6^{c}	0,6^{cd}	1,0^{ab}	0,5^{bcd}	0,4^{bc}	875,0^{ab}	65,5^{ab}	85,7^{b}	13,6^{b}	5,5^{a}	4,3^{abc}	5,3^{de}
ATZ21	117,0^{d}	174,0^{a}	88,6^{a}	46,3^{ab}	4,7^{c}	0,5^{abc}	1,6^{ab}	0,3^{a}	0,2^{a}	138,0^{a}	13,9^{a}	97,8^{abc}	13,4^{b}	5,7^{a}	4,1^{ab}	3,2^{a}
ATZ28	147,0^{f}	174,0^{a}	26,0^{a}	52,0^{ab}	4,6^{c}	0,4^{abc}	1,0^{ab}	0,6^{cd}	0,4^{abc}	67,0^{a}	12,9^{a}	192,4^{g}	13,5^{b}	5,3^{a}	4,5^{bcd}	3,2^{a}
ATZ29	90,0^{a}	174,0^{a}	44,0^{a}	37,9^{ab}	4,2^{a}	0,4^{abc}	3,5^{de}	0,5^{bc}	0,4^{abc}	31,5^{a}	6,0^{a}	178,2^{f}	14,0^{bc}	5,5^{a}	4,4^{bcd}	3,5^{ab}
ATZ36	122,0^{c}	174,0^{a}	31,0^{a}	24,7^{ab}	4,5^{c}	0,5^{bcd}	4,0^{ef}	0,45^{abc}	0,2^{a}	45,0^{a}	7,3^{a}	160,1^{f}	14,2^{bc}	5,6^{a}	4,6^{bcd}	3,7^{ab}
ATZ38	122,0^{c}	174,0^{a}	10,5^{a}	27,6^{ab}	4,4^{a}	0,3^{a}	3,5^{de}	0,5^{bc}	0,4^{bcd}	31,0^{a}	3,4^{a}	101,4^{cde}	13,1^{a}	5,5^{a}	4,4^{bcd}	3,5^{ab}
ELK45	165,0^{g}	215,0^{d}	115,0^{a}	55,8^{ab}	4,5^{c}	0,6^{cd}	2,5^{bc}	0,6^{cd}	0,5^{bc}	311,0^{abc}	35,6^{ab}	102,0^{cde}	19,2^{f}	5,6^{a}	4,8^{cd}	5,4^{de}
ELK46	165,0^{g}	215,0^{d}	113,0^{a}	51,3^{ab}	4,1^{c}	0,6^{cd}	3,5^{de}	0,5^{bcd}	0,4^{bcd}	194,0^{a}	17,9^{a}	89,8^{bcd}	19,7^{f}	5,4^{a}	4,6^{bcd}	3,1^{a}
ELK48	165,0^{g}	215,0^{d}	22,5^{a}	13,2^{a}	4,4^{a}	0,2^{a}	3,5^{de}	0,6^{cd}	0,4^{abc}	29,5^{a}	3,2^{a}	120,8^{b}	19,0^{f}	5,4^{a}	4,3^{abc}	3,1^{a}
ELK49	165,0^{g}	215,0^{d}	118,0^{a}	63,5^{ab}	4,7^{c}	0,6^{cd}	4,0^{ef}	0,6^{d}	0,5^{c}	250,5^{a}	28,1^{ab}	107,6^{cde}	14,9^{bc}	5,5^{a}	4,6^{bcd}	3,3^{ab}
ELK50	165,0^{g}	215,0^{d}	111,0^{a}	28,5^{ab}	5,9^{d}	0,6^{cd}	3,5^{de}	0,6^{cd}	0,4^{abc}	197,0^{a}	13,3^{a}	69,7^{a}	15,8^{bc}	5,6^{a}	4,4^{bcd}	3,6^{ab}
ELK51	165,0^{g}	215,0^{d}	70,0^{a}	26,0^{ab}	6,9^{e}	0,5^{bcd}	3,3^{de}	0,5^{bcd}	0,5^{bc}	121,0^{a}	11,9^{a}	79,3^{ab}	19,7^{f}	5,5^{a}	4,3^{abcd}	3,6^{ab}
Mean	127.5±4.53	193.4±2.83	187.4±31.1	97.8±16.7	5.27±0.1	0.53±0.02	3.53±0.01	0.50±0.01	0.38±0.01	517.2±97.4	51.1±9.11	110.9±3.14	16.2±0.34	6.23±0.13	4.50±0.04	3.50±0.05
Range	89.0-165.0	161.0-215.0	9.00-618.0	5.39-154.7	4.00-7.05	0.29-1.00	2.00-5.00	0.30-0.70	0.20-0.60	21.0-1970.0	3.01-185.7	69.1-193.2	12.9-20.7	5.02-9.06	4.09-4.98	3.01-4.47
CV	22.2	9.13	103.9	106.3	19.3	28.1	16.9	17.5	24.5	117.6	111.4	28.9	14.9	17.7	6.88	10.4
Test F	***	***	***	**	***	*	ns	**	**	***	***	***	***	***	*	*

Significant difference *: (0.01 <P <0.05); **: (0.001 <P <0.01); ***: (P <0.001); ns: not significant difference (P> 0.05)

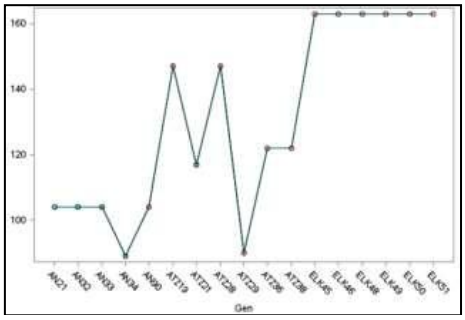

Fig 1a: Genotypes earliness

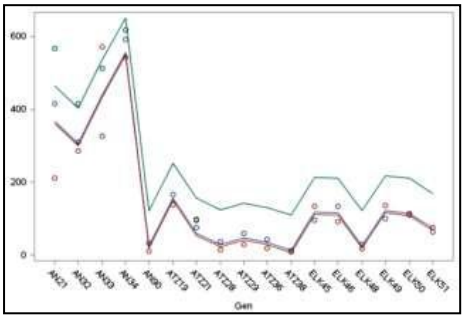

Fig 1b: Number of pods per plant

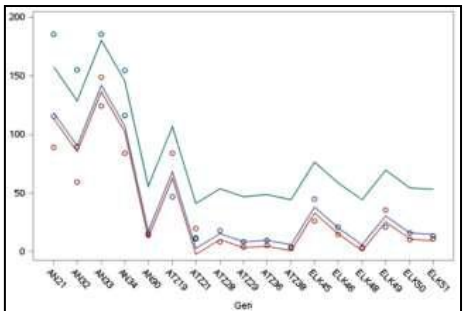

Fig 1c: Seeds yield per plant

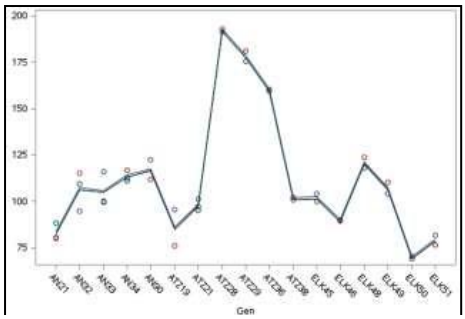

Fig 1d: Weight of 1000-seeds

Figure 1: Agro-morphological quantitative characters variability among seventeen genotypes of pigeon pea

Genotypes clustering

Cluster analysis grouped the evaluated genotypes of pigeon pea in four groups at 82% of similarity. The first cluster grouped four performed genotypes AN21, AN32, AN34, AN33 according to earliness, high plant pods, seed production potential, and seed nutritional quality. The second and the fourth clusters grouped each one six genotypes. Genotypes grouped in second cluster are characterized by a moderate earliness flowering (90 to 122 days), low potential of seed production per plant. While, cluster IV grouped the late flowering genotypes (168 days from sowing) with a low seed yield potential per plant and a high seeds value of crude proteins content. Whereas, the third one grouped a single genotype ATZ19 that was distinguished by late blooming (147 days from sowing), high pods weight, wide pods, high number of seeds per plant and small seed size (Fig. 2).

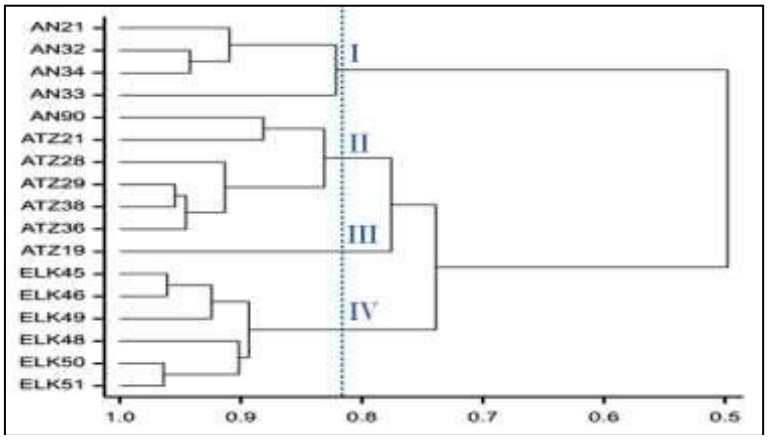

Figure 2: Clustering of seventeen pigeon pea genotypes based on seventeen quantitative traits

Conclusion

The introduction of Pigeon pea crop in the agro-ecological system of Morocco might be a good alternative in marginal lands. As leguminous crop, it may contribute to crop sustainability mainly in climate change context. Furthermore, Pigeon pea seeds contain high nutritive values mainly according to their high level of crude protein that may offer an important source of proteins for vulnerable people and also for animals.

The results showed a large genetic variability based on quantitative characters, from what six promising genotypes showed a high adaptation potential in different Moroccan environments according to their earliness, seed yield potential and nutritive quality. These promising genotypes might encourage research to develop improved varieties adapted to Morocco's edaphic and climatic conditions with both high productivity and nutritional value.

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EFFECT OF OSMOTICUM AND SILICA-GEL DESICCATION ON SOMATIC EMBRYOGENESIS FROM CALLUS CULTURES OF *BIGNONIA ADENOPHYLLA* D.C

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Abstract

Ornamental plants are important source of aesthetic value and the production of medicinal compounds. It is planted along the roads and parks due to its beautiful shape of pods and greenish look of leaves. Leaf and wood extracts of this tree has medicinal properties to control viral diseases. In this paper, we report an efficient protocol for somatic embryogenesis (SE) and plant regeneration of *Bignonia adenophylla*. Seeds were germinated *in vitro* on basic Murashige and Skooge (MS) medium to obtain green cotyledons from sterile seedlings and cultured on MS medium supplemented with thidiazuron or n^6-benzyladenine (BA) alone and with indol-3-acetic acid, α-naphthaleneacetic acid or 2, 4-Dichlorophenoxyacetic acid (2, 4-D) for callus induction. In the first experiment, greenish nodular calluses were grown on 0, 25, 50, 75 or 100% MS salts along with 0, 1.5, 3 or 6% sucrose for four days. In the second experiment, 0, 5, 10, 15 g/L silica gel powder was added in the plain agar for dehydration treatment. Highest (75%) callus induction was obtained with 0.139 g dry weight at BA (2 μM) + 2, 4-D (4 μM) after 36 days. Callus further proliferated and produced 80.33% somatic embryos, having 70.15 number of somatic embryos at 15 g/L silica gel after 15 days. Such somatic embryos germinated up to 11% into cotyledonary stage. We observed that TDZ inhibited the growth of shoot as well as root in the present study. Growth of such habituated cultures was further improved and produced somatic embryos on desiccation (silica gel) medium. The germination of somatic embryos was good enough that may further be improved.

Keywords: *Dehydration treatment, marud phali, somatic embryogenesis, sucrose, thidiazuron*

Introduction

Research curiosity on ornamental and medicinal plants is increasing day by day throughout the world to cater to the aesthetic aspiration and discovery of novel compounds for use in medicine. *Bignonia adenophylla* DC is one such tree that belongs to the family Bignoniaceae. This is a naturally-grown tree in India and other subtropical Southeast Asian regions for improving the aesthetic beauty of roads and parks due to its beautiful shape of pods and greenish look of leaves. In addition to this, leaf and wood extracts of this tree has medicinal properties to control viral diseases. It has been reported that the root extract has been used on snake bite (Jassbi *et al*., 2004). Generally, *B. adenophylla* is propagated through seeds in the field conditions. It produces limited number of viable seeds that might be due to unfavorable climatic conditions for fertilization. Moreover, conventional propagation methods for rooting of cuttings as well as bud sprouting in the nursery conditions are also very difficult. Limitations of conventional methods make this plant endangered in the plain areas of Pakistan. Somatic embryogenesis (SE) is very powerful tool of plant tissue culture for clonal propagation and the conservation of this plant. During SE, somatic cells undergo a series of morphological, physiological and biochemical events to develop into embryos on the tissue culture media containing suitable osmotica (Karami *et al*., 2006). Somatic cells pass through

different developmental phases of globular, torpedo and cotyledonary stages after extensive osmotic and desiccation treatments. Moreover, growth regulators and the amount of sugar in the medium also play role in inducing SE (Kubeš *et al.* 2014). Sucrose is a carbohydrate and being the main carbon source drives cellular metabolism for normal growth and development of *in vitro* plants (Karami *et al*., 2006; Kubeš *et al*., 2014). Optimum level of sucrose in MS medium is 3%, while its variable concentrations have been reported for somatic embryogenesis in different plant species (Yantcheva *et al.* 1998; Karami *et al.* 2006). Silica gel powder is a commonly used lab chemical for thin layer chromatography. This is also used as a desiccator and therefore absorbs moisture in different culture systems. Little information regarding the use of silica gel is available except for carrot somatic embryogenesis in an air tight box (Dumet *et al.* 1993). However, inclusion of silica gel into the culture medium for somatic embryogenesis has not been reported. Therefore, the objective of the present study was to explore conditions for somatic embryogenesis and maturation on various desiccation treatments using in vitro-germinating seedlings as an initial explant material of *B. adenophylla*.

Materials and Methods

Mature seeds of *B. adenophylla* were collected, surface sterilized with ethyl alcohol (65%) for few seconds and then decontaminated in 15% bleach solution (Robin, Reckitt & Colman, Pakistan) for 15 minutes and washed with autoclaved water under the laminar flow cabinet and cultured on MS (Murashige and Skooge, 1962) agar solidified medium added with 0.1% activated charcoal at pH 5.8 and autoclaved under the standard conditions (121 °C and 104 kPa). After inoculation, cultures were shifted to 16 h photoperiod at 25 ± 2 °C.

In vitro seedlings were used for the acquisition of the cotyledonary explants to grow them on MS medium supplemented with 2 µM TDZ (thidiazuron) or BA (N^6-benzyladenine) in combination with 1, 3 or 4 µM indol-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D). The rate of callus induction was calculated after 26 days. Fresh weight (FW) and dry weight (DW) of the calluses was determined.

Calluses pre-cultured on TDZ (2 µM) + 2, 4-D (4 µM) were used for somatic embryogenesis. For this purpose, 0, 25, 50, 75 or 100% MS medium + 0, 1.5, 3 or 6% sucrose were used for 15 days. Silica gel powder at 0, 5, 10 or 15 g/L in plain agar was also tested for somatic embryogenesis for 36 days. Embryogenic cultures were then shifted on to half MS medium with 3% sucrose for maturation. The developing embryos were germinated on MS medium with 3% sucrose.

Data analysis. One way ANOVA was used for mean values compared using DMRT. Simple regression analysis was also used for comparison of the data using SPSS v.16.

Results and Discussion

In the present study, callus induction was observed on all treatments from cotyledons. Fig. 1a and b show callus induction frequency as affected by different auxins in combination with cytokinins after 36 days of initial culture. The rate of callus induction was 44.23% that decreased subsequently with the increasing levels of NAA (Fig. A). This was followed by 2 µM 2, 4-D and 3 µM IAA with 43% and 43.32% callus induction, respectively. However, BAP was most effective when used with all levels of 2, 4-D followed by IAA, for callus induction with a highest (75%) callus induction response at 3 µM 2,4-D + 2 µM BAP after 36 days of initial culture (Fig. 1B). Explants showed different morphological changes on different treatments after 20 days of culture (Fig. 2). Explant on the basal medium did not show any change (no callus induction; Fig. 2A). Fast growth with white nodules were observed on the explant surface as well as from cut margins with 2, 4-D + TDZ (Fig. 2B). IAA was least supportive and resulted in little callus from cut surfaces (Fig. 2C). Within the

same period of time, NAA + TDZ produced brown calluses (Fig. 2D). Initially, the explants on 2, 4-D + BAP became necrotic (Fig. 2E) that subsequently invigorated and formed huge mass of cells. Explants on IAA + BAP or NAA + BAP abruptly developed translucent callus tissues (Fig. 2F, G).

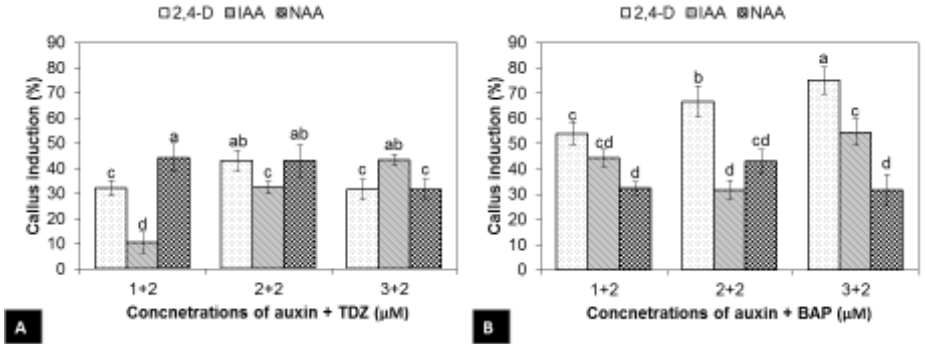

Fig. 1 Effect of different concentrations of auxins along with TDZ (A) or BAP (B) on callus induction in *B. adenophylla* from cotyledons after 36 days of culture.

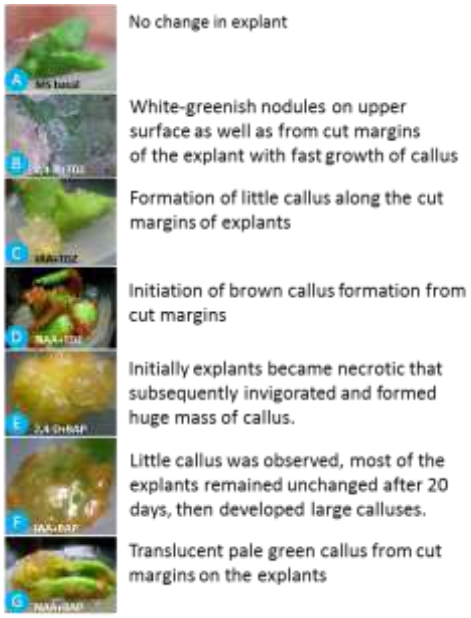

Fig. 2 Morphological characteristics of calluses induced on different treatments in *B. adenophylla* after 20 days of culture.

The highest (2.121 g) fresh weight (FW) of calluses was obtained at BA + IAA as compared to other treatments (Fig. 3). Similarly, the dry weight (DW) of such calluses was also highest (0.339 g) which was 15.98% of its fresh weight.

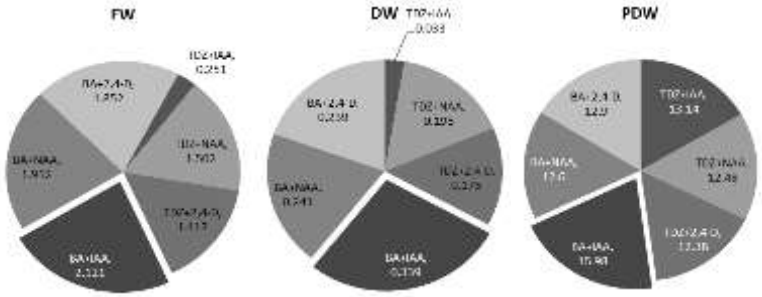

Fig. 3 Pie charts showing the effect of different treatments on fresh and dry weight of callus cultures of *B. adenophylla.* FW: Fresh weight, DW: Dry weight, PDW: Percent dry weight

Fig. 4 Callus induction and somatic embryogenesis from cotyledons of *B. adenophylla*. **A)** Callus formation on the surface of cotyledon at 2 µM BA + 3 µM 2, 4-D after 26 days. **B, C)** Green calluses (36 days old) formed embryo-like structures on the surface (arrows) on plain agar augmented with 15 g/L silica gel. **D, E, F, G)** Development of somatic embryos through globular, sickle, torpedo and cotyledonary stages observed on plain agar added with 15 g/L silica gel pre-cultured on TDZ (2 µM) and 2, 4-D (3 µM). **H)** Germination and further development of somatic embryo.

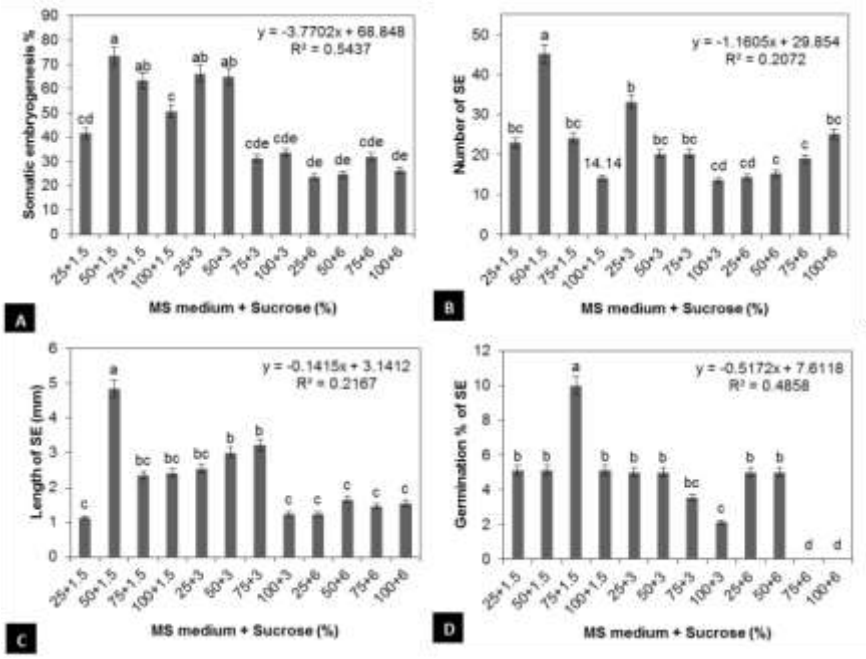

Fig. 5 Induction and further development of somatic embryos (SE) of *B. adenophylla* on various strengths of MS medium supplemented with different levels of sucrose (% values are given) after 36 days.

Callus appeared from cut explant margins (Fig. 4A), however, when transferred to the desiccation medium, it formed embryo like structures on its surface on plain agar medium + 15 g/L silica gel previously cultured on TDZ (2 µM) and NAA (3 µM) after 36 days (Fig. 4 B, C). Various shapes of somatic embryos (SE) were observed, for example, globular (Fig. 4D), sickle (Fig. 4E), torpedo (Fig. 4F) and cotyledonary stages (Fig. 4G) were observed on 3 µM 2, 4-D + 2 µM TDZ. The cotyledonary shaped SE were then germinated into shoots (Fig. 4 H).

Different concentrations of MS medium + sucrose (Fig. 5) as well as silica gel in MS basal medium (Fig. 6) formed and developed SEs. By increasing the levels of sucrose the somatic

embryogenesis was decreased. Sucrose at 1.5% + 50% MS medium formed 73.45% SE (Fig. 5A) with 45.21 numbers (Fig. 5B), 4.85 mm long (Fig. 5C) and 5.11% germination (Fig. 5D) was obtained. This was followed by 1.5% sucrose + 100% MS (63.33% SE, 24.21 number, 2.36 mm length and 10% germination). However, the embryos formed on these treatments were morphologically dissimilar and deprived. SEs were significantly improved by increasing the concentration of silica gel (Fig. 6). Highest (80.33%) somatic embryogenesis (Fig. 6A), 70.15 number (Fig. 6B), 6.16 mm length (Fig. 6C) and 11.01% germination (Fig. 6D) was achieved at 15% silica gel after 36 days of culture. SEs developed on silica gel were stout and vigorous.

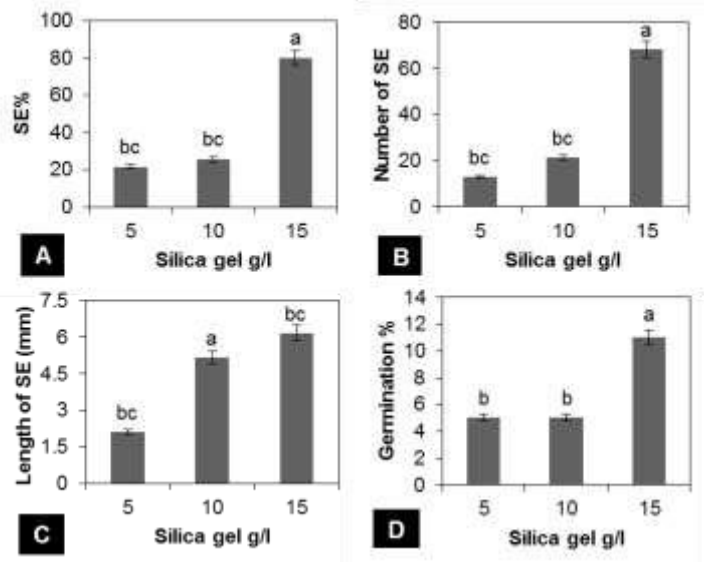

Fig. 6 Induction and further development of somatic embryos (SE) of *B. adenophylla* on different levels of silica gel in the medium after 36 days.

Regression analysis of the individual effect of sucrose, MS medium and silica gel were also compared (Table 1). High correlation (R^2 = 0.78) of somatic embryogenesis induction was observed by silica gel. This was followed by sucrose (R^2 = 0.55) and MS medium (R^2 = 0.06). Number of SE were highly correlated (R^2 = 0.803) by silica gel. The rate of SE germination was also significantly improved (R^2 = 0.72) by silica gel as compared to other desiccants.

Table 1. Factors affecting somatic embryogenesis from calluses on MS + 2 µM TDZ + 4 µM 2, 4-D

Regression analysis	**Constants**											
	Sucrose				**MS medium**				**Silica gel**			
	Dependent variables											
	SE%	NE	LE	Ger	SE%	NE	LE	Ger	SE%	NE	LE	Ger
Coefficients												
B	-15.68	-2.92	-0.59	-1.84	-4.23	-2.25	0.12	-1.56	6.52	5.608	0.08	0.55
Std. Error	2.62	1.76	0.21	0.77	2.72	1.28	0.17	0.55	1.24	1.051	0.12	0.13
Beta	-0.69	-0.27	-0.42	-0.37	-0.25	-0.28	0.11	-0.44	0.88	0.896	0.25	0.85
t	-5.97	-1.65	-2.75	-2.37	-1.55	-1.75	0.67	-2.86	5.11	5.337	0.70	4.32
Model summary												
R	0.74	0.27	0.43	0.37	0.26	0.29	0.12	0.44	0.88	0.86	0.25	0.85
R^2	0.55	0.07	0.18	0.14	0.06	0.08	0.01	0.19	0.78	0.803	0.06	0.72
Adjusted R^2	0.52	0.04	0.16	0.12	0.04	0.06	-0.02	0.17	0.78	0.75	-0.06	0.68
SE estimate	12.86	8.66	1.05	3.79	18.28	8.62	1.15	3.68	14.98	12.86	1.49	1.58
Sig.	0.000 *	0.108 ns	0.009 *	0.023 *	0.130 ns	0.088 ns	0.503 ns	0.007 *	0.001 *	.0001 *	0.507 ns	0.004 *

Generally, somatic embryogenesis is achieved after passing through the cultured tissues on various chemical treatments, osmotic and desiccation stresses. Plant growth regulators (PGR) including cytokinins and auxins also play significant role in somatic embryogenesis. TDZ as a PGR kind of prepares the cultured plant cells to become somatic embryos physiologically by adjusting the optimum level of osmoticum (sucrose) in the medium (Martinez *et al.* 2008). Decreasing the moisture level in the culture medium is also known to push the plant cells towards somatic embryogenesis. For example, the use of 0.75 M sucrose supported the oil palm cells to become somatic embryos after the provision of desiccation with silica gel in the box (Dumet *et al.* 1993). Similarly, initial treatment of abscisic acid (ABA) induced somatic embryos on silica gel in cultured carrot cells (Ikeda *et al.* 2006). ABA in combination with silica gel improved the germination frequency of somatic embryos and survival rate by minimizing (up to 5%) the moisture level in the medium (Dumet *et al.* 1993). These reports support our findings of pretreatment effect.

In our study, TDZ pre-treatment followed by silica gel improved the growth and maturation of somatic embryos. Moreover, TDZ pre-treatment was more effective than BA when used along with auxins. Many other reports also confirmed the validity of our results working on different plants. Kumar *et al.* (2015) demonstrated that 0.5 mg/L TDZ + 20 mg/L picloram + 20 mg/L glutamine induced somatic embryos from calluses of *Pelargonium sidoides*. Chilling treatment improved the germination frequency after short exposure of calluses to TDZ of *Quercus robur* SE (Martinez *et al.* 2008). On the other hand, improved germination (61%) has been reported using BA (0.44 μM) + 0.29 μM gibberellic acid on MS basal medium (Vengadesan and Pijut 2009).

Conclusions

It was observed that TDZ inhibited the growth of shoot as well as root in the present study that may be due to its strong cytokinin activity (Huetteman and Preece 1993; Martinez *et al.* 2008; Akram and Aftab 2016). Such habituated cultures went to maturation and somatic embryogenesis on silica gel medium. The germination of somatic embryos was good enough that may further be improved under such culture conditions.

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THE INFLUENCE OF GROUND PARAMETERS ON SELECTED TRACTION ABILITIES OF AGRICULTURAL VEHICLE

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Abstract

Nowadays, farm tractors are characterized by high versatility – one tractor can be used at various works on different grounds. Different abilities of grounds can cause the differences in traction abilities of agricultural vehicles. In turn, it may make difficult to achieve the optimal pulling abilities of tractor; moreover, in some cases the high energy losses can occur. For this reason, the aim of provided research was to demonstrate the differences in values of selected tractive parameters (wheel slip, pulling force, traction force, tractive efficiency) on two surfaces with different properties. Research was conducted on cultivated soil with two compaction levels and on the pasture turf (with one compaction level). Two tires with similar external dimensions were used in experiment – the difference in this tires concerned their internal construction; 11.2R24 tire had a radial construction (higher flexibility), while 9.5-24 was the bias-ply tire with smaller flexibility. To measure the tractive parameters the measure device mounted o tractor was used. The measure parameters were: pulling force, torque of wheel with tested tire, rolling resistance, theoretical and actual velocities. Then, the values of wheel slip, traction force and tractive efficiency were calculated. Based on obtained results it can demonstrate that the radial tire had a less sensitivity to change of surface of exploitation – the differences in pulling force did not exceed 8% (for the tractive efficiency – 5%). For the bias-ply tire the higher values of pulling force were observed on the soil with higher compaction level (in comparison with the second soil the difference was equal 21%). Moreover, the bias-ply tire achieved the smaller tractive efficiency than radial tire – difference reached 11%. The comparison of the tires on the pasture turf allowed to show that both tires had similar values of pulling force, but the radial tire achieved the higher values of tractive efficiency.

Keywords: *agricultural vehicles, pulling abilities, tractive efficiency, compaction.*

Introduction

Agricultural vehicles, especially farm tractors, are used to different works. One tractor with the same tires can be operated on the grounds with extremely different properties.
It is also important that the variability of substrate conditions can cause differences in traction properties of agricultural vehicles.
Traction abilities are described by a few parameters such as pulling force (or drawbar force), traction force, wheel slip, tractive efficiency (Elwaleed et al. 2006, Zoz and Grisso 2003). From a practical point of view the important parameters are pulling force (it affects on pulling abilities of tractor) and tractive efficiency which describes the degree of recovery of power delivered to the wheels (indirectly it describes the energy losses in wheel-ground system).
The traction parameters are dependent on many factors which can be divide into two main groups:

- construction and maintenance factors: type of tires (radial, bias-ply), tires dimensions, type of a tire tread, vertical load, inflation pressure,

- factors related to the ground: compaction of the soil (am often it is described by the penetration resistance), shear stress strength, soil moisture, the plants coverage of the soil.

For many years, in literature the effects of research of wheel-ground system are presented. Researchers take into account both construction and ground factors. For example, Elwaleed et all (2006) describes the influence of tread high on traction abilities, in Smerda's and Cupera's research paper (2010) there are showed impact inflation pressure on traction performance. Gholkar et al (2009) focuses on simultaneous impact of vertical load and inflation pressure. Thaghavifar and Mardani (2013) presented comprehensive assessment of operational factors on traction properties.

Determination of influence of impact of ground parameters on traction abilities is a complex and difficult process. The main reason of this situation is the variability of ground parameters – on one filed there can be the places with different compaction levels. In the case of the cultivated soil, important factor is the cultivation system (Moitzi et al 2006, Arviddson 2010). Moreover, each change of actual humidity can result in changes in compaction. These factors can cause variability of traction properties of agricultural vehicles (Aday et al 2004, Gharibkhani et al 2012, Inchebron et al 2012). In literature there are showed papers described influence of ground parameters on traction abilities (Xu et al 1991, Lyasko 2010, Narang and Varschney 2006) but am often they concern specific condition and places – their "universal" use is limited. For this reason, it is reasonable to continue the research in this range. The aim of research was to determine the differences in traction properties of two agricultural tires operated on different grounds.

Material and Methods

The experiment based on measurements of tractive parameters for two agricultural tires (the parameters of these tires was shown in Table 1). During the tests the one level of inflation pressure (0,14 MPa) and vertical load (8060 N) were used.

Table 1. Parameters of tested tires

Tire	Construction	External diameter, mm	Width, mm	Rim diameter, mm	Tread high, mm
9.5-24	bias-ply	996	241	610	35
11.2R24	radial	1000	284	610	35

In experiment, the main factor influenced the traction properties was the type of the ground. Research was carried out on the three grounds: cultivated soil with low compaction, cultivated soil with high compaction and the pasture turf. The description of the grounds was shown in Table 2.

Table 2. Description of the grounds

Ground	Actual moisture, %	Compaction, MPa	Shear stress strength, kPa
Compacted soil	13,3	0,82	28,6
Loose soil	22,3	0,56	19,0
Pasture turf	20,7	1,24	66,2

To determinate the traction parameters the special measure stand was used. This stand was mounted on three-point linkage of a Massey-Fergusson 235 tractor. The base of stand was a two-piece frame. The tested wheels were mounted on the shaft which was driven from PTO

through the hydraulic-mechanical transmission. The measure stand was equipped in force sensor (measure accuracy 10 N in range 0-100 kN), torquemeter (accuracy 1 Nm, range 0-3000 Nm) and two rotational sensors for speeds measure. All measured parameters were sent to electronic recording connected with portable computer. Based on values of measured parameters (pulling force, torque, actual speed, and theoretical speed) other traction parameters were calculated, according to formulas 1-6.

$$\delta = 100 \cdot \left(1 - \frac{v_R}{v_T}\right), \quad (1)$$

$$P_T = \frac{M_O}{r}, \quad (2)$$

$$\eta = \frac{P_u}{P_T} \cdot (100 - \delta), \quad (3)$$

where:
δ – wheel slip, %,
v_R – actual speed, ms^{-1} (measured),
v_T – theoretical speed, ms^{-1} (measured),
P_T – traction force, N,
M_O – torque of wheel, Nm (measured),
r – wheel radius, m,
η – tractive efficiency, %,
P_u – pulling force, N (measured).

During the experiment the tested wheel was driven from PTO while the gearbox of tractor was in neutral position. When the measure stand with the tractor was moving, the traction parameters were measured and recorded. In the final part of measurement the tractor was smoothly stopped by brake, then the tested wheel was rolling with increasing slip (at the end the wheel slip was equal 100% and the wheel rotated without linear move). Each of measurements was done in 3 repetitions.

Results and Discussion

According to the aim of research the based traction parameters were determine. Figure 1 shows the values of pulling force, for both tires on three grounds.

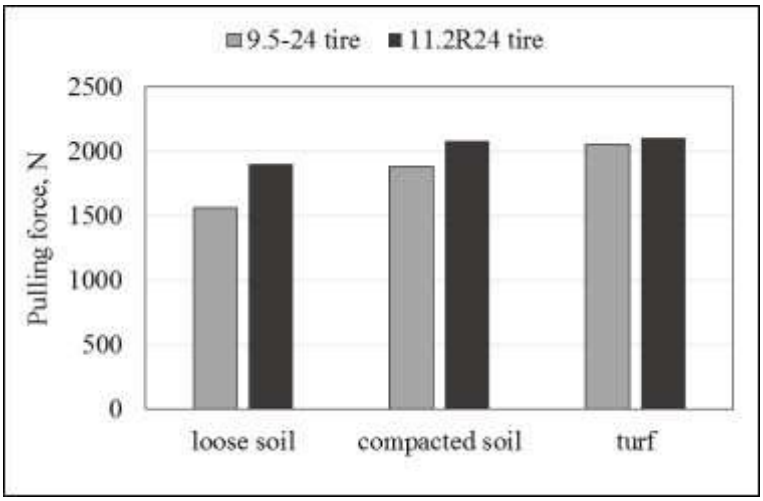

Fig. 1. The values of pulling force (arithmetical means calculated for slip range 0-30%)

In Figure 1 it is visible that the 11.2R24 tire obtained higher values of pulling force than the 9.5-24 tire. This trend was observed in any case of ground, while the highest difference

concerned loose soil (339 N, which is 21,6%). On the pasture turf the differences was the smallest (49 N, 2,3%). These differences can be caused by different construction of both tires. Radial tire (11.2R24) had a better flexibility and it can be better fitted to the ground. Probably, the contact area of radial tire was greater than in the case of bias-ply tire (it can reduce the wheel slip). The advantage of radial tire was shown by other researchers (Materek 2006, Botta et al 2008). It is also visible that the higher compaction causes increase in pulling force. This trend also refers to other research. The similar effects were obtained by Xu et al (1991), Jun et al (2004) and Narang and Varschney (2006).
The second analyzed parameter was the tractive efficiency. The maximum values of this parameter were presented in Figure 2.

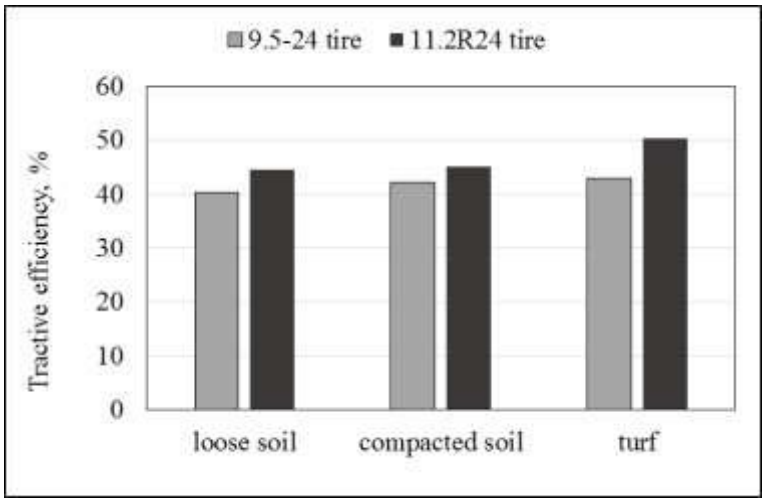

Fig. 2. The values of tractive efficiency (maximum values in the slip range 0-30%)

In accordance with Figure 2 the highest values of tractive efficiency (51,4%) was obtained by the wheel with the 11.2R24 tire on the turf. The lowest tractive efficiency (40,3%) was observed for the bias-ply tire (9.5-24) on the loose soil. The maximum absolute difference between the efficiency for both tires exceeded 11% (relative 27,5%) . For radial tire there is good visible dependence between ground parameters and tractive efficiency; higher compaction (and shear stress strength) allowed to obtain higher tractive efficiency. It refers to other research – Narang and Varschney (2006), Gholkar et al (2009), Wulfsohn and Way (2009) reported that higher soil strength can cause better traction properties. Moreover, it can be found that the bias-ply tire was less susceptible to change of ground parameters, but their traction properties were worse than for radial tire.

Conclusions

Based on obtained results, following conclusions were formulated:
1. The wheel fitted with radial tire obtained higher values of pulling force that wheel with bias-ply tire. The highest difference in pulling force between both tires was observed on loose soil, while lower difference was on the pasture turf.
2. Higher values of tractive efficiency were obtained by the wheel with the radial tire. This trend was observed on all three grounds, but the highest difference between both tires was observed on the pasture turf.
3. Higher strength of the ground (described higher values of compaction and shear stress strength) allowed to obtain better traction properties of both tested tires wherein bias-ply tire was less susceptible to change of ground parameters (for this tire the differences in tractive efficiency on different grounds were smaller than for radial tire).

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MORPHOLOGICAL PROPERTIES OF DIRECTLY SOWED SWEET CORN PLANTS CULTIVATED WITH COVERING TECHNOLOGIES

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Abstract

Aim of experiment set up in 2014 was, to study the evolution of sweet corn growing period with application of some technological elements: propagation time, propagation method, floating row cover. The choosen variety was a conventional, very early ripening sweet corn hybrid, 'Spirit'. The following growing technologies were compared: 1. direct sowing of plants with floating row cover, early period 2. direct sowing of plants without row cover, early period 3. direct sowing of plants with floating row cover, normal period and 4. direct sowing of plants without row cover, normal period (regarded as control).

It was studied the influence of above mentioned technological elements on some important morphological properties of sweet corn plant: evolution of phenological phases, plant height, tassels length, ear weight, height of ear insertion.

Key words: *earliness, sweet corn, vlies covering*.

Introduction

Based on its present growing area, the sweet corn is the vegetable which is grown on the greatest area in Hungary. After dates of Hungarian Fruit & Vegetable Interprofessional Organization in 2003 the maximal growing area was about 38,000 hectares. After 2003 followed a suddenly and sharp decline. After diminishing, the plant returned in rise, in 2006 against over 30,000 hectares. The recession mentioned above affected not only Hungary but also the holdings of the USA and Western Europe. In the case of the former, however, the increase in fresh consumption partly counterbalanced the rate of decrease. Near 50% of world sweet corn production came from US (Slezák *et al.*, 2012). In 2013 the growing area was 34, 000 hectares, less with 1000 ha, compared to 2012 (Avar, 2013).

In order to promote fresh consumption, as well as to maintain and increase the sweet corn exports, it is necessary to promote investigations so as to be able to ensure a further increase in the growing area and yields of sweet corn with the help of the experiences. The literature mainly is concerned with maize growing technology, but a lot of dates could be used by sweet corn. Some sweet corn varieties have much shorter vegetation period, in which they are very sensitive to the weather conditions, especially during germination and emergence as compared to the other subspecies of maize (Dolijanovic et al., 2014). Sowing date of sweet corn is one of the factors which influence both the ear yield and its quality (Rosa, 2014).

Ripening can occur earlier when sowing earlier and using high quality seeds as compared to normal or late sowing. I'só (1969) and Pásztor (1966), after their multi-year sowing date trial, concluded the following: in the case of an earlier sowing seed germination will be more protracted, but silking and harvesting occur sooner than by lately sowing time. After multi-year trial Berzsenyi et al. (1998) have studied the effect of different sowing times on maize development.

Several techniques are known in the art for the purpose of early fresh market shipments: seedling growing or direct seeding with temporary plant cover (Hodossi, 2004).

About the covered early sowing as a technological variation Aylswirth (1986) mentioned, that from an early sowed crop, made in first week of April, arranged in twin rows (42 cm) and covered by plastic, we could harvested marketable cobs by the fourth of July. In case of direct

sowing, as propagation method, another earliness increasing solution is the temporary covering with plastic or vlies, used in different combinations (Hodossi and Kovács, 1996).
Rattin et al., (2006) compared direct sowed and transplanted sweet corn varieties, without covering and concluded no difference, in ear weight and ear length, between transplanted treatments plant's, in comparison to direct sowing treatments plant's.

Materials and methods

The experiment was set up in 2014 on an area eqiupped for irrigation at Tg-Mures, Mures County, situated in Central part of Romania. Conventional, reliable and sufficiently known among growers sweet corn variety, Spirit, was used as a reference variety in the variety comparison trials of the Central Agricultural Office. Hybrid has short growing period of 85 days and yellow kernels. Average height of plants is 159 cm, average ear height is 37 cm, ear length 19.6 cm and average ear weight is 245 g. The variety was granted official recognitions in 1988 and has been the dominant variety of the early ripening category till now. In the year prior to the experiment the area was under wheat.
The following treatments, each with four replications, were applied during the experiment:
P1 = covered direct sowing (April 8th)
P2 = uncovered direct sowing (April 8th)
P3 = covered direct sowing (April 25th)
P4 = uncovered direct sowing (April 25th), (regarded as control).
For the frame structure of the treatments with cover we used ø 4.2 mm zinc coated wire coils. The fleece, 60 cm in width, was stretched over a small tunnel of 40 cm in height and then its edges (25-25 cm, respectively) were covered with soil using a hoe and the its ends were tied to the stakes hammered down. The construction of the frame structure and the setting out of the fleece cover were carried out at the same day as direct seeding and out planting.
At the two propagation times the treatments P1 and P3 were covered with Novagryl floating row cover, having a weight of 19 g/m^2, (using the small tunnel technique) in order to enhance earliness. The stand was created to contain 60,607 plants per hectare, according to the recommendations of the owner of the variety, at a spacing of 110+40x22 cm in twin rows. Each plot had an area of 6x3.5m (8 parallel rows and 16 seeds sown in each row). Sowing depth was 3 cm. The edge was the respective outer rows of the 4 twin rows of the plot.
In October 2013, 35 t/ha of farmyard manure was worked into the soil with ploughing on area. Nitrogen fertilizer (120 kg/ha) was applied at the 6-7 leaf stage, the form of top dressing. The fertilizer application was worked into the soil with a rotary hoe.
During the experiment, we studied plant growth rates and recorded the time of the occurrence of the major phenological stages. For this purpose, we carried out regular observations (every 3 to 5 days) according to the following:
beginning of seed emergence (appearance of first germs),
appearance of tassels (by 50% of the plants),
beginning of tasseling (pollen shed has begun on the axes of tassels),
50% silking (silks have reached a length of 2 cm on half of the ears) "milky stage" (harvest).
highness of plants (cm),
length of tassels (cm),
highness of ear insertion (cm).
Ears, together with the husks, were collected from the four central (two twin) rows and 20 ears of average appearance were selected from each row for following measurement:
unhusked and husked ear weight (gram).
The statistical analysis of the results was carried out by using the programme *RopStat 1.1*. When the standard deviations were identical the mean values were compared by pairs using the *Tukey-Kramer* test, while in the case of the non identical standard deviations the means were compared using the *Games-Howell* test (Vargha, 2007).

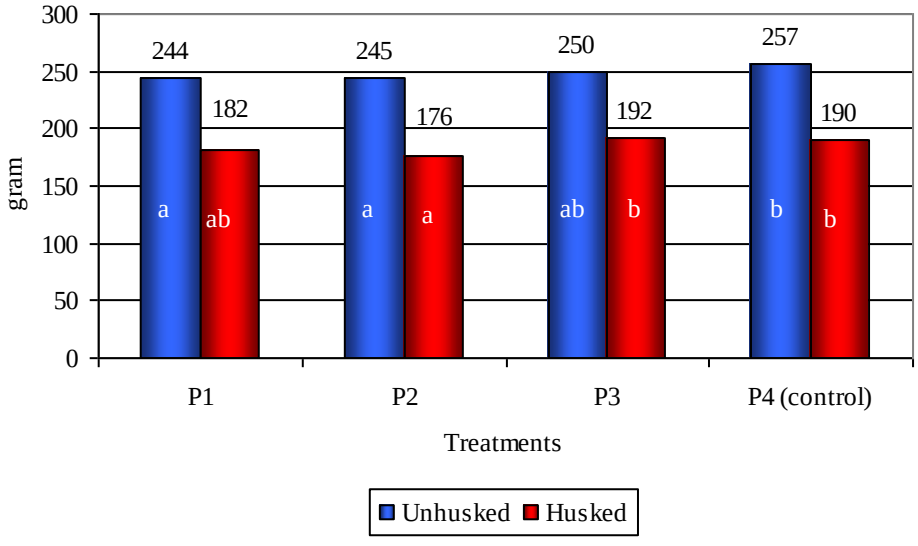
Results and discussion

Table 1. illustrates the times of sowing, emergence as well as the number of days elapsed from the date of direct seeding or transplanting to the different phaenological stages.

Table 1. Rhytm of emergence and generative phaenophases

Treatments (sowing day)	Date of emergence (days)	Tassels appearance by 50% (days)	Stigma appearance by 50% (days)	Starting harvest (days)
P1 (IV. 8)	15 (IV. 23.)	41 (V. 19.)	60 (VI. 7.)	76 (VI. 23.)
P2 (IV. 8)	17 (IV. 25.)	50 (V. 28.)	70 (VI. 17.)	85 (VII. 2.)
P3 (IV. 25)	9 (V. 4.)	52 (VI. 16.)	67 (VII. 1.)	83 (VII. 17.)
P4 (control) (IV. 25)	10 (V. 5.)	57 (VI. 21.)	71 (VII. 5.)	85 (VII. 19.)

It can be observed that time of germination in the treatment had sown at the first sowing date and having no cover had 2 days delay as compared to the germination time of the covered treatment and an significant (9 days) delay in beginning of harvesting time in 2014. Covering helped also in earlier appearance of generative phaenophases (tassels and stigma) by 9 days respectively, by 10 days earliness. In the case of the plants sown at the second sowing date the emergence could be considered practically normal as common. According to our results of experiment, the male flowers (tassels) appeared in the shortest time in the covered treatment. The covering, at this sowing time, resulted in 5 to 4 days earliness in appearance of generative phaenophases (tassels and stigma) compared to the P4 (control) treatment which had been direct sowed, uncovered at a similar time. Compared to the control (P4), the P3 treatment (covered) at the same date, could be harvested 2 days earlier. Earlier sowing time combined with plant covering (P1) treatment resulted, in 2014, an 24 days earliness compared to normal time sowed, covered (P3) treatment, respectively 26 day earlier harvesting compared to normal time sowed, uncovered (P4, control) treatment. Results of the one of the major characteristics in connection with yield rating, unhusked andhusked ear weight, are summarised in Figure 1.



F(4;174)=25,143; F(4;174)=11,836;Sd=99%

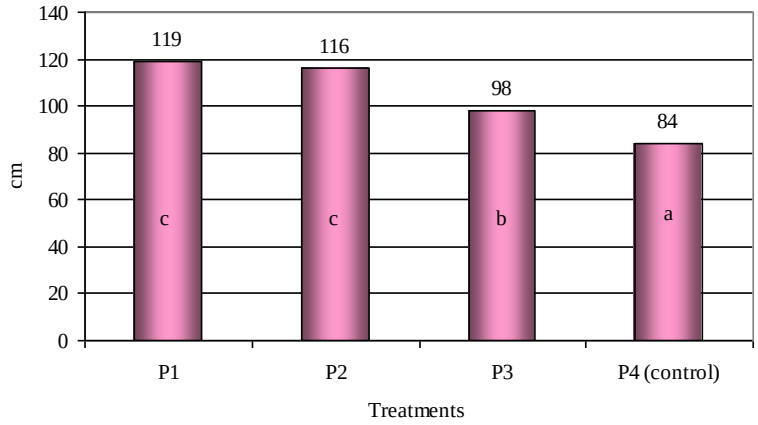
Fig. 1. Unhusked and husked ear weight (g).

Analysing the data measured for unhusked ear yield, it is noticeable that the average weight of

the ears of the normal time sowed, uncovered P4 (control) treatment was significantly (at *p<0.01* level) higher as compared to the earlier sowed, covered and uncovered treatments P1, P2. Though there was some difference between the unhusked ear weight of the normal time sowed, covered P3 treatment and in the same time sowed, uncovered P4 (control) treatment, which fact statistically was not significant.

In case of husked ear weight the normal time sowed, covered P3 treatment and in the same time sowed, uncovered P4 (control) treatment ear weight was significantly (at *p<0.01* level) higher as compared to the earlier sowed, uncovered treatment, P2.

The highness of plants indicator of general condition is represented by Figure 2.

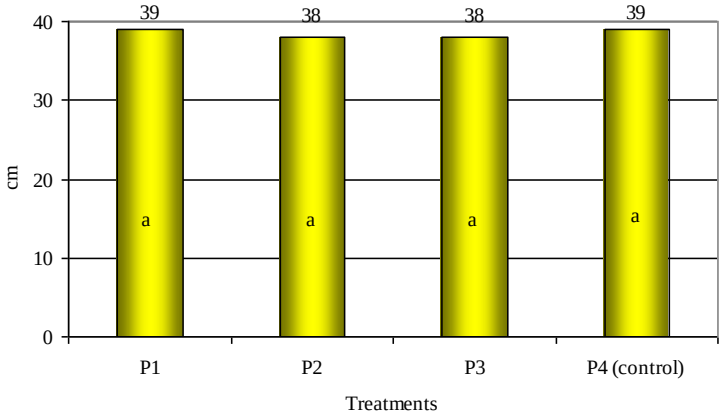


F(4;174)=14,240; Sd=99%

Fig. 2. Height of plants (cm).

The highest value of plant highness was registered by plants of earlier sowed (P1) covered and P2 earlier sowed (uncovered) treatments, difference was significantly (at p<0.01 level), compared to normal sowed (P3) covered and uncovered P4 (control) treatments. Covering (P1) had not favourable on plant highness compared to uncovered (P2) treatment by earlier sowing time.

Evolution of tassels length, supposed to carry more male flowers and implicite better pollination, is summarised on Figure 3.

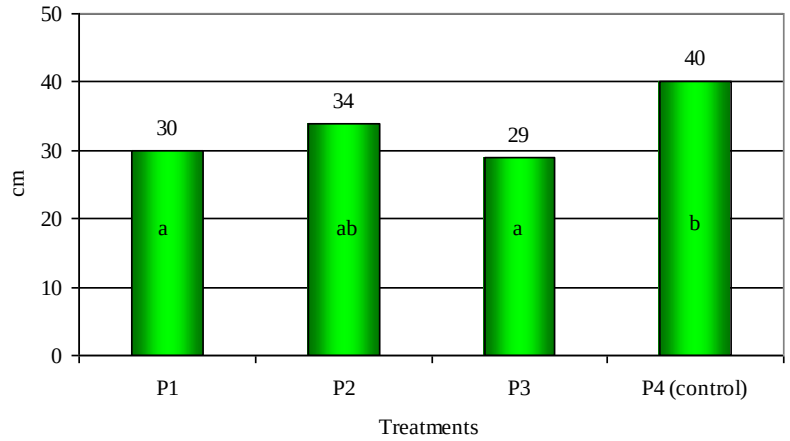


F(4;174)=17,42; Sd=99%

Fig. 3. Tassels length (cm).

In case of tassels length the time of earlier or later sowing and existence of covering had no significantly influence. Plants from earlier sowed, covered (P1) and normal time sowed

uncovered, control (P4) treatments had longer tassels compared to earlier sowed uncovered (P2) and normal time sowed covered (P3) treatments. Difference of tassels length statistically were not significant.
Highness of ear insertion is a very important morphological plant property in case of mechanical harvesting, because adapters of usual harvesting machines can cut only de sweet corn ears inserted higher than 40 cm, is illustrated on Figure 4.



F4;174)=3,481; Sd=99%

Fig. 4. Height of ear insertion (cm).

Obtained data confirmed significantly (at $p<0.01$ level) higher insertion of ears in case of earlier and later sowed, uncovered (P2 and P4) treatments compared to earlier and later sowed, covered (P1 and P3) treatments Covering on earlier sowed (P1) and normal time sowed P4 (control) treatments produced no favourable effect on highness of ear insertion. Mechanical harvesting in 2014 could not be used.

Conclusions

Based on the results of the 2014 year experiment, the following conclusions can be made:
In case to compare the earlier sowed P1 and P2 treatments, covering had 2 days earliness on date of emergence, shortened with 9, respectively 10 days, appearance of generative organs (tassels and stigmina) and shortened harvesting time at least with 9 days between earlier sowed (P1, P2) treatments and shortened harvesting time at least with 2 days between normal time sowed (P3, P4) treatments.
The growing period was significantly shortened with sowing time. Harvest time occurred 17 days earlier in the case of earlier sowed uncovered P2 treatment compared to direct sowed, uncovered, control (P4) treatment.
At the same time the floating plant cover combined with earlier sowing time produce 24 days shortening in the growing season between P1 (earlier sowing of plants with floating row cover) and P4 (normal time sowing of plants with no plant cover) control treatments.
In case of earlier sowed treatment (P1) the effect of covering had positive effect on unhusked, husked ear weight, hight of plants and length of tassels compared to P2 treatment.
In case of normal time sowed treatment (P3) the effect of covering had no important influence among measured morphological properties compared to P4 (control) treatment.
Covering had no significantly positive effect on height of ear insertion.
Mechanical harvesting in 2014 could not be used.

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COMPETITION ON RAPE SEED MARKET IS GETTING STRONGER

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Abstract

The article goal is to identify factors with higher input to the expansion of foreign seeds at the Russian rape seed market. Methods combined economic-statistical, abstract-logical, graphical, expert rating ones. Materials of the State statistics, the National Seed Registers, Russian Ministry of Agriculture, and USDA are used. Oil seeds acreage increased in this country +207% from 4 in 1990 till 12.3 m Ha in 2017 vs majority of cereals and fodder crops to be reduced. While spring rape seed acreage grew up fivefold, winter rapeseed has done fourfold during last 27 years, and is close to 1 m Ha. Farmers switch from cereals cultivation to oilseeds and, in particular, rape seed due to their higher margin, and oil seed processor demand. All-Russia Research Institute of Rapeseed, All-Russia Research Institute of Oil Crops by V.S. Pustovoit and Fodder Crops Institute are leaders on the local rape seed market. Number of listed local rapeseed varieties grown up +44%, from 48 in 2011 till 69 ones in 2017; and non-resident ones did +83% from 89 to 163 varieties for the last 7 years only. Foreign seed companies prefer to commercialize hybrids vs conventional varieties due to their more efficient return of investment to the breeding programs. Rape seed life cycle is assessed as 11 years for winter type varieties, and 13 years for spring ones. Hybrids market share is counted as 56% of spring, and as 76% of winter rape acreage. German seed companies (NPZ-Lembke, Bayer CS, and Deutsche Saatveredelung AG), American ones (Monsanto and Pioneer) and French ones (Euralis semences S.A., and others) domain on local markets. Clearfield and Express production systems (systemic herbicides application in combination with resistant hybrid varieties), improved fatty acid composition, and yellow color of seeds varieties may have competitive advantages.

Key words: *Rape, seeds, hybrid, conventional variety, seed market, breeding program*

Introduction

Oil crop acreage has been growing up at least for the last 25 years due to vegetable oil demand growth related with human prosperity. Even cereals are still main source of food calories for most of people, but they meet competition from oil crops in acreages, yields, productions, and margins. Rape is the second important oil crop worldwide with 36.4 million ha acreage, and 71.9 m tons production in 2017, which is less vs soya beans only (126,1 m ha and 347.9 m tons correspondingly). But it is superior vs sunflower (25,8 m ha, and 46.3 m tons). The article aim was to define some reasons and factors of loosing national seed market share by local breeders.

Materials and methods

The paper based on applied Economic-statistical, abstract-logical, graphical, expert ratings, regression methods, end expert assumptions. Materials of the National statistics, Ministry of Agriculture (www.mcx.ru), and USDA were used. The National Russian Registers of 2017 was analyzed (www.gossort.com).

Results and Discussions

Current commodity market challengers are favorable for growth of oil crop production in Russia (Fig. 1). Export logistic capacities were close to their limits within 2016-2017, but did not solve the problem of carryover stocks, as a result of cereal grain overproduction. State interventions (grain purchases) and subsidies also did not have a significant impact on market conditions. Low grain prices reduced of small grain crop production margin, as a result of which the agricultural producers increasingly began to consider oilseeds as an alternative crop to cereals. Finally, competition of oilseeds and cereals in the country is growing up.

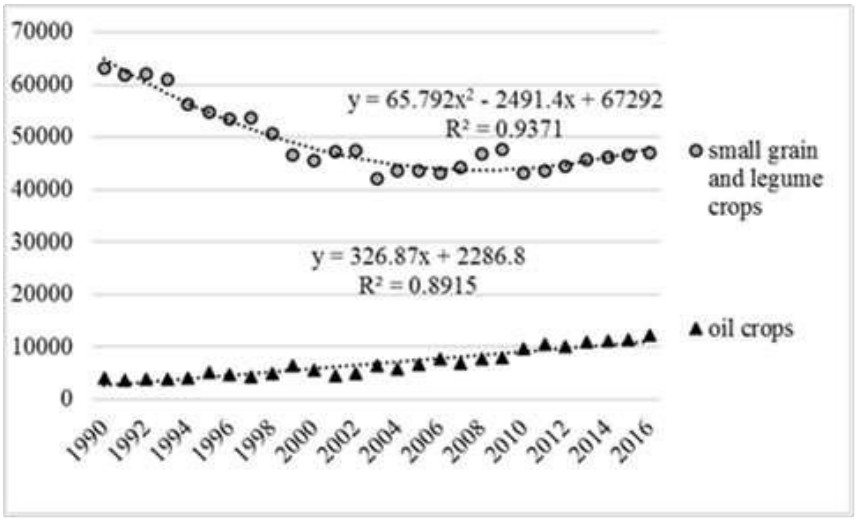

Fig.1. Dynamics of oilseeds, cereals and legume crops acreage in Russia, 000 ha, 1990-2017

Oilseeds (sunflower, soybean and rape) acreage simultaneously increased from 4.0 m ha in 1960 till 12.3 m ha in 2017, i.e. 0.25% on average per annum together with oil extraction plant consolidation and oilseed processing capacity growth in Russia till 24 million tons per annum. Sunflower acreage showed the largest growth dynamics in the Russian Federation, it was planted on 7.6 million hectares (+277% in 2017 vs 1990). Farmer choice in favor of soybean was realized in 330 % acreage growth, and nowadays it is more, then 2.2 m ha. There was a fivefold increase of spring rape planted area, and more than four times of winter rape during last 20 years (Fig. 2).

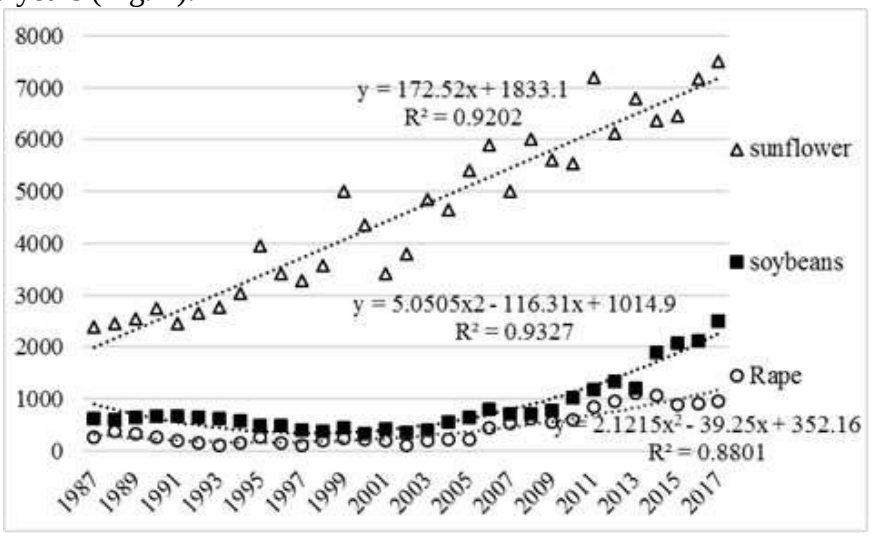

Fig. 2. Harvested acreage of main oil crops in Russia, 000 ha

Russia competes with the United State of America for the 6th place in the ranking of the world top rape seed producers, whose leaders are Canada, India, China and European Union. Russian farmers prefer to cultivate rape especially in traditional commodity areas of oilseed processing plants, and in livestock farms, which need reliable supply of oil meal and oil cake.
The acreage increase of oil crops and, in particular, rape, has required a proportional expansion of the assortment and enlarge of seed production volumes. There were 137 listed in the National register of the Russian Federation rape varieties in 2011, and 65% out of them were applied on behalf of 17 non-resident seed companies. There was a doubling of the listed assortment by 2017 with 232 listed entries, 72% out of them were from 24 foreign applicant. While national breeders increased the number of listed entries from 48 varieties in 2011 to 69 in 2017 (+44 %), the foreign ones did from 89 to 163 (+83 %). External applicants have abandoned conventional varieties in favor of hybrids. As a result, the share of hybrids in the National register is up till 57 % by 2017. Judging by the number of newly listed entries by 2017, such countries as Germany, the USA and France are the leaders in efficiency of rape breeding programs (Fig. 3).

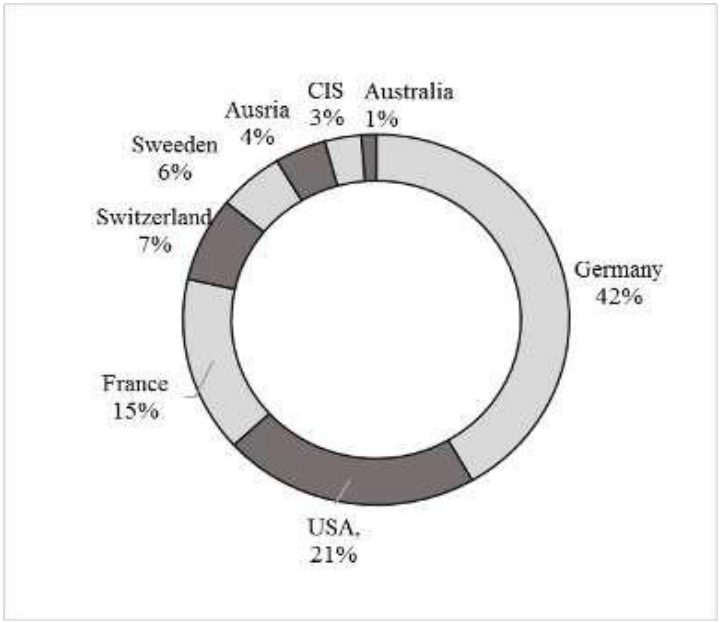

Fig. 3. Shares of listed rape entries from foreign countries, %, 2017

As for winter rape acreage, the share planted with foreign hybrids, has a significant tendency to decrease due to the improvement of local breeding programs, seed production development, as well as due to lower seed prices (Fig. 4).
However, with regard to spring rape, as well as to sunflower, the expansion of foreign genetics trend has not been overcome yet. Even in case of the sunflower hybrid multiplication by foreign seed companies is increasingly organized locally, non-resident rape seeds mainly imported. Rape seeds are multiplied and cleaned in most favorite zones for seed production. Non-resident rape seed share is estimated at 56% of spring rape acreage, 76% of winter rape, 74% of sunflower, and 26% of soybean by 2017.
Rape cultivated areas extension is accomplished with an improvement of seed product range offered in the market by breeding programs, only if the return of investment to breeding is efficient (Fedotov, Goncharov and Savenkov, 2008). Therefore, an expanded seed reproduction takes place. Western seed companies invest in hybrid breeding, but not in conventional varieties. German NPZ-Lembke domains on CIS rape seed market with market share about 40% in Ukraine, and slightly less in Russia.

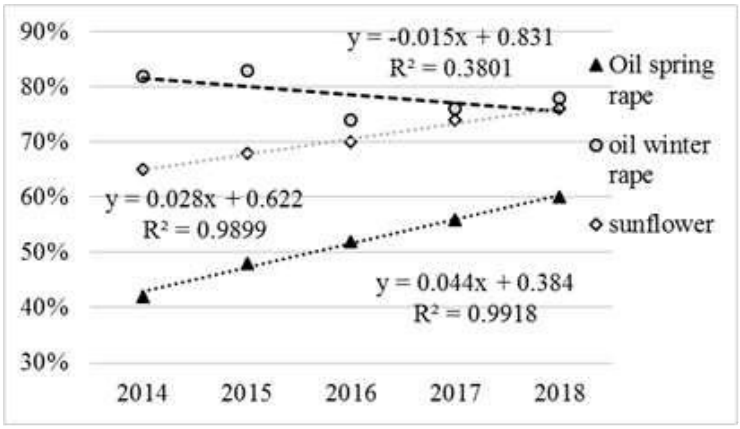

Fig. 4. Market shares of non-resident oil seeds in Russia, %

Other important non-resident players at the rape seed market are Monsanto, Pioneer, Bayer CS (that is currently completing the transaction for Monsanto acquisition), KWS, Deutsche Saatveredelung AG, Euralis semences, and others (Fig. 5). The growth of rape cultivated areas is accompanied with increase in the range of products offered to the market by breeders only in case of proper return of investment.

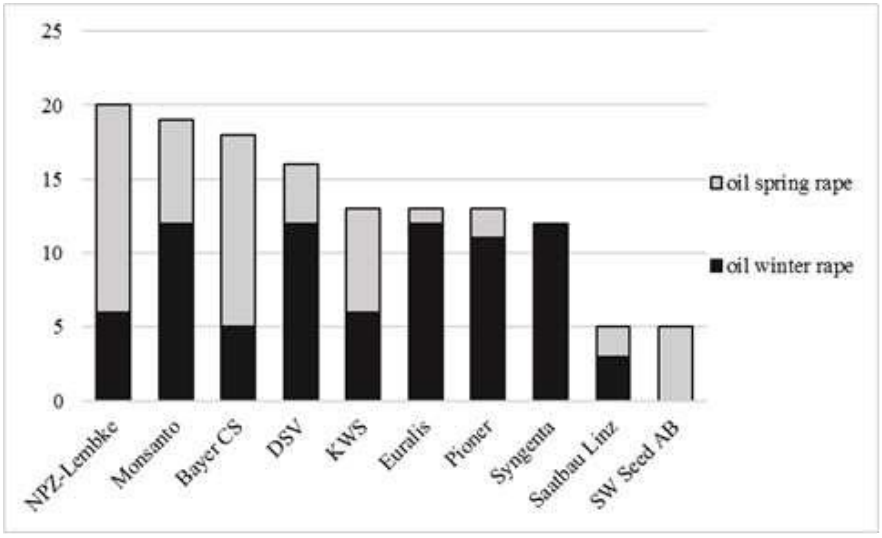

Figure 5. Top 10 foreign breeding companies, by the number of listed entries, %

The stronger competition in the seed market is evidenced by mergers and acquisitions of small seed market players by bigger ones. For example, rape breeding program of Raps GbR was purchased by Bayer CS; nowadays it is in process of selling to BASF. Rape breeding programs of German Saatzucht Diekmann GmbH & CO.KG and Polish Hodowla Roslin Strzelce SP Z O. O both were purchased by Monsanto. Syngenta also has changed the ownership from private shareholders to Chinese ChemChina State corporation.

Domestic Research and Development (R&D) organizations, which are mainly public Scientific Institutions, are funded from budgetary and non-budgetary sources in the ratio close to 50 : 50. In common they were not able to respond properly to market challenges due to their limited resources. Perhaps, it is one of the reasons for long seed life cycle, which is as 13 and 11 years for spring oil rape and winter oil rape. The threefold increase in the acreage of oilseeds after 1990 was not accompanied with a proportional expansion of breeding

programs, with range of varieties and hybrids, and with seed offer. Local All-Russia Research Institute of Oil Crops (Krasnodar), Rapeseed Institute (Lipetsk), and Institute of Feed Crops (Moscow region) have got the biggest market share registered (Fig.5).

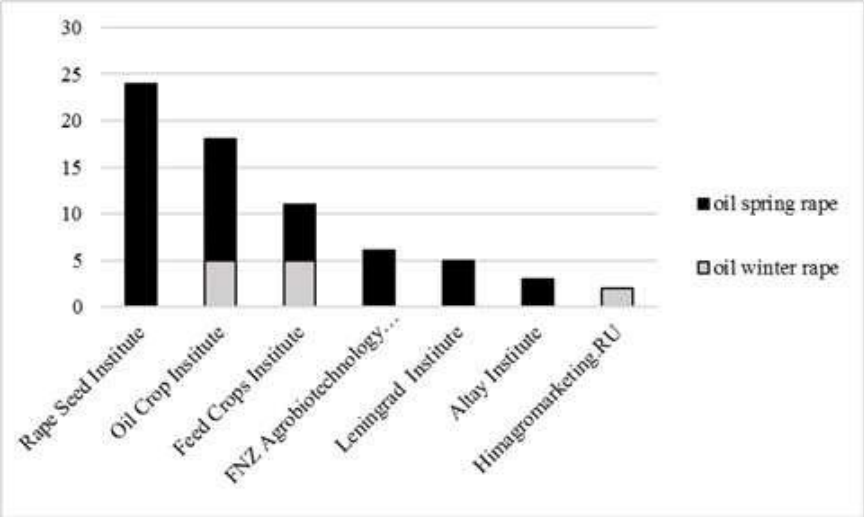

Fig. 5. Ranking of domestic breeding programs by the numbers of listed rape entries, 2017

However, due to geographic localization of rapeseed production in specific climatic zones, breeders tend to focus on breeding only spring or winter oil rape varieties (Goncharov and Gorlova, 2018). It is obvious that it is hard for domestic R&D organizations to compete with foreign seed companies, especially in the context of limited budget funding.

Such rape products with higher adding value are commercialized in this country, as CMS-hybrids, varieties with improved ratio of fatty acids (HOLL – High Oleic Low Linolenic, an oil with a high content of oleic, >70 %, and a low content of linoleic acids, <3%), oil resistance to oxidation during its shelve storage, yellow colored seeds etc. (Gorlova and Gorlov 2016; Gorlova and Bochkareva, 2017). IMI-technology (Clearfield herbicide *imidazoline* groups) in combination with resistant rape hybrids are implemented by farmers too.

Conclusion

There is strong market demand of oilseed commodity and, in particular, rape seeds in this country. Foreign seed companies (NPZ-Lembke, Monsanto, Limagrain, KWS, etc.) expand their activities in the rape seed market in Russia with support of their business models. Unsatisfactory return of investment to local breeding programs has the biggest input to their market share lost.

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EVALUATION OF GENOTYPES OF ARTICHOKE (*Cynara cardunculus* var. *scolimus* L.) AS A SOURCE OF MEDICINAL HERBS - FIRST COMMUNICATION

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Abstract

Artichoke leaf is an important medicinal raw material because it produces preparations that influence blood detoxification and improves blood counts, affect the bile and liver, lower blood cholesterol, and enhance immunity to people after long-term treatment of chronic diseases. In the artichoke plant there are: essential oil, cinarin, glycosides and flavonoids. This plant is rich in fat oils, proteins, minerals and vitamins (A, C and B - complexes). The subject of the research was 4 genotypes: Institutska, Green Globe, Violet and Cardoon. The Experiment was set up at two sites, "Radmilovac" which is an experimental field Faculty of Agriculture of the University of Belgrade (Serbia) and private estate in Batkuša village (Bosnia and Herzegovina, Entity of Republic of Srpska, municipality of Šamac). The density of crops within the plots was different: 70 cm x 10 cm, 70 cm x 20 cm, 70 cm x 30 cm and 70 cm x 40 cm. The plot was set according to the Latin square system (random block system) and three factors were examined: genotype, density of crops and localities for yield of artichoke. The following values were measured: the rosette diameter, the number of leaves in the rosette, the largest leaf area, the weight of the dry plant and the dry leaf randman. Experimental plots at both sites were set at the beginning of April 2017. The results of the first year of the experiment showed that different sites did not significantly affect the tested parameters. The density of crops statistically significantly influenced the differences between the parameters tested. The variety Cardoon proved to be the most relevant, then the variety Institutska, while the worst results were shown by Violet.

Keywords: *Variety, genotype, site, crop density, yield.*

Introduction

Cynara scolymus L. (artichoke) is an ancient herbaceous plant, originating from the Mediterranean area, which today is widely cultivated all over the world. Its flower head is eaten as a vegetable and prepared for different value-added products such as salad, jam, concentrate, and canned beverages. Artichoke was first transplanted in China during the 1990s from Italy and Spain. In China, artichoke can be used for alternative products such as tea and alcoholic beverages. Its leaves have been used for hepatoprotection and as a choleretic and diuretic in traditional European medicine since Roman times (Kirchhoff et al., 1994). In Germany, it is used today as a choleretic (Kraft, 1997) for its lipid-lowering, hepatostimulating, and appetite-stimulating actions. Cynara was a relatively small genus, originating from the Mediterranean area that included two crops, globe artichoke (*Cynara cardunculus* var. *scolymus* L.) and cardoon (*Cynara cardunculus* var. *altilis* DC), as well as their ancestor, wild cardoon (*Cynara cardunculus* var. *sylvestris* L.) as demonstrated (Foti et al., 1999; Portis et al., 2005; Ierna et al., 2010). The earliest description of the Cynara came from the 4th century BC Greek writer Theophrastus. These plants were popular with Greeks and Romans as food and in medicine (Sonnante, 2007; Lattanzio, 2009). Artichoke played an important role in human nutrition, especially in the Mediterranean area (Lattanzio et al., 2009). Furthermore, it contributed significantly to the Mediterranean agricultural economy, where more than 60% of the total world production came from Italy which was classified as

the leading producer (about 474.000 t annually) followed by Spain (215.000 t), France (55.000 t) and Greece (25.000 t) (Ierna et al., 2010). Nowadays, the artichoke is cultivated in many parts of the world, such as the United States, mainly in California, in South America (Argentina, Chile, Peru), North Africa, Near East Turkey and Iran and China (Ierna et al., 2010; Lattanzio et al., 2009; Ceccarelli et al., 2007; Pandino et al. 2011).
Artichoke's commercial production is mainly based on perennial cultivation of vegetatively propagate clones (Lattanzio et al., 2009). The artichoke crop might last six or more years, reaching the maximum productivity in the third year (Ceccarelli et al. 2007).
This plant is genetically robust with a marked tolerance to pathogens. This is due to sesquiterpenes, cynaropicrin, which have antifeedant activities. (Ceccarelli et al. 2007).

Material and Methods

Experiment was set up at two locations, from the Faculty of Agriculture "Radmilovac" and on a private estate in the village of Batkuša (Bosnia and Herzegovina, Republika Srpska, the municipality of Šamac). The density of crop within the plots was different: 70 cm x 10 cm; 70 cm x 20 cm, 70 cm x 30 cm and 70 cm x 40 cm. The dimensions of one trial are 16.5 m x 21 m. Each of the parcels had sixteen plots of dimensions 2.8 m x 4 m. The distance between each of the plots was 1 m. The plot was set according to the Latin square system and three factors were examined: genotypes (Institutska, Green Globe, Violet and Cardoon), density of crops and localities for yield of artichoke. The following values were measured: the rosette diameter, the number of leaves in the rosette, the largest leaf area, the weight of the dry plant and the dry leaf randman by separating leaf nerves from the whole leaf, and then measured on balance scale. Experiment at both sites was set at the beginning of April 2017. The first harvest from both sites was done in the first half of July 2017. The results of the research were established by the method of variance analysis, and the significance of using the Fisher LSD test. The results are presented with graphs and tables.

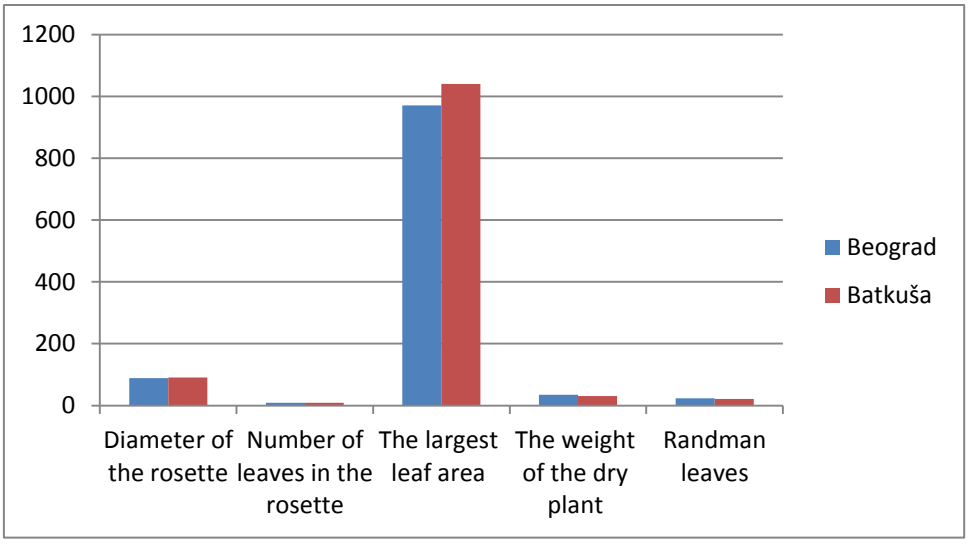
Results and Discussion

The first factor of this study is the location, the first location is Beograd and second is Batkuša. (Table 1, Graph 1). The dry parameter of the dry plant showed a statistically significant difference between Beograd and Batkuša. Better results were achieved in the location Beograd (34,369 g dry weight) than in Batkuša (30,726 g dry weight). Other parameters did not show statistically significant differences among localities.

Table 1. Results of examined parameters at different locations

Location	Diameter of the rosette (cm)	Number of leaves in the rosette	The largest leaf area (cm^2)	The weight of the dry plant (g)	Randman leaves (g)
Beograd	88,45	9,125	970,65	34,369	23,646
Batkuša	90,35	9,375	1040,40	30,726	21,496
LSD test	ns	ns	ns	*	ns

F test – LSD significance difference test; ns – not significant; * - significant; ** - very significant differences



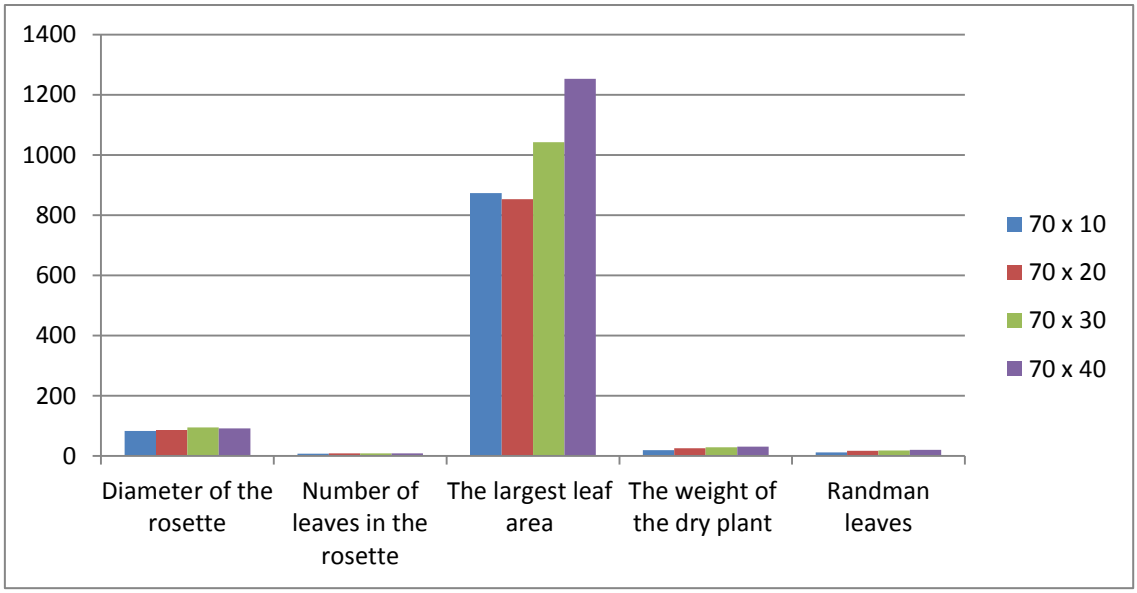
Graph. 1. Graphical presentation of the results of the tested parameters at different locations

Crop density is the second factor in this experiment (Table 2). The differences between the parameters tested were shown to be highly statistically significant. The diameter of the rosette was the largest in plants at a distance of 70 cm x 30 cm (on average 95.525 cm), while the smallest diameter of the rosette had plants that were at a distance of 70 x 10 cm (on average 83.225 cm). The number of leaves in the rosette was lower in plants in the densest assemblage (7.9), while this number was the highest in plants at a distance of 70 cm x 30 cm (9.225). The largest leaf surface in rosette was in plants at a distance of 70 x 40 cm (1252.7 cm^2), while the smallest leaf surface had plants at a distance of 70 x 20 cm (853.03 cm^2). The mass of the dry plant was the highest in plants with the greatest distance (31,371 g), while the smallest mass of dry plants was recorded in plants with the lowest spacing (19,664 g). Randman leaves were the highest in plants with the greatest distance (20,658), while the weakest randman was in the densest plant (12,407).

Table 2. Results of examined parameters in relation to different sowing density

Sowing density	Diameter of the rosette (cm)	Number of leaves in the rosette	The largest leaf area (cm^2)	The weight of the dry plant (g)	Randman leaves (g)
70 x 10	83,225	7,900	873,660	19,664	12,407
70 x 20	86,675	8,800	853,030	25,542	17,353
70 x 30	95,525	9,225	1042,800	29,614	19,006
70 x 40	92,175	9,075	1252,700	31,371	20,658
LSD test	**	**	**	**	**

F test – LSD significance difference test; ns – not significant; * - significant; ** - very significant differences



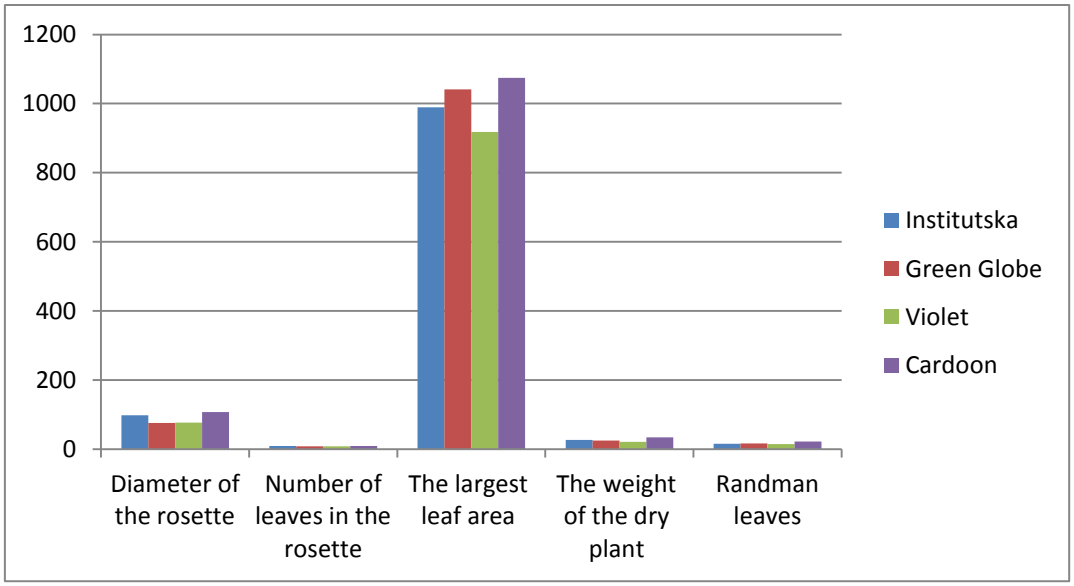
Graph 2. Tested parameters at different sowing density

The genotypes showed highly statistically significant differences in all parameters except for the largest leaf area in the rosette, where the differences are statistically significant (Table 3). The largest diameter of the rosette had a the variety Cardoon (107.15 cm) and the smallest the variety Green Globe (75.4 cm). The largest number of leaves had the Institutska (9,475) variety and the smallest variety Violet (8 sheets). The largest leaf area in the rosette was on average the highest in the tested plants of Cardoon (1074.3 cm^2) and the smallest in the Violet variety (917.49 cm^2). On average, the weight of the dry plant was the highest for Cardoon (33.758 g) and the lowest for Violet (20.725 g). Randman was in the highest ratio with Cardoon (22,207) and the weakest at Violet (14,467 g).

Table 3. Results of examined parameters in relation to different genotypes of artichoke

Genotip	Diameter of the rosette (cm)	Number of leaves in the rosette	The largest leaf area (cm^2)	The weight of the dry plant (g)	Randman leaves (g)
Institutska	98,20	9,475	988,89	26,864	16,036
Green Globe	75,40	8,325	1041,50	24,843	16,714
Violet	76,70	8,000	917,49	20,725	14,467
Cardoon	107,15	9,200	1074,30	33,758	22,207
LSD test	**	**	*	**	**

F test – LSD significance difference test; ns – not significant; * - significant; ** - very significant differences



Graph 3. Graphical presentation of the results of the tested parameters at different genotypes of artichoke

Conclusion

The results of the first experimental year showed that different locations did not significantly affect the parameters tested. Sowing sites showed statistically significant differences. The thicker sowing proved to be worse for all the tested parameters. The variety Cardoon proved to be the best, then the variety of the Institutska, while the worst results were shown by Violet.The density of crops proved to be more cost-effective in the reed assembly. The variety Cardoon had on average the most extensive rosette, the largest surface of the largest leaf in rosette, the largest mass of dry plants and the best randman leaves. Variety Violet was the worst for all parameters except for the diameter of the rosette where the worst results were given by the Green Globe variety. Varieties of the Institutska and Green Globe are approximately uniform in terms of the mass of the dry plant and the randman of the leaves. Preliminary results indicate that the genotype shows the most significant differences in leaf yield. Spacing of sowing shows that a weaker yield in a denser structure could be assumed, since artichoke is a robust plant that requires a large living space. Locations did not show statistically significant differences, which supports the claim that genotype differences are key to yield. So far, it has been established that the most promising variety is Cardoon, which can be successfully cultivated in our agroecological conditions. The largest leaf yield is achieved by sowing at a distance of 70 cm x 30 cm.

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YIELD COMPONENTS AND SEED YIELD OF FOUR RED CLOVER GENOTYPES FROM SOUTHEAST EUROPE

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Abstract

The aim of the study was to analyze the most important yield components and seed yield of selected red clover (*Trifolium pratense* L.) genotypes from Serbia and Bulgaria. Positive traits of individual genotypes could be the use in hybridization and the creation of new varieties, which, in addition to high forage yield potential would have a higher seed yield. The experiment was established in 2012 in Čačak on leached vertisol soil type with acid reaction (pH_{H2O} 4.8). Three genotypes isolated from the local populations and variety of K-39 were planted using a randomized block design with five repetitions, at a distance of 70x40 cm. Weed control was done mechanically. For the analysis of the yield and yield components was used the second cut in the second year of cultivation when usually done the production of seeds. The genotypes differed among themselves in each other's properties, except in a thousand seeds weight. The highest seed yield was achieved variety K-39 (19.8 g plant^{-1}), while the yield of other genotypes varied from 4.4 to 8.6 g plant^{-1}. Also, this variety had the highest values for most yield components. However, the genotype 3, which was isolated from the populations of Rozova dolina, had a significantly higher number of flowers per inflorescence (124.1), compared to the K-39 variety (89.4). The results suggest that certain genotypes could be used in further selection work in order to increase seed yield at already existing culivars.

Key words: *genotypes, red clover, seed yield, seed yield components*

Introduction

Red clover (*Trifolium pretense* L.) is one of the most important legumes for the production of quality forage. It grows in wild forms in meadows throughout Europe and Asia. Red clover seeds yield is mainly determined by the genetic basis of the variety, the ecological conditions of the area, the time of mowing, the presence of pollinator insects, as well as the interaction of the genotype and environment (Steiner et al., 1995). The high variability and genetic plasticity of the species are the result of the extremely xenogamous character of fertilisation and entomophilous pollination (Taylor and Smith, 1979). Due to the high variability and adaptability to the various environmental conditions, natural selection has produced a large number of local ecotypes, superior to the given growing conditions (Helgadottir, 1996).

Numerous studies confirm the advantage of local red clover's ecotypes in relation to materials from other regions, because acclimatized ecotypes achieve higher yields of forage and seeds than weaker acclimatized populations and varieties. Therefore, the choice of domestic varieties of perennial legumes, which are selected in the existing conditions, provides higher and more stable yields during their period of exploitation (Radović et al., 2004). Otherwise, a careful selection of red clover varieties can be one of the most important factors affecting yield, regeneration and potential profit (Drobna, 2009).

The aim of the study was to analyze seed yield and seed yield components of selected red clover genotypes in the area of Serbia and Bulgaria, as well as to select genotypes with higher

values for individual yield components. Such genotypes would represent a good basis for further work on hybridization and the creation of varieties that, in addition to the high potential for forage yield, would also have a higher seed yield.

Materials and methods

A field trial was conducted at the Veterinary Extension Service in Čačak (43°54'39.06" N, 20°19'10.21" E, 243 m a.s.l.) in 2012. The experiment was established on a leached vertisol (pH 4.8), which contained 3.18% organic matter, 0% $CaCO_3$, 22.08 mg extractable P 100 g^{-1} soil and 30.0 mg K 100 g^{-1} soil. Prior to seeding and in autumn, 45 kg ha^{-1} N, 45 kg ha^{-1} P_2O_5, and 45 kg ha^{-1} K_2O were incorporated into the soil.

One factorial experiment was set up according to a completely randomised block design in five replications (with 20 plants per plot at a plant spacing of 70x40cm). A total of four red clover genotypes, including the cultivar K-39 was used. Beside the K-39 cultivar (G_1), two genotypes were extracted from local populations from Bulgaria, Petrič (G_2) and Rozova dolina (G_3), as well as one genotype (G_4) isolated from the local populations in Serbia, the Municipality of Aleksandrovac (Central Serbia). Weeds were controlled mechanically. No irrigation was employed.

The mean annual air temperature in 2012 and 2013 was 13.12°C and 12.99° respectively and amount of annual rainfall 463.5 mm and 582.7 mm respectively. The average annual air temperature for the multi-year period (1992-2002) is 11.97°C, and the average amount of annual rainfall 680.3 mm.

The second growth in the second year (2013) was evaluated under field conditions for the following: stem number per plant, inflorescence number per stem, and inflorescence number per plant, using a sample of five plants per plot. Laboratory evaluation included determination of: flower number per inflorescence and seed number per inflorescence (using ten randomly selected inflorescences). Fertility (ratio between seed number and total flower number per inflorescence) and thousand seed weight (based on the seeds extracted from the same sample) are calculated. Seed yield components (inflorescence number per plant, seed number per inflorescence, thousand seed weight) were used to determine seed yield per plant which was calculated as g $plant^{-1}$.

The obtained results were subjected to a one-factor analysis of variance using SPSS software (1995). The significance of differences between mean values was tested by LSD test.

Results and discussion

The average number of stems per plant in this experiment was 37.7. The G-3 had the highest number of stems per plant, significantly higher than G-2 and G-4, while in relation to the cultivar K-39 it did not differ significantly (Table 1).

The significantly higher number of inflorescences per stem after had the K-39 variety as compared to G-4. The average number of inflorescences per stem was 5.84, and ranged from 4.0 to 7.44. Vasiljević et al. (2010) state that the average number of inflorescences per stem at the cultivar Una was 6.7, in the conditions of rare sowing (60 cm), which is significantly higher in relation to the plantation at a smaller inter-row spacing. A lower number of inflorescences per stem in this experiment is due to poor conditions of growth, first of all in terms of soil.

Thanks to the higher number of stems per plant and the number of inflorescences per stem, cultivar K-39 and G-3 had significantly higher number of inflorescences per plant compared to G-4. The average number of inflorescences per plant was 216.5. According to Montardo et al. (2003), the number of inflorescences per plant is one of the main components of the yield that needs pay attention in selecting red clover for a higher yield of seeds. Herrmann et al.

(2006) point out that the number of inflorescences per plant is the primary component on which seed yield depends.

The largest number of flowers per inflorescence, significantly higher than all other genotypes, had G3. The average number of flowers per inflorescence was 93.9, and it ranged in the interval from 78-124.1. However, G-3, contrary to expectations, did not have the largest number of seeds per inflorescence. The number of seeds per inflorescence in this genotype was significantly lower in relation to the K-39. The reason for this is significantly lower fertility of flowers in G-3 compared to K-39. The cultivar K-39 had a significantly higher fertility of flower, also in relation to the G-2. Oliva et al. (1994) state that the average number of flowers per inflorescence in the Kenland cultivar ranged from 101 to 142. According to Julen (1956) and Miladinović (1978), between red clover genotypes, there are large differences in the terms of number of flowers per inflorescence.

The average number of seeds per inflorescence in this experiment was 30.3. The average number of seeds per inflorescence at the variety Una at the inter-row spacing of 60 cm, according to Đukić et al. (2010) was 105.9. Wilczek and Cwintal (2008) state that the number of seeds per inflorescence at the variety Parada ranged from 61 to 74. The same authors claim that the number of seeds per inflorescence and the fertility of flowers are the most important seed yield components of red clover. Jevtic et al. (2007) and Wilczek and Ćwintal (2008) also show that the presence of insect pollinators has a major impact on the number of seeds per inflorescence. A smaller number of seeds per inflorescence in this experiment is also the result of poorer conditions for growing both in terms of soil and in terms of the lack of precipitation during the vegetation period. This is confirmed by Wilczek and Ćwintal (2008), according to which the fertility of red clover flowers varied from 51.2 to 69.8% in different years, and large amounts of rainfall during flowering can significantly reduce fertility and harvest yield in relation to the potential seed yield.

Table 1. Seed yield and seed yield components of the red clover genotypes

	SP	IS	IP	FI	SI	TSW	F	SP
G 1 K-39	42,8 ab	7,44 a	308 a	89,4 b	39,8 a	1,525	44,9 a	19,8 a
G 2 Petrič	32,0 b	6,34 ab	197 ab	84,1 b	25,1 b	1,318	29,8 bc	6,4 b
G 3 Rozova d.	52,4 a	5,58 ab	268 a	124,1 a	27,5 b	1,494	22,3 c	8,6 b
G4 C. Serbia	23,6 b	4,0 b	93 b	78,0 b	28,7 ab	1,584	36,8 ab	4,4 b
ANOVA 0,05	*	*	**	*	*	ns	**	*

SP – stems per plant, IS – inflorescences per stem, IP – inflorescences per plant , FI – flowers per inflorescence, SI – seeds per inflorescence, TSW – thousand seed weight (g), F - fertility (%), SP – seed yield per plant (g); Values followed by different small letters within columns are significantly different ($P<0.05$) according to the LSD test; **F test significant at $P\leq0.01$; *F test significant at $P\leq0.05$; ns – non-significant

In terms of the thousand seed weight, genotypes did not differ significantly among themselves. The average thousand seed weight was 1.48g.

The largest seed yield per plant as compared to the all other genotypes had K-39 (19.8 g plant^{-1}), while other genotypes did not differ significantly among themselves (their average yield was 6.5 g plant^{-1}). Herrmann et al. (2006) state that the average seed yield per plant in two genotypes of red clover was 5.72 g, with the variation interval of 0.71-11.31g. According to Vasiljević et al. (2000), the coefficients of genetic correlation show that seed yield per plant mostly depended on the number of seeds per inflorescence, the number of flowers per inflorescence and the number of productive stems per plant. The authors also claim that there are significant positive genetic correlations between the number of inflorescence and the

number of internodes per stem and yield of green matter, as confirmed by Steiner and Alderman (2003). Iannucci and Martinello (1998) came to the same conclusion, tracking three populations of the Mediterranean clover. The obtained results suggest that the simultaneous selection to the green forage yield and the seed yield is justified.

The obtained results indicate that the variety K-39 still had the highest seed yield in relation to the genotypes collected from natural populations. However, some genotypes had higher values for individual yield components than the K-39. In particular, the G-3 was highlighted here for the number of flowers per inflorescence. This indicates that in the existing cultivars, it is possible to continue to work on increasing the values for individual yield components in order to increase seed yield.

Conclusion

The red clover genotypes differed significantly among themselves in terms of all seed yield components except for the thousand seed weight. The largest seed yield had K-39 variety (19.8 g per plant). However, some genotypes had higher values for individual components of yield in relation to the cultivar K-39. G-3 here can be mentioned for the number of flowers per inflorescence. The obtained results indicate that with using red clover genotypes from natural populations, it is possible to work further on the increase of values for individual yield components, and thus the total seed yield per plant and per unit area.

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ESTIMATION OF GENETIC DIVERSITY AMONG MAIZE INBRED LINES

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Abstract

One of the most effective method in maize (*Zea mays* L) selection of adapted material is to create synthetic populations from inbred lines of known origin and superior properties. The methods used for inter- and intra-population synthetic improvement are some of the recurrent selection techniques. Success of recurrent selection depends on the choice of parent components and the method that will be used to obtain new inbred lines. The aim of this paper was to apply molecular markers for estimation of genetic variability of inbred lines, as potential parent components of synthetic populations. Molecular characterization of 26 inbred lines was done with 18 polymorphic SSR (*Simple Sequence Repeat*) markers. The total number of obtained alleles was 54, and ranged from two alleles for primers: phi033, phi036, phi087 and umc1013 to five alleles for primer umc1040. Genetic similarity values were calculated using Dice coefficient in the NTSYSpc2 program package. The highest similarity value (0.96) was calculated between inbred lines L22 and L24, while the lowest value (0.26) was between inbred lines L7 and L21. Cluster analysis divided the inbred lines into three groups mostly in accordance with their origin. The variability detected using SSR markers could be useful in selecting best parental combinations in creating synthetic populations.

Keywords: *inbred lines, synthetic population, SSR markers.*

Introduction

Maize is open-pollinated crop species with huge genetic diversity, containing wide variation in morphological traits and large-scale polymorphism on DNA level (Matsuoka *et al*., 2002). Long-term selection as well as development of modern maize hybrids have led to narrowing of the genetic variability of maize populations, i.e. to reducing the pre-conditions for the expression of heterosis. On the other hand, practical application of heterosis has been eliminated significance of the variety production and led to the genetic uniformity of the commercial material. Maize breeders are under constant pressure to create new commercial inbred lines, so they increasingly use F2 populations, reverse crossings and synthetic populations of narrow genetic bases in their programs, which has led to a reduction in the use of local populations as a carrier of high genetic variability (Ho *et al.*, 2005). Maize breeders recognized the importance of the genetic variability of the starting material for the expression of heterosis in the F1 generation. Molecular studies have confirmed that genetic divergence is positively correlated with heterosis (Ajmoone Marsan *et al*., 1998). For the successful program of creating synthetic populations as the source of new inbred lines besides choosing the selection method, the choice of parental components is very important. In order to study the genetic variability of the breeding material, divergence and belonging to heterotic groups, breeders can use data on the origin of the lines, test methods or genetic markers. Recently, in short-term selection programs, F2 generations and narrow base synthetic populations of narrow genetic bases are mainly used as a source of new lines. In contrast, long-term programs use synthetic populations and composites. It is very important to choose the right selection method of working with synthetic populations and parental components for their formation.

The use of molecular markers in diversity estimation and genetic structure is of great help when starting a selection program, creating synthetic populations as a working material and choosing the parents for that purpose. Due to the rapid developments in the field of molecular genetics, various types of techniques are used to estimate genetic diversity (Spooner *et al*., 2005, Semagn *et al.*, 2012, Dao *et al.*, 2014). *Simple sequence repeats* (SSRs) markers provides many advantages that make it especially applicable in studies of diversity and relationships, such as independence from environmental and pleiotropic effects, co-dominant inheritance, high reproducibility, locus specificity and their random distribution across the genome (Morgante *et al*., 2002; Barcaccia *et al*., 2006). The advantages of DNA markers in diversity estimation do not diminish the significance of phenotypic traits, on the contrary, morphological data provide practical information in characterisation of breeding material. In the past decade due to their high informativeness and reproducibility SSR markers become quite useful and frequently used PCR based markers in maize diversity studies (Dubreuil *et al*., 2006; Sharma *et al*., 2010). The objectives of this study were assessment of genetic diversity and relationships of inbred lines, as potential parent components of synthetic populations.

Materials and Methods

A set of 26 maize inbred lines from Maize Research Institute "Zemun Polje", which belongs to diferent heterotic groups and with different genetic background, was analyzed using molecular markers. Genomic DNA for each sample was isolated from seed bulk using the CTAB (cetyl trimethylammonium bromide) procedure according to Doyle and Doyle (1987). Molecular characterization was done with 18 polymorphic SSR markers (Table 1) from the maize germplasm bank (www.maizegdb.org). Polymerase chain reaction (PCR) was carried out in 25μL reaction volume containing: 50 ng of DNA sample, 1xBuffer, 0.8 mM dNTP, 0.5 μM of each primer pair and 1U *Taq* polymerase. The PCRs were performed in Thermocycler Biometra TProfessional Standard 96 using the following touch-down program: an initial denaturation at 95ºC/5min. by 15 cycles each of denaturation at 95ºC/30 s, annealing at 63.5/1min (-0.5ºC/cycle) and extension at 72 ºC /1min; and another 22 cycles of 95ºC/30 s, 56ºC/1min and 72ºC/1min. Final elongation was at 72°C for 4 min. The amplified fragments were separated by vertical electrophoresis (Mini Protean Tetra-Cell BioRad) on 8% polyacrylamide gel, with 100bp ladder as a marker. After staining with 0.5 μg/μL ethidium bromide gels were photographed under UV light on BioDocAnalyse Biometra. SSR profiles were scored as presence/absence of fragments in each sample and the data were assembled into a binary matrix. Genetic similarities between inbred lines were calculated by Dice (1945) and Unweighted Pair Group Method with Arithmetic mean (UPGMA) method was applied for cluster analysis. Statistical NTSYSpc2 program package (Rohlf FJ, 2000) was applied for marker data analyses.

Results and Discussion

Variability of initial genetic material is an important precondition for breeding. One way to increase the genetic variability of the breeding material is to create synthetic populations with a broad genetic base and to further apply the appropriate selection method. To determine the genetic similarity between 26 inbred lines, 18 SSR markers were used. Out of the 22 SSRs, four were not included in data analyses due to absence of amplification product (two primers) and poor amplification (two primers). Total of 54 alleles among the analysed 26 lines were identified. The number of alleles richness varied from two (phi033, phi036, phi087 and umc1013) to five (umc1040) with the average value of 3.15 per locus (Table 1). That is similar to previous study where Bantte and Prasanna (2003) reported 3.25 alleles using 36 SSR loci. Some other investigations have shown considerably higher number of alleles. Lu

and Bernardo (2001) obtained 4.9 alleles with 83 SSR loci, same as Warburton *et al*. (2002) with 85 SSR loci.

Table 1. List of 18 informative primers, with their chromosome position, repeat motif, number of alleles and allele range within analyzed inbred lines

	Probe	Bin	Repeat motif	Number of alleles	Allele range (bp)
1.	umc1013	1.08	(GA)9	2	120-160
2.	umc2047	1.09	(GACT)4	4	120-300
3.	umc1265	2.02	(TCAC)4	3	100-120
4.	umc2129	2.07	(CGC)5	3	90-110
5.	phi036	3.04	AG	2	60-80
6.	umc1350	3.08	(AG)13	3	140-160
7.	umc1418	4.08	(GGAAG)4	3	145-280
8.	umc1109	4.10	(ACG)4	4	100-120
9.	bnlg557	5.03	-	3	100-120
10.	phi087	5.06	(ACC)	2	150-180
11.	phi075	6.00	CT	3	200-220
12.	umc1006	6.02	(GA)19	3	100-120
13.	umc1015	7.03	(GA)45	3	90-110
14.	umc1782	7.04	(GAC)4	3	120-140
15.	bnlg2235	8.02	AG(23)	3	160-200
16.	phi033	9.01	AAG	2	220-240
17.	umc1040	9.01	CT11	5	80-200
18.	umc1492	9.04	(GCT)4	3	130-160

Genetic similarity (GS) values calculated by Dice (1945) between the pairs of all 26 inbred lines ranged from 0.26 (L7 and L21) to 0.96 (L22 and L24), with an average 0.55. The high value of genetic similarity between L22 and L24 can be explained by the same base population derivation. The largest part (53.5%) of obtained GS values was between 0.41 and 0.6 (Figure 1). The estimated average genetic similarity shows relatively high level of diversity among the analysed genotypes.

This study showed 9.8% of pairwise comparisions with GS values of GS greater than 0.7, making them a valuable source in creating synthetic populations. Beside genetic similarity of potential perental lines, their affiliation to heterotic groups should be considered.

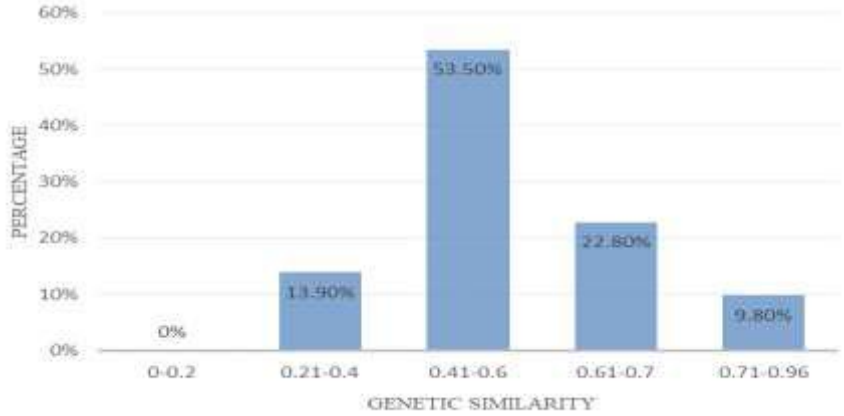

Figure 1. Dispersion og pairwise Dice's genetic similarity values of 26 maize inbred lines obtained from SSR data

The cluster analysis based on Dice′s genetic similarities distributed 26 inbred lines into three clusters (Figure 2) mostly in accordance with their origin. Cluster „A" was consisted of only two inbred lines (L1 and L19) with diferent background, quality protein maize (QPM) line with unknown genetic origin and Lancaster Sure Crop (LSC). QPM lines had unknown genetic orgin, so another goal was to classified them into appropriate heterotic group.
Out of the 26 inbred lines in total, twelve lines were grouped in cluster „B". The related lines belong to QPM lines (L2, L3, L4, L5 and L6), BSSS background mixed with other germplasm (L7, L9, L10, L11 and L25) and mixed local genetic basis and BSSS background (L13 and L18).

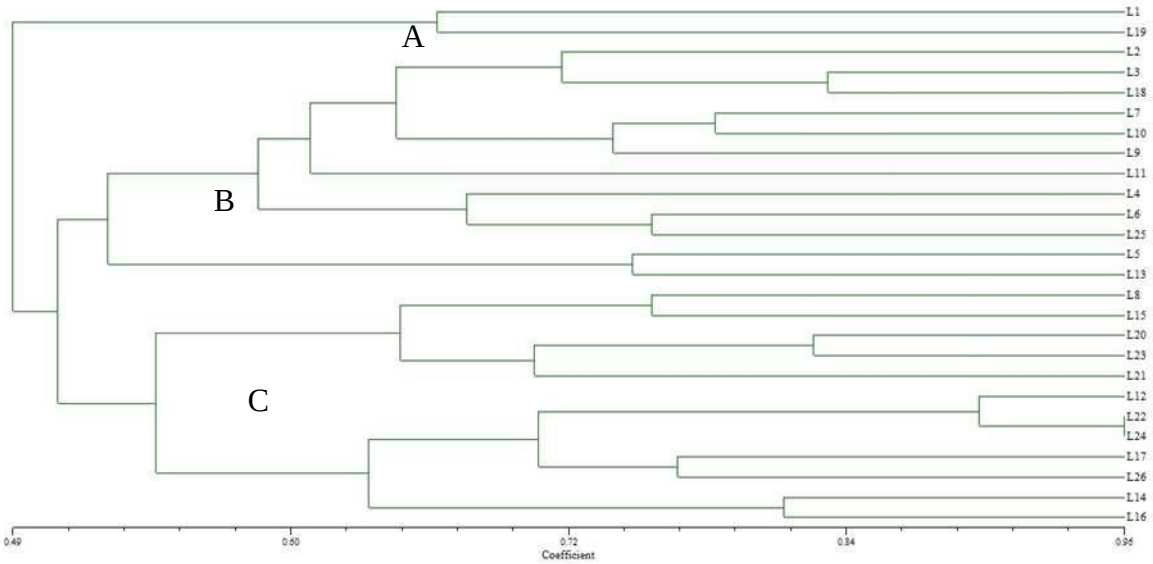

Figure 2. Dendrogram of 26 maize inbred lines constructed using UPGMA cluster analysis of genetic similarity values (Dice, 1945) obtained from SSR data
Remaining 12 genotypes formed cluster „C", which includes eight lines that belong to LSC germplasm (L12, L17, L20, L21, L22, L23, L24 and L26), three lines to BSSS (L8, L14 and L15) and one to local genetic basis (L16).
Using a small number of markers, it may be difficult to clearly evaluate the heterotic grouping due to the absence of some key loci for the differentiation of heterotic groups, indicating the use of larger number of markers.

Conclusions

Molecular marker analysis assigned the most of the inbred lines to their genetic background, substantiate them significant in evaluation of maize diversity. The variability detected using SSR markers could be useful in selecting best parental combinations in creating synthetic populations and new elite lines through breeding programs.

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SOME MORPHOLOGICAL AND PRODUCTIVE TRAITS OF WINTER TRITICALE DEPENDING ON VARIETY AND METEOROLOGICAL CONDITIONS

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Abstract

Thanks to the high genetic potential for yield and favorable nutritional values, triticale is ranked among the perspective plant species.High and stable yields can be achieved under favorable weather conditions and with adequate agro-technology and variety selection.The paper presents the results of examining some morphological and productive traits of winter varieties of triticale cultivated in the north of Montenegro during the two vegetation season (2009/11). The experiment placed on a random block system in three repetitions, included five varieties of winter triticale(Odisey, Kg-20, Triumph, Rtanj and Tango). The applied agro-technology in the production of triticale was standard, with the application of NPK fertilizer combination 120:80:80. The following parameters were observed: height of plants, spike length, number of grains per spike, 1000 grain mass and grain yield of triticale. The results of the study showed significant differences in terms of the morphological and productive traits of winter triticale depending on the variety and weather conditions in the years of research. Variety Tango in both years achieved the highest yield (6841.6 kg ha^{-1}), while the lowest yield was recorded in Kg-20 variety (5216.6 kg ha^{-1}).Also, the variety Tango stood out significantly higher values for most other monitored parameters in both study years. The obtained results indicated that differences in the yields of the cultivars included in the research were the result of varietal specificity, which was mostly genetically conditioned.

Key words: *triticale, variety, morphological traits, productive traits*

Introduction

Triticale (Triticosecale Wittmack), a hybrid created by crossing wheat and rye, was designed with the idea to obtain a cereal, which combines good quality grain yield from wheat parents with tolerance to abiotic and biotic sress (Villegas et al., 2010). Ugarte et al, 2007; Estrada-Campuzano et al., 2008. reported that triticale is an interesting crop for unfavourable conditions where productivity of common crops is more or less limited. Modern triticale cultivars show higher yields and superior adaptation to soil quality and environments than wheat (Erekul and Köhn, 2006; Ugarte et al, 2007; Estrada-Campuzano et al., 2012). Varieties that are able to give high and stable yields in certain conditions have the highest value for production. Therefore, one of the most important tasks of selection and seed production consists of the fact that the proper sortsvarieties by natural ecological regions, taking into account the best adaptation to local conditions of each variety. The basic principle for determining the adaptation of varieties given conditions can be normal growth and development that ensures obtaining high and stable yields per year (Đurić N. et al., 2010).

The quality of triticale depends on the genotype and environmental conditions of their interaction and can be improved by applying nitrogen fertilizers. Biberdžić et al., 2013, point out that the yield was conditioned by the effect of a large number of factors which are most affected by cultivar, cultural practices and weather conditions during the experiment. Also, the same authors state that variety and agroecological conditions have a significant influence as well on the morphological characteristics of triticale.

The goal of our researh was to determine some morphological and productive characteristics of triticale in agroclimatic conditions of north Montenegro, depending on the tested variety.

Material and methods

In the experiment which was carried out in the period 2009-2011, in the north of Montenegro (vicinity of Bijelo Polje ,43º 01' 45" north latitude and 19º 44' 44" east longitude) on the alluvial type of soil, five cultivars of winter triticale were tested (Odysseus, Kg-20, Triumph, Rtanj and Tango).

The experiment was set out in randomized block system with three replications and experimental plot size of 6 m^2. Common technology for the production of triticale was used in the experiment. Sowing was carried out by manual method in optimal term (October). The other technology of production which was applied in the experiment was standard, with using of NPK fertilizer, combination 120:80:80. Phosphorus and potassium were used in equal amounts (80 kg ha^{-1}) before the sowing period, while the ½ of nitrogen was used before the sowing period and the rest of the planned amount was used as a fertilization at the end of March. Triticale harvest was performed in the stage of full maturity. At the same time plant height, spike's lenght, number of grains per spike, 1000 grain mass, hectoliter weight and grain yield were determined. The yield was adjusted to 14% moisture. The obtained results were statistically processed using method of variance analysis, whereby the significance of the average treatments were tested with LSD test, with significance threshold of 1 and 5%.

Soil and climatic conditions

The soil on which experiment was performed belongs to the alluvial type of soil. This soil is weakly calcareous, with the content of carbonate (2,4-2,44%) Based on pH value in saline extract the tested soil is of acid-based reaction (pH KCl 5,01-4,94). The soil is quite humic: 3,35-3,96 % with low phosphorus (5,12 – 4,24 mg/100g soil) and potassium content (7,5-3,8 mg/100 soil).

Table 1. Average monthly air temperature and precipitation amount (Podgorica Weather Bureau)

Year	Months										Sum
	X	XI	XII	I	II	III	IV	V	VI	VII	
Monthly rainfall (mm)											
2009-10	135	94	94	101	80	70	78	80	63	86	**881**
2010-11	65	131	147	36	76	31	46	121	33	79	**765**
1961-90	80	115	91	87	68	60	70	76	72	64	**783**
Average monthly temperatures (°C)									**Average**		
2009-10	9.77	5.95	4.06	1.31	2.4	6.39	10.93	15	18.11	20.95	**9.5**
2010-11	10.12	8.54	2.05	-0.65	0.94	6.03	10.54	14.5	18.9	21.23	**9.2**
1961-90	9.4	4.7	0.2	-1.3	0.7	4.9	9.0	13.3	16.3	17.9	**7.5**

Meteorological conditions, especially temperature and precipitation, are the main non-genetic factors that determine the success of cultivation of winter triticale and other small grains. In the growing season 2009/10 there was 881 mm of rainfall which is 116 mm more than in the second year of the research. Also, in October and November 2009/10 there was 229 mm of rainfall, which is 33 mm more than in the same period of 2010/11. From the data in the table it can be noted that the amount of rainfall in the period from April to June in the first year of research was higher compared to the second year of research. Considering that the amount of rainfall and temperature in these months are extremely important for development of small grains, the first year of research can be characterized as better in terms of weather conditions for growing triticale in this area.

Results and discussions

Morphological characteristics are mostly sort characteristics, although those are highly dependable of production conditions and practices applied (Biberdžić et al., 2013). The

following table shows some morphological and productive traits of winter triticale in regard to variety and climatic conditions in tested years.

Table 2. Some morphological and productive traits of winter triticale varieties

Cultivar (A)	**Winter triticale properties (2009/10)**				
	Plant height (cm)	Spike length (cm)	Number of grains per spike	1000 grain mass (g)	Grain yield kg ha^{-1}
Odisej	119.7	10.3	30.1	45.7	6200.0
Kg-20	116.6	8.2	30.4	33.0	5700.0
Trijumf	114.3	8.5	28.1	44.7	6495.0
Rtanj	129.3	10.5	33.1	43.5	6135.0
Tango	133.3	10.7	41.6	48.7	7140.0
Average B	**122.6**	**9.6**	**32.7**	**43.1**	**6334.0**
LSD 0,05	**1.394**	**0.276**	**0.903**	**0.689**	**82.564**
0,01	**1.966**	**0.392**	**1.281**	**0.978**	**117.092**
Cultivar (A)	**Winter triticale properties (2010/11)**				
	Plant height (cm)	Spike length (cm)	Number of grains per spike	1000 grain mass (g)	Grain yield kg ha^{-1}
Odisej	130.0	9.0	31.5	42.2	5440.0
Kg-20	114.0	8.5	30.0	34.3	4733.3
Trijumf	109.0	8.5	26.9	42.3	5740.0
Rtanj	124.0	8.8	33.9	45.8	6130.0
Tango	125.0	9.3	31.0	48.4	6543.3
Average B	**120.4**	**8.8**	**30.7**	**42.6**	**5717.3**
LSD 0,05	**2.013**	**0.209**	**1.148**	**0.899**	**75.791**
0,01	**2.855**	**0.296**	**1.638**	**1.264**	**108.485**

The plant height of the triticale in our study was influenced by genotype and weather conditions during the growing period through the years of research.

An average plant´s height in the first year of investigation was 122.6 cm, in the second year was 120.4 cm. The average two-year values show that the Tango variety had a significantly higher stem height compared to other varieties included in the research.

Variation of plant height by varieties and years is partly the result of varietal specificities, i.e. genetic differences and part of the agroecological conditions of production. Favorable weather conditions during the winter months and spring part of the vegetation in the first year of the study caused a more intensive increase and a higher height of the stem compared with the height of the stem in the second year of the study. These results are in agreement with the results of Stošović (2009) and Ugrenović (2013) who emphasize that the weather conditions during the vegetation of the grain crops and therefore, the triticale, significantly influence the height of the plants, as well as the overall dynamics of growth and development.

In addition to the important role of the spike as a direct holder of the yield and its participation in the process of photosynthesis is also significant that in his position it least contributes to the shading of the lower parts of the plant. Spike′s length was determined by genotype in both years of research. The data in Tab.2 show that the greatest spike length, in both years of research, was in Tango variety. The Kg-20 and Triumph varieties had significantly shorter spike length than other tested varieties. Starting from the fact that the spike length is considered as an important factor of the fertility, the Tango variety proved to be the best from the aspect of achieving the highest yield in these agroecological conditions. Number of grains per spike represents quite a trait that is largely dependent on agro-ecological conditions, and cultural practices. The results show the existence of highly significant differences in the number of grains per spike among the tested varieties. The largest number of grains per spike was found in the Tango variety (41.6) in the first year of the study, and the smallest in the Triumph variety (26.9) in the second year of the study.

According to the Wiegand and Cuellar (1981) number of spikes and number of grains per spike are very important for yield which needs good weather conditions in different growth stages. 1000 grain mass is an important indicator of physical quality of grains. This is one of the properties that directly affect the grain yield. In average, the first year of investigation achieved higher value of 1000 grain mass than the second year, but there were no statistically significant differences. Also, the results of our research showed that there are significant differences in the values of 1000 grain mass among the investigated genotypes, which is in accordance with Jelic et al. (2002) who pointed out that the mass of 1000 grains is a varietal trait.

Grain yield is a complex feature of polygenic mode of inheritance. It is the resultant of the interaction of the variety, the applied agro-techniques and growing conditions throughout the entire life cycle. An average yield for all of five investigated varieties in the first year was 6334.0 kg ha^{-1}, which represents the difference of 616.7 kg ha^{-1} compared to the second year of studies. The differences in triticale grain yield between the experimental years resulted from the weather conditions. This finding was confirmed by the studies from other researches (Janušauskaitė, 2009, 2013). In our study, we also found significant influence of year on grain yield. Pecio (2010) reported that the highest grain yield was possible due to favorable moisture conditions during the whole growing period and smaller productivity of triticale resulted from worse moisture conditions. In both years of investigation variety Tango had significantly higher grain yield than on other varieties, while the lowest grain yield was found in variety Kg-20.

Conclusions

According to the results of the study the influence of the variety and climatic conditions on the morphological and productive traits of winter triticale, the following can be concluded:

- The plant height was conditioned by the variety, but also by the weather conditions during the vegetation period. The average values show that the Tango variety has achieved a significantly higher stem height in the two-year testing period.
- Favorable weather conditions in the first year of the study caused a significantly large difference in the average value of the spike length compared to the second year. The variety Tango had the highest spike length in both years of the investigation;
- The highest average number of grains per spike (36.3) was in the Tango variety, while on average the least number of grains per spike had the Triumph variety (27.5);
- The average weight of 1000 grains was higher in the first compared to the second year of testing;
- In the two years of the research, the Tango variety had a significantly higher value of the 1000 grain mass in comparison with other tested varieties;
- The average grain yield in the two-year trial was the highest in the Tango variety (6841.6 kg ha^{-1}), while the lowest average grain yield was recorded in the Kg-20 variety (5216.6 kg ha^{-1});
- The Average grain yield was higher in 2009 / 10th year compared to experimental 2010 / 11th year.
- According to all parameters, Tango variety was better than other varieties which were included in the testing. Tango variety is recommended for growing in this area.

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FROM THE GREGOR MENDEL'S GARDEN TO A MOLECULAR MARKER LAB: CUTTING EDGE OF BREEDING GRAIN AND FORAGE CRUCIFERS IN SERBIA

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Abstract

A complex and strategically structured research on oil-rich grain, vegetable and forage crucifers at the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS) is based upon maintaining and sustainably utilising the germplasm collections of each crop. It comprises conventional and molecular breeding, biotechnology, agroecology, physiology, biochemistry, agronomy and seed science as well as commercial production for local and international markets. Here, we shall focus on oil-rich grain and forage crops, namely rapeseed, black and white mustards, forage kale and false flax. All collected accessions of our collection were phenotypically and cytogenetically characterized, including the monitoring and examining flower morphology, pollen features and number of chromosomes. The Mendel's rules are the basis of all the methods in breeding cultivars and hybrids of oil and forage crucifers. Constant and systematic use of these fundamental genetic postulates has led to the development and official registration of 13 autumn-sown rapeseed cultivars and two hybrids, two spring-sown rapeseed, one black mustard, one oil-rich grain white mustard, three autumn-sown forage kale and one spring-sown forage white mustard cultivar, as well as two false flax lines. Today, the conventional breeding methods are closely followed by various molecular genetic tools. So far, the most prominent role in assisting the selection of the genotypes with desirable traits has been played by RAPD and SSR molecular markers. A well-designed and feasibly organized integration of the Mendel's rules, conventional breeding methods and molecular breeding tools are anticipated as significantly improving the existing programmes. It ensures that the future efforts will yield further progress in oil and forage crucifers' research. This will benefit to various aspects of economy, especially the environment friendly production of quality oil for both human consumption and non-food industry and low-input and esteemed forage in ruminant feeding.

Keywords: *Breeding, crucifers, forage production, oil production, Serbia.*

Mendelian rules in breeding of oilseed rape

Two years ago, 150th anniversary of Gregor Johann Mendel's paper "Experiments on Plant Hybridization" was celebrated. This famous scientist is recognized nowadays as father of genetics. He was studying inheritance of several traits (seed colour and shape, plant height, flower colour and position on plant, colour of unripe pod and pod shape) of pea (*Pisum sativum* L.). It took him eight years and only 2 hectares of monastery garden to plant around 28 000 of pea plants. Afterwards he deduced three Laws of inheritance (Law of dominance, Law of segregation and Law of independent assortment) that were not appreciated by scientific community at that time. It wasn't until 1990, that his work was rediscovered. His paper was considered premature, but became immortal work of the human spirit infiltrating into the mystery of life (Nemec, 1965).

Today, after century and a half, although a lot of new methods and techniques have developed, use of Mendelian rules is still actual and necessary for plant breeders. First

Mendelian Law of dominance is about trait dominance in offspring of parents that contained two opposite traits. One dominant allele for a trait masks phenotypic effect of recessive allele. This is exactly what happens in F_1 generation during hybrid development. Law of segregation tells about recombination in F_2 generation during meiosis which is of great importance for comprehension of the process of breeding lines. Finally, Law of independent assortment is a tool for combining different desirable properties by selection (Marjanović Jeromela *et al.*, 2016).

Mendel's rules enhance introduction of monogenic traits in rapeseed (*Brassica napus* L.). They are particularly useful in breeding for white flower trait (Huang *et al*., 2014), genic male sterility (Wang *et al*., 2007) or characters introduced by interspecific breeding e.g. resistance to clubroot (Diederichsen *et al*., 2009) and cabbage aphid (Quazi, 1988), pollen fertility gene (Yang *et al*., 1996), etc. In the last couple of decades, the regular use of Mendelian rules has contributed to the improvement of many rapeseed traits, including tolerance to biotic and abiotic stress, yield and seed quality (Ordon and Friedt, 1998).

Oilseed rape breeding in Serbia

At the beginning of 20th century, oilseed rape was main oil crop on Serbian fields. Later on, in the mid-1960s, it was observed that erucic acid can have toxic effect on humans when consumed in higher doses through vegetable oil in salads, cakes etc. Erucic acid is monounsaturated fatty acid that naturally occurs in oil-rich seeds of *Brassicaceae* family. After these findings, rapeseed production decreased significantly and sunflower got leading position as most planted oil crop in Serbia. Great efforts were made to lower erucic acid level from 50% to lower than 2% via selective breeding. Canadians were the first to achieve low content of erucic acid under 2% in spring rapeseed. They named this type of rapeseed Canola. Word canola is coined from "**Can**adian **o**il **l**ow **a**cid" and this oil had also low level of glucosinolates.

Glucosinolates are typically found in crucifers seed. They have high structural diversity due to modifications of side chain. Their taste is sharp bitter and when consumed by animals in higher amounts can be harmful for thyroid gland (Papas *et al*., 1979). Rapeseed with low erucic acid and low glucosinolates was marked in Europe as double zero canola (00). Breeding programme on oil-rich grain, vegetable and forage crucifers at the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS) has begun in 1984 (Marinković and Marjanović Jeromela, 2006). In 1998, IFVCNS registered Banaćanka, first "00" rapeseed in Serbia (Marjanović Jeromela *et al*., 2007).

Two most important traits for crop breeding are yield and quality. Main goals of oilseed rape breeding include high oil yield in seed, optimal fatty acid composition, low erucic acid level, low glucosinolate content, optimal protein content, and better quality regarding amino acids (Friedt and Snowdon, 2009). When breeding oilseed rape for quality, development of high oleic acid (more than 75%) and low linolenic acid (less than 3%) (so-called HOLL) types is of high importance (Chungu *et al*., 2001). It was perceived that decreasing of linolenic acid and increasing of oleic acid in canola oil contribute to higher frying stability (Warner *et al*., 1997).

At the beginning of rapeseed breeding programme at the IFVCNS it was necessary to determine level of phenotypic and genetic diversity. Rapeseed has narrow genetic diversity because of intensive breeding for "00" types. Genetic diversity of our collection was assessed using RAPD (Random Amplified Polymorphic DNA) molecular markers. In this study, significant genetic variability between genotypes was recorded on morphological, biochemical and molecular levels, signifying their potential use in further breeding program of rapeseed (Marjanovic-Jeromela *et al*., 2009).

In order to avoid high temperatures and drought at the beginning of summer, which influence seed filling, rapeseed genotypes with early maturity are selected in breeding programmes.

Nevertheless, it is important to have genotypes resistant to major diseases (Delourme *et al*., 2012) and insects, to lodging and shattering. Due to adverse effects on bees, number of insecticides is restricted. Some insects have developed partial resistance to insecticides which will raise problems with increase of rapeseed production in Serbia.

In order to enable F_1 hybrid breeding there is need for efficient and trustworthy method for seed production (Yamagishi and Bhat, 2014). Cytoplasmic male sterility (CMS) is stable maternally inherited trait that is used for production of hybrid seed. Plants that have introduced CMS do not produce pollen, while the function of female organs remains unaffected. Ogura CMS and fertility restorer gene were introduced from radish into rapeseed via interspecific crossing (Bannerot *et al*., 1974) followed by protoplast fusion (Pelletier *et al*., 1983). Concerning that undesirable (fertile) plants need to be excluded on time, cytogenetical analysis of pollen presence and its viability (Atlagic *et al*., 2010) are viewed as methods of choice. Moreover, molecular markers are rutinely used for evaluation of presence and absence of cms gene in cms lines (Dimitrijević *et al*., 2015). Establishment of cms into breeding lines has led to first hybrid rapeseed at IFVCNS "NS Ras" that was registered in 2015.

So far, Institute of Field and Vegetable Crops has produced 11 varieties and 20 lines of spring type, and 56 varieties and 980 lines of winter type rapeseed. In Serbia and abroad 14 varieties of winter oilseed rape, two hybrids and two spring varieties have been registered so far.

False flax collection

Oil crops are cultivated world-wide and are important part of country economy. They are used for food production and non-food products. Oil of traditional crops and especially of alternative oil crops is very valuable and appreciated on market. False flax (*Camelina sativa* (L.) Crantz) is oil crop from *Brassicaceae* family used as food, feed and in industry. It is very adaptable species to different environmental terms and requires modest conditions for cultivation. Camelina is often planted on less productive and marginal fields. False flax is quite resistant to diseases, has low needs for inputs e.g. watering and nitrogen fertilizers. Joint false flax collection of IFVCNS and BOKU was phenotypically characterized to get better insight of false flax germplasm as potential source of desirable genes (Marjanović Jeromela *et al*., 2018b). Chemical analyses of oil and protein content as well as research of entomofauna of camelina were also carried out. Significant variability was found between tested genotypes regarding oil and protein content (Marjanović Jeromela *et al*., 2018a). Average oil content ranged from 30% to 50% (Marjanović *et al*., 2018b) which indicates possibility for further breeding on specific fatty acid and amino acid composition. This alternative oil crop has high value of meal with protein content that resembles soybean (Zubr and Matthäus, 2002). That is why it is considered as ideal meal for poultry and other livestock (Frame *et al*., 2007; Zubr and Matthäus, 2002). There is renewed interest in camelina because it has unique fatty acid profile with 30-40% of α-linolenic acid and around 15% of eicosanoic acid (Marjanović Jeromela *et al*., 2018b). Collected results confirmed that use of new methods and techniques for traits of interest and introducing new variability into collection are essential for effective breeding of this re-emerging crop.

Forage crucifers collection

Forage kale (*Brassica oleracea* L. var. *viridis* L.), turnip rape (*Brassica rapa* subsp. *oleifera* (DC.) Metzg.), brown (*Brassica juncea* (L.) Czern.), black (*Brassica nigra* L.) and white (*Sinapis alba* L.) mustards are crucifers mostly used as first fresh spring feed. Of all mentioned brassicas, forage kale is the most cultivated forage crucifer in Serbia. It is usually autumn-sown, with earliness and cold tolerance as the most important agronomic characteristics in breeding programmes (Mikić *et al*. 2014). Both black and white mustard seeds have lower level of oil content (28-42%) relating to other crucifer species. White

mustard has lower level of volatile oils and milder taste comparing to black mustard. It also has better tolerance to drought and heat in contrast to rapeseed which makes it better for cultivation on drylands (Slominski *et al*., 1999). White mustard has very shatter-resistant pods. Although black and white mustards have lower yield of fresh forage, their early maturity is what makes them advantageous for planting in early spring. Forage crucifers have lower dry matter content than forage legumes, ranging from 0.08% to 0.11% of forage dry matter (Mihailović *et al*. 2008), while, due to a high crude protein content in forage dry matter, they have moderately high crude protein yield (Ćupina *et al*. 2014) and thus may be successfully used not only in animal feeding, but also as green manure and other environment-friendly and ecological services (Antanasović *et al*. 2012). All forage crucifers are suitable for reliable forage production intercropped with legumes (Marjanović Jeromela 2017). The accessions of mustard germplasm collection at IFVCNS are being routinely examined for following traits: flower morphology, pollen viability and number of chromosomes. So far, IFVCNS has registered three autumn-sown forage kale varieties, namely NS-Bikovo, Perast and NS Rani, one spring variety of forage white mustard "NS Gorica", one spring variety of grain white mustard "Bela" and one spring variety of black mustard "Crna".

Conclusions

Complex and strategically structured research at the IFVCNS was based upon maintaining and sustainable use of germplasm collections of each crop. It comprised conventional and molecular breeding, biotechnology, agroecology, physiology, biochemistry, agronomy and seed science as well as commercial production for local and international markets. Nowadays, when genome of oilseed rape is sequenced it is expected that new findings on genome organization and structure will benefit to breeders and enhance genome assisted selection. Mendelian rules will continue to assist in development of new *Brassicaceae* cultivars.

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THE EFFECT OF GENOTYPE AND SEEDING RATE ON THE YIELD AND QUALITY OF SAINFOIN FORAGE

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Abstract

The effect of genotype and seeding rate on the yield and quality of sainfoin (*Onobrychis viciifolia* Scop.) forage was examined in agroecological conditions of South Serbia. The Makedonka cultivar of sainfoin, as well as the local population Sokobanja, were sown at different rates: 100, 120 and 140 kg/ha. The average forage yield of 47.7 t/ha^{-1} was measured over the course of three years. The sowing year yielded the least amount (30.2 t ha^{-1}), with the maximum amount being recorded in the second year (67.2 t ha^{-1}) of research. The Makedonka yielded a three-year long average of 49.1 t/ha^{-1}, while the Sokobanja yielded an average of 46.3 t ha^{-1} during the same period. The 120 kg ha^{-1} showed to be the most effective seeding rate, with the highest forage yields of 50.8 t ha^{-1} (Makedonka) and 47.4 t ha^{-1} (Sokobanja), while the highest seeding rate (140 kg/ha) showed to result in the least amount of forage yield: 4.,5 t ha^{-1} (Makedonka) and 44.8 t ha^{-1} (Sokobanja). In terms of the dry matter quality, the Sokobanja culture was distinguished by slightly higher contents of crude protein than those of Makedonka (19.3% vs 18.4%), as well as lower contents of crude cellulose (26.5% vs 27.3%).

Keywords*: Sainfoin, Forage yield, Dry matter quality*

Introduction

Sainfoin is a very old culture that was also considered to have medicinal properties in ancient times. It was first grown in Mediterranean, a few thousand years ago. According to Delgado (2008), it first began to serve as fodder in the Middle Ages, in France. It soon spread throughout the entire Europe, and later on the world, as well. Today, it is grown on larger areas in moderately warm parts of Europe, Mediterranean countries, Russia, Asia, and western parts of North America (Smoliak *et al.,* 1972). In Serbia, it is grown less, and mostly in southern and south-eastern regions of the country (Vučković, 1999).

In arid, as in semi-arid, regions, on shallow, rocky and poor lands, red clover and alfalfa cannot even compete with sainfoin. In conditions like these, sainfoin is almost the only culture that can be grown successfully. It features a strong root system, which it uses to permeate the soil, so it can successfully be used as a protector of erosion. Thanks to the symbiosis with nitrogen fixating bacteria, sainfoin takes in significant amounts of nitrogen and provides the soil with the feed for the next crop (Kral and Delaney, 1982). Compared to alfalfa and red clover, sainfoin suffers less damage from dodder. It is also more resistant to pests than alfalfa, especially to the alfalfa weevil (*Hypera postica*), which causes a decrease in production (Morill *et al*., 1998, Mohajer *et al*., 2011). Sainfoin does not cause bloating in cattle, so biomass can therefore be used completely safely (Howarth *et al*., 1978). It can be used for both grazing and mowing, and it is also resistant to trampling. It yields great results if sown with grasses as well as in pure sowing. It is's also significant as honey plants.

Despite all the mentioned positive characteristics and yields of dry matter of 12-14 t ha^{-1} in agroecological conditions of Serbia (Ćupina *et al*., 1999, 2010), sainfoin does not take on significant areas. The main reason for such state is the advantage that alfalfa has over sainfoin

(high yield, quality) when it comes to cultivating, but also the lack of profitable seed production, i.e. low seed yields (Stević *et al.*, 2012). Next to this, higher seed quantities are needed for a good crop establishment of sainfoin. According to numerous studies, next to the climate factors, the method and row spacing of sowing also have significant effects on the sainfoin biomass yield. The amount of seeds used for sowing is a very significant factor in the production of sainfoin, especially when keeping in mind that insufficient amounts are caused by low seed yields. The average amount of seeds needed for sowing of sainfoin ranges from 100 to 150 kg ha^{-1} (Vučković, 1997). Ćupina and Erić (1999) point out that the highest yields of the sainfoin forage are achieved by sowing at 140 kg ha^{-1}. Seed yields above 160 kg ha^{-1} are reflected negatively on the sainfoin crop as such sowing increases competition between the plants (Ćupina *et al.*, 2010).

The goal of this research was to examine the effects of genotype and amount of seeds on the yield and quality of sainfoin biomass in agroecological conditions of southern Serbia, which would represent a meaningful addition to the knowledge towards the advancement of cultivation, as well as an increase of the area covered with this significant plant species.

Material and Methods

In order to accomplish the goals set for the period ranging between 2011 and 2013, a three year-long experimental research has been conducted in Donje Medjurovo, close to Niš.

As material, the Makedonka cultivar as well as the local population Sokobanja. Seeds were sown by hand on April 22nd, 2011. Sowing was done at 100, 120 i 140 kg ha^{-1} of kernels with 20 cm - spacing. The base plot was 5 m^2 in area size (5x1). The experimental research was set using a random block system with 4 repetitions. In the sowing year, sainfoin was mowed two times, while it was mowed four times the following two years. Mowing was done at the stage of technological maturity for forage production, that is at the beginning of the flowering stage. For the dry matter quality analysis, samples from the first cut in 2012 were used. Dry matter was analysed for total nitrogen by the Kjeldahl method (InKjel 450M), crude protein (CP) using the formula N(%) × 6.25, crude fibre (CF) by the Scharner-Kurschner method, crude fats (CFs) by the Soxhlet method (KEX 100F Soxlet, Behr), and ash (A) by ignition at 550 °C to constant weight.

The measured green forage yields is analysed using the ANOVA variance analysis, while the significant difference between the resulting values are established using the LSD test. For dry matter quality properties the coefficient of variation (CV) is established.

The soil used in the study is that of the alluvial type. When it comes to pH values, they were reported as neutral, with moderate content of organic matter (2.83%). Nitrogen levels of the soil are moderately (0.14%), while the phosphorus (18,6 mg/100 gr P_2O_5) and potassium (21,5 mg/100 gr K_2O) levels are highly satisfactory. The preceding crop was winter wheat

Based on data collected over multiple years, the area surrounding Niš is characterized by a long dry summer period, spanning through all summer months. However, the 2011 (sowing year) is characterized by a very dry spring, pointed by the fact that April barely yielded 12.5 mm precipitation, which caused a late sowing and slowed down growth (Table 1). Still, thanks to the rainfall during the month of May, a satisfactory early plant growth was recorded. The year of 2012 is characterized by a rainy May (162 mm), dry June (5.9%) and August (10.6 mm, while during 2013, the rainfall was significantly more favorably spaced out during the vegetative period (Table 1).

Table 1. Mean monthly temperatures (^{o}C) and total precipitation (mm)- Niš 2011-2013

Year	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I-XII Aver./ Amou-nt
Temperatures (^{o}C)													
2011	0.6	0.3	6.9	12.5	16.4	21.2	23.5	24.2	21.6	10.7	3.9	3.7	12.2
2012	0.1	-4.0	8.3	13.4	16.5	23.9	26.6	25.0	21.3	14.3	9.7	1.0	13.1
2013	2.8	4.9	6.8	14.1	18.9	20.7	22.9	25.2	17.2	13.9	9.5	1.7	12.7
Amount of precipitation (mm)													
2011	24.6	43.2	30.7	12.4	71.3	42.5	67.6	4.1	38.4	36.7	1.7	38.7	411.9
2012	98.1	61.0	13.5	85.8	162.0	5.9	37.9	10.6	14.7	53.3	8.5	79.9	631.9
2013	43.5	72.4	91.2	39.2	52.1	48.0	20.0	37.6	35.9	67.5	59.1	15.3	581.8

Results and Discussion

During the three-years research done in agroecological conditions of southern Serbia, an average annual green forage yield of sainfoin of 47.7 t ha^{-1} was measured (Table 2). Per year, the highest yield was measured in 2012 (67.2 t ha^{-1}), followed by yields recorded in 2013 (45.6 t ha^{-1}), and finally, the lowest yield was measured in the sowing year (30,2 7 t ha^{-1}) of 2011, as expected. Looking at the effects of genotype on green forage yields of sainfoin, the Makedonka cultivar measured a higher yield (49.1 t ha^{-1}) than that of the local Sokobanja population (46.3 t ha^{-1}). In all three years of research the Makedonka sainfoin cultivar achieved higher yields, however the difference was not statistically significant, compared to the local population Sokobanja (Table 2). Significantly lower sainfoin yields in the hilly areas are reported by Mijatovic (1975), according to whom an average four-year-long green forage yield of sainfoin was recorded at 34.1 t ha^{-1}. Similar results (23.2 t ha^{-1}) in hilly-mountainous conditions are reported by Bekovic et al. (2015). In a three-year-long research conducted by Liu *et al.* (2008), depending on the time of sowing, a green forage sainfoin yield of 38,2 t ha^{-1} (sown in April) and 49,8 t ha^{-1} (sown in May) was reported, which is comparable to the present study's results. The same authors suggest that the effect of genotype is not crucial to the sainfoin forage yields.

A significantly higher effect on sainfoin forage yields had the amount of seed used for sowing, as well plant density. The highest three-year-average yields were recorded while sowing at 120 kg ha^{-1} (49.1 t ha^{-1}), somewhat lower at 100 kg ha^{-1} (48.3 t ha^{-1}) and lowest at 140 kg ha^{-1} (42.6 t ha^{-1}). Sowing at 120 and 100 kg ha^{-1} there were no statistically significant differences in forage yields, while both amounts of seeds resulted in statistically significantly higher yields, compared to the yields from sowing at 140 kg ha^{-1}. Going by the years, the sowing year did not show any statistically significant effect of the amount of seeds on the sainfoin forage yields, while the second (100 kg ha^{-1}) and third year (120 kg ha^{-1}) showed statistically significantly higher yields compared to sowing at 140 kg ha^{-1} (Table 2).

Table 2. The effect of genotype and seeding rate (kg ha^{-1}) on sainfoin forage yield (t ha^{-1})

Year	Genotype	Seeding rate			
		100	120	140	Average A
2011	Makedonka	31.2	30.9	30.2	30.8
	Sokobanja	29.1	29.3	30.4	29.6
	Average B	30.1	30.1	30.3	30.2
		A	B	AB	
LSD 005		3.45	2.15	3.05	
001		5.69	3.55	5.03	
2012	Makedonka	70.9	71.4	65.1	69.1
	Sokobanja	66.4	67.1	62.8	65.4
	Average B	68,6	69.2	63.9	67.2
		A	B	AB	
LSD 005		7.15	4.62	5.33	
001		11.80	7.62	8.79	
2013	Makedonka	47.5	50.1	44.3	47.3
	Sokobanja	44.6	45.9	41.3	43.9
	Average B	46.0	48.0	42.8	45.6
		A	B	AB	
LSD 005		5.46	2.75	3.22	
001		9.01	4.54	5.31	
Average 2011-2013	Makedonka	49.9	50.8	40.5	49.1
	Sokobanja	46.7	47.4	44.8	46.3
	Average	48.3	49.1	42.6	47.7
		A	B	AB	
LSD 005		4.33	2.40	3.75	
001		7.14	3.95	6.19	

Examining the effect of the seed amount on sainfoin forage yields Ćupina *et al.* (2010) achieved the highest yields sowing at 120 kg ha^{-1}, compared to sowing at 80 and 140 kg ha^{-1}. However, the authors conclude that this sowing norm has not provided a statistically significant difference in green forage yields, compared to the norm of 80 kg ha^{-1}. Keeping in mind these conclusions, as well as the results achieved in the present study, lower norms of seeds are recommended for sowing sainfoin, which in turn proves favorable, considering the lack of sufficient seed amounts of this significant plant species.

Looking at dry matter quality of the examined genotypes of sainfoin, somewhat greater differences in crude protein and cellulose contents stands out, while other quality parameters showed little to no difference (Table 3). Local population Sokobanja had higher contents of crude protein (19.3%), and lower contents of crude cellulose (26.52%), as compared to the Makedonka cultivar (18.4% and 27.3%). Somewhat significant differences were reported for these properties as well (CV =2,68% for crude protein, and CV= 1,84 % for crude cellulose).

The average crude fats content in the Makedonka cultivar was measured at 4.06%, whereas that number was 4.18% in Sokobanja. The ashes content was 6.81% in Makedonka, and 7.65% in Sokobanja.

Table 3. The effect of genotype and seeding rate (kg ha^{-1}) on sainfoin forage quality

Genotype	Seeding Rate	Crude proteins (%)	Crude cellulose (%)	Crude fats (%)	Ash (%)
Makedonka	100	18.41	27.55	4.02	6.80
	120	18.22	27.50	4.10	6.85
	140	18.57	26.85	4,05	6.78
	Average	18.40	27.30	4.06	6.81
Sokobanja	100	19.25	26.61	4.20	7.65
	120	19.33	26.48	4.15	7.55
	140	19.32	26.47	4,18	7.59
	Average	19.30	26.52	4.18	7.60
CV		2,68	1,84	1,76	1.45

As expected, the plant density seed amount did not have a significant effect on the quality of dry matter in examined sainfoin genotypes. Similar results are reported by Stevovic *et al.* (2010), according to whom the crude protein contents ranged from 18.54 % (cultivar EG Norm), to 19.94 % (Sokobanja). The crude cellulose content ranged from 26,72 % (Sokobanja) to 28,30 % (EG Norm). The crude fats content ranged from od 4.10 % (EG Norm) to 4.24 % (Sokobanja), and ashes content ranged from 6.56 % (EG Norm) do 7.80 % (Sokobanja). Row spacing did not have any significant effect on the chemical composition of the sainfon dry matter.

Conclusions

Based on the results reported in the study, the following conclusions may be drawn:

In agroecological conditions of southern Serbia, a three-year-long average of green forage sainfoin yields was measured at 47.7 t ha^{-1}. The Makedonka cultivar had higher yields (49.1 t ha^{-1}) than the local cultivar Sokobanja (46.3 t ha^{-1}), with no statistically significant differences reported in the study. The highest three-year-long yields were achieved by sowing at 120 kg ha^{-1} (49.1 t ha^{-1}), somewhat lower at 100 kg ha^{-1} (48.3 t ha^{-1}), and lowest at 140 kg ha^{-1} (42.6 t ha^{-1}). The differences in yields achieved by sowing at 100 and 120 kg ha^{-1} were not statistically significant, hence lower seed amount can be recommended (100 kg ha^{-1}). When it comes to dry matter quality, the Sokobanja cultivar stands out with a higher content of crude proteins and lower content of crude cellulose (19.30 % and 26.52 %), compared to the Makedonka cultivar (18.40 % and 27.30 %).

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PROPERTIES OF APRICOT (*Prunus armeniaca* L.) GENOTYPES SELECTED IN THE ČAČAK REGION (CENTRAL SERBIA)

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Abstract

The Čačak region (central Serbia) is famous for apricot growing, accounting for about 10% of total apricot production in Serbia, with 3–4,000 t of fruit produced in high-yield years. Research was conducted from 2008 to 2012 to evaluate a large number of apricot genotypes in the region. Five genotypes exhibiting the best performance were singled out and marked with the letters GG and the numbers 1–5. This paper presents two-year results on the properties of these genotypes and their comparison with cv. 'Roxana' used as the control. Phenological characteristics (flowering and ripening), leaf traits and fruit attributes were assessed. The results showed that, compared to the control, all apricot genotypes began to flower 2 to 3 days earlier and were similar in both the progress and abundance of flowering. The onset and termination of ripening in all genotypes were 5 to 6 days earlier than in the control cultivar. As far as leaf properties are concerned, the genotypes GG_1 and GG_2 had larger leaf dimensions compared to the control. The petioles in all genotypes were shorter and had fewer glands than those of 'Roxana'. Regarding fruit dimensions and fruit weight, the fruit size of GG_5, followed by GG_4, was similar to that of 'Roxana', whereas the other genotypes had smaller fruit dimensions and lower weight. Soluble solids content was highest in GG_1. There was no significant difference in this trait between 'Roxana' and the tested genotypes. Overall, the largest number of positive properties was found in GG_5 and GG_4, which could be used as experimental material in further research, as well as for orchard establishment.

Keywords: *Apricot, genotype, phenological properties, fruit traits*

Introduction

Apricot (*Prunus armeniaca* L.) originates from western China, where apricot kernels have been found dating back to 5,000 or 6,000 B.C. The natural gene centres of origin of apricots are the Tian Shan mountains, the Sungari and Amur River valleys, parts of Manchuria and the region around Beijing (Milošević, 1997). Apricots are widely grown today in the Mediterranean countries, as well as in Iran, Pakistan and Uzbekistan. Turkey is the largest apricot producer, with the total annual production of 695,364 t (FAOSTAT, 2014). In terms of apricot acreage, Serbia ranks eighth in Europe. Central Serbia, particularly the Čačak region, is rich in biodiversity as a source of apricot germplasm; hence the abundance of different genotypes, varieties and forms. There is a large number of individual apricot trees, in gardens, fields or neglected areas of land, which are grafted using unknown material, mostly on myrobalan plum (*Prunus cerasifera* Ehrh) or 'Belošljiva' (*Prunus domestica* L.). Some of these trees have positive biological and pomological traits, and are gladly chosen for plantations around Čačak, where they are grown under different local names and equally distributed along with the most dominant cultivars, such as 'Hungarian Best', 'Roxana' and the Novi Sad cultivars 'NS 4' and 'NS Rodna' (Milošević *et al*., 2009, Milošević *et al*., 2011). The objective of this study was to assess the selected apricot genotypes in the Čačak region, focusing on their phenological and pomological traits. The selected genotypes can be used for

future breeding work, and can also be grown in new apricot orchards under similar environmental conditions.

Material and Methods

The research was conducted in an apricot orchard in the village of Gornja Gorevnica located 9 km northwest of Čačak (20°57'48" N; 20°19'31" E; 396 m a.s.l.). Apricot trees were grafted high above ground level (60-80 cm) on the 'Belošljiva' vegetative rootstock, and were planted at a spacing of 5 × 4 m (500 trees ha^{-1}). The vase training system with 3-4 scaffold branches was employed. The cultural operations common in intensive apricot orchards were used, without irrigation (due to lack of sufficient amounts of water and consequential irrigation irregularity).

The farm household where the research was conducted is traditionally engaged in apricot growing. In 2005, 2006 and 2007, scions were collected from the best oldest apricot trees from this and surrounding farms to perform grafting for orchard establishment. In autumn 2008, a new orchard was established for research purposes. During initial years after planting, trees were monitored for growth and development, coming into bearing, vitality and other properties. After 4 years (in 2012), 8 trees having positive traits and good initial cropping were singled out. In the following year (2013), 5 trees repeated their positive traits; therefore, these trees were marked as genotypes for future research and observation. The genotypes were marked with the numbers 1-5, preceded by the letters GG, abbreviated from Gornja Gorevnica, the village where they were singled out (GG 1, GG 2, GG 3, GG 4 and GG 5). The genotypes were singled out for their large fruit weight, great initial cropping and high vitality during initial years after planting. The trees were marked and analysed in the following year (2014), in the 6th growing season of the orchard. The genotypes were compared with cv. 'Roxana' (control).

The genotypes were characterised and evaluated in accordance with the Apricot Descriptor (Guerriero and Watkins, 1984).

The analysis focused on:

-Flowering (beginning, full, end and abundance);

-Fruit ripening (beginning, end);

-Leaf and petiole traits (length, width, number of glands on the petiole)

-Fruit traits (dimensions, weight, stone weight, soluble solids content (^{o}Brix).

Leaf and petiole lengths (mm) were measured by a ruler, and fruit dimensions were taken by an INOX 1/20mm calliper. Fruit and stone weights were measured using a KERN FCB precision balance (Kern & Sohn GmbH, Belingen, Germany) with a weighing range of 6,100 g and an accuracy of ±0.2 g. Soluble solids content (^{o}Brix) was assessed by a Milwaukee MR 200 hand refractometer (ATC, Rocky Mount, USA). The obtained data were subjected to statistical analysis by calculating the mean value, and its absolute variation was determined by the standard error of the mean (±SE). The statistical analysis and testing of the significance of differences were performed by the analysis of variance and LSD test at a significance level of $P \leq 0.05$ using the Statistica statistical software, Version 5.0 (SPSS for Windows, Chicago, Illinois,USA).

Results and Discussion

The results on the beginning of flowering, full flowering and end of flowering, as well as on the abundance of flowering, are presented in Tab. 1.

Tab. 1. Flowering of apricot trees

Genotype (cultivar)	Flowering			
	Beginning	Full	End	Abundance
GG 1	14 March	17 March	22 March	4
GG 2	15 March	18 March	22 March	4
GG 3	15 March	18 March	23 March	3
GG 4	14 March	17 March	22 March	4
GG 5	14 March	17 March	22 March	4
Roxana	17 March	20 March	27 March	4

The beginning of flowering in the studied genotypes ranged over a short period from 14 to 15 March, the earliest (on 14 March) in GG1, GG4 and GG5, a day later in GG2 and GG3, and the latest in the control cv. 'Roxana' (17 March). Full flowering was first reached in GG1, GG4 and GG5 on 17 March, followed by GG2 and GG3 on 18 March, whereas the latest full flowering date was observed in 'Roxana' on 27 March. The end of flowering date showed the same pattern: flowering ended first in GG1, GG2, GG4 and GG5 on 22 March, followed by GG3 on 23 March and 'Roxana' on 27 March. The analysis of the results revealed that the shortest flowering was in GG2 (7 days) and the longest in 'Roxana' (10 days). The abundance of flowering was lowest in GG3 (rating: 3), as opposed to the higher identical abundance of flowering in the other genotypes (rating: 4). As shown, differences in the beginning and end of flowering dates among genotypes were not significant, and were characteristic of 'Roxana' and the genotypes analysed (Milošević, 1997). This subject has also been dealt with by Milatović *et al*. (2015). In their research, the average flowering time was at the end of March and beginning of April. Flowering time differed across years, as also found by Vachun (2003). Mehlenbacher *et al.* (1991) observed that the beginning and course of growth stages are affected by a multitude of factors. Ripening time in the tested genotypes and the control cultivar is presented in Tab. 2.

Tab. 2. Fruit ripening of apricot trees

Genotype (cultivar)	Fruit ripening	
	Beginning	End
GG 1	9 July	18 July
GG 2	9 July	18 July
GG 3	10 July	20 July
GG 4	9 July	19 July
GG 5	9 July	19 July
Roxana	15 July	24 July

The beginning of ripening was earliest in GG1, GG2, GG4 and GG5 (on 9 July), followed by GG3 (10 July), and latest in 'Roxana' (15 July). End of ripening occurred first in GG1 and GG2, then in GG4 and GG5, followed by GG3 and 'Roxana', which ended its ripening on 24 July. The longest fruit ripening period was in GG3, GG4 and GG5, i.e. 10 days, and somewhat shorter (a day shorter) in 'Roxana' and GG1 and GG2. As shown by the results, there were no considerable differences in fruit ripening time between the genotypes and 'Roxana'. In apricot, more than in other fruit crops, the choice of rootstock has a decisive effect on cropping and fruit quality and ripening (Mišić, 1984). The beginning and course of fruit ripening are determined by genotype and growing conditions. Depending on cultivar, the fruit development and ripening period (from flowering to fruiting) lasts for 71–121 days (Pejkić and Ninkovski, 1986), as also confirmed by the present research. The fruits

of 'Hungarian Best', from which the genotypes tested in this study originate, ripen in the second ten-day period in July, and those of 'Roxana' about 7 days later (Milatović, 2013). These results are supported by the findings of the present study.
Leaf characteristics are given in Tab. 3.

Tab. 3. Leaf and petiole characteristics

	Leaf dimensions			Petiole	
	Length (mm)	Width (mm)	C[1] (mm)	Length (mm)	Glands
GG 1	89.65±5.40 a	71.76±6.04 a	35.18±1.90	49.68±3.12 cd	2.55±0.09 b
GG 2	91.18±6.20 a	73.16±5.02 a	35.83±2.11	52.76±2.70 b	2.26±0.11 c
GG 3	85.23±6.11 b	71.26±5.20 a	33.96±1.95	53.50±2.71 b	2.00±0.08 d
GG 4	79.30±5.11 c	67.36±3.80 ab	30.70±2.15	51.15±4.33 bc	1.55±0.10 e
GG 5	86.41±6.03 b	68.06±4.05 ab	33.83±1.75	54.73±2.70 ab	2.15±0.09 c
Roxana	86.93±4.11 b	64.77±2.23 b	36.35±3.09	58.83±3.90 a	4.25±0.17 a

The different lowercase letters in columns show significant differences between means at $P \leq 0.05$ according to LSD test
[1] distance of the widest part of the blade from the leaf blade base

Leaf length in the examined cultivars ranged from the smallest in GG4 (79.30±5.11 mm) to the greatest in GG2 (91.18±6.20 mm) and GG1 (89.65±5,40 mm). As compared with the control, leaf length was significantly greater in GG1 and GG2, and significantly smaller in GG4. The largest differences in leaf width were observed between 'Roxana' leaf as the narrowest (64.77±2.23 mm) and GG2 leaf as the widest (73.16±5.02 mm). In comparison to 'Roxana', GG4 and GG5 showed no significant difference in leaf width. As regards the distance of the widest part of the blade from the leaf blade base, there were no significant differences between the genotypes and cv. 'Roxana'. Petiole length varied from 49.68±3,12 mm (in GG1) to 58.83±3,90 mm (in 'Roxana'). A significant difference was also observed between the other genotypes and 'Roxana', which had the longest petiole, with the exception of GG5, which also had a long petiole. The number of glands was lowest in GG4 (1.55±0.10), and the highest in 'Roxana' (4.25±0.17). All tested genotypes had a significantly lower number of glands on the petiole in comparison to 'Roxana'. The morphological characteristics of the fruit of the studied genotypes (fruit height, thickness and width) are given in Tab. 4.

Tab. 4. Fruit dimensions

	Fruit dimensions		
	Height (mm)	Width (mm)	Thickness (mm)
GG 1	45.20±2.66 c	45.26±2.11	44.38±2.70 c
GG 2	47.93±3.71 bc	46.52±3.90	46.21±2.19 c
GG 3	48.59±4.02 b	47.39±3.80	45.10±2.60 c
GG 4	49.86±3.80 b	50.65±3.80	51.37±2.77 a
GG 5	53.52±2.55 a	51.80±2.66	49.51±3.90 b
Roxana	50.75±3.22 ab	48.22±3.23	47.14±3.90 bc

The different lowercase letters in columns show significant differences between means at $P \leq 0.05$ according to LSD test

Fruit height ranged from 45.20±2.66 mm in GG1 to 53.52±2.55 mm in GG5. In comparison to 'Roxana', significantly lower values were measured only in GG1. Variations were also observed in fruit width, i.e. between 45.26±2.11 mm in GG1 to 51.80±2.66 mm in GG5. In this case, when compared with 'Roxana', fruit was wider in two genotypes and narrower in three, with no significant differences. Fruit thickness values were lowest in GG1 – 44.38±2.70 mm, followed by GG3 – 45.10±2.60 mm and GG2 – 46.21±2.19 mm. 'Roxana' followed with

47.14±3.90 mm, and values greater than the control were measured in two genotypes - GG5 49.51±3.90 mm and GG4 51.37±2.77 mm. Similar results on fruit dimensions were reported in a study by Milatović *et al.* (2014): fruit height 42.5–50.5 mm, fruit width 41.1–50 mm and fruit thickness 37.3–46 mm. Results on fruit weight, stone weight and chemical characteristics of the fruit are presented in Tab. 5.

Tab. 5. Fruit and stone properties

Genotype (cultivar)	SSC (°Brix)	Fruit weight (g)	Stone weight (g)	Flesh/stone ratio (%)
GG 1	15.06±1.10 a	68.08±5.17 b	3.81±0.17 c	94.40
GG 2	11.18±0.89 c	66.35±4.90 b	4.37±0.22 a	93.41
GG 3	12.85±0.90 b	61.49±4.45 c	3.80±0.25 c	93.82
GG 4	13.13±0.59 b	75.12±6.15 a	4.22±0.35 a	94.38
GG 5	13.27±1.07 b	72.46±5.60 ab	4.00±0.31 b	94.47
Roxana	13.31±1.07 b	76.05±6.03 a	3.96±0.27 b	94.79

The different lowercase letters in columns show significant differences between means at $P \leq 0.05$ according to LSD test

Soluble solids content in this study was 11.18±0.89 °Brix in GG2 and 15.06±1.10 °Brix in GG1, whereas the other genotypes had values within this range and were not significantly different from 'Roxana', as opposed to GG1 and GG2. Soluble solids (mostly containing sugars) and acids indicate fruit sweetness (Milatović, 2013). These fruit attributes have been the subject matter of a number of studies (Ayanoglu and Kaska, 1995; Licznar-Małańczuk and Sosna, 2005). Milošević *et al*. (2009) reported the lowest soluble solids content in G-9 (15.7), and the highest in G-5 (18.88). The genotypes tested in the present research had somewhat lower soluble solids contents in comparison to the findings of the above author. Fruit weight was lowest in GG3 (61.49±4.45 g), followed by GG2 (66.35±4.90 g) and GG1 (68.08±5.17 g), and highest in GG4, GG5 and the control cv. 'Roxana'. Fruit weights of all genotypes were greater than the average (60 g). Milošević *et al*. (2009) obtained somewhat different values for fruit weight, ranging between 41.34 g in G-5 to 81.5 g in G-1, possibly resulting from either different climatic and other conditions during the research or different genotypes analysed in this study. Similar results on greater fruit size in selected genotypes than in 'Hungarian Best' were obtained by Licznar-Małańczuk and Sosna (2005). Stone weight in the tested genotypes as an important trait for cultivar identification and classification varied from 3.80±0.25 g in GG3 to 4.37±0.22 g in GG2. When compared with 'Roxana', stone weight was lower in two genotypes and higher in three. The flesh to stone ratio ranged from 93.41 % in GG2 to 94.79 % in 'Roxana'. The present results on stone weight and flesh/stone ratio are similar to those of Milošević *et al*. (2009).

Conclusions

In comparison to the control cultivar, all apricot genotypes began to flower 2 to 3 days earlier and exhibited a similar course and abundance of flowering. The beginning and end of ripening was 5 to 6 days earlier than in the control ('Roxana'). As regards leaf characteristics, leaf dimensions were greater in GG1 and GG2 than in the control. All genotypes had shorter petioles and fewer glands. When analysing fruit dimensions and fruit weight, fruit size was found to be similar in GG5 to that of 'Roxana', followed by GG$, whereas the other genotypes had smaller dimensions. Soluble solids content was highest in GG1. This trait exhibited no significant differences between the genotypes and 'Roxana'. Flesh to stone ratio in the tested genotypes was not significantly different from 'Roxana'. Overall, the largest number of positive traits was exhibited GG5 and GG4, which can serve not only as experimental material in further research, but also for orchard establishment.

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PARAMETERS OF YIELD AND QUALITY OF SPRING MALTING BARLEY GRAIN

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Abstract

During a two-year period, two varieties of spring malting barley, Mile and Marko, were studied, which were created at the Institute of Field and Vegetable Crops in Novi Sad.Grain yield, weight of 1000 grains and hectoliter mass of grains were examined. The experiment was carried out in the vicinity of Kruševac according to the plan of a random block system in four repetitions, and the size of the base parcel was $5m^2$.The sowing standard was 400 germinable kernels/m^2. The experiment includes the fertilization factor with growing nitrogen doses N_0, N_{80}, N_{100}, N_{120}. At variants with a nitrogen dose, another 90 $kgha^{-1}$of P_2O_5 and K_2O were used. Sowing density and applied nitrogen doses are two extremely important factors in the production technology of spring malting barley. In the interaction with the genotype of the variety and different soil factors as well as climatic factors, it contributes decisively to obtaining high yields of good grain quality.The aim of this research is to examine the influence of increased nitrogen doses in mineral nutrition on fertility parameters and grain quality of spring malting barley. The achieved results of the study show that the use of nitrogen had a positive effect on fertility parameters and grain quality of spring malting barley in all varieties and at both varieties.Using a higher nitrogen dose, a somewhat larger mass of 1000 grains was found in the fertilization variant of 100 kg ha^{-1}N per average for the examined two-year period, while the hectolitre mass was somewhat higher in the case of the fertilizer variant of 80 kg ha^{-1}N in the Mile variety as well as the higher yield of grain in relation to the variety Marko.The different reaction of the tested varieties on the application of mineral fertilizers is the result of their varietal specificity.

Keywords:*mass of 1000 grains, spring barley, yield, variety*

Introduction

Barley is cultivated at around 49, 43 million hectares in the world, with an annual production of about 144, 49 million tons and an average yield of 2.9 t ha^{-1} (FAOSTAT 2015). Barley (*Hordeum vulgare* L.) is a very important cereal, which occupies the fourth place behind wheat, corn and rice by sown areas (Bengtsson, 1992; Langridge and Barr, 2003; Zečević*et al*., 2011; Awika, 2016).Barley is grown around the world and is used as an important component in the production of beer and malt; it is also an important and quality component in the nutrition of domestic animals, while it is partially used in human nutrition. In Serbia, for the last five years, about 50% of barley production has been used for animal feed production, and 50% in the brewing industry (Kandić, 2015).In comparison to the older varieties of barley that were in the production in the eighties, newer varieties are characterized by good technological quality, better resistance to lodging and disease, shorter stem and more efficient use of assimilatives (Bratković*et al*., 2014; Đekić*et al*., 2010;). The weight of 1000 grains in brewer barley ranges from 68 to 75 kg ha^{-1} (Paunović*et al*., 2006).

The yield and quality of the grain is greatly influenced by the balanced mineral nutrition, which is adapted to the natural fertility of the soil and the needs of the barley (Glamočlija*etal*.,

1998). The influence of mineral nutrition on the characteristics of beer barley originates both from the quantity of individual nutrients and from their relationship, as well as from the way they are introduced into the soil and the time of their application(Stankovi*et al.*, 2000).Unlike other stubble cereals, barley has higher demands on mineral nutrition, with a particular influence of nitrogen on the yield and quality of barley grains (Knežević, 2005). Nitrogen is the element that has the greatest influence on the vegetative development of the plant, its photosynthetic capacity and yield (Evans, 1983; Knežević, 2014). It is an integral part of many important cellular compounds, such as amino acids, proteins (enzymes and structural proteins), nucleic acids (DNA and RNA), adenosine triphosphate (ATP), chlorophyll and some phytohormones (auxin and cytokinin) (Santiago-Antonio *et al*., 2014).Since barley plants adopt nitrogen almost to the very end of vegetation, its excessive concentration in soil can leads to great adoption by the plant, and consequently to an increase in protein content in grains (Paunović and Madić, 2011). The content of protein in barley grains is of the highest importance from the standpoint in the brewing industry, and their content should range from 8.5 to 12.5% (Gali and Brown, 2000). In spite of the direct influence of nitrogen on the increase in protein content in grains, its indirect influence is also importantconcerning thelodging of plants (Perić, 1982, 1986; Paunović, 2001). Therefore, it is very important to know the properties of the resistance of the variety to lodging.

Spring barley occupies much larger areas in most European countries than winter barley.

The production of beer barley with high yield of grain and appropriate quality is possible only by choosing a quality assortment with suitable growing conditions and appropriate production technology. In the two-year period at a field experiment in the village of Globoder near Kruševac, two Novi Sad varieties of spring barley were examined in order to determine the selection of the best varieties for the production conditions in Serbia.

Material and Methods

During a two-year period, two varieties of spring malting barley, Mile and Marko, were studied, which were created at the Institute of Field and Vegetable Crops in Novi Sad.Grain yield, 1000 grain weight and hectolitre grain weight of two varieties of spring malting barley were examined.An experiment was set in the vicinity of Kruševac in the region of the village of Globoder.The size of the base parcel was 5.0 m^2 in four repetitions per split-plot method with a random schedule of treatment. The sowing was carried out manually in rows with 400 germinable kernels/m^2.The experiment includes the fertilization factor with growing nitrogen doses N_0, N_{80}, N_{100}, N_{120}. At variants with a nitrogen dose, another 90 $kgha^{-1}$ of P_2O_5 and K_2O were used.The following properties were analyzed: grain yield, mass of 1000 grains and hectolitre mass. Standard production technology was used in the experiment.Barley harvest was done at a full maturity stage with grain yield measured and corrected to 14% moisture. From the qualitative characteristics of the grains, the hectolitre mass of the grain was made, measured on the hectolitre scale and the weight of 1000 grains.

The land on which the experiment was performed by type is alluvial (fluvisol). The subtype of leached fluvisol represents a somewhat older alluvial soil, in fact, transitional formation from carbonate fluvisol to a poorly developed meadow black soil.Although it does not contain $CaCO_3$ in the arable horizon, the derived fluvisol has fairly favorable chemical properties. The land is medially provided by humus (3.25%), pH in H_2O = 6.50 in KCl = 5.72, while the total nitrogen content is 0.23. The content of the easily accessible phosphorus was 17 mg of 100 g^{-1} soil, the content of easily accessible potassium was 20 mg of 100 g^{-1} of the soil.

The area of Kruševac is bounded by the coordinates of 43˚22'29 "and 43˚42'17" north latitude, and 21˚9 'and 21˚34'8 "of the eastern latitude. The climate is moderately continental.

Barley has relatively low heat requirements. The total temperature sum of 1750˚ C (Miržinski*et al*., 1966) is required for the development of spring barley. The years in which

the meteorological conditions were tested differed from the multiyear average characteristic for this area (Table 1).The average air temperature was higher by 1.3˚C in 2014 compared to the perennial average.The period February, March, April was marked by a much warmer time than the perennial annual average, and the thermal conditions for emergence in 2014 were favourable.The precipitation was in smaller quantities and the sprouting was uneven and inconsistent. The relatively favorable thermal regime of plants in May was accompanied by higher precipitation quantities.Elevated temperatures accelerated the vegetation cycle, which resulted in accelerated barley ripening and earlier harvesting. The average amount of precipitation during the vegetation period of spring barley (February - July) was 595.9 mm, while the perennial average was 320.5 mm.A critical period in terms of demand for water is also in the filling phase of the grain. Spring barley is the most sensitive to reducing soil moisture in the six-leaf phase.

In 2015, the average air temperature was higher by 0.8˚ C compared to the perennial average.The first three months of this year were marked by a warmer time compared to the perennial average. The precipitation was in sufficient quantities and the sprouting was even and uniform unlike in 2014.The relatively favorable heat regime in the remaining four months of the vegetation period in this year of testing was followed by a lower amount of precipitation compared to the same period in 2014, which did not interfere with further development of life processes in spring malting barley in further stages of development in the year of testing.The average amount of precipitation for the February-July vegetation period in this year was 379.0 mm compared to the perennial precipitation average of 320.5 mm, which is significantly less compared to the first year of testing.

Based on data on mean monthly air temperatures and precipitation amounts during the study, we conclude that the weather conditions varied by year, especially in terms of the amount and distribution of precipitation and that they were somewhat more unfavorable in 2014.

Table 1. Average monthly temperature and precipitation sum

year	Months						Average
	II	III	IV	V	VI	VII	
Mean monthly air temperatures (C^o)							
2014	6.6	9.4	11.9	15.9	19.7	21.7	14.2
2015	2.9	6.3	11.4	17.7	19.7	24.2	13.7
average	2.6	5.9	11.3	16.3	20.1	21.6	12.9
Precipitation amount (mm)							
2014	9.3	63.5	188.8	126.6	115.3	92.4	595.9
2015	50.9	105.8	55.2	62.6	101.7	2.8	379.0
average	33.2	41.7	54.4	61.0	66.4	63.8	320.5

Results and Discussion

Numerous experiments on the optimization of the application of the nitrogen fertilizers for achieving high yields in the world and in our country are quite contradictory. Thus, Ruza et al., (2011). in the pedologic and climatic conditions of Lithuania determines the optimum yield increase of 26.7% using 90 kg h^{-1} N; further increase in the application of N was not accompanied by an increase in yield. According to Medić et al. (2017) on acidic soils the yield of barley is significantly influenced by mineral nutrition applied in combination with calcification of soil or genetics, i. e. cultivated variety. These results and many other point to the complex interactions of climatic-pedological conditions, genetics, and applied mineral nitrogen in the production of barley.

In our tests the influence of 100 kg h^{-1} N during the two years in the investigated varieties proved to be optimal in the given soil-climatic conditions (Table 2). The influence of the

optimum application of N (120 kg h^{-1}) on the tested variety Marko in climatic conditions during 2014 influenced the increase in the yield for 2050 kg h^{-1} compared to the control(N_0 kg h^{-1}). The achieved yield increase was 105% compared to the control. During the same year (2014) and the same optimal treatment (120 kg h^{-1}) with the Mile variety, an increase of 1530 kg h^{-1} was achieved, which increased the yield by 66% compared to the control (N_0 kg h^{-1}). In contrast, with Mile variety in the climatic conditions that were present in 2015 when the optimal application of N (120 kg h^{-1}) was realized, there was an increase in the yield of 1900 kg h^{-1} and with Marko variety 1590 kg h^{-1}, indicating all the complexity of the interaction of the variety x environment, or selection of a variety for certain climatic conditions.

Table 2. Yield (kg h^{-1}) by varieties, fertilization variants and years

variety	Year	Fertilization variants				
		0	80	100	120	Average
Mile	2014	2.657	3.677	4.210	3.900	3.611
	2015	2.100	3.420	4.000	3.768	3.322
	average	2.379	3.549	4.105	3.834	3.467
Marko	2014	1.950	3.512	4000	3.968	3.358
	2015	2.300	3.300	3.890	3.618	3.277
	average	2.125	3.406	3.945	3.793	3.317
average		2.252	3.478	4.025	3.814	3.392

Lsd	•	(B)	(AxB)	(C)	(AxC)	(BxC)	(AxBxC)
0,05	249,2	249,2	517,3	172,4	356,5	356,5	633,3
0,01	333,2	333,2	676,5	232,8	475,5	475,5	961,1

A-variety, B-fertilizer dose, C-the year of study

The influence of the variety, the dose of nitrogen application in the two years studied, influenced the absolute mass of 1000 grains by 4.96 g. (variety Marko, application of N 80 kg h^{-1}, 2015 36,07 g and maximum value of 41,03 g on the same variety without application of N during 2014). However, the impact of the individual influence of the variety, the application of mineral nitrogen and the year influenced little to the absolute weight of 1000 grains (Table 3).

Table 3. Absolute mass of 1000 grains (g) by varieties, fertilization variants and years

Variety	year	Fertilization variants				
		0	80	100	120	average
Mile	2014	39.05	39.00	38.02	38.09	38.54
	2015	39.25	39.22	41.06	39.04	39.64
	average	39.15	39.11	39.54	38.57	39.09
Marko	2014	41.03	38.00	38.40	40.05	39.37
	2015	37.15	36.07	38.90	38.16	37.57
	average	39.09	37.04	38.66	39.11	38.46
average		39.12	38.08	39.10	38.84	38.78

Lsd	(A)	(B)	(AxB)	(C)	(AxC)	(BxC)	(AxBxC)
0,05	1,04	1,04	2,08	0,73	1,47	1,47	2,94
0,01	1,38	1,38	2,76	0,97	1,95	1,95	3,90

A-variety, B-dose of fertilizer, C-the year of studyest

For all variants of the application of nitrogen fertilizer and the year Mile variety had a higher hectolitre mass by 0.82 kg hl^{-1}(Table 4). In general, thehectolitre mass by 0.82 kg hl^{-1} , depending on the variety, fertilization variants during the two years varied by 6.05 kg hl^{-1}

(variety Mile, application of N 100 kg h^{-1}, in 2015 68.83 kg $hl^{-1}$and the maximum value of 74.85 kg hl^{-1}on the same variety with the application of 80 kg h^{-1} of N during 2014).

Table 4. Hectolitre mass (kg hl^{-1}) by varieties, fertilization variants and years

Variety	year	Fertilization variants				
		0	80	100	120	average
Mile	2014	73,01	74,85	72,06	73,05	73,24
	2015	70,60	73,60	68,83	72,16	71,30
	Average	71,81	74,23	70,45	72,61	72,28
Marko	2014	71,25	73,35	70,13	72,34	71,77
	2015	68,14	73,05	73,22	70,18	71,15
	average	69,70	73,20	71,68	71,26	71,46
average		70,76	73,72	71,07	71,94	71,87

Lsd	(A)	(B)	(AxB)	(C)	(AxC)	(BxC)	(AxBxC)
0,05	0,99	0,99	1,99	0,70	1,41	1,41	2,82
0,01	1,32	1,32	2,64	0,93	1,87	1,87	3,74

A-variety, B-dose of fertilizer, C-the year of study

Using the simple correlation coefficients significant ($p \leq 0.05$) negative correlation between yield and absolute mass of 1000 grains (r=-0.662) was found as well as between the absolute mass of 1000 grains and the hectolitre mass in Mile variety. There was no significant ($p \geq 0.05$) interrelation between the traits found in Marko variety (Table 5).

Table 5. Correlation coefficients between tested properties (n=10)

Variety						
	Mile			Marko		
property	I	II	III	I	II	III
yield (kg h^{-1}) I	-	-0.662*	0.077 NS	-	-0.102 NS	0.422 NS
Absolute mass of 1000 grains (g) II		-	-0.662*		-	0.170 NS
Hectolitre mass (kg hl^{-1}) III			-			-

Statistical significance levels: *$p \leq 0.05$, NS – not significant ($p \geq 0.05$).

Conclusions

The investigated varieties behaved differently according to the climatic conditions in which the yield was formed, as well as for the interdependence of grain yield and quality parameters: the absolute mass of 1000 grains and the hectolitre mass. On the basis of the coefficient of correlation, it was established that significant interdependence was not found in variety Marko, while in variety Mile significant ($p \leq 0.05$) negative interdependence was found between the yield and the absolute mass (r=-0.662) and between the absolute mass of 1000 grains and the hectolitre mass (r=-0.648).This points to all the complexity of choosing a variety to achieve maximum yields and grain quality.

In given pedological climatic conditions, the application of nitrogen of 100 kg h^{-1} clearly proved to be optimal for both tested varieties and during both years.

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EFFECTS OF ZEOLITE ON GERMINATION OF SOYA BEAN SEED AND ITS USE AS A SUBSTRATE

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Abstract

Zeolite is a mineral of volcanic origin with negatively charged crystalline sieve that, according to many studies, has the ability to exchange ions. Mechanical processing - grinding of zeolite results in the increase of the particle surfaces and thereby its ability to adsorb heavy metals, mycotoxins, positively charged ions of sodium, potassium, nitrogen. The aim of the present study was to determine the effect of natural zeolite on seedlings and their development, as well as on total soya bean seed germination under laboratory conditions. The use of zeolite in the combination with soil and sand, as methods for seed germination, was observed in the study. The test was conducted on three samples of soya bean seeds at the Seed Testing Laboratory of the Maize Research Institute, Zemun Polje. Particles (0.3-1 mm) of natural zeolite, mixed with soil and sand in the concentration of 1% and 3%, were used in the study. The soil type was degraded chernozem taken form the ploughing layer of the maize field and prepared according to the standard procedure: 4x100 seeds of soya bean were tested by the methods filter paper + soil, filter paper + sand (standard), filter paper + soil + zeolite (1% and 3% weight percentages) and filter paper + sand + zeolite (1% and 3% weight percentages). Seeds were germinated in the germination cabinet at the alternating temperature of 20<=>30^{o}C (16 h : 8 h / light : dark photoperiod) for 8 days. The use of zeolite increased germination of soya bean seeds, while different concentrations of zeolite did not significantly increase germination.

Key words: *zeolite substrate, germination of soya bean seeds*

Introduction

Zeolite is a mineral of volcanic origin found in 1756. This mineral has the capacity to intensively lose water during heating, due to which was named *zeolite* from the Greek language zéō – meaning to boil and lithos – meaning stone, Polat et al. (2004). There are over 50 types of naturally occurring zeolites. The most important and most observed is clinoptilolite, which has a three-dimensional structure with the ability to exchange ions, due to which it is a good absorbent. Due to electric charge of the aluminium-silicate surface, clinoptilolite is known as a "molecular sieve", Ramôa (1993) and is used in industry, agriculture, environmental protection, medicine, decontamination in the military industry.

The use of zeolite in agriculture began in 1960 on farms in Japan. Japanese farmers used to apply zeolites for years to control the soil moisture content, improve the soil structure and ph. Mineral zeolite possesses three main properties of great importance in agriculture: high capacity of cation exchange, high water holding capacity and high absorption capacity, Mumpton (1999). Alongside water, nutrients are also retained in the zone of the root system, so that plants can use them when needed.

There are many studies dealing with the effects of zeolites on seedlings, the development of shoots and roots. Trinchera et al. (2009) have observed effects of various zeolite granular sizes on maize seedlings. It was established that the application of granular and micronised zeolites in the substrate favoured the occurrence of secondary roots and root hairs. On the other hand, the use of micronised zeolite positively affected the development of maize shoots. The highest increase in the length to weight ratio in primary roots of maize has been

obtained by the treatment with micronised clinoptilolit in the amount of 0.1%, while the lowest increase was gained by the treatment with granular clinoptilolit, which indicates the effect of different granular sizes of clinoptilolit on the maize root.

Furthermore, the effect of zeolite on plants by its activity and by the combinations of various substrates, has been also studied. Yilmaz et al. (2014) have concluded that the zeolite, turf and perlite mixtures improved the cucumber seedling quality, hence zeolite can be successfully used in the seedling production.

Oilseed rape seedlings growing under stress conditions of drought without zeolites have a less dry weight and a lower total germination percentage. Moreover, the ratio of above ground parts to root is less favourable and therefore the chances to survive stress conditions of drought are smaller. As a result of this, quality and quantity of produced seeds have been decreased, Armandpisheh et al. (2009).

Khan et al. (2009) have observed effects of zeolites on soya bean seeds and established the increase of seed quality and germination when zeolite was applied at the rate of 1 and 2%. The zeolite addition to soya bean crops increased germination and vigour, and decreased the level of seed infection caused by *Phomopsis* sp.

Material and methods

The study was performed in the Seed Testing Laboratory of the Maize Research Institute, Zemun Polje on seeds of three soya bean cultivars. Zeolite provided by the company "Zeomedik", Altina-Zemun, was of the following composition: clinoptilolit - 94%, quartz - 4% and phlogopite (mica) - 2%. Granulation of the used zeolite ranged from 0.3 to 1mm.

The effects of zeolite in the mixture with the soil in the mass concentration of 1-3% and quartz sand in the same concentrations were observed. Both substrates were combined with the filter paper. Soil and sand without zeolite, also combined with the filter paper, served as control variants.

Prior to their use, all substrate were tested according to the ISTA Handbook of Seedling Evaluation (2013). The used soil was degraded chernozem collected from a maize field with the following parameters: pH - 7.5, moisture - 17.9%, moisture capacity - 48%. The sand fraction size was 2.6, moisture 0.033%, pH 7.5, moisture capacity 29%. The properties of the filter paper were as follow: weight - 110 g, pH 7.5, moisture 4.7 and moisture capacity 37%. *Festuca rubra* seeds, susceptible to substrate toxicity, were germinated and it was determined that soil, sand and filter paper did not contain toxic substance and that they met requirements of the standard.

A total of 4x100 seeds were studied by their placing on filter paper + soil, filter paper + soil + 1% zeolite and paper + soil + 3% zeolite, filter paper + sand, filter paper + sand + 1% zeolite and filter paper + sand + 3% zeolite. Seeds were germinated in the germination cabinet at the alternating temperature of 20<=>30^{o}C under light: dark photoperiod of 8 h : 16 h. The final count was read after 8 days, International Seed Testing Association (2017). The obtained values were expressed in the percentage participation of normal seedlings.

Results were statistically processed by a computer program MSTAT. The two factorial analysis of variance and the least significant difference (LSD) test were applied, Hadživuković (1991).

Results and discussion

The analysis of variance showed the statistically significant differences in obtained values of total seed germination and the applied test method (factor A) and in tested cultivar seeds (factor B). Total seed germination did not significantly differ over replications and interactions between observed factors (Table 1).

Table 1. Statistical significance of effects of observed factors on total seed germination

Factor	Degree of freedom (DF)	Mean squares (MS)	F value
Replication	3	33.037	0.9044^{ns}
Factor A (method)	5	407.689	11.1612^{**}
Factor B (cultivars)	2	561.556	15.3736^{**}
AB	10	21.956	0.6011^{ns}
Error	51	36.527	

ns – not significant; ** - significant at the 0.01% level

Table 2 shows total seed germination over methods and cultivars expressed in % – the soil /filter paper/zeolite (1% and 3%) combination. The lowest, i.e. highest value of total seed germination for all three methods was in the third, i.e. the first cultivar, respectively.
The addition of 1% zeolite increased total seed germination by 7-9% in relation to the control variant. The number of abnormal seedlings was decreased by 5-7%, while the percentage of dead seeds remained approximately the same. Moreover, the addition of 3% zeolite significantly increased total seed germination in comparison to the control method. Total germination increased by 11-13% over cultivars on the account of abnormal seedlings. The percentage of dead seeds decreased insignificantly (by 2-3%).
The zeolite concentration of 3% resulted in the increase of total germination, similar as with the concentration of 1%. The comparison of zeolite concentrations shows that seed germination was higher by 2-6% when the higher concentration of zeolite was applied.
In the course of the germination evaluation it was determined that the soil, with or without zeolite, was not suitable substrate for soya bean seed germination. Tender soya bean shoots penetrate soil with difficulty, therefore seedlings in the middle of the role are brittle, smaller and discoloured, which resulted in breakage of hypocotyls. Unlike hypocotyls, the root was strong and well developed in normal seedlings. Due to the zeolite property to hold water, the soil was compact, which made the extraction and the evaluation of seedlings more difficult.

Table 2. Values of total seed germination over methods in % – soil/zeolite combination

Cultivar	Control FP + soil			Method FP + soil + 1% zeolite			Method FP + soil + 3% zeolite		
	Normal seedlings	Abnormal seedlings	Dead seeds	Normal seedlings	Abnormal seedlings	Dead seeds	Normal seedlings	Abnormal seedlings	Dead seeds
1	61	24	15	68	19	13	74	14	12
2	63	23	14	72	16	12	73	15	12
3	56	27	17	63	20	17	65	21	14

Table 3 shows total seed germination over methods and cultivars expressed in % – the soil /filter paper/zeolite (1% and 3%) combination. The lowest, i.e. highest value of total seed germination for all three methods was in the third, i.e. the first cultivar, respectively.
The addition of 1% zeolite increased total seed germination by 3-9% in relation to the control variant. The number of abnormal seedlings was decreased by 4-9%, while the percentage of dead seeds remained approximately the same. The addition of 3% zeolite increased total seed germination by 6-8% in comparison to the control method. The percentage of dead seeds decreased insignificantly (by 1-2%).
The comparison of zeolite concentrations shows that total seed germination was higher by 2-4% when higher concentrations of zeolite were applied.
In the course of the germination evaluation it was observed that sand was a better substrate for soya bean seed germination than soil. The use of sand as a substrate in all three methods (with

different zeolite concentrations, or without zeolite) resulted in robust seedlings with the strong root system and well developed hypocotyls. Sand in the combination with zeolite provided a loosened germination medium, which enabled the easier evaluation and extraction of well developed seedlings.

Table 3. Values of total seed germination over methods in % – sand/zeolite combination

Cultivar	Control FP + sand			Method FP + sand + 1% zeolite			Method FP + sand + 3% zeolite		
	Normal seedlings	Abnormal seedlings	Dead seeds	Normal seedlings	Abnormal seedlings	Dead seeds	Normal seedlings	Abnormal seedlings	Dead seeds
1	74	18	6	83	9	8	81	15	4
2	73	19	8	77	12	11	79	12	9
3	64	17	19	67	13	20	71	12	17

The analysis of results by the LSD test for the interactions among cultivars shows the significant difference in total seed germination. The lowest difference was in the cultivar 3, while there were no significant differences in remaining cultivars (Table 4.).
The highest and the lowest values of total seed germination were obtained by the methods: sand + filter paper + 3% zeolite and soil + filter paper, respectively. A statistically significant difference was achieved by the method 6, with the highest total seed germination and by the method 1 with the lowest total seed germination. There were no statistically significant differences in remaining methods (Table 5).

Table 4. LSD test for observed cultivars

Cultivar	Total germination
1	73.17^{A}
2	72.17^{A}
3	64.33^{B}

Table 5. LSD test for applied methods

Ordinal number	Test methods	Total germination
1	Soil + filter paper	60.00^{D}
2	Soil + filter paper + 1% zeolite	67.33^{C}
3	Soil + filter paper + 3% zeolite	70.50^{BC}
4	Sand + filter paper	70.33^{C}
5	Sand + filter paper + 1% zeolite	75.33^{AB}
6	Sand + filter paper + 3% zeolite	75.83^{A}

Conclusion

According to gained results it can be concluded that sand is a better substrate than soil for soya bean seed germination. This was confirmed by the comparison of results of total germination of control methods in soil and in sand. It was established that the best substrate to germinate soya bean seeds is sand in the combination with 3 % zeolite. The highest total germination was achieved by this method, and the presence of zeolite provided a loosened and sufficiently wet medium that provided easer extraction of seedlings. In the course of the germination evaluation, seedlings were strong with well developed roots and hypocotyls.
Soil in the combination with zeolite was not such a good substrate, because zeolite bound water and soil became sticky and cloddy, so extraction of brittle and breakable seedlings was

difficult. On the other hand, the increase in the zeolite percentage in the combination with soil improved total seed germination.

Results obtained on total seed germination showed that the application of both lower and higher concentrations of zeolite significantly affected its increase. The comparison of results gained on concentrations shows that better effects were achieved by the higher zeolite concentration. According to these results the recommendation would be to apply 3% zeolite with seed in the sowing season.

Sand is used for germination tests of soya bean seeds in the Seed Testing Laboratory. The substrate sand + zeolite could be applied in poor quality soya bean seed lots in order to obtain maximum seed germination necessary for seed trade.

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FORAGE QUALITY AND *IN VITRO* DRY MATTER DIGESTIBILITY OF PEA:OAT MIXTURES DEPENDING ON STAGE OF GROWTH

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Abstract

The production and utilization of intercropped, complementary legume : cereal bi-crops as ruminant feeds is beneficial for many reasons. The nitrogen fixation by the legume benefits the cereals and decreasing the need for N fertilization. In turn, the cereal supports the legume, thereby preventing the lodging that typifies pure stands of mature forage pea. The investigations were carried out to evaluate the quality of oat (*Avena sativa* L.) and pea (*Pisum sativum* L.) in pure stands as well as in mixtures of 75:25, 50:50 and 25:75 ratios at the Institute for forage crops to address the problem of nutritious green forages for livestock in the region. The samples were harvested at the beginning of pea flowering – 10% of flowering and at the forming green seeds in 2/3 pea pods. Green forage samples were assayed for CP (Crude Protein), Crude Ash, Ether Extract, NDF (Neutral detergent Fiber), ADF (Acid Detergent Fiber), Hemicellullose, ADL (Acid Detergent Lignin) and IVDMD (*in vitro* Dry Matter Digestibility). Oat:pea mixtures produced higher crude protein content than oat pure stand. 75:25, 50:50 and 25:75 oat : pea mixtures resulted in 46.4%, 57.0% and 80.5% higher crude protein content than oat pure stand, respectively. The highest forage quality parameters were achieved when pea was grown as a monoculture or when at a high proportion (75%) in mixture. The results showed that mixture of pea and oat at the 75:25 seeding ratio was obtained the highest forage quality.

Keywords: oat*:pea mixtures, forage quality, dry matter digestibility*

Introduction

Bi-crops of various grain legumes and cereals have received much attention because of their high yields (Salawu et al., 2001). In particular, bi-crops with pea varieties or mixtures with high grain to straw ratios are considered to have a good balance of energy and protein contents (Anil et al., 1998). Legumes are rich in terms of protein concentration, whereas cereals have higher carbohydrate contents, and cereal benefit from the nitrogen fixed by legumes when they are grown together. Several researches reported that annual legume-cereal mixtures resulted in high yields and high nutritional quality as compared with cereal alone (Yolcu et al., 2009; Balabanli et al., 2010; Kocer et al., 2012). The goal of most dairy farmers is to use the available land to produce cheaper but high quality feed with adequate protein content for lactating cows in order to maximize milk yield (McKenzie and Spaner, 2002). From the nutritional point of view, pea-oat intercrops offer the possibility of reduced importation of protein concentrates in ruminant diets as well as increased value of harvested product because of increased protein content.

Pea forages have higher crude protein and *in vitro* digestible organic matter in DM, and lower neutral detergent fiber (NDF) and acid detergent fiber (ADF) than wheat (Salawu et al., 2001), and higher CP contents than oat (Faulkner, 1985). However, adding pea to wheat, oat or barley increases forage CP concentration and decreases NDF and ADF (Salawu et al., 2001). Mustafa and Seguin (2004) found that whole-crop pea silage has similar forage yields, but higher CP and lower NDF contents than pea-cereal silages and that the *in vitro* DM

digestibility of pea silage is higher than that of pea-cereal mixtures at earlier harvest, but the difference is reduced at later harvest (Mustafa and Seguin, 2004).
However, it can be difficult to determine the best time to harvest whole crop forages to optimise their nutritive value without compromising yield. The quality of grain legumes (e. g. peas), whole crop cereals and their bi-crops, do not decline with age in the same manner as grasses and legumes, because of the additional influence of the grain and pod yields (Salawu et al., 2001). This makes it more difficult to determine the appropriate harvest time for these crops to use indairy herd feeds.
The main aim of the present study was to determinate the nutritive value and the *in vitro* dry matter digestibility of oat and pea sown in pure crop and in bi-crop mixtures depending on different seeding rate and harvested at different time.

Materials and methods

The experiment was designed with three replications according to a randomized complete block. Experiment was established in autumn in 2012, on October the 20th and the samples were taken in spring in 2013. Pea and oat were grown in binary mixtures at the experimental field of Institute for forage crops, Kruševac – Serbia (21º19´35´´ E, 43º34´58´´ N). The pea and oat were tested at five different mixture rates: 100% pea + 0% oat; 0% pea + 100% oat; 25% pea + 75% oat; 50% pea + 50% oat and 75% pea + 25% oat. All mixtures were sown on plots of 20 m^2. Initial soil test from 0-30 cm soil depth before the trial commenced showed 0.16% N, 4.9 mg P_2O_5 / 100 g of soil, 23.1 mg K_2O / 100 g of soil, 3.5% organic matter and a pH of 5.7 in N KCl. One level of fertilizer was applied, 300 kg ha^{-1} NPK (15:15:15) before the seeding. Plant samples were taken in spring 2013, at the beginning of pea flowering – 10% of flowering and at the forming green seeds in 2/3 pea pods.
Standard procedures described by the AOAC (1990) were used to determine ash (AOAC, 942.05), crude protein (CP; AOAC 984.13) and ether extract (EE, AOAC 954.02). NDF assayed with heat stable α-amylase (aNDF), acid detergent fiber (ADF), hemicellulose (HCL) and lignin content were determined according to Van Soest et al. (1991). Two stage pepsin-cellulase method was used for *in vitro* DM digestibility (De Boevar et al., 1986).
The experimental data were analyzed by a two-way analysis of variance for silage samples using a model that accounted for the main effects of pea:oat mixtures and time of harvest. Effects were considered different based on significant ($p< 0.05$) F ratio. The significance of differences between arithmetic means was tested by LSD test (STATISTICA 6, Stat. Soft. 2006).

Results and discussion

An analysis of variance found statistically significant differences among cutting stages for investigated forage quality parameters (Table 1). Results of this study showed that CP, ash, ADF and DMD decreased, whereas EE, NDF, hemicellullose and ADL contents increased with advancing plant growth and development. The increasing NDF and ADL content with plant development affected decreasing of DMD from 706.3 g kg^{-1} DM to 600.1 g kg^{-1} DM. With advancing the plant maturity CP content decreased by 9.2%, whereas NDF and ADL content increased by 7.9% and 11.5%, respectively. Many researchers reported similar rasults (Uzun and Asik, 2012; Turk et al., 2007; Tan and Serin, 1996). The forage quality of grasses and legumes declines with age due to reductions in the leaf – to – stem ratio in conjuction with a decline in the stem component nutritive value. Therefore, the strategy for obtaining good quality forage is to harvest when the leaf – to – stem ratio is high. In contrast, the quality of grain legumes (e. g. peas), whole crop cereals and their bi-crops do not decline with age in the same manner as grasses and legumes, because of the additional influence of the grain and pod yields (Salawu et al., 2001).

Table 1. Nutritive value of oat-pea mixture depending on the seeding rate and time of harvest

		B_1	B_2	B_3	B_4	B_5	$\overline{X}$ B
CP, g kg^{-1}	A_1	195.6^{b}	102.1^{f}	156.4^{d}	176.8^{c}	192.7^{b}	**164.7^{A}**
	A_2	213.1^{a}	97.1^{f}	135.2^{e}	136.0^{e}	166.8^{c}	**149.6^{B}**
	$\overline{X}$ A	**204.3^{A}**	**99.6^{E}**	**145.8^{D}**	**156.4^{C}**	**179.8^{B}**	
Ash, g kg^{-1} DM	A_1	92.2^{d}	111.4^{b}	115.8^{a}	117.3^{a}	106.7^{c}	**108.7^{A}**
	A_2	77.5^{f}	95.9^{d}	90.6^{de}	88.4^{e}	86.5^{e}	**87.8^{B}**
	$\overline{X}$ A	**84.9^{C}**	**103.7^{A}**	**103.2^{A}**	**102.9^{A}**	**96.6^{B}**	
EE, g kg^{-1} DM	A_1	26.2^{cd}	23.4^{e}	25.1^{d}	27.8^{c}	31.6^{b}	**26.8^{B}**
	A_2	27.8^{c}	36.8^{a}	27.9^{c}	32.4^{b}	25.8^{d}	**30.2^{A}**
	$\overline{X}$ A	**27.0^{BC}**	**30.1^{A}**	**26.5^{C}**	**30.1^{A}**	**28.7^{B}**	
ADF, g kg^{-1} DM	A_1	377.9^{e}	404.5^{b}	390.4^{c}	418.9^{a}	383.1^{d}	**395.0^{A}**
	A_2	336.9^{g}	396.6^{c}	401.0^{b}	393.3^{c}	361.8^{f}	**377.9^{B}**
	$\overline{X}$ A	**357.4^{C}**	**400.6^{A}**	**395.7^{A}**	**406.1^{A}**	**372.5^{B}**	
aNDF, g kg^{-1} DM	A_1	429.4^{h}	627.0^{a}	515.4^{g}	550.0^{d}	434.3^{h}	**511.2^{B}**
	A_2	541.7^{e}	578.8^{b}	559.6^{c}	548.7^{d}	530.5^{f}	**551.9^{A}**
	$\overline{X}$ A	**485.5^{C}**	**602.9^{A}**	**537.5^{B}**	**549.4^{B}**	**482.4^{C}**	
HCL, g kg^{-1} DM	A_1	51.5^{e}	222.5^{a}	125.0^{d}	131.1^{d}	51.2^{e}	**116.2^{B}**
	A_2	204.8^{a}	182.2^{b}	158.6^{c}	155.4^{c}	168.7^{c}	**174.0^{A}**
	$\overline{X}$ A	**128.2^{C}**	**202.4^{A}**	**141.8^{B}**	**143.3^{B}**	**109.9^{D}**	
ADL, g kg^{-1} DM	A_1	68.4^{d}	61.3^{f}	68.3^{d}	70.5^{d}	66.1^{e}	**66.9^{B}**
	A_2	64.3^{e}	90.0^{a}	83.4^{b}	75.9^{c}	73.0^{c}	**77.3^{A}**
	$\overline{X}$ A	**66.3^{B}**	**75.7^{A}**	**75.9^{A}**	**73.2^{A}**	**69.6^{B}**	
DMD, g kg^{-1} DM	A_1	761.8^{a}	589.7^{e}	725.2^{b}	701.7^{c}	753.2^{a}	**706.3^{A}**
	A_2	727.9^{b}	536.1^{g}	553.3^{f}	563.7^{f}	619.3^{d}	**600.1^{B}**
	$\overline{X}$ A	**744.9^{A}**	**562.9^{D}**	**639.3^{C}**	**632.7^{C}**	**686.3^{B}**	

A_1- beginning of pea flowering – 10% of flowering; A_2- forming green seeds in 2/3 pea pods. B_1-100% pea + 0% oat; B_2-0% pea + 100% oat; B_3-25% pea + 75% oat; B_4-50% pea + 50% oat; B_5-75% pea + 25% oat. CP-Crude Protein; EE-Ether Extract; ADF-Acid Detergent Fiber; aNDF-Neutral Detergent Fiber; HCL-Hemicellulose; ADL- Acid Detergent Lignin; DMD-Dry Matter Digestibility. Different letters denote significantly different means ($P< 0.05$).

Åman and Graham (1987) found that in pea plants the most dramatic changes in the distribution of chemical constituents occur during the pea filling process, a rapid exponential phase of pea growth. The same authors indicated that nutrients, especially carbohydrates and protein are translocated from the vegetative parts of the plant to the pea grains, while the leaves and stems gain cellulose, hemicellulose and lignin. They also concluded that the gross chemical composition of the whole crop remains remarkably constant, with the exception of the transformation of soluble sugars to starch and the increased content of cell walls. Trevino et al. (1987) pointed out to the fact that in forages harvested at early pod fill stage the leaf component constitutes the largest part of the total plant dry weight, but at later harvest the fully developed peas constitute around half of the whole crop (Åman and Graham, 1987), and after an initial increase the CP remains relatively uniform, as does the lignin content. On the another hand, Jaster et al. (1985) found that in pea-oat bi-crop forage the CP content declines during maturity, but the DM, CP, starch, NDF content and *in vitro* digestible organic matter of pea forages increase with maturity.

Crude protein content of forage is one of the most important criteria for forage quality evaluation (Assefa and Ledin, 2001). In all mixtures, the CP content increased as field pea

seeding proportion increased (P< 0.05), (Table 1). Monoculture field pea had the highest CP content (204.3 g kg^{-1} DM), followed by the mixture of pea with oat (75 : 25) (179.8 g kg^{-1} DM). Monoculture oat had the lowest content of CP (99.6 g kg^{-1} DM) and pea-oat mixtures (25 : 75, 50 : 50 and 75 : 25) had 46.4%, 57.0% and 80.5% more CP respectively than monoculture oat.

The ash and EE were also affected by composition of pea-oat mixtures. The highest ash and EE contents were determined in pure oat (103.7 g kg^{-1} DM and 30.1 g kg^{-1} DM, respectively). Increasing pea proportion in the mixtures affected decreasing the ash content, but EE content did not changed in the same way (Table 1).

The lowest ADF content was determined in the pure pea (357.4 g kg^{-1} DM) and with increasing pea seeding rate in the pea-oat mixtures ADF content increased from 395.7 g kg^{-1} DM (25 : 75 pea-oat mixture) to 406.1 g kg^{-1} DM (50 : 50 pea-oat mixture). These ADF values did not differ significantly from pure oat ADF content (400.6 g kg^{-1} DM). In all pea-oat mixtures the content of ADF was higher than in pure oat, and according to the pea seeding rate in the mixture was the lowest in 75 : 25 pea-oat mixture (372.5 g kg^{-1} DM). The highest NDF content was determined in pure oat (602.9 g kg^{-1} DM), but with decreasing seeding rate of oat in the mixture NDF content increased from 537.5 g kg^{-1} DM in 25 : 75 pea-oat mixture to 549.4 g kg^{-1} DM in 50 : 50 pea-oat mixture. The NDF content in the 75 : 25 pea-oat mixture was lower than NDF content in pure oat. Content of hemicellulose changed in the same way as NDF with increasing pea seeding rate in pea-oat mixture (Table 1).

Kocer and Albayrak (2012) pointed out that ADF and NDF contents increased as the pea seeding ratio decreased in mixtures. These authors also indicated that monoculture pea had the lowest values of ADF (25.81%) and NDF (38.27%) whereas the highest ADF and NDF values were observed in the monoculture of oat (34.61% and 59.12%). Van Soest (1996) indicated that under similar growth conditions legumes have lower NDF and ADF than cereals, wich are in agreement with the present study.

Results of the present study showed that the lowest ADL content was determined in pure pea (66.3 g kg^{-1} DM), and the highest ADL content was in pure oat (75.7 g kg^{-1} DM) and in 25 : 75 pea-oat mixture (75.9 g kg^{-1} DM). With increasing pea seeding ratio in pea-oat mixtures, content of ADL decreased from 75.9 g kg^{-1} DM to 69.6 g kg^{-1} DM. DMD was significantly different among the experimental treatments, with the exception of 25 : 75 and 50 : 50 pea-oat mixtures. The highest DMD was recorded in pure pea (744.9 g kg^{-1} DM) and the lowest in pure oat (562.9 g kg^{-1} DM). DMD decreased in mixtures with increasing pea seeding rate from 25:75 to 50:50 pea-oat mixture. The reason could be found in the fact that 50 : 50 pea-oat mixture had high level of NDF and ADF content.

Conclusions

According to the data obtained from this research work, it was realized that pea and oat can be planted succesfully for herbage and hay production. Forage quality of pea-oat mixtures was generally affected by stage of plant development and seeding rate of pea and oat. Cultivating pea and oat in mixtures were found to be advantageous in increasing forage quality. Growing oat in a mixture with pea significantly increased forage quality – CP and DDM, compared to pure oat. This confirms that growing leguminous forages like pea in a mixture with oat addresses dietary advantages especially in terms of CP and DDM on ruminal animal production. The lowest quality was deterrmined in 50 : 50 pea-oat mixture because this mixture had the highest NDF and ADF contents and consequently the lowest DDM.

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THE INFLUENCE OF THE SOIL TYPE ON TOTAL NUMBER OF MICROORGANISMS IN UGAR AND SOWN MAIZE

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Abstract

Various groups of microorganisms live in the soil: aminoheterotrophs, aminoautotrophs, oligonitrophilic bacteria, cellulolizators, *Azotobacter* sp., actinomycetes, fungi, sporogenic bacteria and others. The mentioned groups of microorganisms have a precisely determined and irreplaceable role in the processes of matter cycling in the soil, which lead to mineralization and repeated synthesis of organic matter whereas nutrients become accessible to plants. In this work, the total number of microorganisms is determined, as the most significant indicator of the soil fertility, in two types of soil: "cernozem" (at the locality Zemun Polje) and "gajnjaca" (at the locality Rača Kragujevačka) in Serbia. The total number of microorganisms is determined depending on the soil type, the manner of its use (ugar and sown maize) and the quantity of the applied N fertilizer. It is examined how the rising quantities of nitrogen fertilizers, N_{30}, N_{60}, N_{120}, N_{160} kg ha^{-1}, affect the total number of microorganisms in relation to the control variation, which was without applied fertilizer. The soil samples for this microbiological analysis are taken on the depth of 0-30 cm. The number is determined by the standard indirect method of sowing of diluted soil samples on the nutritious substratum tripton-soya agar TSA (10^{-5}) and it is expressed in grammes of completely dry soil. The results of the research are analysed by the descriptive statisics method. The total number of microorganisms is different in different types of soil and it shows the dependance on examined factors.

Key words: *microorganisms, different types of soil, nitrogen, maize.*

Introduction

Maize have an important place in crop rotation (Popović, 2010; Živanović et al., 2017; Erić et al., 2018). Microorganisms make a living part of soil (Govedarica et al., 1997) and together with other organisms in soil, they are a component of the most dynamic ecosystems in nature. Different ecophysiological groups of microorganisms live in soil and each of them has a very important and irreplaceable role in the processes that are led within it.

Therefore, heterotrophs as a source of carbon and energy use complete organic compounds, enabling their mineralization in soil (Đukić and Jemcev, 2000). Aminoheterotrophs are a very numerous group of soil microorganisms. On the basis of the research results, Šarčević-Todosijević and Živanović (2017), say that the number of aminoheterotrophs in "cernozem" Zemun Polje varies from $17.4 – 671.5x10^5 g^{-1}$.

In nitrogen cycling, it is especially notable to emphasize the role of *Azotobacter* sp., an organism from the group of free azotofixators, which adopt elementary nitrogen and reduce it to ammonia form and then turn it into organic one (Madigan et al., 1997; Đukić and Jemcev, 2000). Actinomycetes and germs are numerous in soil microflora and have a significant part in a transformation of soil organic matter. Šarčević-Todosijević et al. (2018) say that the

number of actinomycetes in "cernozem" of Zemun Polje is from 0.1 – 17.7x$10^4 g^{-1}$, and in "gajnjača" at the locality Rača Kragujevačka that number is from 2.1 – 9.0x$10^4 g^{-1}$. The total number of microorganisms depends on the soil type, organic matter and water contents, climate, season and antropogenic influences.

In this work, it is determined the total number of microorganisms considering the soil type and quantity of applied nitrogen fertilizer on "ugar" and under the sown maize.

Material and methods

The research of the influence of the soil type and quantity of nitrogen on the total number of microorganisms is carried out in two areas: Eastern Srem (Maize Research Institute, Zemun Polje) and Central Šumadija (Rača Kragujevačka) in Serbia. Microfield testings are done by method of split plots in four repetitions. The research included the three following factors:

1. Soil type (A)
   A_1 – "cernozem" (Zemun Polje)
   A_2 – "gajnjaca" (Rača Kragujevačka)

2. Quantity of nitrogen (B)
   B_1 – Control (without fertilizer)
   B_2 – $P_{90}\,K_{60}\,N_{30}$ kg ha^{-1} (basis, phon)
   B_3 – $P_{90}\,K_{60}\,N_{60}$ kg ha^{-1}
   B_4 – $P_{90}\,K_{60}\,N_{120}$ kg ha^{-1}
   B_5 – $P_{90}\,K_{60}\,N_{180}$ kg ha^{-1}

3. The manner of using soil (C)
   C_1 – "ugar", C_2 – under crop.

The applied agrotechnique during the observations was standard, as the same as for regular maize production.

The soil samples for microbiological analyses were taken on the depth of 0 to 30 centimeters two times, in the phase of a cob blooming and in the phase of physiologic reapness of maize grains. The number of microorganisms was determined by a standard indirect method of sowing diluted soil samples on a nutritious substratum, which was after wards incubated on the temperature of 28^0C (Knežević-Vukčević and Simić, 1999; Đukić and Mandić, 2003). The total number of microorganisms on the nutritious substratum TSA was determined. The number is expressed in grammes of completely dry soil ($10^5 g^{-1}$). The results of the research are analyzed by descriptive statistics method.

Results and discussion

The total number of microorganisms presents a general soil biogenecy (Đukić and Jemcev, 2000) and, as an indicator of fertility, it is in significant and very significant correlative bonds with a number of different ecophysiological groups of microorganisms in soil, as well as with other soil fertility indicators, microbiological biomass of carbon and nitrogen, enzyme activity of soil (Đorđević, 1998; Hajnal-Jafari, 2010; Šarčević, 2010). By the variable analysis, it is confirmed that the total number of microorganisms is significantly affected by all the examined factors and their interactions, Table 1 and 2.

In a phenophase of a maize cob reaping (Table 1), the total number of microorganisms was expressed in "gajnjaca" (108.9 x $10^5 g^{-1}$), in comparison to "cernozem" (100.0 x $10^5 g^{-1}$), but without a statistically significant difference.

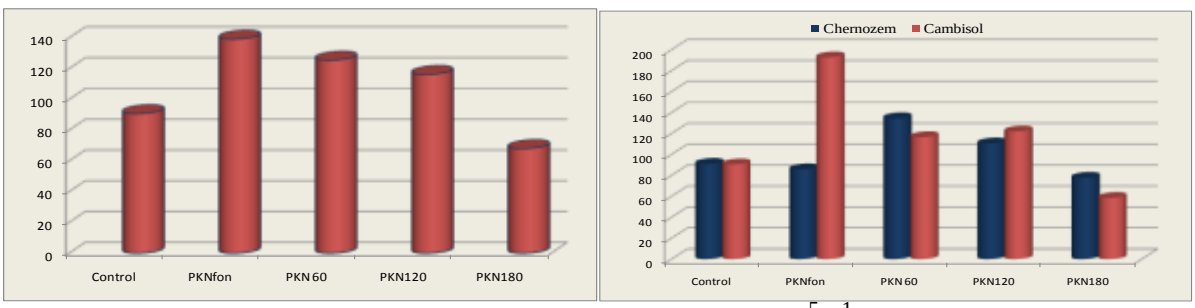
The results show that the manner how the soil was used ("ugar" or crop) had a significant impact on the total number of microorganisms. In a phenophase of maize cob blooming, a higher number of microorganisms was determined on "ugar" (121.1 x $10^5 g^{-1}$) than under the sown maize (92.4 x $10^5 g^{-1}$), and the difference was statistically very significant (Table 1).

Table 1. The influence of soil type, quantity of nitrogen and the manner of using soil on the total number of microrganisms ($10^5 g^{-1}$) in a phenophase of a maize cob blooming

Soil type (A)	Quantity of nitrogen (B)	The manner of using soil (C)		Average AB	Index (%)
		"Ugar"	"Under crop"		
"Cernozem"	Control	89.9	92.0	91.0	106.1
	PKN_{phon}	95.4	76.1	85.8	100.0
	PKN_{60}	**139.4**	**129.7**	**134.6**	156.9
	PKN_{120}	137.0	83.9	110.5	128.8
	PKN_{180}	72.3	83.1	77.7	90.6
	Average AC	106.8	93.0	100.0	-
	Index (%)	100.0	87.1	-	100.0
"Gajnjaca"	Control	114.4	66.6	90.5	47.0
	PKN_{phon}	**279.3**	106.1	**192.7**	100.0
	PKN_{60}	97.4	**135.6**	116.5	60.5
	PKN_{120}	123.3	120.6	122.0	63.3
	PKN_{180}	62.1	54.9	58.5	30.4
	Average AC	135.3	96.8	108.9	-
	Index (%)	100.0	71.5	-	108.9
Average BC	Control	102.2	79.3	90.8	65.2
	PKN_{phon}	**187.4**	91.1	**139.3**	100.0
	PKN_{60}	118.4	**120.3**	125.6	90.2
	PKN_{120}	130.2	102.3	116.3	83.5
	PKN_{180}	67.2	69.0	68.1	48.9
Average C		**121.1**	92.4	108.0	-
Index (%)		100.0	76.3	-	-

L S D	A	B	C	AB	AC	BC	ABC
0.05	10.32	22.61	14.76	40.97	21.78	39.54	72.72
0.01	13.89	36.19	19.87	45.07	30.12	43.43	75.29

The variable analysis confirmed significant differences in the total number of microorganisms between different applied quantities of nitrogen. In a phenophase of maize cob blooming (Table 1; Graph. 1a and b), the highest number of microorganisms in comparison with control, in average, was in a variation of applying PKN_{phon} (139.3 x $10^5 g^{-1}$), and the least one was in a variation of applying 180 kg ha^{-1} of nitrogen (68.1 x $10^5 g^{-1}$). The differences in the number of microorganisms were highly statistically significant. Lower quantities of applied mineral fertilizers had a stimulative effect on the number of microorganisms and soil biogenecy, where as high concentrations showed an inhibitory effect of nitrogen fertilizers on the number of microorganisms.



Graph 1. a) The total number of microorganisms in soil ($10^5 g^{-1}$) in comparison with the nitrogen quantity, b) The influence of soil type and nitrogen quantity on the total number of microorganisms ($10^5 g^{-1}$) in a phase of maize cob blooming

The highest number of microorganisms, under "ugar", was in a variation of applying PKN_{phon} (139.3 x $10^5 g^{-1}$), whereas the highest number under crops was in a variation of applying 60 kg ha^{-1} of nitrogen (125.6 x $10^5 g^{-1}$). The differences in the number of microorganisms were not statistically significant. In compliance with our research, were the researches of many authors. Milošević (1990) points out that lower concentrations of applied mineral fertilizers have a stimulative effect on the number of microorganisms and soil biogenecy, while high concentrations show an inhibitory effect. Šarčević-Todosijević et al. (2018) mention the noticed effect of stimulation of lower quantities of nitrogen fertilizers on the number of actinomycetes, which confirms similar observation of Epanchinov (1975). Đorđević (1998) mentions that mineral fertilizers, applied in higher or lower quantities on "cernozem" Zemun Polje, very notably affected the total number of microorganisms in a way that lower quantities increased, and higher quantities decreased their number in comparison with control.

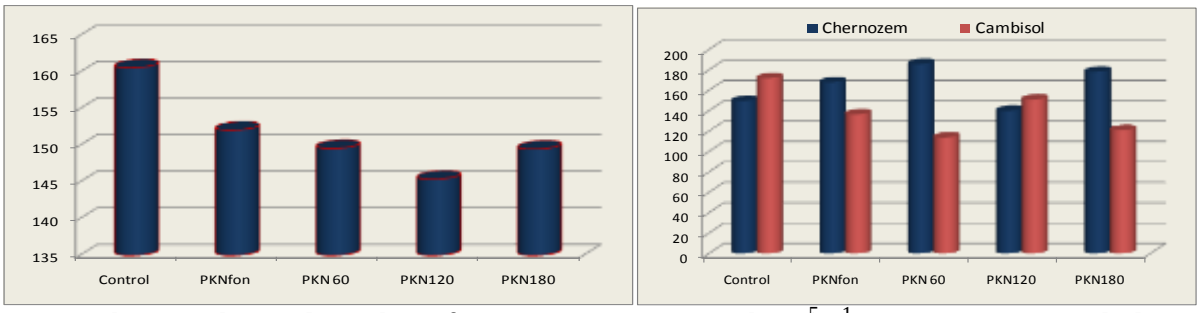
Table 2. The influence of soil type, nitrogen concentration and a manner of using soil on the entire number of microorganisms in a phase of physiological reapness of maize grain

Soil type (A)	Quantity of nitrogen (B)	The manner of using soil (C)		Average AB	Index (%)
		"Ugar"	"Under crop"		
"Cernozem"	Control	170.4	128.5	149.5	89.1
	PKN_{fon}	169.6	165.9	167.8	100.0
	PKN_{60}	196.7	174.3	185.8	110.7
	PKN_{120}	134.2	145.6	139.9	83.4
	PKN_{180}	152.0	204.1	178.1	106.1
	Average AC	164.6	163.7	**164.1**	-
	Index (%)	100.0	99.5	-	100.0
"Gajnjaca"	Control	204.5	139.0	171.8	126.0
	PKN_{fon}	119.6	153.1	136.4	100.0
	PKN_{60}	103.1	124.3	113.7	83.4
	PKN_{120}	184.5	117.3	150.9	110.6
	PKN_{180}	125.8	116.0	120.9	88.6
	Average AC	147.5	130.0	**138.7**	-
	Index (%)	100.0	88.1	-	84.5
Average BC	Control	187.5	133.8	160.6	105.6
	PKN_{fon}	144.6	159.5	152.1	100.0
	PKN_{60}	149.9	149.3	149.6	98.4
	PKN_{120}	159.4	131.5	145.4	95.6
	PKN_{180}	138.9	160.1	149.5	98.3
Average C		156.0	146.8	151.4	-
Index (%)		100.0	94.1	-	-

L S D	A	B	C	AB	AC	BC	ABC
0.05	11.94	30.67	24.24	48.71	35.77	26.54	60.20
0.01	16.08	43.00	32.64	62.11	49.47	37.53	72.10

Microorganisms take part in a competition with plants for biogenic elements including them also in biosynthesis of a plasm of their cells (Kuzyakov et al., 2000), so the total number of microorganisms under "ugar" can be the consequence of the absence of such relations. Also,

the role of ugar in soil fertility renewal, accumulating of humus and nitrogen and repression of harmful phenomena which are the result of many years' antropogenic influences and causes of soil strain, was noticed a long time ago (Vukadinović, 1998; Popović, 2010). On the basis of the research results, Šarčević-Todosijević et al. (2016; 2017) and Šarčević-Todosijević and Živanović (2017) say that the number of different ecophysiological groups of soil microorganisms was more expressive on "ugar" than under the sown maize. The difference in the number of microorganisms, in a phase of physiological reapness of maize grain, between "cernozem" and "gajnjaca" statistically was very significant. The total number of microorganisms was more expressive in "cernozem" (164.1 x 10^5g^{-1}) in comparison with "gajnjaca" (138.7 x 10^5g^{-1}), Table 2. In a phase of physiological reapness of maize grain, the total number of microorganisms between treatments under "ugar" and sown maize, was higher under "ugar" (156.0 x 10^5g^{-1}), but the difference was not statistically significant, Table 2, Graph 2a. All the applied quantities of nitrogen induced decreasing of the total number of microorganisms in a phase of physiological reapness of maize grain, in comparison with control (105.6 x 10^5g^{-1}), Table 2, Graph 2b.



Graph 2. a) The total number of microorganisms in soil (10^5g^{-1}) in comparison with the nitrogen quantity, b) The influence of soil type and nitrogen quantity on the total number of microorganisms (10^5g^{-1}) in a phase of physiological reapness of maize grain

The highest number of microorganisms under "ugar" was in a control variation (187.5 x 10^5g^{-1}), whereas the highest number, under crops, was in a variation of applied 180 kg ha^{-1} of nitrogen (160.1 x 10^5g^{-1}). The differences in the number of microorganisms between variations of fertilizers and soil type were statistically significant, Table 2, graph 2. Many researches point to the highest biogenecy in "cernozem", which is conditioned by chemical features of this soil type, humus abundance and mineral clay, lime and absorbed calcium, because of which pH reaction of soil is neutral.

Picture 1. *Bacillus* sp. isolated from the samples of soil type "cernozem" in Zemun Polje

Then follow "gajnjaca", "ritska crnica", where as the lowest values of biogenecy indicators were confirmed in "pseudogley" and "smonica" (Milošević and Govedarica, 1997).

Šarčević-Todosijević et al. (2017; 2018) mention statistically remarkably higher number of *Azotobacter* sp. and actinomycetes in "cernozem" in comparison with "gajnjaca".

Conclusion

The total number of microorganisms in "cernozem" varied from 72.3–204.1 x $10^5 g^{-1}$ and in "gajnjaca" these values were from 54.9 – 184.5 x$10^5 g^{-1}$. By the variable analysis, it is confirmed that the total number of microorganisms was significantly influenced by all the examined factors and their interactions.

A significantly higher total number of microorganisms was confirmed in "cernozem" in comparison with "gajnjaca", in a phase of physiological reapness of maize grain.

The applied quantities of nitrogen had a significant impact on the total number of microorganisms in comparison to control.

The higher total number of microorganisms under "ugar" was confirmed in comparison with maize crops.

Although in soil live different ecophysiological groups of microorganisms, and in different number, they are all united by their feature of enabling developing biogeochemical series of matter cycling which are the base of keeping life on the planet.

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PARENTAL POLYMORPHISM ANALYSIS IN MARKER ASSISTED SELECTION FOR β-CAROTENE RICH MAIZE

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Abstract

Marker assisted selection (MAS) is widely implemented into modern grain breeding programs. Molecular markers are used in foreground selection to control the target gene, as well as in background selection to accelerate the reconstruction of the recurrent parent genotype. The best results have been achieved with the qualitative traits, regulated by the action of a single or several genes and clearly phenotypically defined. One successful example of MAS is the improvement of β-carotene content using *crtRB1* specific molecular marker. Maize Research Institute "Zemun Polje" has a breeding program aimed at conversion of standard maize to β-carotene rich genotypes adapted to temperate regions. The objectives of this study were to test the utility of gene-specific SSR marker in foreground selection and to identify polymorphic markers between parental lines to be used in background selection. Genetic variability between two standard and three high β-carotene parental inbred lines was analyzed with 40 SSRs distributed over the maize genom. Total number of alleles detected with 30 informative markers was 77, average being 2.57. The genetic similarity values calculated on Dice coefficient ranged from 0.49 to 0.66. Parental polymorphism for *crtRB1* showed a 543 bp fragment in donor lines, whereas a distinct 296 bp amplicon and a faint 1221 bp amplicon were generated in the recurrent parents. This marker will be used as foreground selection marker for the *crtRB1* gene in the conversion of standard maize to β-carotene enriched lines for growing in temperate regions.

Keywords: *β-carotene, crtRB1 gene, maize, marker assisted selection, SSR.*

Introduction

Maize is an important source of food and nutritional security for millions of people in the developing world, especially in sub-Saharan Africa, Latin America and in many countries in Asia (Shiferaw et al., 2011). However, most of the maize varieties do not contain enough provitamin A (ProVA), the precursor that leads to the formation of vitamin A. Consequently, diets mainly relying on maize may lead to vitamin A deficiency (VAD), primarily in nutritionally demanding periods of life, such as early childhood and pregnancy (Song et al., 2017). The World Health Organization has classified VAD as a public health problem, the leading cause of preventable childhood blindness, anaemia, and weakened host resistance to infection, which can increase the severity of infectious diseases and risk of death (WHO, 2009). Improvement of nutritional quality in food crops (biofortification) is a promising strategy to fight undernutrition, particularly among the rural poor in developing countries (Shiferaw et al., 2011). One of the most important breeding objective is improving the ProVA content of maize grain. Research under the HarvestPlus, Consultative Group on International Agricultural Research Challenge program designed to use breeding for crop biofortification, has provided significant leads for developing ProVA enriched maize (Harjes et al., 2008; Yan et al., 2010). Two genes have the most significant effect on ProVA concentrations in maize grain. One gene, *lcyE* on chromosome 8, encoding lycopene epsilon cyclase is associated with variation in the ratio of various carotenoids in the α- to β-branches of the carotenoid biosynthesis pathway (Harjes et al., 2008). Another gene, *crtRB1* on chromosome 10, encodes

β-carotene hydroxylase enzyme and it has a significant impact on variation for β-carotene concentration in the endosperm (Fu et al. 2013). Babu et al. (2013) reported that *crtRB1* gene had a much larger effect on ProVA concentration than *lcyE*.

CrtRB1 converts β-carotene into β-cryptoxanthin, whose ProVA activity is theoretically only half that of β-carotene. Natural genetic variation in *crtRB1* gene has been reported by Yan et al. (2010). That one favourable allele (*crtRB1* 3′TE) alone is responsible for effecting 2- to 10-fold increase of β-carotene concentration in maize kernel (Babu et al., 2013). The 3′TE (transposable element) polymorphism of the gene that spans the 6th exon and the 3′-UTR (untranslated region) generates three alleles associated with altering β-carotene accumulation: *allele 1* (543 bp; without TE insertion), *allele 2* (296 bp+875 bp; with 325 bp TE insertion) and *allele 3* (296 bp+1221 bp+1880 bp; with 1250 bp TE insertion). *Allele 1* of the *crtRB1* gene is favourable and increases the level of β-carotene, whereas *allele 2* and *allele 3* cause unfavourable effects (Yan et al. 2010).

Muthusamy et al. (2014) reported accelerated development of β-carotene enriched maize, using marker assisted selection (MAS) that holds immense promise as it selects desirable plants precisely and eliminates large scale biochemical estimation in the segregating generations. The introgressed inbreds possessing favourable allele of *crtRB1* can be used in the biofortification program. Moreover, improved hybrids with immense increase of b-carotene (>15 µg/g) can be directly utilised in alleviating VAD worldwide.

Maize Research Institute "Zemun Polje" (MRI) has a breeding program aimed at conversion of standard maize to β-carotene rich genotypes adapted to temperate regions. The main objective of the research presented herein was polymorphism analysis of parental lines used in MAS for β-carotene rich maize. The utility of the SSR marker specific to the *crtRB1* gene in foreground selection was tested. Also, genetic variability between parental lines was analyzed and a set of polymorphic SSR markers was selected to be used in background selection.

Material and Methods

Plant material

Three maize inbred lines provided by the International Maize and Wheat Improvement Center (CIMMYT) were used as the donor parents (DP_1, DP_2 and DP_3) of the favorable allele *crtRB1*-3′TE of the *crtRB1* gene. Two MRI commercial inbred lines were used as the recurrent parents (RP_1 and RP_2). These lines are adapted to the local environmental conditions in Serbia and due to their excellent combining abilities they are components of the leading MRI hybrids.

DNA extraction

Genomic DNA was isolated according to Doyle and Doyle (1987) from the four-weeks-old plants. Bulks were prepared by pooling an equal amount of leaf tissue taken from 20 leaves per line. The DNA was quantified using biospectrometer (BioSpetrometer kinetic, Eppendorf) and diluted to a working concentration of 20 ng/µl. The samples were stored at -20^{0}C until their application in polymerase chain reaction (PCR).

Polymorphism analysis for *crtRB1*

The set of primers used in polymerase chain reaction for the marker specific to the *crtRB1* is presented in Table 1.

Table 1. The set of primers used as a foreground selection marker for the *crtRB1* gene

Primer	Sequence
crtRB1-3′TE-F	5′-ACACCACATGGACAAGTTCG-3′
crtRB1-3′TE-R1	5′-ACACTCTGGCCCATGAACAC-3′
crtRB1-3′TE-R2	5′-ACAGCAATACAGGGGACCAG-3′

Polymerase chain reaction was carried out in 20 µl reaction volume containing: 1×enzyme buffer, 200 µM dNTP, 0.5 µM primers, 1U *Taq* polymerase and 20 ng DNA template. Amplifications were performed in thermocycler Biometra TProfessional Standard 96 with the following program: an initial denaturation at 95ºC/5min, followed by 19 cycles each of denaturation at 95ºC/1min, annealing at 60ºC/1min (-0.5ºC/cycle) and extension at 72ºC/1min; another 19 cycles of 95ºC/1min, 55ºC/1min and 72ºC/1min with final elongation at 72°C for 10 min. The amplified fragments were resolved by 8% polyacrylamide gel electrophoresis (Mini Protean Tetra-Cell, BioRad) at 80 V for 1.5 h. After staining with 0.5 µg/µl ethidium bromide, they were visualized under UV transilluminator and documented in gel documentation system (BioDocAnalyze, Biometra). The size of the amplification products was determined comparing with the 100 bp molecular weight ladder.

Genetic similarity analysis

Simple sequence repeat (SSR) analysis was done with 40 primer pairs spanning over the whole genome. PCR was carried out in 25 µl reaction volume containing: 1×enzyme buffer, 200 µM dNTP, 0.5 µM primers, 1U *Taq* polymerase and 20 ng DNA template. The following touch-down program (thermocycler Biometra TProfessional Standard 96) was performed: an initial denaturation at 95ºC/5min, followed by 15 cycles each of denaturation at 95ºC/30 s, annealing at 63.5ºC/1min (-0.5ºC/cycle) and extension at 72ºC/1min; another 22 cycles of 95ºC/30 s, 56ºC/1min and 72ºC/1min with final elongation at 72°C for four min. The PCR products were separated by electrophoresis on 8% polyacrylamide gel in 1 x TBE buffer, with 100 bp molecular weight ladder as a marker. Gels were run on small format (7.3 x 10 cm) vertical gel system (Mini Protean Tetra-Cell, BioRad), at 80 V for 1.5 h. After staining with 0.5 µg/µl ethidium bromide they were photographed under UV light using Biometra BioDocAnalyze gel documentation system. SSR profiles were converted into a binary matrix based on the presence (1) or the absence (0) of a specific allele. Genetic similarity (GS) was calculated in accordance with Dice (1945): $GS_{ij} = 2a/2a+b+c$; where:

a - the number of fragments present in both variety *i* and *j* (1.1),

b - the number of fragments present in *i* and absent in *j* (1.0),

c - the number of fragments absent in *i* and present in *j* (0.1).

Marker data analyses were performed using statistical NTSYSpc2 program package (Rohlf, 2000).

Results and Discussion

The results presented here are a part of the MRI project on conversion of standard maize to β-carotene enriched lines for growing in temperate regions trough marker assisted backcross breeding. Being the carriers of the favorable allele *crtRB1*-3′TE, three maize inbred lines provided by CIMMYT were used as the donor parents (DP_1, DP_2 and DP_3). As the recurrent

parents, to be the recipients of favourable allele and to improve the β-carotene content, two comercial lines were selected (RP_1 and RP_2).

The first goal of this study was to test the utility of SSR marker specific to the *crtRB1*gene in foreground selection. In any particular cross, it is necessary to run the markers first on the two parents, to confirm the polymorphisms (size differences) for the marker alleles. Similar to Muthusamy et al. (2014), distinct marker polymorphism was observed between the donor and recurrent parents for *crtRB1* gene. The donor parents carried the favorable allele with a 543 bp band (*allele 1*), while the recurrent parents exhibited the unfavorable allele with a 296 bp+1221 bp band (*allele 3*). An illustration of the amplification with this gene specific SSR marker is given in Figure 1.

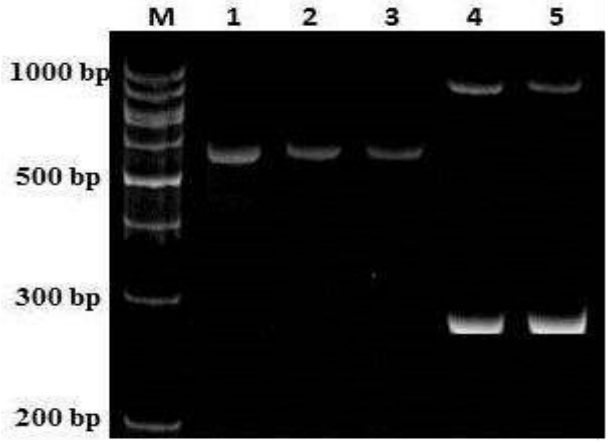

Figure 1. Parental polimorphism with the *crtRB1*-specific SSR marker. M: 100 bp DNA ladder, 1-3: donor parents DP_1, DP_2 and DP_3, 4-5: recurrent parents RP_1 and RP_2.

Being the codominant marker, *crtRB1*-3′TE can detect homozygous dominant, heterozygous and homozygous recessive plants separately. Therefore, it will be used in marker assisted selection for the *crtRB1* gene. Heterozygous plants (*allele 1*/*allele 3*) will be selected in the BC_1 and BC_2 generations, while homozygotes (*allele 1*/*allele 1*) will be selected in the BC_2F_2 generation. Plants without favourable allele will be discarded prior to pollination, hence the size of the breeding population will be reduced. As stated in Choudhary et al. (2016), assessment of genetic relationships among the inbreds is key to success of hybrid breeding programme. Genetic diversity analyses based on molecular markers proved to be very effective in this assessment. This can be extremely helpful for identifying the most appropriate parental lines to be crossed (Ribaut and Hoisington, 1998). Consequently, another goal of this paper was to estimate genetic similarity between parental lines selected for MAS for β-carotene rich maize. Donor and recurrent parent lines were analyzed using 40 SSRs distributed throughout the maize genome. Total number of alleles detected with 30 informative markers was 77, average being 2.57, which is similar to those previously reported in maize inbreds (Maphosa et al., 2011; Bante and Prasanna, 2003; Choudhary et al., 2016). These polymorphic SSR markers will be employed for identification of the genotypes with the highest proportion of recurrent parent genome in BC_2 generation. The pairwise genetic similarity values calculated on Dice coefficient ranged from 0.49 (D_2/RP_2) to 0.66 (D_2/RP_1) (Table 2). These results enable identification of potential high β-carotene hybrid combinations for the biofortification programme. As concluded in Choudhary (2016), genetically diverse and contrasting inbred lines can be used in developing segregating mapping populations for identifying modifier genetic loci influencing β-carotene accumulation.

Table 2. The pairwise genetic similarity values calculated on Dice coefficient between donor (DP_1, DP_2 and DP_3) and recurrent parent lines (RP_1 and RP_2).

	D_1	**D_2**	**D_3**
RP_1	0,62	0,66	0,65
RP_2	0,57	0,49	0,65

Conclusions

The *crtRB1*-3′TE marker showed codominant polymorphism between the donor and recurrent parents. Therefore, it will be used in foreground selection for the *crtRB1* gene. Selected polymorphic SSR markers will be employed in background selection to recover the recurrent parent genome. The results of this analysis constitute the framework for marker assisted introgression of the favourable allele of *crtRB1* into the local maize genotype.

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PROPAGATION OF *COTONEASTER MULTIFLORUS* BUNGE. BY SOFTWOOD CUTTINGS

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Abstract

This deciduous large shrub, with long arching branches and very abundant white flowers followed by scarlet-red fruit, is suitable for landscape use as an ornamental plant. It is considered as a drought tolerant species that can grow on clay soils. The aim of this study was establishing quick and effective method for vegetative propagation of selected elite genotypes of *Cotoneaster multiflorus* growing in the Belgrade area (Serbia). Two types of softwood cuttings were used: cuttings with current season wood and cuttings with two year old wood. The cuttings were divided into two groups, with cuttings treated with 1% IBA (indole-3-butyric acid) and with cuttings without auxine treatment. After 7 weeks of sticking, rooting percentage as well as number and length of primary roots, and presence and number of secondary roots were determined. Obtained results showed that there were no statistically significant difference in rooting rate among current season wood cuttings (49.3%) and two year old wood cuttings (36.7%), although current season wood cuttings had a better rooting percentage and higher number and length of primary roots. Results obtained in a control treatment without IBA were poor, indicating that auxine treatment was necessary in propagation of this species, and that obtained results could be improved investigating the use of different auxine treatments.

Keywords: *Many-flowered cotoneaster, vegetative propagation, cuttings, greenwood, auxine.*

Introduction

Cotoneaster multiflorus is deciduous large shrub, native to Kazakhstan and Northwest China. It grows up to 4 m tall, with a greater spread up to 4.5 m wide. Its arching, cascading branches are 3-5 m long, developing a mounding habit. The dark-green, simple leaves are alternate, with short petioles, entire margins, up to 5 cm long, turning an attractive yellow in autumn. It flowers from May to June, forming 3-12 flowers per cluster (corymb), that are white and showy, five-petaled, 1 cm wide. In summer, Many-flowered Cotoneaster produces fleshy, ornamental, bright red, globose fruits, about 1 cm in diameter, containing 1-2 seeds (Phipps *et al.*, 1990; Fryer and Bertil, 2009; Dickoré and Kasperek, 2010).

This plant prefers a sunny to half-shady location and fertile, moderately moist, well-drained soil. It can grow on a wide range of soils, from sandy to loamy clay soils, and once established it tolerates drought very well. *C. multiflorus* forms shallow roots and it is suitable for rooftop gardens, medium-high cut hedges and for free-growing high hedges. This plant also provides shelter for birds (Dirr, 1983; Fryer and Bertil, 2009). For these reasons, this ornamental and low-maintenance species should be planted more often in urban green areas, but it is important to select and propagate appropriate genotypes that have been adapted to microclimatic conditions in Belgrade area.

Cotoneaster spp. can be propagated by seed, cutings or grafting (Hartmann *et al.* 2002). Seeds should be soaked for 90 minutes in concentrated sulfuric acid, rinsed, and then stratified for 3 to 4 months at about 4°C. Cuttings should be taken in spring or summer and rooted under mist. Khatamian and van der Hoeven (1997) investigated the influence of IBA concentrations on rooting of *C. multiflorus* softwood cuttings, using quick dip method. Besides their report,

there is no available data for vegetative propagation of *C. multiflorus*. For this reason we decided to conduct research in order to establish rapid and effective method for vegetative propagation of selected elite genotypes of *C. multiflorus* growing in the Belgrade area, in Serbia.

Material and Methods

The elite, vigorous and healthy *C. multiflorus* shrub growing in the urban green area in Belgrade was selected. At the end of May, long, well developed branches were cut and brought in a greenhouse of the Faculty of Forestry in Belgrade. From these branches, 2 groups of green cuttings were taken: nodal with current season's wood only, and nodal from 2-year-old shoots. Cuttings belonging to both groups were 10-15 cm long, each cutting with 4-6 nodes. Before sticking in a sand as a rooting substrate, the cuttings were treated by 0,15% dilution of fungicide Previcur, and their base was dipped in dust preparation of 1% (indole-3-butyric acid) IBA. In the control the auxine treatment was omitted. The rooting was performed under intermittent mist (10 seconds mist with 15 minutes interval).

A total of 600 cuttings (three replications of 50 cuttings per treatment) were used. A randomized experimental design was applied. After 7 weeks, a state of cuttings was determined and the following parameters were measured: a number and length of primary roots, and a number of secondary roots. The significance of differences between the means was determined by the analysis of variance (ANOVA, $p < 0.05$) and the least significant difference (LSD) test. Before the statistical analysis, arcsine transformation was used to convert the percentage data.

Results and Discussion

After 7 weeks, the rooting percentage of cuttings treated with IBA was 36.7% and 49.3% and rooting of non-treated cuttings in a control treatment was significantly lower (Table 1). The cuttings with current season wood rooted poorly in a control treatment, but relatively high percentage of them (21.3%) compared to 2-year-old cuttings (1.3%) was recorded with root primordias, thus indicating that cuttings taken from young shoots have a higher rooting potential, which can be confirmed by the results of numerous anatomical studies of woody species (Lovell and White, 1986).

The high number of cuttings developed a callus that may delay or inhibit the formation of adventitious roots (Hartmann *et a*l. 2002). Wounding of basal callus tissues and applications of rooting hormones can result in rooting of callused cuttings (Silva *et al*., 2002), but effectiveness of this treatment should be additionally investigated.

Table 1. State of cuttings seven weeks after sticking

Cutting type	Hormone	State of cuttings				
		rooted (%)	with root primordias (%)	unchanged (%)	callusing (%)	necrotic (%)
1-year-old	1% IBA	49.3^{a}	3.3^{b}	2.0^{b}	16.6^{b}	28.6^{a}
1-year-old	control	14.7^{bc}	21.3^{a}	14.6^{ab}	45.3^{a}	4.0^{b}
2-year-old	1% IBA	36.7^{ab}	1.3^{b}	0.6^{b}	36.0$^{a\ b}$	25.3^{a}
2-year-old	control	8.6^{c}	0.0^{b}	24.0^{a}	42.0^{a}	25.3^{a}

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

The root system of cuttings treated with IBA was well developed, both for cuttings with current season wood and cuttings taken from 2-year-old shoots (Table 2). There was no significant difference between these two types of cuttings regarding mean number of primary and secondary roots, but the younger cuttings developed longer roots.

Table 2. Number and length of roots

Cutting type	Hormone	Mean No. of primary roots	Mean length of primary roots (cm)	Mean No. of secondary roots
1-year-old	1% IBA	4.8 [a]	11.3[a]	10.5 [a]
1-year-old	control	1.4 [b]	0.3[c]	1.3 [b]
2-year-old	1% IBA	4.1 [a]	5.7[b]	6.3 [a]
2-year-old	control	2.3 [b]	0.7[c]	1.3 [b]

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

These results indicate that auxine treatment is necessary for rooting, and a similar finding was reported by Khatamian and van der Hoeven (1997). However, they applied IBA using quick dip method, and the best result (100 % rooted cuttings) was recorded using 5000 ppm IBA for 10 seconds, which is high concentration. Kroin (1992) stated that recommended concentration for rooting softwood cuttings using the quick dip method for the majority of wooden species is 1000 ppm IBA, and for hardwood cuttings is 2000 ppm IBA. For difficult to root hardwood cuttings Kroin (1992) suggests using 5000 ppm IBA up to 20000 ppm IBA.

In addition, Khatamian and van der Hoeven (1997) performed rooting in a mixture of 30% Canadian sphagnum peat and 70% perlite, and the substrat composition can also affected the success of rooting, including rooting rate, development of roots and/or callus (King *et al*., 2011; Witcher and Pounders, 2016).

Results obtained with other *Cotoneaster* species, also suggest importance of IBA use. Thus Kalyoncu *et al.* (2017) researching the rooting of *C. horizontalis* apical softwood cuttings obtained high rooting rate (100%) in all treatments, including control, but cuttings tretaed with 4000 ppm IBA developed significantly higher root number. However, Hocking and Thomas (1981) reported occurence of basal necrosis of *C. conspicua* cuttings when concentration of IBA was increased from 0.4% to 2%.

Conclusions

Selected elite genotype of *C. multiflorus* suitable for growing in the conditions of Belgrade area can be vegetatively propagated by nodal softwood cuttings, taken from current season shoots, treated with 1% IBA using a dry dip method. However, obtained rooting percentage can be improved using a quick dip method of IBA application, which should be further investigated by additional research. Also, use of different rooting media and apical cuttings should also be considered.

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THE INFLUENCE OF THE SUBSTRATE COMPOSITION ON ROOTING OF HARDWOOD CUTTINGS OF LYCIUM BARBARUM L.

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Abstract

Lycium barbarum is a deciduous shrub that has been used for centuries in China as a traditional medicinal and food supplement. Today, it is widely grown as a fruit as well as a landscape ornamental species, including some ornamental varieties. It is easily grown plant, suitable for hedges, and it endures pruning. Goji has well developed root system and it can be planted for erosion control or to stabilize sandy soils. It can be propagated by seed, cuttings, layering and division. Propagation by cuttings is the most convenient method for mass production of uniform plants. In order to establish an efficient production system, the influence of different substrates on rooting of hardwood cuttings was examined. Six substrate mixtures were tested: sand, sand and compost (1: 2), sand and peat (1: 2), pine bark and compost (1: 1), pine bark and peat (1: 1), pine bark and sand (1: 1). Except for rooting percentage, success of rooting was determined using following parameters: the number and length of primary and secondary roots, number and length of shoots. The best results were obtained in sand. The rooting percentage was the highest (66%), and the length of primary roots, number and length of shoots were higher compared to other treatments. The influence of pH value of substrates on rooting performance was also discussed.

Keywords: *Goji, vegetative propagation, cuttings, rooting medium.*

Introduction

Lycium barbarum (wolfberry, goji) is a deciduous shrub growing to 2.5 m, spreading up to 4 m, developing the long arching branches, with the side branches often reduced to a short leafless spines. It flowers from June to August, and the fruit is orange or dark red berry, 2 cm in diameter, ripen from August to October (Flint, 1997; Maughan and Black, 2015). Goji has been used for centuries in China as a traditional medicinal and food supplement, and today it is becoming popular worldwide due to its medicinal proprieties and benefits for consumption (Kulczyński and Gramza-Michałowska, 2016; Gao *et al.*, 2017).

Wolfberry is also easily grown, low-maintenance, landscape ornamental plant. It succeeds in nutritionally poor soil, and can be planted to stabilize sandy banks. Goji is suitable for planting in groups or in informal hedges and it is very tolerant of pruning and can regrow from old wood (Huxley and Griffiths, 1992). Goji is also salt tolerant species (Juan *et al.*, 2004). It can be easily propagated by seed, but vegetative methods are more convenient for clonal production of desired genotypes. Goji can be propagated by division of suckers in late winter and by layering (Huxley and Griffiths, 1992; Maughan and Black, 2015), but these methods are suitable for small scale production. Also, there are some reports regarding micropropagation of this species (Cao *et al.*, 1999; Hu *et al.*, 2008; Fira and Clapa, 2011; Osman *et al.*, 2013*;* Ciorchină *et al.*, 2018), but it is expensive method and it is not widely used. The most convenient method of large scale production of uniform wolfberry plants is by cuttings (Huxley and Griffiths, 1992). The majority of research regarding optimization of vegetative production of Goji by softwood, semi-hardwood and hardwood cuttings was conducted in China (Shen and Chen, 1990; Qiong, 2011; Zheng *et al.*, 2012; Zong-Cai *et al.*, 2012; Wang *et al.*, 2016). These studies examined the effect of different growth regulators on

rooting and development of Goji cuttings taken from local genotypes. Besides commonly used, auxine based growth regulators (such as IBA - Indole-3-butyric acid), they investigates the effect of plant growth regulators invented in China, including ABT rooting powder series (invented by Chinese Academy of Forestry). However, one of the important factors that influence rooting performance of cuttings is a composition of the rooting media (Altman and Freudenberg, 1983; Caspa *et al.*, 2009; Gehlot *et al.*, 2014, 2015; Asanica *et al.*, 2016). For this reason, we decided to test the influence of different substrates (commonly used in Serbian nurseries) on rooting of Goji hardwood cuttings in order to establish an efficient, cost-effective and applicable production system.

Material and Methods

At the end of February, the cuttings were taken from 3-year old container plants with multiple shoots developed, grown outdoors. In this time, the stock plants didn't have leaves, and the temperature during a day didn't drop below 5°C. The average length of nodal hardwood cuttings was 9.5 cm, and diameter was 2.8 mm. The cuttings were randomly placed in the 104-cell plugs containing different substrate mixtures: sand, sand and compost (1: 2), sand and peat (1: 2), pine bark and compost (1: 1), pine bark and peat (1: 1), pine bark and sand (1: 1). The pH value of each media mixture was measured (Table 1) according to Bacto Laboratories methodology (http://www.bacto.com.au/measurement-of-ph-in-soil/).

Table 1 Rooting substrates used in the research

Substrate mixture	pH value
sand	7.0
sand and compost (1: 2)	7.0
sand and peat (1: 2)	5.5
pine bark and compost (1: 1)	6.0
pine bark and peat (1: 1)	5.0
pine bark and sand (1: 1)	5.0

The cell plugs with cuttings were placed in the cold frame greenhouse of the Faculty of Forestry and kept 14 months before examining the results. Irrigation was adjusted according to a daily temperatures, using manual irrigation during cold months (March, April, September, October) and a mist system during summer months. At the begining of April next year, following parameters were determined: sum of primary root lengths, number of primary and secondary roots, sum of shoot lengths, number of shoots and number of cuttings with proliferated buds. Three replications of 33 cuttings per treatment were used, for each treatment 99 cuttings (total 594 cuttings). Obtained results were analysed statistically and the significance of differences between the means was determined by the analysis of variance (ANOVA, $p < 0.05$) and the least significant difference (LSD) test. Before the statistical analysis, arcsine transformation was used to convert the percentage data.

Results and Discussion

The rooting medium strongly influenced rooting percentage (Table 2). The best result was obtained in sand (66.6%), whereas mixture of equal parts of sand and pine bark was the most unfavorable with the only 22.2% rooted cuttings, and with only 0.7% of cuttings with proliferated buds. The longest shoots also developed from cuttings rooted in a sand. Besides composition of rooting medium, the pH value can also influence rooting (Kumar *et al.*, 2011;

Fisher *et al.*, 2016). However, rooting percentage in the mixture of sand and peat (1: 2) with pH 5.5 was similar to rooting in media with pH 7, and significantly higher than medium with pH 5.0 (pine bark and sand). Kumar *et al.* (2011) found that pH influence effectiveness of auxine for induction and growth of adventitious roots of *Gmelina arborea*, and in their research addition of IBA was ineffective at pH 5.5–7.0 and inhibitory at pH 8.5. However, our research was conducted without auxine treatment.

Table 2. Rooting of hardwood Goji cuttings

Substrate mixture	Rooting percentage (%)	Cuttings with proliferated buds (%)	Sum of shoot length per cutting (cm) ± SE
sand	66.6^{a}	32.0^{ab}	$11.5^{a} \pm 1.33$
sand and compost (1: 2)	53.5^{ab}	34.0^{a}	$6.3^{bc} \pm 0.99$
sand and peat (1: 2)	51.5^{ab}	23.0^{bc}	$8.6^{b} \pm 0.31$
pine bark and compost (1: 1)	46.4^{b}	21.0^{c}	$4.0^{cd} \pm 1.01$
pine bark and peat (1: 1)	42.4^{b}	21.0^{c}	$4.6^{cd} \pm 1.29$
pine bark and sand (1: 1)	22.2^{c}	0.7^{d}	$2.6^{d} \pm 1.58$

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

The highest number of primary roots was formed in mixture of sand and compost (Table 3). However, the cuttings rooted in a sand had the highest number of secondary roots and they also developed significantly longer roots.

Table 3. Number of primary and secondary roots and average sum of primary root length

Substrate mixture	No of primary roots ± SE	No of secondary roots ± SE	Sum of primary root length per cutting (cm) ± SE
sand	$1.4^{b} \pm 0.15$	$3.4^{a} \pm 0.53$	$19.4^{a} \pm 1.06$
sand and compost (1: 2)	$2.7^{a} \pm 0.45$	$1.4^{bc} \pm 0.63$	$6.5^{b} \pm 1.90$
sand and peat (1: 2)	$1.3^{b} \pm 0.16$	$1.7^{b} \pm 0.57$	$5.1^{b} \pm 0.99$
pine bark and compost (1: 1)	$1.2^{b} \pm 0.33$	$1.1^{bc} \pm 0.54$	$4.7^{b} \pm 2.10$
pine bark and peat (1: 1)	$1.4^{b} \pm 0.48$	$0.7^{cd} \pm 0.31$	$7.7^{b} \pm 3.81$
pine bark and sand (1: 1)	$0.5^{c} \pm 0.24$	$0.11^{d} \pm 0.243$	$2.1^{b} \pm 1.69$

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

The obtained results showed importance of rooting media in a vegetative production of Goji. However, Qiong (2011) stated that the use of plant growth regulators (PGRs) is necessary for successful propagation of this plant. Nevertheless, the application of PGRs should be carefully investigated. For example, Asanica *et al.* (2016) obtained 60% rooted Goji cuttings (0.4 - 0.8 cm diameter) in a control treatment in a peat and sand (1: 1) mixture, 50% rooted cuttings after treatment with 1500 ppm IBA, and only 40% rooted after treatment with 500 ppm IBA. However, their results with thiner cuttings (0.1 - 0.3 cm) were significantly different, reaching 90% rooting of cuttings treated with 500 ppm IBA, rooted in a same substrate mixture. Gehlot *et al.* (2015) showed that success of hardwood cuttings rooting depends of several factors and their interactions, including cuttings diameter, PGRs type and concentration and a rooting medium. Genotype is also important factor, and each clone could

have a different response to a treatments, and the rooting rate can vary from 30% to 100% depending on genotype (Asanica *et al.*, 2016).

The number of roots obtained in our research was relatively low, up to average of 2.7 primary roots per cutting. Wang *et al.* (2016) investigated the effect of ABT-6 rooting agent on the hardwood cuttings of *L. barbarum* var. *implicatum.* They obtained the best results using quick dip method (400 mg/L ABT-6), with high rooting percentage (82.3%) and very high number of shoots (6.6) and roots (8.9), which was long and well developed. For this reason, the use of different PGRs should also be considered for the successful vegetative propagation of Goji.

Conclusions

The rooting media composition significantly influenced the success of rooting of wolfberry hardwood cuttings. The best results, with highest rooting percentage (66%), and the longest shoots and roots was obtained in sand. However, the use of different PGRs and should be considered in order to improve production effectiveness of this valuable ornamental, edible and medicinal species.

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GRAIN YIELD AND STABILITY PARAMETERS OF ZP MAIZE HYBRIDS GROWN IN CENTRAL SERBIA IN THE PERIOD 2014-2017

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Abstract

Central Serbia, besides Vojvodina, is the most important crop production region in Serbia. The climate is the main difference between central Serbia and the Autonomous Province of Vojvodina. The first region is characterised with somewhat lower precipitation sums during the growing season and with temperatures a bit lower during the whole year. Due to this, every year, the Maize Research Institute, Zemun Polje performs production trials with its most distributed commercial hybrids in both regions. In this study, 10 commercial ZP maize hybrids were tested in 85 locations in central Serbia in the period 2014-2017. The number of locations per year amounted to: 26, 22, 23 and 14 in 2014, 2015, 2016, 2017 respectively. The average maize grain yields were recorded in 2014 and 2015, while 2016 was exceptionally high yield year. On the other hand, drought in 2017 reduced the yield. Yield stability was estimated by the method developed by *Eberhart* and *Russel*. The lowest four-year average yield (7.199 t ha^{-1}) was recorded in the hybrid ZP 341. This hybrid was also unstable (b_i=0.729). The highest yield was recorded in the hybrid ZP 600 (9.506 t ha^{-1}). The highest yield stability was estimated in the hybrid ZP 606 (b_i=0.954), which was also high yielding (8.688 t ha^{-1}). The most yielding hybrid ZP 600 was at the same time the least stable (b_i=2.483) and adjustable to favourable growing conditions.

Key words: *maize, grain yield, stability performance.*

Introduction

Maize is one of the most important crops not only in our country but also worldwide. It was domesticated in Central America approximately 7700 years ago. Maize represents a plant species that is evolutionarily most developed in the entire plant kingdom (Glamočlija, 2004). Maize is one of the leading species in the global agricultural production ranking high in the world economy. It ranks first according to total production quantity and grain yield per area unit and second according to harvested areas in the world (FAOSTAT, 2013). The revolution in maize production began with the discovery of heterosis and cultivation of hybrids in the USA during the 1930s (Duvick 2005). Besides selection for high and stable yields, maize growing practices has also changed. It is believed that about 60% increase in grain yield ~~is~~ results from the development of new hybrids, while the remaining 40% is a result of new technological solutions and education of maize producers (Jocković et al. 2010).

Breeding and selection of maize hybrids of high genetic yield potential have been performed at the Maize Research Institute, Zemun Polje for the past 70 years. All maize hybrids developed at the Institute can be classified into six cycles of selection (http://www.mrizp.rs/en/research-l/breeding). Each cycle of selection produced hybrids that have been better adopted to agro-ecological conditions and they have been characterised by a higher genetic potential of the yield.

Central Serbia, besides Vojvodina, is the most important crop production region in Serbia. The climate is the main difference between central Serbia and Vojvodina. The first region is characterised with somewhat lower precipitation sums during the growing season and with temperatures a bit lower during the whole year. Due to this, every year, the Maize Research

Institute, Zemun Polje performs production trials with its most distributed commercial in both regions.

The aim of this study was to observe yields of Zemun Polje (later ZP) commercial maize hybrids and to establish grain yield stability not only in central Serbia but also in its three production regions: Mačva and Podrinje (the Drina river basin), Podunavlje (the Danube river basin) and Braničevo and central and eastern Serbia.

Materials and methods

In this study, 10 commercial ZP maize hybrids of different maturity groups (FAO 300-600) were tested in 85 locations in central Serbia in the period 2014-2017. All grown hybrids are commercial and widespread through the whole of Serbia. Production trials were set up at: 26, 22, 23 and 14 locations in 2014, 2015, 2016, 2017 respectively.

The elementary plot size amounted to 0.1 ha. Sowing and harvest of the trial was done mechanically. The plant density per hectare varied over FAO maturity groups and it ranged from 70,000 (FAO 300-400) to 60,000 plants ha^{-1} (FAO 500-600). Grain yield was calculated in t ha^{-1} at 14% moisture.

The average grain yield was estimated cumulatively for all 85 locations, and separately for Mačva and Podrinje (the Drina river basin), Podunavlje (the Danube river basin) and Braničevo and central and eastern Serbia, as they are two separate agro-ecological environments for maize cultivation. The method after Eberhart and Russell (1966) was applied to determine stability parameters for both all 85 locations cumulatively and Mačva and Podrinje (the Drina river basin), Podunavlje (the Danube river basin) and Braničevo and central and eastern Serbia separately.

Eberhart and Russell method is represented by the following equation:

$Y_{ij} = m + b_i I_j + d_{ij}$

Y_{ij} - the average yield of the i^{th} genotype in the j^{th} location

m - the average yield of the i^{th} genotype at all locations

b_i – standardised regression coefficient of the environment and genotype, genotype relationship to the environment

I_j - index of the environment as the average of all genotypes in the j^{th} location, reduced from the total average

d_{ij}-deviation from regression of the i^{th} genotype in the j^{th} location

The standardised regression coefficient (b_i) shows the response of genotypes to environment. When b_i=1, then the average adaptability and stability of the tested genotypes was considered uniform under the influence of favourable and poor environment conditions; when $b_i > 1$, genotype was considered stable and good only in favourable environment conditions, and when the $b_i < 1$, genotype was considered to perform better than the average in less favourable growing conditions and environments. All calculations were done in the Excel programme.

Results and discussion

The average maize grain yields were recorded in 2014 and 2015, while 2016 was exceptionally high yield year. On the other hand, drought in 2017 reduced the yield.

In central Serbia the lowest four-year average yield (7.199 t ha^{-1}) was recorded in the hybrid ZP 341. This hybrid was also unstable (b_i=0.729). The highest yield was recorded in the hybrid ZP 600 (9.506 t ha^{-1}). The highest yield stability was estimated in the hybrid ZP 606 (b_i=0.954), which was also high yielding (8.688 t ha^{-1}). The most yielding hybrid ZP 600 was at the same time the least stable (b_i=2.483) and adjustable to favourable growing conditions (Table 1).

Table 1. Grain yield and stability parameters of ZP hybrids in 85 locations in central Serbia for the 2014-2017 period.

Hybrid	Grain Yield (t/ha)	Rank according to grain yield	bi	Rank according to b_i
ZP 341	7.199	10	0.729	9
ZP 366	7.589	9	0.739	7
ZP 388	7.684	7	0.801	6
ZP 427	7.782	6	0.809	5
ZP 434	7.609	8	0.748	8
ZP 555	8.150	5	0.909	3
ZP 560	8.323	3	0.919	2
ZP 600	9.506	1	2.483	10
ZP 606	8.688	2	0.954	1
ZP 666	8.231	4	0.908	4
Average	8.076		1	

The highest average grain yields in the region of Mačva and Podrinje were recorded in hybrids ZP 600 (8.725 t ha^{-1}) and ZP 560 (8.636 t ha^{-1}), while the lowest yield was established in the hybrid ZP 341 (7.442 t ha^{-1}), which was, at the same time, the least stable hybrid (b_i=0.892). Values of the regression coefficient show that the hybrid ZP 434 (b_i=0.994) was the most stable in this growing region, while the hybrid ZP 666 (b_i=0.966) ranked second, and also had a very low grain yield (7.840 t ha^{-1}) (Table 2).

Table 2. Grain yield and stability parameters of ZP hybrids in locations in Mačva and Podrinje for the 2014-2017 period.

Hybrid	Grain Yield (t/ha)	Rank according to grain yield	bi	Rank according to b_i
ZP 341	7.442	10	0.892	10
ZP 366	7.964	6	0.921	9
ZP 388	8.107	4	1.038	3
ZP 427	7.849	8	0.959	4
ZP 434	7.876	7	0.994	1
ZP 555	8.051	5	1.067	8
ZP 560	8.636	2	1.048	5
ZP 600	8.725	1	1.055	6
ZP 606	8.494	3	1.060	7
ZP 666	7.840	9	0.966	2
Average	8.099		1	

The highest yields in the regions of Podunavlje and Braničevo were recorded in hybrids ZP 600 (11.290 t ha^{-1}) and ZP 606 (8.744 t ha^{-1}). Furthermore, the hybrid ZP 341 had the lowest yield in this region too (7.038 t ha^{-1}). The highest yield stability was expressed by hybrids ZP 606 and ZP 666 (b_i=0.820 and b_i=0.800, respectively), while the top yielding hybrid ZP 600 was the least stable (b_i=3.964) and adjustable to favourable growing conditions (Table 3).

Table 3. Grain yield and stability parameters of ZP hybrids in locations in Podunavlje and Braničevo for the 2014-2017 period.

Hybrid	Grain Yield (t/ha)	Rank according to grain yield	bi	Rank according to b_i
ZP 341	7.038	10	0.589	6
ZP 366	7.399	7	0.553	8
ZP 388	7.391	8	0.584	6
ZP 427	7.783	6	0.640	5
ZP 434	7.365	9	0.559	7
ZP 555	8.102	4	0.738	4
ZP 560	8.044	5	0.752	3
ZP 600	11.290	1	3.964	9
ZP 606	8.744	2	0.820	1
ZP 666	8.486	3	0.800	2
Average	8.164		1	

In the region of central and eastern Serbia, the hybrids ZP 606 and ZP 666 were top yielding (8.842 t ha^{-1} and 8.368 t ha^{-1}, respectively), while the lowest grain yield was recorded in hybrids ZP 341 (7.116t ha^{-1}) and ZP 366 (7.388 t ha^{-1}). The hybrids ZP 427 (b_i=0.993) and ZP 388 (0.972) were the most stable, while hybrids ZP 341 and ZP 434 were the least stable (b_i=0.825 and b_i=0.837, respectively) (Table 4).

Table 4. Grain yield and stability parameters of ZP hybrids in locations in Podunavlje and Braničevo for the 2014-2017 period.

Hybrid	Grain Yield (t/ha)	Rank according to grain yield	bi	Rank according to b_i
ZP 341	7.116	10	0.825	10
ZP 366	7.388	9	0.911	5
ZP 388	7.558	8	0.972	2
ZP 427	7.704	6	0.993	1
ZP 434	7.600	7	0.837	9
ZP 555	8.326	3	1.091	6
ZP 560	8.308	4	1.129	8
ZP 600	8.202	5	1.039	3
ZP 606	8.842	1	1.122	7
ZP 666	8.368	2	1.082	4
Average	7.941		1	

Conclusion

The average maize grain yields were recorded in 2014 and 2015, while 2016 was exceptionally high yield year. Drought in 2017 reduced the yield. Thus, agro-ecological conditions in these four years were ideal for testing stability and adaptability of hybrids. ZP maize hybrids showed good results related not only to grain yields but also to yield stability. The hybrid ZP 600 was top yielding (9.506 t ha^{-1}) in all 85 locations, and at the same time expressed the lowest yield stability and good adaptability to more favourable growing conditions. While testing hybrid stability, it is possible that the highest yielding hybrids show a below-average stability (Babic, 2006). The highest yield stability was estimated in the hybrid ZP 606 (b_i=0.954), which also ranked second by its yield (8.688 t ha^{-1}). The most yielding hybrid ZP 600 was at the same time the least stable (b_i=2.483) and well adjusted to favourable growing conditions. Based on the four-year average it can be concluded that hybrids of earlier maturity groups (FAO 300-400) had lower grain yields than medium late and late maturity hybrids, which is not in accordance to results obtained by Pavlov et al. (2011), hence they might be recommended for cultivation under agro-ecological conditions of central Serbia.

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CORRELATION BETWEEN AGGRESSIVENESS AND SYNTHESIS-ABILITY OF MYCOTOXIN ISOLATES OF *Fusarium graminearum* IN MAIZE IN SERBIA

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Abstract

Fusariosis is one of the most common and most important diseases of maize. Every year, *Fusarium graminearum* (Schwabe) reduces grain yield and quality in both commercial and seed production. This species is probably one of the most destructive pathogens. The damages it causes increase due to mycotoxin synthesis-ability. The determination of a correlation between aggressiveness and synthesis-ability of deoxynivalen and zearalenone in isolates originating from wheat and maize grain was the aim of this study. The pathogenicity of 30 isolates of *F. graminearum* was tested after Reid et al. (1996). Artificial inoculation of ears was done by injecting 2 ml of conidial suspension ($1x10^5$) into silk channels on the second day after maize silked. The estimation of the degree of pathogenicity was based on the determination of symptoms of fusarium maize ear rot on the 1-7 scale. The mycotoxin concentration was determined by the ELISA according to the manufacturer's instructions (Tecna, Italy, CelerDON, ZEA Test Kit). Isolates exhibited pathogenicity on maize ears and synthesised both mycotoxins. The correlation between aggressiveness and DON, i.e. ZEA synthesise-ability was statistically significant, i.e. not statistically significant, respectively.

Keywords: *Fusarium graminearum*, maize, deoxynivalenon, zearalenone.

Introduction

Small grains and maize dominate the overall crop production in Serbia, both per area unit and total yield. However, the cereal production is endangered by a large number of pathogens, particularly of species of the genus *Fusarium*, which are widely distributed in all parts of the world. This genus is the most important genus in agriculture, whose pathogenic species infect plants and synthesise mycotoxins (Stanković et al., 2007). Due to great variability, the species of this genus are adapted to different agroecological conditions of cultivation (Stanković et al., 2007). Although the data on fusarioses exist for more than two centuries, more attention was paid to them only during the last decades of the 20th century (Logrieco et al., 2002). Fusarioses of plants are present to a certain percentage every year, and in years of epidemics they can reduce the yield by 70-90% (Stanković et al., 2012).

Agroecological conditions in Serbia favour, to a greater extent, the development of pathogenic and toxigenic species of the genus *Fusarium*. The most common species of this genus belong to the sections *Discolor* (*Fusarium graminearum* Schwabe and *F. avenaceum* (Fr.) Sacc.) and *Liseola* (*F. verticillioides* Sheldon) (Stanković et al., 2007). During the past two decades, the species *Fusarium graminearum* has been standing out as one of the most destructive causal agents of diseases on cereals, as it can cause yield losses of several billion dollars (Clear and Patrick, 2000; Windels, 2000). *F. graminearum* is one of the most isolated species from infected maize plants in Europe (Logrieco et al., 2002). The importance of *F. graminearum* is not only reflected in yield reductions, but also in the reduction of grain quality due to synthesis-ability of mycotoxins in infected plants and the adverse impact on human an animal health.

Fusariotoxins belong to the group of the most commonly identified mycotoxins in nutrients. *Fusarium* species synthesise a broad spectrum of mycotoxins, but their concentration is not always proportional to the intensity of fusarioses (Champeil et al., 2004; Wegulo**,** 2012; Krnjaja et al., 2015). The origin of over 300 toxic fungal metabolites, of which 139 are fusariotoxins, have been identified (Logrieco et al., 2002; Leslie and Summerell, 2006). The most common among them are trichothecenes (deoxynivalenol-DON), followed by zearalenones (ZEA) and fumonisins (FB1), which differ in the chemical structure, mechanism of action and characteristic symptoms of diseases caused in humans and animals. *F. graminearum* synthesises over 17 types of mycotoxins: ZEA, DON, mono-acetyldeoxynivalenol, nivalenol (NIV), trichothecene type A, culmorin, fusarin C, fuzarohormanon and steroids (Logrieco et al., 2002; Leslie and Summerell, 2006).

The species of the genus *Fusarium*, exhibit a high degree of interspecies and intraspecies variability relating to both toxigenic and pathogenic properties. Isolates may vary from non-pathogenic to very pathogenic to a certain plant species. Moreover, one species can be pathogenic to one and non-pathogenic to another plant species. Many authors have established the existence of variability in the degree of pathogenicity or aggressiveness of the *F. graminearum* isolates in the world (Carter et al., 2002; Brennan et al., 2003; Mesterházy et al., 2005; Krnjaja et al., 2007, 2008). Due to toxigenic properties for humans and animals, certain species of the genus *Fusarium* are given greater importance as more potentially toxigenic than as pathogenic species.

Materials and methods

Thirty isolates of *F. graminearum* originating from the maize and wheat kernels were used in the study. The pathogenicity of isolates was tested after Reid et al. (1996). Artificial inoculation of maize ears was done by injecting 2 ml of conidial suspension ($1x10^5$) into silk channels on the second day after maize silked. The inoculum was prepared of the 14-day culture isolate cultivated on the SNA (synthetic nutrition agar) under the combination of fluorescence and ultra-violet light at 20ºC. The pathogen was directly inserted into the host tissue by a sterile syringe. Five plants in four replicates were inoculated per each isolate. The same procedure was applied to control plants, but instead of the inoculum, sterile distilled water was used. The estimation of the degree of pathogenicity was performed at the stage of maize harvest maturity based on characteristic symptoms of gibberella ear rot of maize on the 1-7 scale (Reid et al., 1996).

The sample preparation for qualitative and quantitative mycotoxicological analyses was done on sterile maize kernels in accordance with the method described by Logrieco et al. (1995). Samples were analysed for the presence of DON and ZEA using ELISA (Enzyme linked immunosorbent assay). The quantitative determination of mycotoxin concentrations was done at the wave number of 450 nm by ELISA reader spectrophotometer (BioTek EL x 800^{TM}).

The Pearson Product-Moment Correlation Coefficent was used to establish the relationship between aggressiveness and mycotoxin concentrations.

Results and discussion

The pathogenicity of all tested *F. graminearum* isolates was confirmed after artificial inoculation of the maize ear. The obtained results indicated that all tested isolates were pathogenic, because they caused the occurrence of characteristic symptoms of gibberella ear rot. Depending on the aggressiveness of the isolates, ears were completely or partially infested by fungal mycelia. Husk was tightly adhered to ears in isolates that expressed stronger pathogenicity. Symptoms of the disease did not occur on ears that were inoculated with sterile distilled water as a negative control. The average assessment of the infection intensity varied in maize and wheat from 2.16 (isolate 1368) to 4.47 (isolate 699) and from 2.11 (isolate 795)

to 4.99 (isolate 1485), respectively. The comparison of average values shows that isolates originating from maize expressed stronger aggressiveness (3.14) than isolates originating from wheat (2.79) (Table 1). Studying the variability of pathogenic properties of *Fusarium* spp. originating from kernels of maize and wheat grown in Serbia, Tančić et al. (2009) observed that intraspecies variability in aggressiveness was most pronounced in *F. graminearum* isolates. Moreover, these authors stated that *F. graminearum* isolates derived form wheat kernels had expressed less pathogenicity than isolates derived from maize kernels. The pathogenicity of *F. graminearum* isolates has been studied in many countries throughout America and Europe (Brennan et al. 2003; Fernandez and Chen 2005; Broders et al., 2007; Kuhnem et al., 2015). Fernandez and Chen (2005) have analysed the pathogenicity of *Fusarium* species in Canada and established that species *F. graminearum* and *F. culmorum* were the most pathogenic on wheat, while species *F. equiseti* and *F. poae* were least pathogenic. Aggressiveness under *in vitro* conditions of 112 of *F. graminearum* isolates originating from 30 locations in 13 countries showed that all observed isolates were very pathogenic on maize seeds and moderately to highly pathogenic on soya bean seeds (Broders et al., 2007). The aim of studies carried out by Kuhnem et al. (2015) was to determine whether *F. graminearum* isolates originating from wheat and maize had been more aggressive on host plants and whether the aggressiveness further affected the production of trichothecenes. These results indicated that the aggressiveness of isolates had widely varied in both wheat and maize, and that there had not been statistically significant differences in aggressiveness among isolates of different origins.

Table 1. Pathogenicity and production ability of *F. graminearum* isolates

Maize-derived isolates	Rate	Mycotoxin		Wheat-derived isolates	Rate	Mycotoxin	
		DON (µg/g)	ZEA (µg/kg)			DON (µg/g)	ZEA (µg/kg)
699	4.47	334.4	68.93	203	2.56	40.2	12.93
762	3.0	65.7	31.55	618	2.21	91.5	46.73
971	4.82	168.3	123.17	677	2.65	21.7	38.36
1030	3.15	88.9	32.06	681	3.16	170.1	45.21
1133	3.45	97.7	90.12	744	2.28	95.0	64.94
1195	2.88	38.6	14.84	749	2.46	58.2	32.33
1211	2.96	23.3	22.49	763	2.29	23.8	9.27
1249	2.58	17.5	24.02	767	2.25	16.6	4.26
1268	4.57	41.2	6.58	789	2.77	51.6	9.67
1368	2.16	79.1	35.08	795	2.11	65.7	78.74
1419	2.96	19.8	4.77	866	3.7	14.2	21.87
1554	2.36	97.5	38.49	1337	2.69	79.8	7.84
1696	2.34	47.3	21.03	1343	2.89	98.2	18.99
2624	2.42	84.5	46.53	2820	2.9	44.2	29.52
2812	2.95	72.6	1.12	1485	4.99	184.6	117.98
average	**3.14**	**85.09**	**37.38**	**average**	**2.79**	**70.36**	**45.21**

The ELISA was used to determine the synthesis-ability of ZEA and DON of the observed *F. graminearum* isolates, as the most important toxigenic property. All studied isolates expressed synthesis-ability of mycotoxins DON and ZEA. The ZEA concentration varied among isolates originating from both the same host and different hosts. The range of variation in concentrations in maize-derived isolates was greater (1.12-123.17 µg/kg) than in wheat-derived isolates (4.26-117.98 µg/kg) (Table 1). The analyses of synthesis-ability of mycotoxins of species of the genus *Fusarium* in the world have produced very divergent

results. It is considered that the reason for this is the different origin of isolates and climatic conditions of the given environment, as well as, nonconforming methods and standards applied in the mycotoxin analyses (Lević, 2008). Wheat-derived isolates produced greater average concentrations of ZEA (45.21 μg/kg) than maize-derived isolates (37.38 μg/kg). However, Tančić et al. (2015), studying the variability in the ZEA production in different species of the genus *Fusarium*, observed that there were no significant differences between average concentration and the range of concentrations in *F. graminearum* isolates derived from wheat and maize in Serbia. The average concentrations of ZEA synthesised by isolates observed in this study were far lower that the average ZEA synthesis in *F. graminearum* isolates (30.600 μg/kg) analysed by Tančić et al. (2009). Studies of the variability of species of the genus *Fusarium* and fusariotoxins in Serbia indicate that *F. graminearum* had the greatest variability in synthesis-ability of ZEA. Obtained results on ZEA synthesis are in accordance with the results on synthesis-ability of *F. graminearum* isolates gained by Jajić et al. (2007) (from the detection threshold to 220 μg/kg). Kuhnem et al. (2015) studied synthesis-ability of ZEA of the *F. graminearum* isolates derived from wheat and maize and established a great range of variation in concentrations of this mycotoxin (100 to 97.520 μg/kg). By studying toxigenic potential of *F. graminearum* originating from maize, Cvetnić et al. (2005) established high concentrations of ZEA ranging from 60.000 to 180.000 μg/kg, while Logrieco et al. (2003) observed significantly higher ZEA concentrations (1.500.000 μg/kg).

Results of the quantitative analysis of DON of studied *F. graminearum* isolates showed that maize-derived isolate 699 had synthesised DON with the highest concentration (334.4 μg/g), while the lowest concentrations (14.2 μg/g) were produced by the wheat-derived isolate 866 (Table 1). The comparison of total average concentrations of DON originating from various hosts shows that *F. graminearum* isolates derived from maize synthesised the highest concentrations (85.09 μg/g), while those wheat-derived isolates synthesised DON concentrations of 70.36 μg/g (Table 1). However, previous studies showed that *F. graminearum* isolates derived from wheat had synthesised greater DON concentrations in comparison with isolates derived from maize kernels (Tančić et al., 2015). The greatest variation range in concentrations of this mycotoxin was observed in maize-derived isolates: 17.5 μg/g (isolate 1249) to 334.4 μg/g (isolate 699) (Table 1). On the other hand, this variation in wheat-derived isolates ranged from 14.2 μg/g to 184.6 μg/g. Tančić et al. (2015) established in their studies on synthesis-ability of *F. graminearum* isolates that the span between the minimum and maximum synthesis of toxins was identical for both wheat- and maize-derived isolates in Serbia. Stanković et al. (2008) studied the potential of the synthesis of *F. graminearum* isolates originating from wheat kernels sampled under various agroclimatic conditions in Serbia and established that all isolates had the ability to synthesise DON up to 45.26 μg/g. Contrary to these results, Tančić et al. (2015) recorded significantly lower average concentrations of DON (10.1 μg/g) synthesised by *F. graminearum* isolates. Concentrations of synthesised DON in this study ranged from 14.2 to 334.4 μg/g. Jajić et al. (2007) established similar values of DON concentrations in wheat kernels (from the detection threshold to 465.9 μg/g). When observing DON synthesis-ability in *F. graminearum* isolates derived from maize, Harris et al. (1999) detected a significantly greater variation range of concentrations (160-1302 μg/g).

Table 2. Correlation between pathogenicity and production ability of mycotoxins by *F. graminearum* isolates

Pathogenicity	Mycotoxin			
	DON (μg/kg)		ZEA (μg/kg)	
	maize	wheat	maize	wheat
maize	0.55*		0.49	
wheat		0.53*		0.48

*Statistically significant difference (p<0.05)

The results obtained in the study about pathogenicity of *F. graminearum* isolates on maize ears under field conditions and the qualitative analyses of mycotoxins pointed out to the existence of a statistically significant correlation between isolate aggressiveness and synthesis-ability of mycotoxin DON. However, it was noticed that there was no statistically significant correlation between isolate aggressiveness and ZEA-producing ability of maize- and wheat-derived isolates (Table 2). There are many different data in literature regarding the correlation between isolates pathogenicity and their ability to synthesise mycotoxins (Carter et al., 2002; Mesterházy, 2002; Gilbert et al., 2002; Kuhnem et al., 2015). By studying pathogenicity and chemotypes of *F. graminearum* isolates**,** Carter et al. (2002) established that different chemotypes were equally pathogenic to wheat, while the NIV chemotype was significantly more pathogenic to maize, pointing out to the possibility that the type of produced toxin could affect the degree of isolate pathogenicity, as well as that the plant host could affect the expression of these properties. Mesterházy (2002) observed that the aggressiveness of the species *F. graminearum* and *F. culmorum* depends on the DON synthesis, that is, the ability to synthesise mycotoxins was closely related to the degree of aggressiveness. Furthermore, it was determined that the resistance of wheat cultivars significantly affected the DON synthesis. The same author concluded that the amount of a synthesised mycotoxin was positively correlated with its aggressiveness, while the cultivar resistance had a stronger effect on the mycotoxin synthesis. By analysing 15 *F. graminearum* isolates, Kuhnem et al. (2015) observed that there was a significant correlation between the DON synthesis and aggressiveness of isolates of this species on wheat and maize. Only one isolate did not synthesise DON on wheat and maize kernels, although it expressed pathogenicity in both hosts. The studies performed by the same authors showed that nine of 15 isolates had synthesised ZEA on maize kernels, while this mycotoxin was not detected in wheat kernels. The amount of synthesised ZEA was not correlated with the degree of aggressiveness on maize. Contrary to these studies, Gilbert et al. (2002) studied 15 *F. graminearum* isolates originated from Canada and noticed that the intensity of the disease in wheat was not correlated with the amount of synthesised DON.

Conclusion

Small grains and maize are exposed to numerous diseases, while fusarioses are the most common and most important diseases occurring on these plant species. The harmful effect of the *F. graminearum* is enhanced by the ability to produce metabolites that are toxic to humans and animals. Having sufficient information on all factors that directly or indirectly affect the development of the disease is a necessary prerequisite for successful prevention of damages caused by this pathogenic species. Studies have shown that all observed *F. graminearum* isolates derived from maize and wheat were pathogenic on maize ears. The isolate aggressiveness ranged from 2.11 to 4.99. Results of the quantitative analysis of mycotoxins obtained by the ELISA showed that all studied isolates had ability to synthesise mycotoxins DON and ZEA in the range of 14.2-334.4 μg/g and 1.12-123.17 μg/kg, respectively. A statistically significant correlation was established between the pathogenicity of *F.*

graminearum isolates and the DON synthesis-ability, while there was no a statistically significant correlation between the aggressiveness and ZEA synthesis-ability.

Climate factors together with numerous physical and cultural conditions affect the geographical and seasonal variation of the appearance of toxigenic species, as well as the level of the mycotoxin synthesis. In recent years, global climatic changes have caused variability of agroclimate conditions, which can contribute to the synthesis of higher concentrations of mycotoxins in wheat kernels during the growing season and can cause economic losses in production, as well as an increased risk to human and animal health. The stated reasons indicated the need for continuous monitoring of these toxigenic species in the cereal production.

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CORRELATION BETWEEN GRAIN YIELD AND YIELD COMPONENTS IN TRITICALE (*x Triricosecale* Wittmack)

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Abstract

Grain yield of small grain cereals is mostly determined by the number of spikes m^{-2}, number of kernels per spike and kernel weight, which are, therefore, direct yield components. The objective of the study was to assess the effect of mineral fertilisers, particularly phosphorus, manure and liming, on grain yield and yield components in triticale cultivars grown on an acidic soil, and evaluate their interactions. The results confirmed significant differences among cultivars and showed a positive effect of mineral fertilisers, manure and liming on grain yield and yield components in all cultivars. There were significant positive correlations at ($P \leq 0.05$) between grain yield and yield components, as well as among yield components. Grain yield exhibited the strongest correlation with number of spikes per m^{-2}, plant height and number of kernels per spike. Number of kernels spike was very strongly correlated with spike length, kernel weight per spike and main stem weight. Interactions between grain yield and yield components under different agro-environmental conditions serve not only as a guide to making a proper choice of cultural practices but also as a selection criteria in choosing cultivars for less favourable production conditions.

Keywords: *Triticale, correlation, grain yield, yield components*

Introduction

Triticale (*x Triticosecale*) is a cereal crop developed by crossing wheat (*Triticum* sp.) and rye (*Secale cereale*) to combine the positive traits of wheat (yield and quality) and rye (resistance/tolerance to biotic and abiotic stresses). Breeding has mostly improved triticale traits such as nutritional value, earliness, grain fill and grain yield. As the result of its positive characteristics, triticale has become an important crop, with its world acreage (5 million ha) increasing (Đurić *et al.*, 2015). Most triticale cultivars developed in Kragujevac are generally early maturing and highly drought resistant (Milovanović *et al*., 2006). The high tolerance of triticale to arid conditions has also been confirmed by the research conducted in Egypt (Hassan, 2003). Triticale is suitable for regions where wheat and other cereals give low yields, particularly on less fertile and acidic soils, which account for 30–40% of total world soil area (von Uexkull and Mutert, 1995). Acidic soils are deficient in macronutrients, particularly readily available P and increased levels of toxic H and Al ions (Samac and Tesfaye, 2003). In terms of resistance to Al toxicity, there is high variability among species as well as among cultivars within species. A relatively high range of Al resistance has been determined in wheat (de Sousa, 1998) and rye (Aniol *et al*., 1980). Triticale has good tolerance to low pH (about 5.0) and many triticale genotypes have shown better adaptation to acidity in comparison with wheat cultivars (Oettler *et al.*, 2000). Triticale produces satisfactory yields under unfavourable weather conditions (Nožinić *et al.*, 2009) and at high altitudes (Stošović *et al.*, 2010). Triticale exhibits high tolerance to acidic soils and good productive performance on sandy soils (Madić *et al.*, 2013; Đekić *et al.*, 2014). The objective of this study was to analyse

the effect of mineral and organic fertilisation and liming on grain yield and yield components in two triticale cultivars grown on an acidic soil, and determine their correlation.

Material and Methods

A field trial with two triticale cultivars, 'Favorit' and 'KG-20', was conducted on the experimental field of the Secondary School of Agriculture in Kraljevo from 2013 to 2015 (43°43′00′′N, 20°40′60′′E, 198 m altitude). The soil used for the trial was characterised by unfavourable physical properties, a low content of humus and an acid pH (pH_{H2O} = 4.7). In both experimental years, crop rotation was used, with maize as the preceding crop. The trial was established in a randomised plot design with three replications and a plot size of 10 m^2. Seeds were sown mechanically, at a spacing of 12 cm between rows and 3 cm within rows.

Complex NPK fertilisers (N_8: P_{24}:K_{16}), superphosphate (17% P_2O_5) with different amounts of phosphorus (I–0; II–80; III–160; IV–80 kg ha^{-1}) and a top dressing of ammonium nitrate 34.4% N (120 kg ha^{-1}) were used. Phosphorus and potassium fertilisers and one third of nitrogen were incorporated during seedbed preparation. The remaining amount of nitrogen was used for top dressing at the beginning of spring. In addition to mineral fertilisation, "Njival Ca" lime (98.5% $CaCO_3$, 1% $MgCO_3$) was applied in treatment IV. At full maturity, 30 plants were sampled from each plot for analysis of plant height (cm), spike length (cm), kernels per spike, kernel weight per spike (g) and grain yield (kg ha^{-1}). Number of spikes/m^{-2} was determined at the end of the growing season during sampling by counting spikes per m^{-2}. After harvest, grain yield per plot was measured and calculated as yield.

The results were subjected to a two-way analysis of variance (with cultivar and fertiliser as factors), and the significance of differences between means was assessed by the LSD test using SPSS software (1995). The correlation between yield and yield components was expressed by the simple correlation coefficient.

Results and Discussion

The results of the analysis of variance revealed significant differences in the analysed traits between the cultivars and across fertilisation treatments (Table 1). Stem height is mostly determined by the genetic makeup of cultivar, production method and climatic conditions (Kirchev *et al.*, 2012). Stem height in the experimental years showed significant differences, which indicated a significant effect of different rates of phosphorus fertilisers and liming. Significantly higher values for stem height in both years were produced by liming (treatment IV) and increased P rate (treatment III), whereas no differences were observed between the years. Spike length and number of kernels per spike in all treatments showed significant differences in comparison to unfertilised treatment (Table 1). Kirchev *et al.* (2012) found that spike length and number of spikelets per spike are largely determined by genotype. Kernel weight per spike in both years was lowest in the control (treatment without mineral fertilisation), significantly higher after mineral fertilisation, and highest under mineral fertilisation and liming treatments.

The number of kernels per spike and number of kernels per plant are the result of the number of spikelets and number of flowers per spikelet, on the one hand, and fertilisation and seed set success on the other, indicating that the number of kernels is largely dependent on additive and non-additive genetic action (Madić *et al.*, 2014) and environmental conditions (Kirchev *et al.*, 2016; Kirchev and Georgieva, 2017).

Number of kernels m^{-2} was lowest in the treatment without mineral fertilisation, significantly higher in treatment II and treatment III, and highest in treatment IV (treatment with mineral fertiliser and lime). Regardless of the type of fertiliser used, in both years, cv. 'Favorit' had significantly higher values for all traits, except for grain yield in 2014. Differences in grain yield in both years were significant among treatments: the lowest in treatment I, and the

highest in treatment IV. Averaged across cultivars, grain yield increased almost threefold in the first and the second year under liming and mineral fertiliser treatment (regardless of cultivar), compared to the unfertilised control, whereas increased amounts of P (treatments II and III) resulted in an almost twofold increase in grain yield in both years. The response of cultivars to liming was more pronounced than their response to increased P fertilisation. As 'Favorit' exhibited a better response to liming, its yield in treatment IV in both years was higher than that of cv.'KG-20'. Harmoney and Thompson (2005) reported that triticale grown for green forage showed a favourable response to small incremental levels of N and P. Moinuddin and Afridi (2008) also reported that triticale grain yield was positively affected by increasing NP rates up to a level of 200 kg N + 40 kg P ha^{-1}, whereas negative effects occurred with a further increase.

Table 1. Means for stem height (SH), spike length (SL), number of kernels per spike (NKS), kernel weight per spike (KWS), number of spikes m^{-2} (NS) and grain yield (GY), for triticale cultivars under different fertiliser treatments in 2013/14 and 2014/15.

Year			SH (cm)	SL (cm)	NKS	KWS (g)	NS	GY (kg ha^{-1})
13/14	Cultivar	Favorit	112.9a	11.4a	45.8a	1.84a	473a	4475
	(A)	KG-20	100.3b	8.5b	35.7b	1.19b	410b	4079
	Fertilisation	I	97.2b	7.9b	31.6b	0.99c	298c	2038d
		II	107.3ab	9.7a	42.7a	1.59b	325c	4055c
	(B)	III	112.8a	9.8a	43.5a	1.60b	393b	4814b
		IV	114.4a	9.9a	44.3a	1.95a	575a	5925a
	ANOVA	A	**	**	**	**	**	ns
		B	*	**	**	**	**	**
		AB	ns	**	ns	ns	ns	ns
14/15	Cultivars	Favorit	111.7a	10.19a	46.8a	1.97a	499a	4453a
	(A)	KG-20	98.4b	7.87b	36.3b	1.58b	399b	3775b
	Fertilisation	I	87.6c	7.2b	31.5b	1.09c	342c	2112d
		II	103.9b	9.3a	42.9a	1.84b	399b	3774c
	(B)	III	113.8a	9.7a	44.7a	1.90ab	445b	4808b
		IV	114.4a	9.8a	46.8a	1.99a	595a	5997a
	ANOVA	A	**	**	**	**	**	**
		B	**	**	**	**	**	**
		AB	ns	**	ns	**	ns	ns

Means across columns for cultivars and fertilisation treatments in the same year followed by the same lower-case letters are not significantly different at 95% by the LSD test

** F –test significant at 0.01; * F –test significant at 0.05; ns –non-significant

The coefficients of simple correlations between grain yield and yield components, as well as those among yield components had positive significant values at $P \leq 0.01$, except between grain yield and spike length ($P \leq 0.05$) (Table 2). Grain yield was most strongly correlated with number of spikes m^{-2}, stem height and number of kernels per spike. Number of kernels per spike was very strongly correlated with spike length and kernel weight per spike. Kozak *et al*. (2007) found that the strength of correlation between grain yield and yield components, and the strength of correlation among yield components cannot be explained by yield structure; therefore, their correlation, as reported, is mostly determined by genotypic factors. In their analysis of 271 triticale lines, Giunta *et al.* (1999) determined that spikes per m^{-2} is a

limiting grain yield component in winter triticale. Mandry *et al*. (1994) and Oettler *et al*. (2000) also observed a strong correlation between spikes m^{-2} and grain yield.

Table 2. Correlation coefficients among grain yield (GY), stem height (SH), spike length (SL), number of kernels per spike (NKS), kernel weight per spike (KWS) and number of spikes m^{-2} (NS)

	SH	SL	NKS	KWS	NS
DK-*SL*	0.80**				
BZK-*NKS*	0.82**	0.89**			
MZK-*KWS*	0.75**	0.81**	0.79**		
BK-*NS*	0.52**	0.60**	0.54**	0.66**	
PZ-*GY*	0.61**	0.38*	0.61**	0.54**	0.71**

*significant at $P \leq 0.05$; **significant at $P \leq 0.01$

The significant positive correlation between number of kernels per spike and kernel weight per spike is consistent with the results of Fischer (1985), who found that number of kernels per unit of biomass is constant for genotypes in a wide range of environmental variation. Wallace and Zobel (1994) reported that the environment modifies the expression of yield components, thus changing grain yield and the relative contribution of yield components to grain yield.

Conclusions

Triticale cultivars 'Favorit' and 'KG-20' exhibited a better response to liming in comparison to increased levels of phosphorus fertiliser, which can be associated with the greater availability of macro- and micronutrients as soil pH increased, i.e. with phosphorus immobilisation in the strongly acidic environment. As 'Favorit' responded better to mineral fertilisation and liming, it produced significantly more kernels per spike and spikes per m^{-2}, and hence larger grain yields, under these treatments in all years, in comparison to cv. 'KG-20'. Significant positive correlations were generally found among yield components as well as between grain yield and yield components, except between grain yield and spike length. Grain yield was most strongly correlated with number of spikes m^{-2}, plant height and number of kernels per spike. In addition to providing guidelines for making a proper choice of cultural operations, the correlation between grain yield and yield components under varied agroenvironmental conditions can serve as a selection criterion for choosing cultivars for less favourable production conditions.

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KINETICS OF DRY MATTER CONTENT DURING DRYING OF CV 'ČAČANSKA RODNA' FRUITS

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Abstract

Drying kinetics of plum fruits can be presented with diifferent drying curves (curves of fruit mass, moisture content on a wet base, moisture content on a dry base…), as well as with appropriate drying rate curves. This paper presents the curve of dry matter content change of the fruits of CV 'Čačanska Rodna' during the drying process. Fruits of the plum cultivar Čačanska rodna taken from the Fruit Research Institute Čačak 'Preljinsko brdo' site have been used for the examination. Tests were conducted using convective procedure in the hot air flow at the constant drying temperature of 90 ºC throughout the experimental drying chamber. Prior to drying experiments, fresh fruit mass, content of stone and dry matter content were determined. Mass share of the stone in the fruit was determined based on the fruit and stone mass. Before the drying process, the fresh plum fruits with pre-defined characteristics were distributed on the trays. Then the trays were placed in a drying chamber with the pre-adjusted drying air temperature. During the drying process, fruit masses were measured on the trays at the respective time intervals, thereby monitoring the kinetic process. It was noted that dry matter content in fruits increased during the drying process and the curves of dry matter content change were suitable for presenting the kinetics of drying process.

Key words: *Convective drying, Drying curves, Dry matter content, Fresh fruit characteristics.*

Introduction

Prunes are the most important dried fruits in the Serbia. In order to obtain dried fruits of high quality (i.e. appropriate appearance, color, consistency, taste and odour), it is important to select suitable varieties for drying (Cinquanta et al., 2002; Mitrović et al., 2013a). Also, it is important to select fresh fruits with adequate technological properties (Kandić et al., 2017).

The process of convective drying is a process of evaporation of water from the fruits with heated air, which is still the most common and the most widespread way of drying plums in Serbia. But this does not mean that the dried fruits of high quality can be obtained by any convective drying process. This is a complex simultaneous process of heat and mass transfer. Heated air simultaneously transfer heat and receive the evaporated water at the same time. Therefore, in order to define the optimal technology of convective drying of the plum, the kinetics of the drying process of plums in experimental laboratory or semi-industrial (pilot-scale) devices are carried out (Barbanti et al., 1995; Newman et al., 1996; Mitrović et al., 2013b). Drying kinetics can be presented by different curves (curves of fruit mass, moisture content on a wet base, moisture content on a dry base…) that represent changes of basic material (plums) physical characteristics by time.

The aim of this study was to determine suitability of curves of the dry matter content changes for displaying the kinetics of the plum drying process.

Material and methods

The tests were carried out in 2016, in an experimental dryer for testing the kinetics of the process of convective drying. The most important segment of this device is the drying chamber with trays (Kandić et al., 2007; Kandić et al., 2014).

As testing material in the experiments, the fruit of the Čačanska rodna variety from the experimental orchard of the Fruit Research Institute Čačak (Serbia), from the locality Preljinsko brdo, was used.

Analyses of fresh plums before the drying process included:

- mass of fresh fruits (g),
- stone ratio (g/g),
- total dry matter (g/g).

Before the start of the drying process, the total mass of fresh plums on every tray was measured. In all experiments, the total mass of fresh fruits on the trays was about 3,500 g.

The experiments were carried out in such a way that the trays were placed in a drying chamber. Temperature of the heated air was 90 °C, before passing through plum layers on the trays. At certain time intervals, during the drying process, the mass of the fruits on the trays were measured and in this way the process kinetics was monitored.

Table 1: Experimental results

Experim.	M_0 (g)	Number Fruits	M_P (g)	G_{K0} (g/g)	G_{SM0} (g/g)	G_{SMz} (g/g)	τ (h)
[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
E-01	3,503	**79**	**44.34**	0.0323	0.2420	0.7438	**10**
E-02	3,499	**79**	**44.29**	0.0323	0.2390	0.7556	**10**
E-03	3,504	**79**	**44.35**	0.0323	0.2210	0.7480	**9**
E-04	3,497	**79**	**44.27**	0.0323	0.2210	0.7567	**9**
E-05	3,502	**79**	**44.33**	0.0323	0.2210	0.7604	**9**
E-06	3,496	**79**	**44.25**	0.0323	0.2210	0.7593	**9**
E-07	3,503	**77**	**45.49**	0.0332	0.1975	0.7592	**10**
E-08	3,502	**80**	**43.78**	0.0345	0.2222	0.7579	**9**
E-09	3,506	**80**	**43.82**	0.0345	0.2222	0.7610	**9**
E-10	3,502	**82**	**42.70**	0.0334	0.2350	0.7483	**9**
E-11	3,498	**82**	**42.66**	0.0334	0.2350	0.7549	**9**
E-12	3,503	**82**	**42.72**	0.0334	0.2350	0.7561	**9**
E-13	3,503	**85**	**41.21**	0.0362	0.2321	0.7455	**9**
E-14	3,504	**85**	**41.22**	0.0362	0.2321	0.7521	**9**
E-15	3,501	**86**	**40.71**	0.0362	0.2321	0.7529	**9**
E-16	3,504	**86**	**40.74**	0.0362	0.2321	0.7526	**9**
E-17	3,501	**87**	**40.24**	0.0362	0.2321	0.7558	**9**
E-18	3,502	**86**	**40.72**	0.0323	0.2370	0.7561	**9**

Results and Discussion

Results of 18 experiments were shown in Table 1.

Column [1] lists the experiment labels. The other columns show the values of the following experimental parameters:

- M_0 [g] - Total mass of fresh fruits on the tray (column [2]);
- Number of fruits on the tray (column [3]);
- M_P [g] - Average mass of fresh individual fruits (column [4]);
- G_{Ko} [g/g] - Average mass fraction of the stones in fresh fruits (column [5]);
- G_{SMo} [gSM/g] - Content of total dry matter in edible part of fresh fruits (total dry matter of fresh fruits) (column [6]);

- G_{SMz} [gSM/g] -The total dry matter content in the edible part of the dried fruits (total dry matter of the dried fruits) (column [7]);
- τ_Z [h] - Total duration of the drying process on the tray (column [8]).

By analyzing the values of the characteristics of the fresh fruits shown in Table 1, the following can be noted:

Total mass of fresh fruit on the tray M_0 in all 18 experiments had approximately the same value of 3,500 g (values were from 3,496 g to 3,506 g).

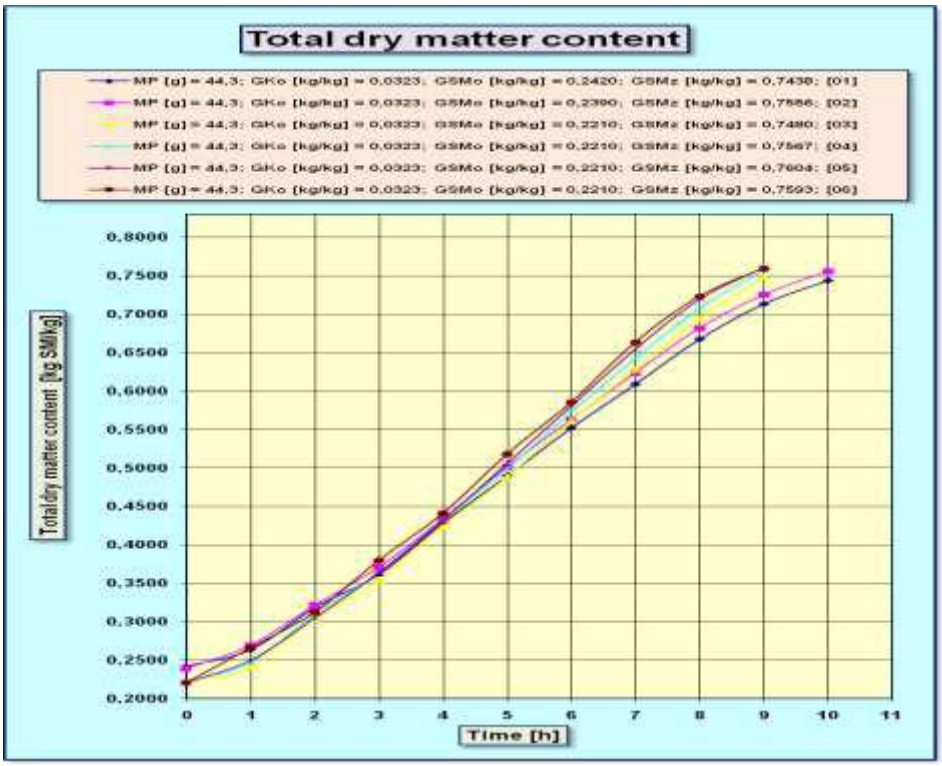
The 18 experiments presented are divided into three groups of 6 experiments.

- The first group contains data for 6 experiments with the same characteristics of fresh fruits. In all 6 experiments 79 fresh individual fruits were distributed on the trays. The average mass of these fruits was $M_P \approx 44.3$ g and the average mass fraction of the stones in fresh fruits was $G_{Ko} = 0.0323$ g/g. The total dry matter content of fresh fruits in 4 experiments was $G_{SMo} = 0.2210$ g DM/g. Only, in the first two experiments of this group the total dry matter content was $G_{SMo} \approx 0.24$ g DM/g.
- The second group contains data for 5 experiments with almost the same characteristics of fresh fruits. In three experiments 82 fresh individual fruits were distributed on the trays. The average mass of these fruits was $M_P \approx 42.7$ g, the average mass fraction of the stone in fresh fruit was $G_{Ko} = 0.0334$ g/g, and the total dry matter content was $G_{SMo} = 0.2350$ g DM/g. In two experiments 80 fresh individual fruits were distributed on the trays. The average mass of these fruits was $M_P \approx 43.8$ g, the average mass fraction of the stone in fresh fruits was $G_{Ko} = 0.0345$ g/g, and the content of the total dry matter was $G_{SMo} = 0.2222$ g DM/g. Only the first experiment in this group had different characteristics of fresh fruits. In that experiment 77 fresh individual fruits were distributed on the tray. The average mass of these fruits was $M_P \approx 45.5$ g, the average mass fraction of the stone in fresh fruits was $G_{Ko} = 0.0332$ g/g, but content of total dry matter was lower than 0.2 g DM/g i.e. $G_{SMo} = 0.1975$ g DM/g.
- In the third group, data for 6 experiments with almost the same characteristics of fresh fruits. In five experiments, the average mass fraction of the stone in fresh fruits was $G_{Ko} = 0.0362$ g/g and the total dry matter content was $G_{SMo} = 0.2321$ g DM/g. In the first two experiments from this group, 85 fresh individual fruits were distributed on the trays. The average mass of these fruits was $M_P \approx 41.2$ g. In the next two experiments 86 fresh individual fruits were distributed on the trays. The average mass of these fruits was $M_P \approx 40.7$ g. In the fifth experiment 87 fresh individual fruits were distributed on the tray. The average mass of these fruits was $M_P \approx 40.2$ g. In the last, sixth experiment in this group 86 fresh individual fruits were distributed on the tray, but with somewhat different characteristics of fresh fruits from others in this group. The average mass of these fruits was $M_P \approx 40.7$ g, the average mass fraction of the stone in fresh fruits was $G_{Ko} = 0.0323$ g/g and the total dry matter content was $G_{SMo} = 0.237$ g DM/g.

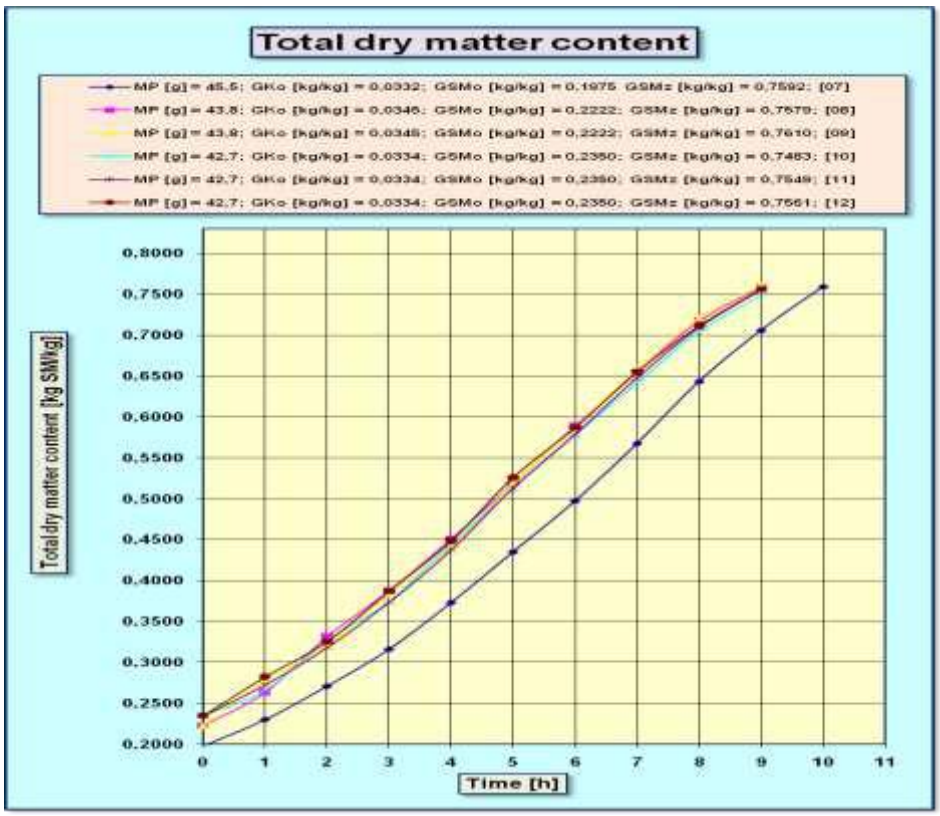
The dependence of the duration of the drying process on the initial characteristics of the plum fruit (average individual mass of fresh fruits, average mass fraction of the stones in fresh fruits and total dry matter of fresh fruits) is shown in Table 1. When the fruits dry up to approximately a constant value of the total dry matter content of GSMz of about 75% and if the initial characteristics of the fruits are similar, then the drying time of the fruit at 90 °C was 9 hours. This is consistent with the results of testing the kinetics of drying plums cv. Čačanska rodna which were obtained in the previous studies (Mitrović et al., 2007; Kandić et al., 2014).

In this work, three graphs of the curves of total dry matter content changes during drying for three groups of experiments are shown. It is interestingly that in 15 experiments there were the excellent matching of all drying curves when the drying time was 9 h. There was a deviation in the first two experiments of the first group and in the first experiment of the

second group, where the drying time was 10 h, which is due to somewhat different values of the initial characteristics of the fruits.



Graph 1: Total dry matter content - group 1



Graph 2: Total dry matter content - group 2

Graph 3: Total dry matter content - group 3

Conclusion

It was concluded that the duration of the drying process depends on the initial characteristics of the plum fruits: the average individual weight of the fresh fruits, the average mass fraction of the stem in fresh fruits and the total dry matter of fresh fruits. If these initial characteristics are approximate values and if the fruits dry up to a near constant value of the total dry matter dry matter, then the drying time of these fruits is the same. It can also be noted that the values of the content of the dry matter of the fruit are increased during the drying process and that the curves of the changes in the dry matter content are suitable for displaying the kinetics of the drying process.

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VARIABILITY AND PATH ANALYSIS FOR YIELD COMPONENTS OF DIFFERENT WHEAT GENOTYPES

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Abstract

The study was carried out to investigate the genotypic and phenotypic variability, heritability and relationship between wheat yield components. A randomized complete block design experiment was conducted, with sixteen wheat genotypes (Dukat, Dunavka, Fundulea 4, Iskra, Jedina, Jugoslavija, Kavkaz, Mačvanka 1, Marija, NS-5804, Pitoma, Poljana, Skopjanka, Tamiš, Vali PKA-7114 and Zvezda), in Novi Bečej (Vojvodina, Serbia), during 2016 and 2017 growing seasons. Grain weight per plant had the highest genotypic and phenotypic variability (15.45 and 20.58%, respectively), while spike length had the lowest ones (5.68 and 6.78%, respectively). High broad sense heritability was observed for plant height (H^2=86.19%) and spike length (H^2=71.73%). Heritability was low in the case of spike weight (H^2=38.82%) and grain weight per spike (H^2=26.56%), which indicates that environmental factors had higher impact on expression of these traits in relation to genetic factors. Path analysis revealed that spike weight and spike length had the highest significant direct positive effect on the grain weight per plant, while thousand grain weight had the highest significant negative effect. The grain weight per spike, number of grains per spike and spike length had a significant indirect effect, through spike weight, on grain weight per spike.

Key words: *Heritability, variability, direct effect, indirect effect*

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most widely planted crops worldwide and provide about 20% of total food calories for the people of the world (Nukasani *et al*., 2013).

The study of genetic variability and correlation of morpho-agronomic traits with grain yield provides information necessary for successful selection (Ali *et al.,* 2008). It is particularly important to examine the genetic variability of varieties in different agroecological conditions and different soil categories to determine the degree of phenotypic variation and identify the sources of this variability (Petrović *et al*., 2007). Thus, an ideal variety, characterized by high yield or a high value of another desired trait, should exhibit a genetic potential with a low variance value in different environmental conditions (Zečević *et al*., 2008).

It is necessary to separate the total variation into heritable and non-heritable components with the help of genetic parameters, i.e. the genotypic and phenotypic coefficient of variation, and heritability (Paul *et al.,* 2006). Higher heritability values make the selection process more effective, by helping breeders in predicting the interactions of genes in the succeeding generation (Waquas *et al.,* 2014; Saleem *et al.,* 2016). Higher heritabilities indicate that the given trait is under relatively minor influence of the environment (Zerga *et al.,* 2016).

Many researchers have investigated the relationship between yield components and found that knowledge of correlations between traits which determine yield can help in the indirect selection of yield components (Zečević *et al*., 2004a, Hristov *et al*., 2011). In accordance with Rohani and Marker (2016), correlations between yield and its components give a confusing picture due to the mutual relations between the yield components themselves. Thus, mutual

correlations represent only the degree of interaction between given characteristics, while Path analysis quantifies and separates the relationships between yield components into their direct and indirect effects on grain yield (Ashfaq *et al*., 2003).
Path analysis is widely used in plant breeding to determine the nature of the relationship between grain yield and its components, and to determine which yield components with a significant yield effect can be potentially used as selection criteria (Naghavi *et al*., 2014).
The aim of this investigation was to analyze the variability, heritability and direct and indirect effects of yield components on the grain weight per plant. A secondary goal was to determine which traits could be used as yield phenotypic selection markers in a breeding process.

Material and Methods

The experimental material consisting of sixteen diverse wheat genotypes: Yugoslavia, Jedina, Fundulea 4, Iskra, Dunavka, Tamis, Caucasus, Skopjanka, Dukat, Pitoma, Poljana, Marija, NS 58-04, Mačvanka 1, Vali PKA 7114 and Zvezda. Selected genotypes are genetically divergent, which could be used for the development of germplasm in the future plant breeding. The experiment was carried out in a randomized block design with three replications, during two growing seasons (2015/2016 and 2016/2017) in Novi Bečej (Vojvodina, Serbia). The plot size was 2 m^2, where sowing density was 650 grains per m^2. Normal agronomic practices were applied to the experiment throughout the growing season. Ten plants per replication were used for recording plant height (cm), spike length (cm), spike weight (g), number of grain per spike, grain weight per spike (g), grain weight per plant (g) and thousand grain weight (g).
The genetic parameters of variability, estimation of heritability and parameters of variance were computed according to the method suggested by Falconer (1981). The direct and indirect effects of yield components on grain weight per plant were measured by Path analysis as described by Dewey and Lu (1959).

Results and Discussion

The high broad sense heritability of plant height (H^2 = 86.19%) indicates that the analyzed traits had been mostly conditioned by genetic factors, which can help breeders to more easily predict phenotypes of the progeny, based on the genotypes of the parents. This is in agreement with results obtained by Zečević *et al*. (2004a), Petrović *et al*. (2007), Ali *et al*. (2008) and Bhushan *et al*. (2013). The low genotypic and phenotypic coefficients of variation for plant height (GCV = 8.18% and PCV = 8.81%) indicate low variability of this trait, which could be related to the fact that the variation in plant height in European wheat varieties is controlled by the presence or absence of major dwarfing genes (Rht-D1 and Rht-B1) (Würschum *et al*., 2015) (Table 1).
Heritability of spike length was high (H^2 = 71.73%), which is in accordance with results obtained by Ali *et al*. (2008) (76.2%) who noted that the high heritability was contributed by additive gene effects. Thus, selection for this trait can be effective in the early generations. Kotal *et al*. (2010), Rahman *et al*. (2016), Sabbit *et al*. (2017) also found high heritability for spike length. Genotypic and phenotypic variation of spike length was not statistically significant (GCV = 5.68%, PCV = 6.71%), which was confirmed by its small proportion of ecological variance (Table 1).
The low broad sense heritability for spike weight (H^2 = 38.82%) showed that this trait would make the selection process more difficult. Also, Sabbit *et al*. (2017) found low heritability for spike weight. Due to the significant influence of ecological variation, a very significant difference was observed between genotypic and phenotypic variabilities, (GCV = 7.96% and PCV = 11.3%) (Table 1).

Grain weight per spike was a trait with very low broad sense heritability (H^2 = 26.56%). Thus, it would be very difficult to perform effective selection for this trait. This finding is in agreement with results obtained by Zečević *et al*. (2010). The PCV was 12.84%, which was almost twice the GCV (6.67%) (Table 1).

Table 1. Genotypic and phenotypic variance and broad sense heritability of wheat yield components

Yield components[1]	Mean values	Estimates of variance components[2]				CV (%)	GCV (%)	PCV (%)	H^2 (%)
		σ^2_g	σ^2_{ph}	$\sigma^2_{g\times y}$	σ^2_E				
PH	89.26	53.37	61.92	15.86	8.549	6.37	8.18	8.81	86.19
SL	10.18	0.335	0.467	0.228	0.133	10.22	5.68	6.71	71.73
SW	2.58	0.033	0.085	0.093	0.052	22.50	7.96	11.3	38.82
GWS	1.97	0.017	0.064	0.085	0.047	26.10	6.67	12.84	26.56
NGS	47.41	22.93	35.93	22.89	12.992	19.99	10.1	12.64	63.82
GWP	13.86	4.67	8.14	6.098	3.47	34.99	15.45	20.58	57.37
TGW	41.26	5.543	9.65	6.002	4.107	4.39	5.69	7.528	57.44

[1]PH – plant height, SL – spike length, SW – spike weight, GWS – grain weight per spike, NGS – number of grains per spike, GWP – grain weight per plant, TGW – thousand grain weight; [2] σ^2_g – genetic variance, σ^2_{ph} – phenotypic variance, $\sigma^2_{g\times y}$ – genptype and phenotype variance, σ^2_E – environmental variance

Heritability in the broad sense for grain number per spike was moderate (63.82%) (Table 1), which is in accordance with results obtained by Zečević *et al*. (2010). Significant GCV and PCV were found for number of grains per spike (10.1 and 12.64%, respectively) (Table 1). Also, Knežević *et al*. (2012) showed a significant phenotypic variability for number of grains per spike, and concluded that it is very advantageous to perform selection for this yield component.

Moderate heritability was recorded for grain weight per plant (H^2 = 57.37%), which confirms a significant proportion of ecological variance within the total phenotypic variance (Table 1). This is in accordance with results obtained by Ali *et al*. (2008), Kotal *et al*. (2010) and Sabbit *et al*. (2017). GCV and PCV for the trait were 15.45 and 20.58%, respectively, showing high variability for grain weight per plant, which, with moderate heritability, could facilitate the selection of parents. The large difference between GCV and PCV indicates a significant contribution of ecological variance to total phenotypic variance (Table 1).

Thousand grain weight exhibited a moderate heritability (57.44%), which is consistent with results reported by Eid *et al*. (2009), who analyzed heritability in arid conditions, while at favorable environmental conditions, they established a higher heritability. Thousand grain weight had low variability (GCV = 5.69%, PCV = 7.53%) (Table 1).

Spike length had a very significant ($p<0.01$) and positive direct effect (0.156**) on grain weight per plant (Table 2). The results obtained are consistent with those reported by Baranwal *et al*. (2012), where they found that spike length had a significant direct and positive effect on grain yield. Path analysis showed that spike length had a significant positive indirect effect (0.229), through spike weight, on grain weight per plant (Table 2). Zečević *et al*. (2004b) found that spike length had the highest positive indirect effect on grain weight per plant through number of spikelets per spike. Spike weight had the strongest direct and positive effect (0.674**) on grain weight per plant, while the indirect effect, through other traits, was not significant (Table 2). These results indicate that spike weight can be a good selection criterion in wheat breeding. Grain weight per spike had a very high positive effect on grain weight per plant, though the greatest influence was contributed by the indirect effect that this

property had through spike weight (0.665). These findings suggest that indirect selection can be made for higher grain weight per spike by selecting plants with a higher average spike weight.

Table 2. Direct and indirect effects of yield components on grain weight per plant

Yield components[1]	**Direct effect**	**Indirect effect**						**Total indirect effect**	**Total effect**
		PH	SL	SW	GWS	NGS	TGW		
PH	0.021	-	0.026	0.032	0.001	-0.023	0.005	0.041	0.062
SL	0.156**	0.004	-	0.229	0.010	0.024	0.012	0.280	0.435
SW	0.674**	0.001	0.053	-	0.010	0.073	0.012	0.172	0.846
GWS	0.034	0.001	0.048	0.665	-	0.075	-0.013	0.777	0.810
NGS	0.090	-0.005	0.042	0.545	0.028	-	-0.001	0.609	0.699
TGW	-0.156**	0.000	-0.010	-0.042	0.002	0.000	-	-0.050	-0.246
R^2=0.745								1.828	2.607
Dependent variable: grain weight per plant									

** $p<0.01$

[1]PH – plant height, SL – spike length, SW – spike weight, GWS – grain weight per spike, NGS – number of grains per spike, TGW – thousand grain weight

The positive influence of number of grains per spike was reflected through the indirect positive effect that this trait had through spike weight (0.545). Therefore, selection for more grains per spike would not necessarily guarantee a higher grain weight per plant. In this case, the selection of plants with a higher number of grains per spike could be done indirectly by selecting for spike weight, all to increase grain weight per plant. Khaliq *et al*. (2004) found that number of grains per spike had a very high and positive indirect effect on grain yield, through spike length. Thousand grain weight had a highly significant and negative direct effect on grain weight per plant (-0.156 **). Kashif and Khaliq (2004) established that thousand grain weight had a significant negative direct and indirect effect on grain yield. Similarly, Nukasani *et al*. (2013) found that thousand grain weight had a negative direct effect on yield, though without statistical significance.

Path analysis showed that plant height had no significant direct or indirect effect on grain weight per plant (Table 2). Nukasani *et al*. (2013) established a positive, but non-significant, direct effect of plant height on grain yield.

Conclusion

High heritability was observed for plant height and spike length. In these traits, ecological variance and genotype × environment interaction made minor contributions to the total phenotypic variance, which could help breeders to predict phenotypes of the progeny based on the genotypes of the parents. Very low heritability was recorded for spike weight and grain weight per spike, which could complicate the process of breeding. Also, significant genotypic and phenotypic variability was observed, especially in grain weight per spike. Spike weight and spike length had the highest significant direct positive effects on grain weight per plant, while thousand grain weight had the highest significant negative effect. Thus, the grain weight per plant could be increased by direct selection for spike weight and spike length. Grain weight per spike, number of grains per spike and spike length had significant indirect effects, through spike weight, on grain weight per spike. Thus, it could be concluded that spike weight

was the trait which contributed most to the increase in grain weight per plant amongst these diverse wheat genotypes.

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A 10-YEARS ANALYSIS OF GRAPE PRODUCTION IN SERBIA

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Abstract

Grapes and grape wine have played an important part in Serbian history, stretching back for thousands of years. Unfortunately, during the last decade the Republic of Serbia had devastating results in regard to the total production of grape. Statistics shown on Statista,astatistic portalshows the global leading grape producing countries in 2016/2017. For that period,China is ranked first and the United Statesfifth in grape production. Serbia is not in the top-ten and the possible reason forthiscan betheshrinking of harvested areas during the last decade. This paper presents an analysis of grape production in the Republic of Serbia, using the index comparison method, i.e. using the statistical data on previous years, comparing them with the data on 2017. The data available on the website of the FAOSTAT statistical database and the Statistical Office of the Republic of Serbia (RZS) were used for the comparison and analysis. The analysis of the data on the period from 2007 to 2017 showed that the total production of grapes and harvesting area was diminishing as the time went on. The biggest harvested area was measured in 2007 and it was 59,068 ha. On the other hand, the harvested area in 2017 was 21,201 ha. The best results in grape production were achieved in 2009. In comparison to 2017, the Republic of Serbia had bigger production of grapes in 2009 by 160%. Theworst results were made in 2014 and the production was around 26% lower than the production in 2017. In the last few years, the harvested area has stopped diminishing and onecan see a little improvement in the production.

Keywords: *grape, production, harvested area.*

Introduction

Serbia is located between 42° and 46° northern latitudes, which is among the most suitable areas for viticulture. Despite the fact that suitable natural conditions for cultivation of grapevines exist in Serbia, grape production is lagging behind the countries with similar conditions (Denda S. and Denda B., 2016). After solving the phylloxericcrisis and renewing vineyards (after 1880),grapevine nurseries were developed in Serbia,producingseedlings by grafting local vines on American grapevine rootstocks. In Serbia, as in all of Central and Eastern Europe in the early nineteen-sixties, high breeding and wide rows, usually 3 x 1 m with 3,333 vines per hectare, were introduced. Over the last twenty years, a quality improvement in viticulture occurred in Serbia, particularly on private properties.A new technology was introduced into viticulture. The spacing between the vines in a vineyard is reduced to 2.2-2.7 m, and the vine spacing within a row is reduced to 0.6-0.8 m, so that the number of plants per hectare is 4000-6000 (Ivanišević *et al*., 2015). According to the method of cultivation, there are plantation and extensive vineyards. Wine varieties are dominant, compared to table ones. Statistical data, after the 2012 census, indicated that there were about 25,000 ha under vines on the entire territory of the Republic of Serbia. Wine grape varieties were grown in Serbia onaround 75.7% of the total vineyard area. The varieties of grapes that were intended for fresh consumption werecultivated on 24.3% of the total area under vines (Ivanišević and Jakšić., 2014). In 2013, vineyards covered 1.0% of the total agricultural area

in the Republic of Serbia. On the basis of statistical data,in the nineteen-eighties, there were 100,000 hectares of vineyards in Serbia, and today there are 22,150 hectares (Ivanišević and Jakšić, 2015; Korać *et al*., 2014). In Serbia, growing organic vineyards is in the initial development, at the surface of around 6 hectares (Vukosavljević*et al*., 2016).The share of grapes in the total amount of agricultural production in Serbia in 2002 amounted to 4.6% (Republički zavod za statistiku, 2007). Later, until 2017, there was a decline in the share of grapes inagricultural production and it was around 2.0%, with slight variations from year to year (Republičkizavod za statistiku,2010).With passing the Law on Plant Protection Products (Zakon o sredstvima za zaštitu bilja, 2009), "Official Gazette of the Republic of Serbia," No. 41/09, plant health and the rights of cultivators were partially aligned with the EU directives and regulations. Large gaps have remained, especially when it comes to implementation and control. Part of the Law on Seeds ("Official Gazette the Republic of Serbia," No. 45/05 and 30/10 – other law), referring to fruits and grapes, is partially harmonized with the EU legislation (ИПАРД програмзаРепубликуСрбијузапериод2014-2020 (2017)).

ProkopijeBolić is considered to be the first Serbianampelographer.Heprovided ampelographic descriptions of 35 vine varieties that were grown in the late eighteenth and early nineteenth centuries in Serbia. Most of these varietiesare extinct;those were native *pontica, balcanica* varieties (Klještanović, 2012; Ivanišević *et al*., 2015). At the end of the nineteenth century, resistant varieties were created, based on the principle that one genotype could combine resistance and quality. So far a greater number of wine and table grape varieties have been created (Klještanović, 2012). The grape assortment in Serbia is divided into the following three groups: native and regional varieties; international and domestic varieties, created varieties (Korać, 2012). In an interview, prof. DjordjePapric said: "When assessing a favorable assortment, one should bear in mind that, out of all grape varieties now we have clones adapted to our climate and they should be grown." (Boarov, 2002)

Grape derivatives are usually wine and brandy, for example, the production of wine in 2013 g. amounted to 45,000 liters (Statistički godišnjak Republike Srbije, 2014). According to the manner of grape use, varieties can be classified into the following groups: wine-grape varieties; alcoholic beverage varieties; table varieties; dried grape varieties; varieties for the production of sweet compotes, marinades, candied fruits, and the like; varieties formakingnon-alcoholic grape juice and concentrates and for the production of colors – teinturiervarieties (Serbian Brandy, 2012).Natural factors make wines in a specific region unique; in wine-making, this term is called *terroir*. These factors include local climatic conditions, the vineyard location (altitude and slope) and soil characteristics. In wine production, fruits are the best when the climate is moderate and provides enough light and nights without frost and porous soil. Vines grow best on soils that are loose, sandy, permeable and rocky (Processing of Raisins and Wine Making, 2000). The same grape varieties can lead to different tastes of wine depending on the place where they were grown. Different soil types and different climatic conditions give products of unique wine styles out of the same grape variety. This phenomenon is called *terroir*. Practically, *terroir* is the connection between the qualitative characteristics of wine and the geographic origin (Ranković-Vasić, 2013). Wine contains alcohol called ethanol. It is present in an amount sufficient to kill microorganisms that cause diseases. It was believed that drinking wine was more useful than drinking water and milk (McGovern, 2007).Wine contains around 85-89% of water, around 10-14% alcohol and less than 1% of fruit acids. Wine characteristics depend on numerous factors, including the grape variety, the place of growing and processing techniques in the winery (Anderson, F. S. and Anderson, D. 1989).The trend in demand for high-quality wines with the geographic origin continues and white wine grape varieties are dominant in modern, young plantations: Chardonnay, Sauvignon, Pinot Blanc, Rhine Riesling, Italian Riesling, Pinot Gris,etc. Currently, vineyards with red wine grape varieties, Cabernet Sauvignon and Merlot, are being

planted to a great extent, and to a lesser extent, vineyards with varieties, Pinot noir, Frankovka and Portugizer (Ivanišević*et al*., 2015). The importance of vines is not only in grapes and grape derivatives. Vines are significant because they can be grown onsoilswhere a number of other crops would not be cost-effective or itwould not be possible to grow them (Burić,1972).In addition to wines and spirits, grapes seeds can be used for the production of oil. The oil content in grape seed samples is relatively low, and it ranges from 7.53 ± 0.07 to 10.88 ± 0.40% on the dry matter. The limiting condition of seed quality, as a raw material for the production of cold pressed oil, is the acid value,which is the highestin samples after distillation(Bjelica*et.al*., 2017).The share of private farms in the total vineyard capacities in the Republic of Serbia in 2006 and 2009 was approximately 92%, and the number of fruit-bearing trees for grapes was about 95% compared to the property of companies and cooperatives (Republičkizavod za statistiku,2007; Republičkizavod za statistiku, 2010).
Based on the research results in 1999, conducted by Simic (1999), it can be noted that a crisis existed in viticulture at the time, where despite favorable climate and soil conditions for the growing of vines, the natural advantages were not utilized. The author (Simić, 1999) points to the extensive nature of production, outdated plantations with nontopicalassortment and low productivity. After this period,after the year 2000, an increase in the production started to happen in orcharding and viticulture, but the livestock production began to decrease (Gulan, 2013).The influence of the soil pH on the quality (taste) of wine is an important factor (Retallack and Burns, 2015).

Material and Methods

Based on the data collected from FAOSTAT for the period from 2007 to 2016 and the 2017 data of the Statistical Office of the Republic of Serbia (Table 1), the index comparison of the total vine production in the Republic of Serbia for the given period was processed. A review of the index comparison for total production is presented in Figure 1.Because of significant changes in harvested area in the last decade there is also a review of the index comparison for total harvested areathat is presented in Figure 2.

Table 1. The area under vines and the production in the Republic of Serbia for the 10-year period (2007-2017) (FAO); * source: RZS (2017)

Year	Grapes - Area harvested (ha)	Grapes – Production (tones)
2007	59068	353343
2008	58324	372967
2009	57540	431306
2010	47366	330070
2011	45804	324919
2012	35719	263419
2013	21201	199955
2014	21201	122489
2015	21201	170647
2016	21201	145829
2017*	21201	165568

Results and Discussion

On the basis of Figure 1, we can conclude that the total vine production in the period from 2009 to 2017 relatively decreased gradually with minor fluctuations during the period from 2014 to 2017.

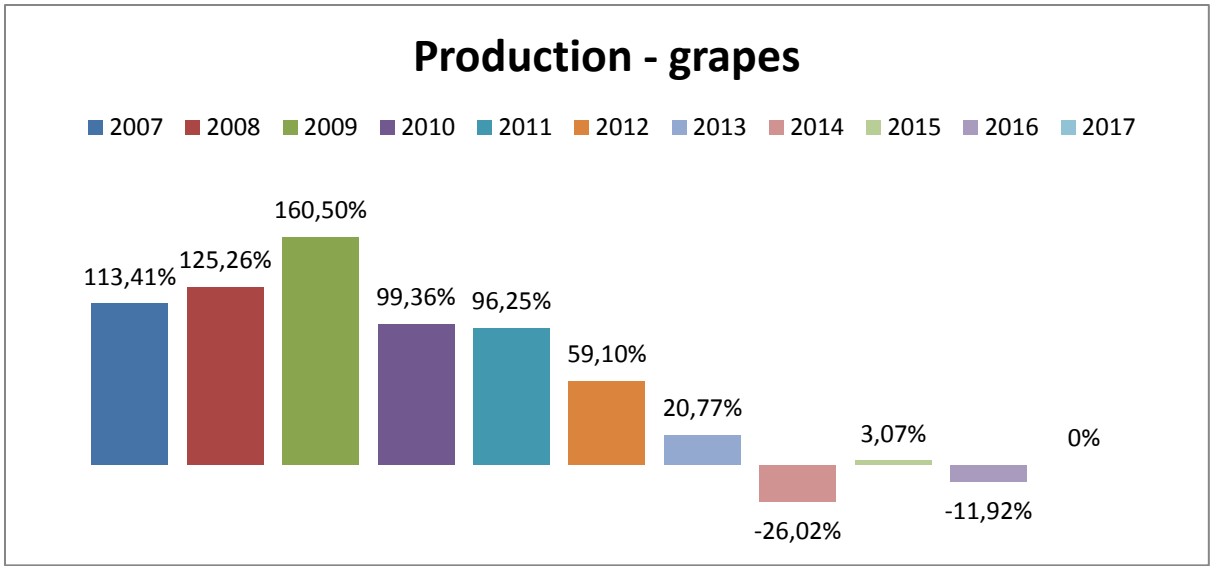

Figure 1. The index comparison of the vine production in the Republic of Serbia for the 10-year period (2007-2017)

The maximum total production was achieved in 2009, when it stood at 431,306 t and when it was higher by 160.5% compared to the total production of the year 2017, which amounted to 165,568 t. The lowest total vine production was achieved in 2014, when it amounted to 122,489 t, which was around 26% lower compared to the total production in 2017.
The total production gradually increased from 2007 to 2009, although there was a slight reduction in the harvested area under vines (Figure 2), and afterwards it was in decline until 2015. In 2015, the achieved production was 29.09% higher than the one in 2014 and 3.07 higher compared to 2017. Thereafter, in 2016, a decrease in the total production by 11.92% occurred in 2016, compared to the production in 2017.
Regarding the harvested areas under vines, based on the FAOSTAT data,Figure 2 was created,presenting an index comparison of the harvested areas in the observed time period.

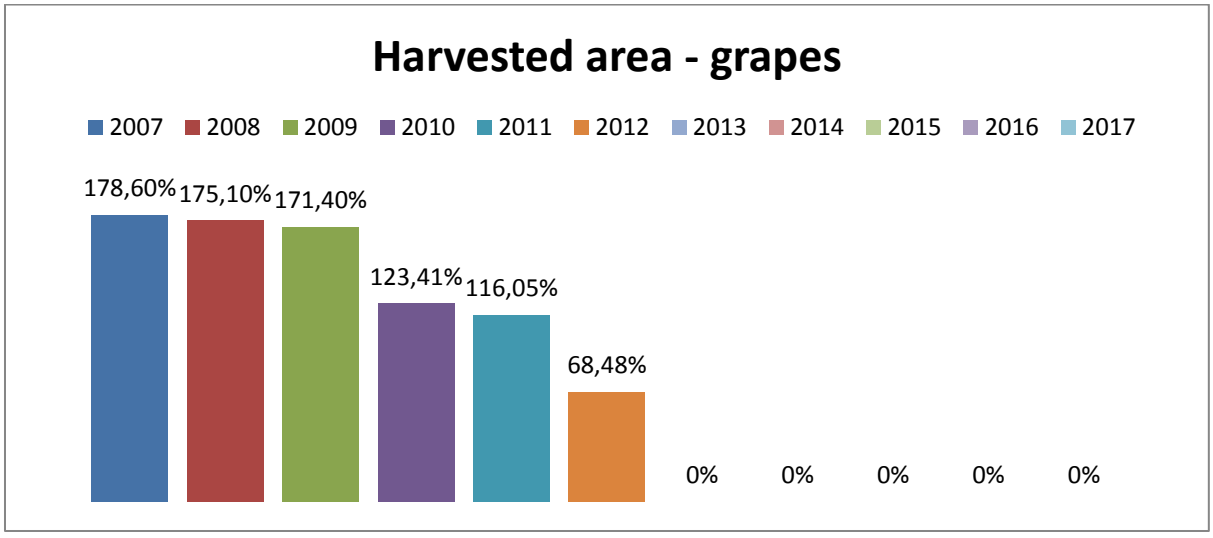

Figure 2. The index comparison of harvested areas under vines in the Republic of Serbia for the 10-year period (2007-2017)

Their clear decreasefrom 2007 to late 2013 can be noted. After this one, there was a period up to 2017, in which neither an increase nor a decrease in thearea under vines occurred. For the observed time period, the Republic of Serbia hadthelargest harvested area under vines in

2007, when it owned 59,068 hectares, which was a larger area by 178.6% compared to 2017. The most visible decrease was noted firstly in 2010 compared to 2009, when the area was reduced by 48%. In 2009, the Republic of Serbia had 57,540 ha under vines, and in 2010 it had 47,366 ha.Thereafter, a more visible decrease in the total harvested area occurred in 2012 compared to 2011, when it was 47.57%. In 2012, the total harvested area was 35,719 ha, while in 2011 it was 45,804 ha. In 2013 (21,201 ha) as compared to 2012 (35.719 ha), the vine areawas smaller by 68.48%, which was also the most visible decrease in the total harvested area under vines during the observed period.

Conclusions

The FAOSTAT results show that the production of vines in the Republic of Serbia from 2009 to 2017 gradually decreased. The maximum total production was achieved in 2009 and the lowest total production of vines was achieved in 2014.When it comes to the harvested area under vines, based on the FAOSTAT data, we can see a decrease from 2007 to 2013. After this, there was a period in 2017, in which there was a stagnation of the area under vines. The Republic of Serbia had the biggest harvested area under vines for the observed time period in 2007, when it owned 59,068 hectares, which was 178.6% a larger area compared to 2017. The most visible decrease was observed firstly in 2010, compared to 2009, when the area was reduced by 48%.

The risk of growing new varieties unadjusted to Serbian agroecological conditions is borne by manufacturers themselves. On Serbian areas favorable for growing vines, with recommended varieties, rootstocks, breeding forms and other information for each of these areas, we can achieve great results in the production of grapes and wine. Since there are exceptional pedological characteristics of the soil, a sufficient number of sunny days, the continental climate, sites of great exposition, there are ecologically clean and healthy areas ideal for vine growing. To intensify viticulture in Serbia, it is necessary to continue with favorable incentives for planting certified seedlings and technology modernization.

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THE INFLUENCE OF WEATHER CONDITIONS AND FERTILIZING METHOD ON PLANT HEIGHT AT DIFFERENT CULTIVARS OF WINTER WHEAT

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Abstract

Examinations were performed at stacionary field experiment with fertilization that had been applied for many years (over 30) at the property of Center for Small Grains in Kragujevac (Serbia). The experiments were performed in three years period. The experiment on which examinations have been performed includes also six variants of fertilization: 1) N_0 P_0 K_0; 2) $N_{80.\ 120}$ P_0 K_0; 3) $N_{80.\ 120}$ P_{60} K_{60}; 4) $N_{80.\ 120}$ P_{100} K_{60}; 5) $N_{80.\ 120}$ P_{60} K_0; 6) $N_{80.\ 120}$ P_{100} K_0; 7) $N_{80.\ 120}$ P_0 K_{60}. Individual fertilizers were used: KAN as the nitrogen fertilizer, superphosphate as the phosphate fertilizer, 60% potassium saltas the potassium fertilizer. The experiment included also seven different of winter wheat cultivars: Takovchanka, Ana Marija, KG 100, Lazarica, KG 56S, KG 4 and KG 5. The aim of this work was to examine the influence of various dosages and relation of mineral fertilizers on plant height of different cultivars of winter wheat. The height of plant was the varietal characteristics, but in a great extentlargely depends on the entered fertilizers and weather growing conditions. In the three year average, minimum height of plant for examined varieties of the winter wheat was on the control. The use of fertilizer had a highly significant increase in the height of winter wheat. The biggest height of plants of winter wheat in period of triennial examination, obtained in cultivar KG 4 (80 cm) at NP_1K variant of fertilization, at higher nitrogene dose.

Keywords: *Wheat, Plant Height, Fertilizing Variants, Cultivars*

Introduction

The height of the plant is a varietal feature, but it also depends largely on mineral fertilizers introduced and weather conditions during growing (Malešević, 1989), (Zečević *et al*., 2005). Of all the elements of mineral nutrition on plant height, nitrogen has the greatest influence (Lazović, 1997), as well as other elements of mineral nutrition, phosphorus and potassium (Jevtić and Malešević, 1988). The impact of precipitation and temperature on the plant height depends not only on their quantity, but also on the intensity, but especially on their distribution at certain stages of plant growth and development (Sarić i Jocić, 1993).

According to the research (Pavlović 1997), the share of variety and ecological factors affecting the height of the stem is 48:52% and points out that the factors of the external environment and the genotype/environment interaction significantly influence the height of the stem.

The aim of these tests was to determine the influence of different doses and the ratio of mineral fertilizers to the height of the plant of various varieties of winter wheat.

Material and Methods

The tests were carried out on a stationary field experiment with fertilization, which is performed for many years (over 30) on the property of the Center for Small Grains in Kragujevac (Serbia). The tests were performed in a three-year period as follows: 2005/2006, 2006/2007 and 2007/2008. The experiment in which the tests were performed besides the control includes six fertilization variants: 1) N_0 P_0 K_0; 2) $N_{80,\ 120}$ P_0 K_0; 3) $N_{80,\ 120}$ P_{60} K_{60}; 4) $N_{80,\ 120}$ P_{100} K_{60}; 5) $N_{80,\ 120}$ P_{60} K_0; 6) $N_{80,120}$ P_{100} K_0; 7) $N_{80,120}$ P_0 K_{60}. The individual

fertilizers were used as follows: KAN as nitric, superphosphate as phosphorus and 60% potassium salt as potassium fertilizer. In addition to the mentioned variants of mineral nutrition, seven different Kragujevac winter wheat varieties are included in the experiment: Takovchanka, Ana Morava, KG 100, Lazarica, KG 56 S, KG 4 and KG 5. Takovchanka is a medium early variety, highly resistant to low temperatures. The average height is about 90 cm. Ana Morava belongs to a group of medium early varieties. It has excellent resistance to low temperatures. It is characterized by the medium height. KG 100 is a medium early variety, resistant to low temperatures. Plant height is 70-73 cm. Lazarica is a medium early variety. The height of the stem is about 80 cm. KG 56 S is a medium late variety, extremely resistant to low temperatures. It has a stem of medium height of about 85 cm. KG 4 is a medium early variety of excellent resistance to low temperatures. KG 4 is of medium height, over 85 cm. KG 5 is a medium early variety, of the middle-height of the stem of 82.5 cm. All the investigated varieties in our research were created in the Center for Small Grains in Kragujevac, Serbia.

The surface of the basic experimental plots is 100 m^2 for fertilization with phosphorus and potassium and for fertilization with two nitrogen doses of 80 and 120 kg ha^{-1}, the basic plot was is divided into two sections of 50 m^2 each.

The soil on which the experiment is carried out belongs to the type of vertisol, in the process of degradation (Table 1).

Table 1. Facts of soil fertility at experimental field

fertilizing variats	profound (cm)	humus (%)	pH		N overall (%)	P_2O_5	K_2O
			H_2O	KCl		mg/100 g soil	
O	0 - 20	2,13	5,85	4,37	0,12	2,60	18,67
N		2,10	5,83	4,26	0,14	2,20	17,60
NP_1K		2,39	5,58	4,27	0,15	8,17	27,47
NP_2K		2,25	5,72	4,28	0,14	9,83	24,00
NP		2,34	5,63	4,15	0,15	9,00	17,40
NK		2,24	5,73	4,22	0,16	2,83	23,53

The height of the plant in our research is measured from the soil to the top of the spike.

The experiment was set in a randomized block design with 5 replications. Standard agro-technology was applied to the experiment. Statistical data processing of winter wheat was done using the variance analysis method.

Results and Discussion

The plant height depends on the genetic features of the wheat variety, agrotechnical and climatic conditions during the growing period (Božović, 1993). Weather conditions during the wheat vegetation significantly affect the height of the plants, as well as the overall dynamics of growth and development (Pešić *et al*., 2005), (Mićanović *et al*., 1994).

Based on the data in (Table 2) about mean monthly temperatures and precipitation amounts during our testing we can conclude that the weather conditions were different by the years of testing. The most favorable weather conditions were in the first vegetation period of 2005/06, where the largest total rainfall was recorded with the best distribution according to months.

The temperatures were also optimal for growing wheat, especially during the most important stages of its development. The vegetation period in 2007/2008 was favorable for winter wheat

cultivation, while the most unfavorable weather conditions for growing winter wheat were in the second year of testing.

Table 2. Temperature and water in the course of the vegetation in 2006-2008

Month	Temperature t ºC				Water lm^{-1}			
	Year			Average	Year			Average
	2005/ 2006	2006/ 2007	2007/ 2008	вишег. 1961/99	2005/ 2006	2006/ 2007	2007/ 2008	вишег. 1961/99
September	16,2	17,4	17,7	16,7	31,0	57,4	115,6	50,5
Oktober	14,7	11,5	13,3	11,3	50,1	16,7	49,0	42,8
November	6,8	5,6	7,6	6,5	90,7	13,7	54,8	46,4
December	3,0	3,3	3,5	1,1	170,0	51,9	47,1	46,8
January	1,5	- 1,7	6,1	- 1,8	36,6	45,3	27,9	38,3
February	- 1,5	1,5	6,3	3,0	66,9	32,1	38,1	35,7
Mart	4,5	5,6	9,1	6,5	178,7	62,9	116,1	40,4
April	11,6	12,7	12,1	11,3	72,3	3,6	29,6	53,1
May	16,4	16,4	18,2	16,3	70,2	118,4	86,3	66,7
Jun	19,2	19,7	22,8	19,0	39,1	25,3	84,8	80,3
July	21,6	23,0	24,8	21,1	86,2	10,1	22,4	70,6
(IX - VII)	10,6	10,6	12,9	10,4	891,8	437,4	671,7	571,6

In the three-year average, the lowest plant height of the varieties examined was in the control. The use of fertilizers resulted in a significant increase in the height of winter wheat (Savić, 2009) (Table 3).

Using only N the plant height increased by 19.72 cm, which was a significant increase in relation to control (Savić *et al*., 2007). The addition of P and K fertilizers (NPK variant) resulted in a significant increase of plants compared to the control and N fertilizer variant (Jaćimović *et al.*, 2012). The plant height on all fertilized variants, except for NP_2K, was significantly higher at higher than lower nitrogen doses.

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The varieties on a three-year average showed certain differences in this property, which were naturally caused by differences in their genetic constitution.

The varieties of winter wheat achieved a very different plant height with the same varieties of mineral nutrition. The highest height of the cultivars was Takovchanka (72,31 cm) and KG 4 (71,31 cm), and the difference in height between these two varieties was not significant.

The lowest variety was KG 100 (57.90 cm), which had a significant decrease in plant height compared to all other varieties examined. The highest plant height in the three-year experiment period was achieved by KG 4 (80 cm) on fertilizer variant NP_1K at a higher nitrogen dose.

Table 3. The plant height (triennial average 2006-2008)

Variant fertlizing	Dosage N	C u l t i v a r s								
		1.	2.	3.	4.	5.	6.	7.	Average	
O		54	47	41	42	47	47	47	46,76	46,76
N	N_1	72	69	55	59	64	71	65	65,19	66,48
	N_2	73	72	58	61	69	73	65	67,76	
NP_1K	N_1	74	68	65	65	74	74	71	70,54	72,02
	N_2	77	77	64	66	77	80	72	73,48	
NP_2K	N_1	74	75	62	67	77	77	68	71,76	71,69
	N_2	76	74	64	67	74	74	71	71,62	
NP_1	N_1	77	75	59	66	77	75	69	71,43	72,14
	N_2	76	75	63	68	78	78	70	72,86	
NP_2	N_1	73	71	56	65	73	74	69	69,10	70,64
	N_2	74	74	63	65	75	79	72	72,19	
NK	N_1	77	69	59	61	71	73	68	68,62	68,79
	N_2	77	72	58	62	71	74	67	68,95	
Average	N_1	71,95	68,05	56,95	61,10	69,33	70,29	65,76	66,20	66,93
	N_2	72,67	70,62	58,86	62,00	70,52	72,33	66,62	67,66	
Average	N_1/N_2	72,31	69,33	57,90	61,55	69,33	71,31	66,19	66,93	

Conclusions

The obtained results show that the use of mineral fertilizers had a positive effect on the height of plants in almost all varieties of fertilization and in all wheat varieties examined. The plant height on all fertilized variants, except for NP_2K, was significantly higher at higher levels than at lower nitrogen doses. The greatest height had the varieties Takovchanka (72.31 cm) and KG 4 (71.31 cm), and the difference in height between these two cultivars was not significant. The lowest variety was KG 100, which had a significant decrease in plant height compared to all other varieties examined. The greatest plant height in the three-year experiment period was achieved by KG 4 (80 cm) at fertilizer variant NP_1K at a higher nitrogen dose.

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ESTIMATION OF ABOVEGROUND BIOMASS AND GRAIN YIELD OF WINTER WHEAT USING NDVI MEASURMENTS

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Abstract

Aboveground biomass of wheat is considered as one of the most important crop parameters and correct estimation of aboveground biomass can help improve crop monitoring and grain yield prediction. Remotely sensed vegetation indices such as NDVI (Normalized Difference Vegetation Index) represent one of the most promising tools for application in field phenotyping with potential to provide complex information on different traits of wheat. The objective of this study was to evaluate the potential of different NDVIs derived from field reflectance measurements in identification of a specific growth stage in which proximally or remotely sensed data showed the highest correlation with aboveground biomass and grain yield of 24 winter wheat genotypes. The NDVI was determined using an integrated proximal sensor GreenSeeker (NTech Industries Inc., Ukiah, California, USA) and hyperspectral camera (Ximea Corp., Lakewood, CO USA) at four growth stages of wheat: full flowering, medium milk, early dough and fully ripe. The hyperspectral NDVI indices were calculated from two-band combinations between red (600-700 nm) or far-red (700-750 nm) and near-infrared (756-955 nm) regions. Highly significant correlations were found between different NDVIs and both examined traits at medium milk growth stage, with r values of up to 0.69. The strong positive relationship implies that medium milk stage is optimal for wheat traits assessment in semiarid or similar growing conditions. The overall results indicated that hyperspectral camera provided alternative spectral combinations for different NDVIs which could be successfully used in assessing aboveground biomass and grain yield of a large number of wheat genotypes.

Keywords: *GreenSeeker, hyperspectral, NDVI, wheat, yield*

Introduction

Being adapted to a broad range of latitudes, temperatures, water regimes and nutritional levels, wheat is one of the most widely grown crops and the future productivity of wheat will arguably have more influence on global food security than any other crop (Reynolds et al., 2012a). Beside of high potential for yield of current wheat cultivars, weather conditions are becoming increasingly unstable due to climate change and grain yields of wheat fluctuate more widely from year to year (Hristov et al., 2012). Since that the pressure put on modern crop production to maximize the yield and minimize the inputs, timely monitoring of crop growth status early in the growing season could be of vital importance for in-season site-specific crop management, especially in regions characterized by climatic uncertainties (Henik, 2012). Large agricultural lands are usually monitored using proximal and remote sensing technology, which allows for instantaneous data acquisition over vast areas (Kostić et al., 2016). Recent studies suggest that remote sensing technology possesses a great potential for estimation of yield and yield related traits, both in precision farming, as well as in large breeding programs (Duan et al., 2017). Agricultural remote sensing is based on canopy light

reflectance indices, which rely on the fact that plants absorb the light at specific wavelengths associated with specific plant traits. These indices could provide large-scale evaluation of germplasm in a rapid and nondestructive manner (Reynolds et al., 2012b; Araus and Cairns, 2014; Morgunov, 2014). Among various vegetative indices, Normalized Difference Vegetation Index (NDVI) is one of the most widely used for crop monitoring, since it is related to leaf development, amount of chlorophyll, amount of photosynthesis, stay-green traits, grain yield and aboveground biomass of wheat (Lopes and Reynolds, 2012). Based on the principle that green vegetation strongly absorbs solar radiation in the red (R) part of the visible electromagnetic spectrum, while strongly reflecting radiation in the near-infrared (NIR) region, NDVI is defined as the difference between the red and near-infrared reflectance divided by their sum (Tucker, 1980). GreenSeeker is the one of the most widely used active hand-held sensors for measuring NDVI due to its invariance to light conditions and the time of day, but limited by its use of only two central wavelengths (Yao et al., 2013). Reflectance in visible and near-infrared regions is strongly dependent on both structural and biochemical properties of the canopy, varies with the growth stage and environmental conditions (Kumar et al., 2001). Hence, it is a challenge to develop a unique and optimal two-waveband combination for NDVI exclusively sensitive to targeted grain yield traits. Hyperspectral data could provide extensive information about plants and can serve as a basis for finding the most indicative wavebands for assessment of targeted crop parameters (Kaur et al., 2015). Therefore, the present study was carried out with two main objectives. The first objective was to find the optimal combination of hyperspectral wavebands in R and NIR regions, for aboveground biomass and early grain yield assessment of 24 winter wheat genotypes. The second objective was to determine the specific growth stage where the correlation between the yield traits and NDVI acquired by GreenSeeker sensor and hyperspectral camera is highest and therefore the most suitable for use in both proximal and remote sensing.

Material and Methods

The present study was carried out at the experimental field of the Institute of Field and Vegetable Crops (45°19′51′ N, 19°50′59′ E) in Novi Sad, Serbia, in typical semiarid conditions, during the 2015–2016 growing season. The experimental material was comprised of 24 winter wheat (*Triticum aestivum* L.) genotypes, namely, Ubavka (G1), Matuška (G2), Javorka (G3), Brazda (G4), Efrosinija (G5), Doroteja (G6), Obala (G7), Vlajna (G8), Kala (G9), Mila (G10), Azra (G11), Nafora (G12), Pudarka (G13), Ljubica (G14), Petrija (G15), Futura (G16), Ilina (G17), NS 40S (G18), Zvezdana (G19), Simonida (G20), Rapsodija (G21), Renesansa (G22), Evropa (G23) and Pobeda (G24). The cultivars were sown in 2 m long rows with 20 cm of inter-row spacing and 10 cm spacing between plants in the row. The trial was sown in a randomized block design, with three replications. Examined cultivars were grown using common agronomic practice. Meteorological data, values of temperature and precipitation throughout the season were obtained from Rimski Šančevi Meteorological Station, located near the experimental field (Table 1). At the stage of full maturity, grain yield (t ha^{-1}) of 24 winter wheat genotypes was estimated, while plants in one square meter of each genotype were harvested from the experimental plots individually to record aboveground biomass (g m^{-2}). For each of the 24 winter wheat genotypes NDVI was measured at four different growth stages: full flowering (BBCH 65), medium milk (BBCH 75), early dough (BBCH 83) and fully ripe (BBCH 89). For NDVI measurements, the GreenSeeker handheld (NTech Industries Inc., USA) proximal sensor and Ximea hyperspectral camera xiSpec MQ022HG-IM-SM5X5-NIR (Ximea Corp., USA) were used.

Table 1. Temperature and precipitation values for the growing seasons 2015–2016 and multi-year averages (1981-2010) for field trial region

	2015–2016		Multi-year average	
Month	Temperature °C	Precipitation mm	Temperature °C	Precipitation mm
October	11.3	74.6	11.7	47.6
November	7.8	56.1	5.9	51.2
December	3.2	3.6	1.5	46.2
January	1.2	51.6	-0.5	37.3
February	7.3	49.0	1.8	31.8
March	7.9	65.3	6.4	37.1
April	14.3	74.2	11.4	48.8
May	16.7	84.6	16.8	59.6
June	22.6	143	19.9	85.7
Average	10.14	67.32	8.3	49.5
Total	91.3	669.32	74.9	615.5

GreenSeeker, as an active hand-held sensor, emits light and measures the reflectance at 660 nm (R) and 770 nm (NIR) (Tremblay et al., 2009). In-field reflectance measurements were taken by holding GreenSeeker sensor about 60 cm horizontally above the crop canopy and scanning the central part of each wheat plot. For each plot, 10 NDVI readings were collected and averaged to obtain a single value per plot. Hyperspectral camera was mounted at a height of 1 m above canopy on a mobile tripod, which corresponded to an image area of 2 m^2 while the spatial resolution was 0.5 × 0.5 mm per image pixel. Its output was consisted of 66 images, each corresponded to one channel between 600 and 955 nm. As the pixel values are proportional to the existing amount of sunlight, each image was calibrated using Sphere Optics Zenith reflectance sheet. For calibration and calculation of NDVI was used MATLAB (MathWorks Inc., US). As NDVI requires measurements in RED and NIR domains, candidates for the red domain were hyperspectral channels in visible red (600–700 nm) and far-red (700–750 nm) part of the spectrum, while candidates for NIR channel were hyperspectral channels between 756 nm and 955 nm. This gave the aggregate of 3 (red), 22 (far-red and near-infra red) and produced 66 different NDVI indices. The NDVI measurements from both instruments were made close to noon, between 10:00 am and 2:00 pm on sunny, cloud-free days when the plant canopy and soil surface were dry. Pearson correlation coefficient (*r*) was used as a measure of correlation of NDVI with aboveground biomass and grain yield. Besides observing the correlation based on absolute NDVI values, the relationship between the yield traits and the decline in NDVI between BBCH 65 and BBCH 75, BBCH 83 and BBCH 89 was analysed. The relative decline (Dj) in NDVI served as a measure of magnitude of reduction in NDVI from BBCH 65 to BBCH 75, BBCH 83 and BBCH 89, calculated as follows:

$$\mathrm{Dj} = \frac{\mathrm{NDVI_i} - \mathrm{NDVI_j}}{\mathrm{NDVI_i}} \times 100\%$$

where *i* present NDVI measured at BBCH 65 growth stage of wheat, while *j* presents NDVI measured at BBCH 75, BBCH 83 and BBCH 89 growth stage of wheat. In this way we introduced three relative decline measures: D1, D2 and D3, respectively. All statistical analyses were carried out using software STATISTICA, version 13 (StatSoft Inc., USA).

Results and Discussion

The presented results revealed wide range between the minimum and maximum values for observed traits of wheat and the greatest values varied on overall basis. The overall mean values of aboveground biomass of the 24 winter wheat genotypes varied from 300 g m^{-2} for genotype G8 to 700 g m^{-2} for genotype G10. The mean grain yield values varied from 9.2 t ha^{-}

[1] for genotypes G8, G15 and G21 to 11.2 t ha^{-1} for genotype G9. In respect of NDVI values, the results showed that maximum values of NDVI were observed during BBCH 65, when photosynthesis was at the highest point and the leaf area was largest. Through the later growth stages, NDVI values gradually declined reaching the minimum values at BBCH 89. In chronological order, the sensor GreenSeeker based NDVI score ranged from 0.61 to 0.72, 0.40 to 0.61, 0.24 to 0.36 and 0.15 to 0.20 at BBCH 65, BBCH 75, BBCH 83 and BBCH 89 stage of wheat, respectively (Table 2). These results indicated that through the process of senescence toward the end of the season, reflectance of visible wavelengths increases and reflectance of NIR decreases as a consequence of less absorption of visible light in the leaves (Reynolds et al., 2012b; Sultana et al., 2014). The variability of NDVI throughout the genotypes was lowest at the BBCH 65, because the NDVI was mainly affected by saturation effects, which is well known at high crop densities (Erdle and Schmidhalter, 2013). Since that all observed wheat genotypes showed similar and the largest NDVI values at BBCH 65, it becomes clear that this growth stage could not prove to be indicative for aboveground biomass and grain yield assessment. With respect to NDVI values derived from hyperspectral imagery, the results show that two-band combinations respond in different manner to variations in yield traits, but the mean values displayed the same tendency as the GreenSeeker based NDVI values (Table 2). With respect to the association between grain yield and aboveground biomass of wheat it was observed the strong positive relationship (r = 0.88**). Positive relationship between grain yield and aboveground biomass of wheat is mandatory for a system intended to predict yield traits early in the season by assessing the canopy growth (Marti et al., 2007). The strong positive relationship between the grain yield and aboveground biomass of wheat (r = 0.88**) suggested that higher biomass production during the green up stage, particularly during the grain filling period have an advantage for the yield increase in wheat because translocation of assimilates from the vegetative to generative parts of a plant contribute significantly to yield. Significant positive association between aboveground biomass and grain yield of wheat has been confirmed by several researchers (Bogale and Tesfaye, 2016). In present study a high and significant positive correlation between grain yield and aboveground biomass of wheat was observed, which justified approach of predicting the yield by measuring NDVI of the canopy. With respect to the association between measured traits and NDVI from both devices and all hyperspectral two-waveband combinations, the result revealed that the significant and positive association was observed at the medium milk stage of wheat. The GreenSeeker based NDVI showed the correlation of 63%** with aboveground biomass and 58 %** with grain yield. Positive, but not significant correlations were observed at BBCH 83, while the correlations in the stages BBCH 65 and BBCH 89 were lower or even negative with none of them being significant. It can be noted that in aboveground biomass assessment, the NIR wavelength used by GreenSeeker (770 nm) was the optimal one for hyperspectral NDVI, as well, while the R wavelength was slightly longer (674 nm instead of 660 nm). The optimal R wavelength for grain yield prediction remained the same, while the optimal NIR wavelength changed to 909 nm (Fig. 1). Beside of significant positive correlation between NDVI and both traits found at BBCH 75, it was also observed that wheat genotypes which displayed high NDVI values at BBCH 75 tended to have high yield, as well as aboveground biomass. This indicates that the delay in senescence extends the grain-filling period and increase grain yield (Hawkesford et al., 2015). This result agrees with the previous findings of several researchers (Morgounov et al., 2014; Gonzalez-Dugo et al., 2015). Since that optimal stage for measuring NDVI depending on the germplasm and environmental conditions, contradictory findings have been reported.

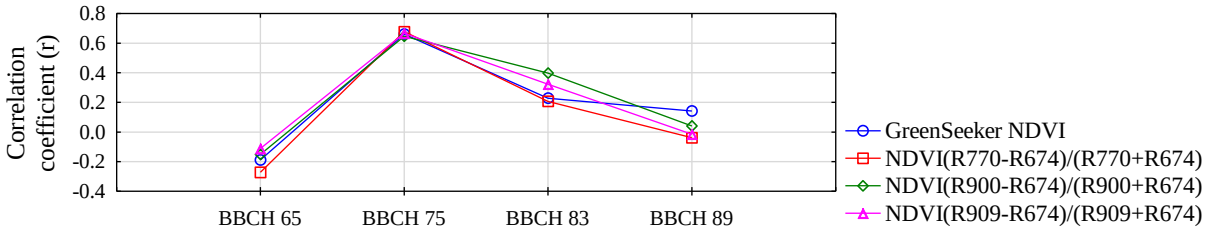
Table 2. The mean values of aboveground biomass, grain yield, best selected NDVI and magnitude of reduction in NDVI from BBCH 65 to BBCH 75 of 24 winter wheat genotypes

			GreenSeeker NDVI					NDVI (R770-R674)/(R770+R674)				
G[a]	GY[b]	AB[c]	BBCH 65	BBCH 75	BBCH 83	BBCH 89	D_1	BBCH 65	BBCH 75	BBCH 83	BBCH 89	D_1
G1	10.8	660	0.71	0.54	0.26	0.18	23.9	0.68	0.50	0.22	0.16	27.0
G2	10.6	580	0.69	0.57	0.25	0.15	17.4	0.67	0.53	0.21	0.13	21.3
G3	9.8	560	0.68	0.58	0.25	0.16	14.7	0.66	0.54	0.21	0.14	19.0
G4	11.1	680	0.67	0.58	0.28	0.21	13.4	0.64	0.54	0.23	0.22	15.7
G5	10.7	570	0.71	0.54	0.36	0.20	23.9	0.69	0.50	0.32	0.20	28.5
G6	11.1	670	0.62	0.55	0.34	0.16	11.3	0.58	0.53	0.30	0.14	8.8
G7	11.0	650	0.67	0.57	0.31	0.20	14.9	0.68	0.50	0.28	0.27	26.3
G8	9.2	300	0.72	0.40	0.28	0.16	44.4	0.68	0.34	0.24	0.14	50.3
G9	11.2	620	0.65	0.58	0.28	0.18	10.8	0.62	0.54	0.24	0.18	11.8
G10	11.0	700	0.71	0.61	0.29	0.15	14.1	0.67	0.57	0.25	0.01	15.3
G11	9.9	460	0.72	0.58	0.24	0.18	19.4	0.67	0.54	0.20	0.20	19.7
G12	10.2	500	0.70	0.61	0.25	0.17	12.9	0.68	0.55	0.21	0.15	18.2
G13	10.9	480	0.71	0.52	0.31	0.18	26.8	0.69	0.49	0.27	0.20	30.0
G14	9.8	370	0.61	0.50	0.26	0.19	18.0	0.64	0.46	0.20	0.18	28.8
G15	9.2	310	0.71	0.48	0.27	0.16	32.4	0.67	0.40	0.23	0.09	40.7
G16	9.3	340	0.72	0.51	0.29	0.19	29.2	0.69	0.47	0.25	0.19	31.8
G17	10.9	550	0.69	0.51	0.30	0.19	26.1	0.67	0.48	0.26	0.18	27.9
G18	10.5	660	0.72	0.55	0.35	0.17	23.6	0.70	0.53	0.31	0.14	24.6
G19	10.5	580	0.69	0.54	0.31	0.18	21.7	0.67	0.51	0.29	0.17	23.8
G20	9.4	450	0.66	0.53	0.26	0.20	19.7	0.65	0.52	0.22	0.20	20.4
G21	9.2	310	0.71	0.49	0.32	0.19	31.0	0.69	0.44	0.28	0.17	36.4
G22	10.0	360	0.70	0.56	0.23	0.16	20.0	0.68	0.52	0.21	0.14	23.7
G23	9.3	320	0.68	0.51	0.30	0.16	25.0	0.65	0.48	0.26	0.18	26.9
G24	9.7	350	0.69	0.54	0.31	0.16	21.7	0.67	0.50	0.27	0.16	25.9
G	GY	AB	NDVI(R900-R674)/(R900+R674)				D1	NDVI(R909-R674)/(R909+R674)				D1
G1	10.8	660	0.78	0.65	0.43	0.36	16.2	0.80	0.63	0.46	0.38	21.2
G2	10.6	580	0.77	0.64	0.31	0.34	16.7	0.77	0.56	0.39	0.35	27.5
G3	9.8	560	0.76	0.63	0.42	0.34	17.3	0.77	0.70	0.43	0.40	9.5
G4	11.1	680	0.77	0.67	0.40	0.41	13.4	0.78	0.71	0.44	0.42	8.6
G5	10.7	570	0.79	0.65	0.45	0.40	18.1	0.81	0.62	0.48	0.43	23.1
G6	11.1	670	0.72	0.64	0.44	0.29	11.9	0.72	0.65	0.45	0.36	9.5
G7	11.0	650	0.77	0.66	0.43	0.43	14.4	0.77	0.68	0.47	0.44	11.1
G8	9.2	300	0.79	0.46	0.36	0.35	41.6	0.78	0.47	0.40	0.39	40.4
G9	11.2	620	0.75	0.67	0.42	0.39	10.5	0.77	0.68	0.46	0.41	11.2
G10	11.0	700	0.77	0.65	0.43	0.28	16.2	0.79	0.69	0.44	0.33	12.1
G11	9.9	460	0.76	0.64	0.38	0.37	15.1	0.77	0.66	0.44	0.41	15.0
G12	10.2	500	0.78	0.62	0.32	0.35	20.5	0.79	0.68	0.38	0.34	14.4
G13	10.9	480	0.79	0.63	0.41	0.35	19.8	0.81	0.70	0.41	0.31	13.5
G14	9.8	370	0.74	0.58	0.38	0.40	22.6	0.79	0.59	0.43	0.39	25.1
G15	9.2	310	0.77	0.50	0.36	0.27	35.6	0.78	0.51	0.40	0.29	34.5
G16	9.3	340	0.77	0.61	0.43	0.39	21.4	0.79	0.64	0.45	0.42	19.2
G17	10.9	550	0.77	0.55	0.44	0.36	28.4	0.79	0.65	0.47	0.40	17.6
G18	10.5	660	0.79	0.64	0.49	0.37	18.3	0.81	0.68	0.48	0.38	15.6
G19	10.5	580	0.76	0.55	0.46	0.35	26.6	0.78	0.66	0.49	0.39	15.4
G20	9.4	450	0.76	0.58	0.37	0.41	23.9	0.77	0.62	0.43	0.36	19.4
G21	9.2	310	0.79	0.61	0.40	0.35	23.3	0.79	0.57	0.44	0.40	27.6
G22	10.0	360	0.77	0.59	0.39	0.30	23.7	0.80	0.66	0.39	0.37	17.2
G23	9.3	320	0.75	0.55	0.43	0.41	27.0	0.77	0.58	0.46	0.45	24.6
G24	9.7	350	0.78	0.65	0.40	0.34	17.2	0.79	0.60	0.46	0.41	24.1

[a]G: Wheat genotypes, [b]GY: Grain yield t ha^{-1}, [c]AB: Aboveground biomass g m^{-2}, [c]D1: Relative decline, reduction in NDVI from BBCH 65 to BBCH 75 for GreenSeeker based and hyperspectral NDVI measured at (R770-R674)/(R770+R674), (R900-R674)/(R900+R674) and (R909-R674)/(R909+R674), respectively (%).

Contrary to these results, previous studies demonstrated that grain yield can be estimated at earlier stages, such as stem elongation and booting stage (Marti et al., 2007; Kaur et al., 2015). The contrasted and variable climate could explain the inability to estimate yield before grain filling, as this period is critical for yield formation, as well as under most conditions 90–95% of the carbohydrate in grain is derived from carbon dioxide fixation after flowering stage (Gonzales-Dugo, 2015). With better growing conditions the grain-filling period extended and the amount of available nutrients increased, the grain achieves a higher yield. Although, the average temperatures during grain filling in early June was 22.6°C, frequently short periods (3–5 days) of hot and dry weather were reflected in NDVI, as well as in grain yield losses. In the stage of full maturity, significant correlation between both traits and NDVI was not observed since in this stage canopy dries out and NDVI drops to minimum.

a)



b)

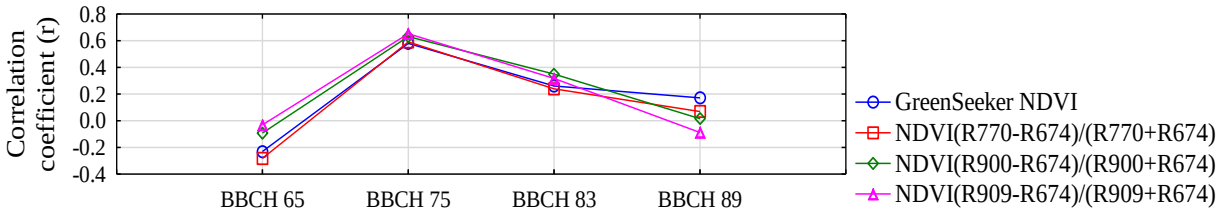
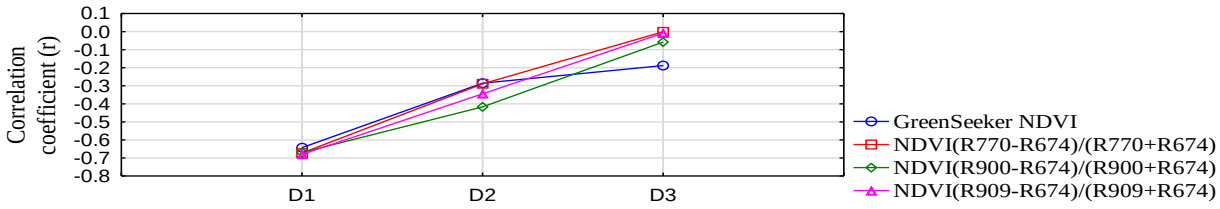


Figure 1. Pearson's correlations coefficients (r) obtained between the best selected NDVI and aboveground biomass (a) and grain yield (b) of wheat at 4 growth stages: BBCH 65, BBCH 75, BBCH 83 and BBCH 89

Besides the positive and significant correlation between the absolute NDVI and examined traits at BBCH 75, the significant negative correlation between reduction in NDVI from BBCH 65 to BBCH 75 and both traits was observed (Fig. 2). NDVI values acquired by both devices displayed similar tendency as they both showed the sharpest decline between BBCH 65 and BBCH 75 and expressed the greatest negative correlation with the examined traits. The relative decline displayed the largest, significant negative correlation with the trait aboveground biomass (r = -0.66**) for GreenSeeker based and hyperspectral NDVI (r = -0.71**). The largest significant negative correlation for GreenSeeker based (r = -0.60**) as for hyperspectral NDVI (r = -0.66**) with grain yield was also observed (Fig. 2). Considering this correlation, hyperspectral camera generally outperformed GreenSeeker sensor, by 5% in aboveground biomass prediction for wavelengths (674 nm – 770 nm) and by 7% in grain yield prediction, for wavelengths (674 nm – 909 nm). The magnitude of reduction in NDVI from BBCH 65 to BBCH 75 proved to be different for genotypes and showed a strong negative correlation with measured traits. Based on the magnitude of reduction in NDVI from BBCH 65 to BBCH 75, wheat genotypes G9, G6 and G10, which displayed the lowest reduction (10.8, 11.3 and 14.1 %), could be considered as genotypes with the highest potential for aboveground biomass and grain yield. Corresponding reduction from 44.4, 32.4 and 31.0 % indicated that wheat genotypes G8, G15 and G21 could be considered as genotypes with lower aboveground biomass and grain yield, as seen in Table 2. These results revealed that with progressing senescence, when the temperatures were higher, the quality of differentiating cultivars increased and with certain exceptions, high grain-yielding wheat cultivars maintained higher NDVI during the BBCH 75 than in other stages. A slow decline in NDVI

during the BBCH 75 of certain genotypes as a consequence of delayed senescence increased the wheat grain yield.

a)



b)

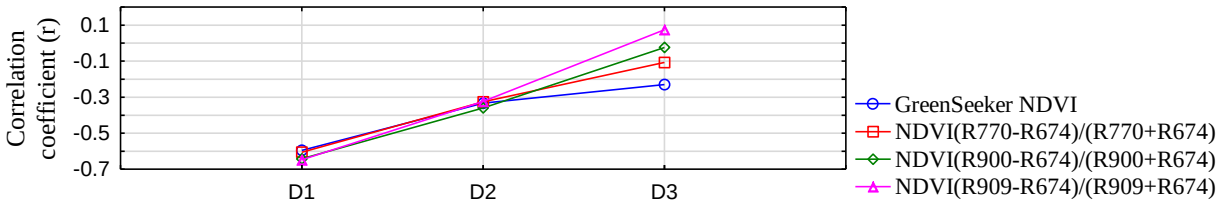


Figure 2. Pearson's correlations coefficients between the values of relative decline of best selected NDVI and aboveground biomass (a) and grain yield (b), where D1 present relative decline in NDVI from BBCH 65 to BBCH 75, D2 relative decline in NDVI from BBCH 65 to BBCH 83, D3 relative decline in NDVI from BBCH 65 to BBCH 89

Conclusions

Reliable estimation of aboveground biomass and grain yield of winter wheat based on NDVI is attained measuring during the medium milk stage. Besides observing absolute values of NDVI in different growth stages, observing the change in NDVI throughout the stages may be very indicative. With certain exceptions, high yielding wheat cultivars maintained higher NDVI during the BBCH 75 than in other growth stages. Furthermore, NDVI acquired with hyperspectral camera was found to be more indicative than NDVI acquired with GreenSeeker sensor since it provides additional waveband combinations for NDVI more sensitive to examined traits. The findings of this study give promising results which can be used as a basis for development and improvement sensing devices with alternative spectral combinations which could be successfully used in assessing important traits of winter wheat.

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TESTING AND SELECTING NEW, PROMISING MAIZE HYBRIDS

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Abstract

Every year, the Maize Research Institute, Zemun Polje, Belgrade, Serbia organizes the strip trials in a great number of locations throughout Serbia, in order to establish a proper regional distribution of hybrids and to select commercial maize hybrids best adapted to particular production regions. These trials are usually organized in approximately 50 locations per year and they cover all major regions of maize growing in our country. In addition, in 10-12 locations every year, the trials are set up not only with commercial hybrids, but with newly developed hybrids with good traits that have shown promising results in the process of their releasing. The trials were set up to check and compare values of newly developed hybrids with already existing commercial hybrids and to select new hybrids for future production and commercialization. The production value of new hybrids has to be higher than the value of current commercial hybrids within the same maturity group. The results, obtained in 37 locations in the 2014-2017 period, are presented in the present paper. Based on gained four-year results, the medium early maturity hybrids ZP 366 and ZP 388 were selected for commercialization, as they had results better than the commercial hybrid ZP 341.

Key words: *commercial maize hybrids, new hybrids, strip trials*

Introduction

Maize is the most important field crop in Serbia. It has various purposes in nutrition of humans and animals and is a very important factor in the export of agricultural products (Pavlov *et al*., 2011). There are several different regions of maize production in Serbia and each of them is characterized by different agro-ecological conditions. Northern part of the country is more suitable for intensive maize production, while production in central and southern part is more extensive, due to drier and harsher climate and harder soil types (Camdzija *et al*., 2013). Maize breeding program in Maize Research Institute Zemun Polje was established more than 70 years agoand more than ZP 700 hybrids were developed and registered in Serbia so far. Each season 10-15 new ZP hybrids have been registered in Serbia. Every year, the Maize Research Institute, Zemun Polje organizes the strip trials in a great number of locations throughout Serbia, in order to establish a proper regional distribution of hybrids and to select commercial maize hybrids best adapted to particular production regions. These trials are usually organized in approximately 50 locations per year and they cover all major regions of maize growing in our country. In addition, in 10-12 locations every year, the trials are set up not only with commercial hybrids, but with newly developed hybrids with good traits that have shown promising results in the process of their releasing. The trials were set up to check and compare values of newly developed hybrids with already existing commercial hybrids and to select new hybrids for future production and commercialization. The production value of new hybrids has to be higher than the value of current commercial hybrids within the same maturity group.In addition to high productivity, it is important that new hybrids have good stability parameters, which means that hybrids have good performances in both, optimal and poor agro ecological conditions (Babic *et al*., 2006, Stevanovic *et al*., 2011). The aim of this work was to evaluate performances and select new maize hybrids for further commercialization, based on the results obtained in strip trials.

Material and methods

Twelve maize hybrids from FAO groups 300-600 were evaluated at 37 locations in strip trials from 2014-2017. Testing locations represent the most important growing regions in Serbia. Plot size per each hybrid was 0.1 ha. Sowing and harvesting were performed mechanically. Grain yield (GY) is calculated to the moisture content of 14%. Planting densities applied in this study were 69500 plants/ha^{-1} for hybrids FAO 300-400, 65000 plants/ha^{-1} for FAO 500 and 59500 plants/ha^{-1} for FAO 600. Majority of the hybrids used in this study are widely grown, commercial maize hybrids, but also new, promising maize hybrids were included in trials. Hybrids belonging to FAO 500-600 used in this work are already commercial, but in FAO 300-400 only ZP 341, ZP 427 and ZP 434 are commercial, while other hybrids are selected according to the promising results during registration period.

Stability parameters were estimated by model proposed by Eberhart and Russel (1966). As described by this model, the behavior of the cultivars was assessed by the model $Yij=m+biIj+dij+eij$, where Yij = observation of the i-th (i =1,2,..., g) cultivar in the j-th (j=1,2,...n) environment, m=general mean, bi=regression coefficient, Ij=environmental index obtained by the difference among the mean of each environment and the general mean dij the regression deviation of the i-th cultivar in the j-th environment and eij=effect of the mean experimental error (Scapim *et al*., 2000).

Regression coefficient (bi) measures the response of genotypes to environments. When bi=1 there is average stability and adaptability to both poor and good environments, when bi>1 genotypes give above average stability only in good environment. Whereas, when bi<1, it indicates genotypes adaptation to poor environment (Aremu *et al*, 2009).

Results and discussion

Average grain yields and moisture content of the examined hybrids in each year of testing are presented in Table 1.

Table 1: Average grain yields and moisture content of the examined hybrids during 2014-2017. period

	2014		2015		2016		2017	
Hybrid	12 locations		10 locations		10 locations		5 locations	
	GY (kg/ha)	Grain Moisture (%)	GY (kg/ha)	Grain Moisture (%)	GY (kg/ha)	Grain Moisture(%)	GY (kg/ha)	Grain Moisture(%)
ZP 333	10007	17	7928	16.1	9366	16.8	6888	14.0
ZP 341	10409	17.2	8027	16.5	9847	17.2	7422	14.0
ZP 366	10470	17.6	8128	16.3	10211	17.6	7594	14.5
ZP 388	10916	17.8	7860	16.9	10217	17.7	6966	14.4
ZP 4007	10781	17.4	7938	16.5	9985	17.3	6895	14.4
ZP 427	10878	17.3	8225	16.2	10215	16.8	7789	14.2
ZP 434	10415	17.4	7949	16.0	10435	17.4	7242	14.3
ZP 555	10955	19.3	7560	17.5	10720	18.6	6779	16.4
ZP 560	11862	18.5	7702	17.3	11131	18.0	7429	15.9
ZP 600	11724	19.2	7973	18.0	10900	18.8	7677	15.9
ZP 606	12910	20.3	8380	18.0	10961	19.3	7496	16.6
ZP 666	11784	19.5	7814	18.2	10782	19.3	7198	16.5
Average	11093	18.2	7957	17.0	10398	17.9	7281	15.1

In 2014 and 2016. average grain yields were higher compared to other two years of testing, due to enough quantity and good distribution of precipitations. On the other side, 2015. and especially 2017. were characterized by lack of precipitation and very high temperatures during pollination and grain filling period and obtained grain yields were lower. For this study we used results only from five testing locations in 2017, since results from some locations were not useful due to extreme weatherconditions. In order to evaluate yield stability of maize hybrids it is desirable to test hybrids in optimal, but also in poor agro ecological conditions.
In FAO 300, hybrid ZP 366 overyielded ZP 341 in all four seasons of testing. In 2014., 2015. and 2017. grain yield of this hybrid were slightly higher than ZP 341, while the best results it obtained in 2016. Hybrid ZP 388 had the highest grain yield among all hybrids from FAO 300 in 2014. and 2016., which were very suitable for maize production. On the other side, in 2015. and 2017, which were more dry, it had lower yield compared to ZP 341 and ZP 366.
When it comes to the hybrids FAO 500-600, hybrid ZP 606 had the highest yield in 2014., 2015. and 2016., while ZP 600 has the highest grain yield in 2017. In these maturity groups also some new hybrids were examined, but none of them were tested during the 2014-2017 period, therefore results are presented only for the hybrids which were tested in all four years of testing.
In Table 2 average grain yields based on the results from 37 locations during the 2014-2017 as well as stability parameters are presented.

Table 2: Average grain yields, moisture content and stability parameters for the four year period of testing (Source: Author's elaboration based on obtained results from strip trials)

Hybrid	GY kg/ha	Moisture %	Rank GY	bi	Rank bi
ZP 333	8850	16.3	12	0.9533	8
ZP 341	9210	16.6	11	0.9920	3
ZP 366	9378	16.8	7	1.0101	5
ZP 388	9367	17.1	8	1.0089	4
ZP 4007	9272	16.7	10	0.9987	1
ZP 427	9564	16.5	5	1.0302	7
ZP 434	9325	16.6	9	1.0044	2
ZP 555	9410	18.2	6	1.0135	6
ZP 560	9941	17.7	2-3	1.0707	10
ZP 600	9941	18.3	2-3	1.0707	10
ZP 606	10427	18.9	1	1.1231	11
ZP 666	9821	18.7	4	1.0578	9
Average	9542	17.4			

Medium-early matured hybrids had *bi* value lower than 1, which means that they have better adaptability to poor environments, while late maturity were better adapted to good environments and had *bi* value higher than 1. Similar results were reported by Camdzija *et al.*, 2012, who also found that late maturity hybrids have better adaptability to good environments.
When it comes to the grain yields of medium-early maturity hybrids, in FAO 300 hybrid ZP 366 had the highest grain yield (9378 kg/ha), followed by ZP 388 (9367 kg/ha). Both hybrids had higher yield than standard commercial hybrid ZP 341, so they can be recommended for further commercialization. The most stable hybrid was ZP 4007, but on the other side this

hybrid had yield at the same level as check hybrid ZP 434. Hybrids ZP 434, ZP 341, ZP 388 and ZP 366 also showed above average stability. The most unstable hybrids were ZP 606, ZP 600 and ZP 560.On the other side, three unstable hybrids showed the highest yields over four year period. Other authors also noticed that the highest yielding hybrids have the lowest stabilityand prefer good environments (Delic *et al*., 2009, Stevanovic *et al*., 2011).

Conclusion

Based on four year results it can be concluded that in FAO 300 two hybrids (ZP 366 and ZP 388) outperformed standard commercial hybrid ZP 341, so those can be proposed for further commercialization. Hybrid ZP 366 had higher average yield compared to ZP 341 in all four years of testing, in optimal and also in poor agro ecological conditions. According to these results, ZP 366 can be recommended for growing in both, intensive and extensive conditions. On the other side, hybrid ZP 388 had very high yields in optimal conditions in 2014. and 2016., while in dry seasons 2015 and 2017. it had lower yield than ZP 341 and ZP 366. Thus, this hybrid can be recommended for intensive maize production.In FAO 400 commercial hybrids ZP 427 and ZP 434 were highest yielding and there is a need to include new, promising hybrids in these trials. In FAO 500-600, during 2014-2017some new hybrids were tested, but none of them in all four years of testing. There are several new, recently registered ZP hybrids which will be included in strip trials and compared with commercial ones.

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THE EFFECT OF MOVEMENT OF TRACTORS AND MOBILE SYSTEMS ON SOIL COMPACTION AND THE YIELD OF VARIOUS MAIZE HYBRIDS IN THE CONDITIONS OF SOUTHERN SERBIA

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Abstract

Movement of tractors and mobile systems during the plant production can be divided into the movement of the interior of the plot and the movement on the headlands. Both movements result in soil compaction of various intensity, whereby the compaction is more expressed on the headlands, unlike the interior of the plot due to lower speeds in turning. The consequences of compaction create unfavorable conditions for growth and development of cultivated crops, permanent soil damage, reduction of yields and increase production costs. The paper shows the results of measuring the impact of tractors and mobile systems movement on soil compaction changes and the yield of various maize hybrids. The tests were carried out in the conditions of the Jablanica County, and the soil compaction was measured by the Eijkelkamp 6.0 penetrologer in accordance with EN 5140, while the soil moisture was measured by the Theta probe. Cone resistance was measured at the inner part and the edges of the plot at the beginning of the vegetation (emergence phase) and at the end of the vegetation (harvesting phase). The aim was to determine the effects of changes in soil compaction, on the growth, development and yield of various maize hybrids. The obtained results showed that the soil compaction in the emergence phase of maize on the headlands was on average higher by more than 92% in relation to the interior of the plot. In the harvesting phase on the headlands, more intensive soil compaction values were measured in relation to the plot's interior, and the soil compaction increased more than 57%. Due to the more intensive soil compaction on the headlands, the yields of maize on average were reduced more than 60%, compared to the plots interior.

Keywords: *Soil compaction, tractor, maize, yield.*

Introduction

Soil compaction is one of the main forms of degradation and it is present in total degradation with 11%, while in Europe it is the cause of degradation of 33 million hectares of agricultural land (Akker and Cararache, 2001; Lynden, 2000). The threat of soil compaction is greater today than in the past because of the dramatic increase in the size of farm equipment (Sjoerd, 2004). On the headlands compared to inner parts of plots, at all the plants because of slower speeds of machinery and longer exposing to the normal charges, soil compaction is more intensive. On a headland were measured high values of soil compaction, 4.35, or 4.50MPa (Savin *et al.*, 2004; Nikolić *et al.*, 2007).The increase of soil compaction is especially expressed at a depth of up to 50 cm, with more difficult water and nutrients absorption, an increased risk of erosion and increased energy consumption for soil cultivation (Yavuzcan *et al.*, 2005; Šeremešić *et al.*, 2005; Gajić, 2006; Manuwa *et al.*, 2011; Jerzy and Leszek, 2012). The negative consequences of soil compaction are multiple and significantly affect the reduced yield (Ishaq *et al.*, 2001; Friton, 2001; Nikolić *et al.*, 2002 Savin *et al.*, 2003; Hamza and Anderson, 2005; Ramazan *et al.*, 2012). By applying lighter mechanization, soil

compacting can be significantly reduced with reduced energy consumption (Mouazen and Palmqvist, 2009). The soil compaction changes with the depth of measurement, and the cone resistance when measuring soil compaction at a depth of 20-30 cm was 2 MPa (Yavuzcan *et al.*, 2002). In the emergence stage of maize, the soil compaction on the headlands was higher for 102.87% in relation to the interior, and in the harvesting phase for 59.37% (3.30 MPa - 35 cm depth). In the inner part of the plot the yield of maize grains was higher for 42.81 % in relation to the headlands (Jarak *et al.*, 2005).

Material and Methods

During 2017, in the vicinity of Leskovac (43°04'18.7" N 21°46'52.9" E and 43°01'08.0" N 21°56'11.0" E) the influence of the movement of tractors and mobile systems on changes in the soil compaction and the yield of different maize hybrids on two soil types were carried out. In production of maize, the usual production technology was applied, and all the tools were aggregated to the tractor of power 36.9 kW. The tests were carried out on Vertisol and Aluvium soil, and maize hybrids were represented NS 4051, AGR DIAN, ZP 666, AS 534 and Bećar. The soil compaction was measured in the inner part of the plot and on the headlands, in the maize sprouting phase and in the collection phase. In order to make a consideration of the right headlands, the parcels were selected along the path, so that the turning of the tractor and mobile systems was done only on a plot where the proper headland were formed Width of each experimental plots was 20 m, and length of 100 m. A penetrologer was used to measure soil compaction - Eijkelkamp hardware version 6.0, software version 6.03. Soil compaction was measured at the depth of 0-35 cm, by pressing the cone of the surface 1 cm^2 with the tip of the cone 60^0, in accordance with the standard NEN 5140, the penetration rate of 2 cm sec^{-1}, with no deviation greater than 0.5 cm s^{-1}. The used embossing cone was of standard size, and the standard is defined according to ASAE standard (ASAE S313.1). During the measurement, the penetrologer inclination did not exceed 3.5^0 in relation to the vertical (the position was monitored by the penetrologer libel), while the penetration rate was followed by a speed indicator in a display that was close to the median position (position between "S" and "L"). The soil moisture at the moment of compression measurement was determined by the Theta probe and expressed in % vol. On the soil type Vertisol, in the emergence phase of maize, soil moisture in the interior of the plot was on average 27%, and on the slopes 23%, and in the harvesting phase 19% and 17%. On the soil type Aluvium, in the emergence phase, the soil moisture was 26% in the interior of the plot and 23% on the slope, while in the harvesting phase it was 24% in the interior and 20% on the slopes. The cone resistance was measured in 5 repetitions with a distance of 2 m between the measuring points, with the centre point located in the middle of the headland.

Results and Discussion

On Figure 1 and 2 the soil compaction values are shown during the production of different maize hybrids during the emergence phase, during the harvesting phase of maize in the interior of the plot and on the headlands. Based on the results in Figure 1, it is noticed that the soil compaction in the interior of the plot during the maize sprouting phase was significantly lower in relation to the headland, and with the increase in the depth of measurement, the compaction increased. Soil moisture during the measurements in the inner part of the plot amounted to an average of 27%, and on the headlands of 23%. The lowest soil compaction of Vertisol type was measured at the stage of maize sprouting in the interior of the plot at 0-5 cm and it was on average 0.19 MPa, while on the headland at the same depth the compression was 0.37 MPa, which is the increase of soil compaction from 94.74 %. The highest compression in this phase of measurement was measured on the headland at a depth of 35 cm and amounted to 4.46 MPa, while the inner part of the plot composed at the same depth 2.32

MPa, which is a difference of 92.24%. Similar differences in the soil compaction were observed in other measurement depths in the maize emergence phase (Figure 1). In the phase of harvesting the maize with the depth of measurement, soil compaction increases, where the soil is significantly more compact on the headlands than in the inner part of the plot.

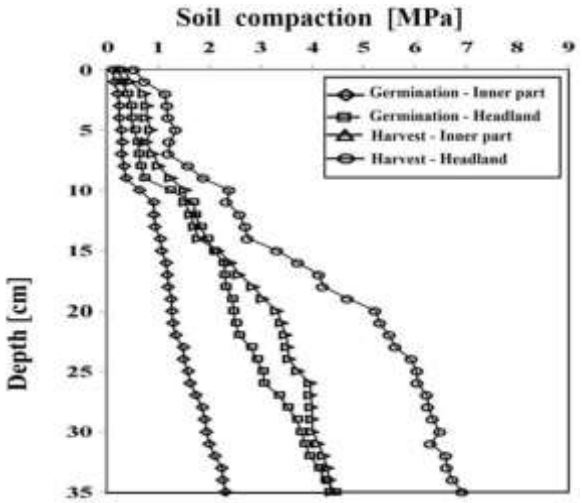

Figure 1. Vertisol's compaction in the inner part of plots and on the edges in the stage of sprouting and harvesting of maize [MPa]

At a depth of 0-5 cm in the interior of the plot, the average soil compaction of 0.65 MPa was measured, while at the same depth on the headland the compression was 1.01 MPa, an increase of 55.38%. On the headlands, the highest values of soil compaction were measured at a depth of 35 cm, and 6.54 MPa, while in the inner part of the plot the compaction was 3.98 MPa, which is a difference of 64.32%.The soil moisture in the maize harvesting phase was 19% in the inner part of the plot, and 17% on the headlands.

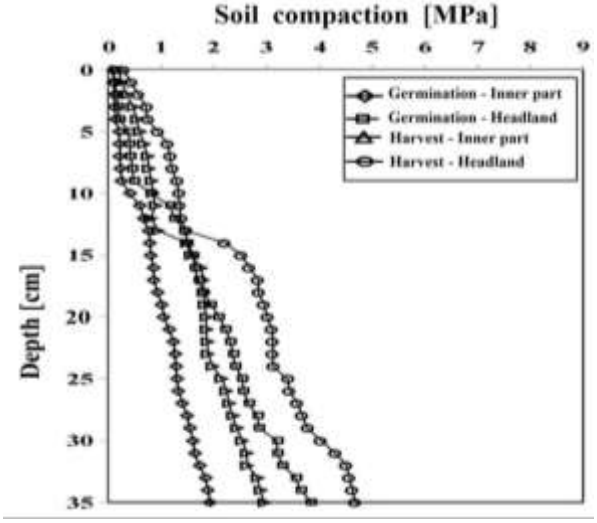

Figure 2. Soil compaction of Alluvium soils in the inner part of the plot and on the headlands in the stage of sprouting and harvesting maize [MPa]

The results of the measurements shown in Figure 2 show that the lowest soil compaction in the Aluvium in maize sprouting phase was in the inner part of the plot at a depth of 0-5 cm, on

average 0.11 MPa, while on the headlands at the same depth the soil compaction amounted to 0.20 MPa. The highest values of soil compaction were measured at a depth of 35 cm 3.84 MPa, while in the inner part of the plot at the same depth the compaction amounted to 1.93 MPa, which makes a difference of 98.96%. Similar differences in the soil compaction were also observed in other depths of measurements in the maize sprouting phase (Figure 2). The average soil moisture in the maize sprouting phase was 26% in the interior of the plot and 23% on headland. At the stage of harvesting the maize, the lowest compaction was measured in the interior of the plot at a depth of 0-5 cm, on average 0.38 MPa, while on the same depth, on the headland, the compaction amounted to an average of 0.55 MPa (Figure 2). On this type of soil, during the maize harvesting phase, the highest soil compaction values were measured at a depth of 35 cm, so the increase in soil compaction on the headlands related to the inner part was 58.82%. Similar differences are observed in other measurement depths. The soil moisture at the stage of harvesting the maize was 24% in the inland plot and 20% on the headland.

Extremely high values of compaction of soil type Vertisol, in the harvesting period, can be explained as a result of unfavourable climatic condition during the measuring, as well as the result of soil properties of this soil type.

The results of our research agree with the findings of other authors (Savin *et al*., 2004; Yavuzcan *et al.,* 2005; Šeremešić *et al.,* 2005; Gajić, 2006; Nikolić *et al.,* 2007; Jerzy and Leszek, 2012; Ramazan *et al.*, 2012; Manuwa *et al.*, 2011).

Due to the intensive soil compaction, the yield on the headland decreased related to the inland of the plot, so that the maize yield on the Vertisol-type soil on the headlands was 3.12 t ha^{-1} to 3.43 t ha^{-1}. In the interior of the plot the yield of maize varied from 5.45 t ha^{-1} to 5.95 t ha^{-1} (hybrid AGR DIAN, or NS 4051). A similar influence of the compaction on the yield was recorded in other maize hybrids (Figure 3).

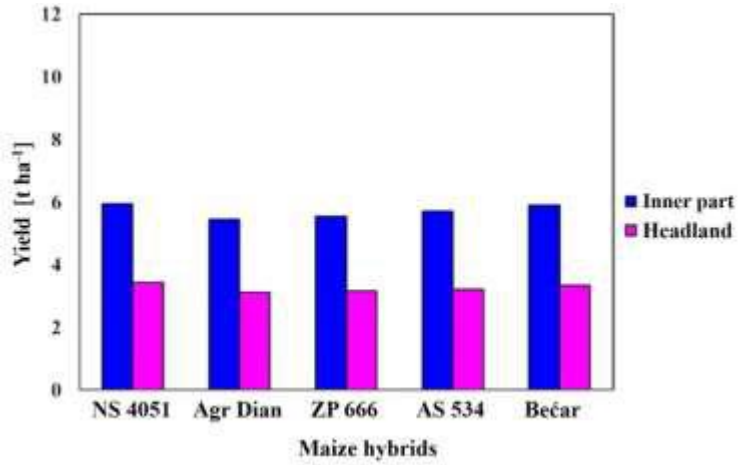

Figure 3. Average values of maize yields realized on Vertisol type

More intensive soil compaction on the headland compared to the inland of the plot showed a significant influence on Aluvium, so that the yields achieved were significantly lower and varied in the range of 5.24 t ha^{-1} to 6.70 t ha^{-1} (hybrid ZP 666). Inside the land plot yields were within the range of 9.18 to 11.90 t ha^{-1} (hybrids NS 4051, ZP 666) (Figure 4). In the case of other maize hybrids, a similar effect of changes in compression on the yields was recorded.

The obtained results show the negative impact of more intensive soil compaction on the headlands compared to the inland of the plot, so that the yields of maize in the examined

conditions on both types of soil on the headland in relation to the inland were lower by more than 60% on average.
Similar results in their research are also reported by other authors (Ishaq *et al.*, 2001; Friton, 2001; Nikolić *et al.*, 2002; Savin *et al.*, 2003; Hamza and Anderson, 2005; Jarak *et al.*, 2005; Ramazan *et al.*, 2012).

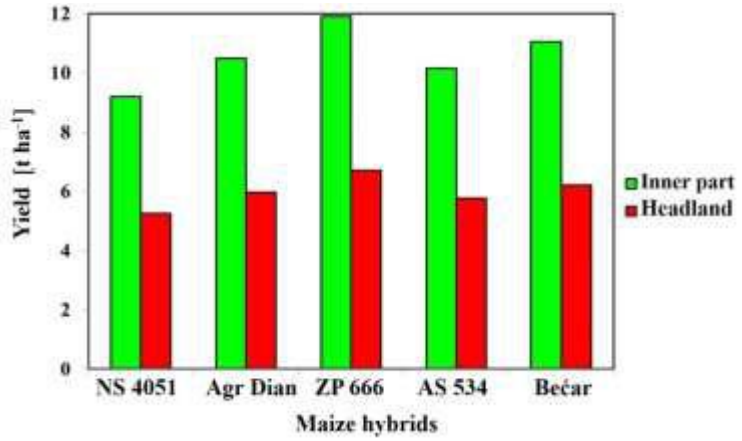

Figure 4. Average values of maize yields realized on Alluvium type

Conclusions

On the basis of the obtained results, it can be concluded that in the inland of the plot, the soil compaction is lower in relation to the headland in both crops, with the lowest values being measured in the phase of emergence on both types of soil. Soil compaction increased in the depth of measurement during all stages of measurement. The lowest compaction in the maize-sprouting phase was measured on the Aluvium at a depth of 0-5 cm and an average of 0.11 MPa, and the highest on Vertisol at a depth of 35 cm on average 4.46 MPa. Soil compaction on the headlands was, on average, higher by over 92% compared to the interior of the plot on both types of soil individually at all depths of measurement. In the harvesting phase, the lowest compaction was measured at Aluvium at a depth of 0-5 cm, it was an average 0.38 MPa, and the highest on Vertisol at 35 cm 6.54 MPa. On the headlands of both soil types at all depths of measurements, compaction was more than 57% higher. The mutual differences in the compactness of both soil types between the plot's interior and the headlands in the maize harvesting phase are less expressed than in the emergence phase, with less generalized compaction values on Aluvium than on Vertisol. As a result of intensive compaction, yields on the headland were reduced related to the inner part of the plot, so the lowest yields were measured on the headlands (Vertisol) 3.12 t ha^{-1} (hybrid AGR DIAN). The highest maize yields were achieved in the interior of the plot (Aluvium) 11.90 t ha^{-1} (hybrid ZP 666). The average reduction in the yield of maize on the headland in relation to the interior of the plot for all the hybrids is over 60%

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MICROPROPAGATION OF CHRYSANTHEMUM CULTIVARS IN SERBIA

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Abstract

Micropropagation represents the plant tissue technique for rapid multiplication of valuable plant material. Last two decades there has been a continuous research effort in Institute for Biological Research "Siniša Stanković" (Belgrade, Serbia) for development of fast and efficient protocol for micropropagation of chrysanthemum cultivars. Recently, the focus of our research is on implementation of developed protocol in practice. The aim of this work was to evaluate potential for mass plant production by classical micropropagation approach of five commercially growing cultivars of chrysanthemums in one growing season from single shoot as a starting plant material. Aseptic stem segment cultures (88 explants/cultivar) were established from all investigated cultivars (12-54%) in January. Shoot multiplication was evaluated during next three subcultures with shoot multiplication index of 2.8-6.1 newly developed shoot/explant depending on cultivar and culture media. The best shoot multiplication was obtained on nutritional medium supplemented with α-naphthalene acetic acid (NAA) and 6-benzylaminopurine (BAP). Regenerated shoots were rooted (100%) in following three weeks on plant growth regulator free medium. About 3500 micropropagated chrysanthemum plantlets were rooted and planted in field in nurseries during May. Plants were successfully (100%) acclimatized *ex vitro* and flowered during autumn. As a final result we evaluated the cost of *in vitro*-derived chrysanthemum plantlets produced by our protocol for micropropagation.

Keywords: *stem segment culture, plant production, in vitro.*

Introduction

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) represents a significant group of cut flowers which belong to the *Asteraceae* family. Besides rose, chrysanthemums are the second economically most important ornamental crop in the world (Teixeira da Silva, 2004). Name chrysanthemum originated from the Greek words for gold flower (lat. *krus anthemon*). Chrysanthemums are also called »autumn roses«, because they were in the past used as cut flower during late summer and whole autumn. Nowadays, because of their ornamental values and many new cultivars, they have grown and have been available on the market during the whole year. Plant tissue culture techniques, especially micropropagation, as a method for rapid production of clonally identical plants, is very useful method for large-scale production of chrysanthemum cultivars. Also, it is essential for breeding of new chrysanthemum cultivars (Teixeira da Silva and Kulus, 2014). Conventionally chrysanthemum cultivars are commonly propagated by root suckers and terminal cuttings. Micropropagation as a method for shoot proliferation from different type of initial explants provides a useful tool for production of genetically uniform plants originated for donor plant. Plant regeneration of chrysanthemum cultivars has been reported, for the first time about fifty years ago (Hill, 1968). There are numerous reports of adventitious shoot regeneration from various explants of chrysanthemum (summarized in Teixeira da Silva, 2004). Investigation of different ways for *in vitro* plant regeneration of more than 30 chrysanthemum cultivars has studied in Institute for Biological Research "Siniša Stanković" Belgrade, Serbia in last more than 20 years of research (Radojević *et al*., (1994, 2000), Jevremović and Radojević (1995, 2004). Successful plant regeneration was achieved in culture of nodal and internodal segments, but also the culture of

leaf tissue (Trifunović *et al*., 2005). Shoots were induced by the adventitious shoot development on initial explants with very short callus phase which implicate high level of true-to-typeness of regenerated plantlets. The clonally fidelity of some cultivars was confirmed even after ten days of multiplication in culture *in vitro*. The flower shape and colour of 'spider' type chrysanthemum cultivars were not changed after long term micropropagation (Jevremović *et al*., 2006, 2012). Also, chrysanthemum cultures can successfully survive cold storage at 4 °C up to six months without subculturing which is very important for planning of plant production during the year (Trifunović et al., 2006).
Main objective of this work is to evaluate the potential for plantlet regeneration from one initial plant shoot/cultivar during one growing season, from January to October. Additional value of this study is the evaluation of the cost of *in vitro* derived chrysanthemum plantlets according to our protocol for plant regeneration.

Material and methods

As a starting plant material we used one single shoot of the donor plants of five different chrysanthemum cultivars collected from the growers from village Vinča near the Belgrade (Table 1). For early flowering cultivar with 'Cremist' flower type we used one shoot for variety with white and one shoot for variety with yellow flowers.

Table 1. List of *Chrysanthemum morifolium* cultivars used in our study.

Cultivar code	Time of flowering	Type	Colour
RH2	Early autumn	'Margaret'	white
RH3	Early autumn	'Cremist'	white, yellow
KH1	Late autumn	'Spider'	yellow
KH2	Late autumn	'Viking'	yellow
KH4	Late autumn	'Daisy'	magenta

Nodal and internodal stem segments were cut in peace (5 cm) and washed for one hour in tap water. After this period of time, for surface sterilization stem segments were replaced to 100 ml glass bottles filled with 30% solution diluted in sterile water of commercial bleach ('Varikina') with 4% NaOCl. After 20 min the solution was drained off and stem segments were washed three times for five min with sterile water. For shoot induction sterilized stem segments were cut transversely on 0.5 cm peace and placed on culture medium for shoot induction. Basal culture media (BM) used in our study were the same for all culture media and composed of Murashige and Skoog mineral solution and vitamins (Murashige and Skoog, 1962) solidified with 7% agar with 3% sucrose and 100 mg/L myo-inositol. Surface sterilized nodal segments were cultured on BM medium for shoot induction which were supplemented with plant growth regulators: α-naphthalene acetic acid (NAA, 0.5 mg/L) and 6 benzylaminopurine (BAP 1.0 mg/L). Shoot multiplication was tested on four culture media supplemented with two auxins indole acetic acid (IAA) and NAA supplemented in two concentrations (0.1 and 0.5 mg/L) and BAP 1.0 mg/L during three successive four weeks long subculture. For shoot rooting, 2 cm long apical shoots were grown on BM medium without plant growth regulators for 3 weeks. Nutritional media were sterilized in an autoclave for 20 min at 114 °C. All cultures were grown with photoperiod 16h day/8h night at 23 ± 2 ° C.
Rooted plantlets were washed of adhering agar under warm water before planting. Rooted plantlets are potted in peat and perlite (3:1) before grown in the field conditions. Survival of initial explants was measured seven days after initiation of culture as number of green aseptic stem segments without any sign of contamination. The shoot multiplication index was measured as a number of *de novo* formed shoots on initial explants regenerated after four

weeks of culture. Shoot multiplication was evaluated in a period of February-May. All data were subjected to statistical analysis using STATGRAPHICS and analysis of variance (ANOVA) using least significance (LSD) tests.

Results and discussion

Micropropagation represents a method for mass production of disease-free and true to type planting material within a short duration and limited space. It is performed under aseptic and controlled environment. Plant regeneration of many chrysanthemum cultivars has reported up to date and an establishment aseptic culture is a first and crucial step for evaluation of successful protocols for micropropagation of different plant species (Teixeira da Silva, 2014). After seven days of culture 12.2-54.0% initial stem segments survived depending on cultivar and were free of contamination (Table 2). The rest of the cultures were lost due to mostly fungi contamination. Relatively high level of contamination is not unexpected since we used donor plants which were grown in the field.

Table 2. Establishment of aseptic cultures of chrysanthemum cultivars.

Cultivar code	EXPLANTS	
	Cultivated	Aseptic (%)
RH2	56	14 (25.0)
RH3	190	32 (16.8)
KH1	74	9 (12.2)
KH2	71	20 (28.2)
KH4	50	27 (54.0)
Total	441	102

Aseptic internodal and nodal chrysanthemum segments (Fig. 1A) were placed on new culture medium and first adventitious shoots were developed within the next four weeks (Fig. 1B). All regenerated shoots were multiplied during three successive subcultures by activation of axillary shoots by nodal segment culture without callus phase (Fig. 3C). *De novo* induced axillary shoos formed 6-11 nodal segments after four week of subculture, which were used for shoot multiplication in next subcultures. We tested the effect of two auxins in two concentrations on shoot multiplication of chrysanthemum cultivars. The best shoot multiplication was achieved on culture media supplemented with NAA as auxin where the highest number of newly developed shoots was recorded in all cultivars (Table 3). Chrysanthemum cultivar with white 'Margaret' type of flowers showed the greatest regeneration potential (6.1 shoot/explant) on medium where the ratio of plant growth regulators (NAA:BAP) was 1:10. For all other cultivars culture medium with 1:5 ratio of plant growth regulators was more appropriate for shoot multiplication.

Table 3. The effect of culture media on shoot multiplication of chrysanthemum cultivars.

Cultivar code	SHOOT MULTIPLICATION			
	IAA		NAA*	
	0.1	0.5	0.1	0.5
RH2	2.7 ± 0.5^{a}	2.9 ± 0.3^{a}	$\underline{6.1 \pm 1.0^{b}}$	2.8 ± 0.5^{a}**
RH3	1.5 ± 0.1^{a}	1.5 ± 0.1^{a}	1.7 ± 0.1^{a}	$\underline{4.0 \pm 0.2^{b}}$
KH1	2.0 ± 0.1^{a}	1.7 ± 0.05^{a}	1.6 ± 0.2^{a}	$\underline{3.2 \pm 0.2^{b}}$
KH2	$2.1 \pm 0.2^{a,b}$	1.4 ± 0.3^{a}	1.5 ± 0.2^{a}	$\underline{3.2 \pm 0.4^{b}}$
KH4	$2.2 \pm 0.3^{a,b}$	1.4 ± 0.1^{a}	1.1 ± 0.1^{a}	$\underline{4.9 \pm 0.4^{b}}$

*Medium was supplemented with 1 mg/L BAP and different concentrations of auxins IAA or NAA. The concentrations of auxins were in mg/L ** Mean ± standard error. Values signed in rows with different letter are significantly different according to LSD test.

The combination of plant growth regulators NAA and BAP are important for shoot growth of many chrysanthemum cultivars (Kaul *et al*., 1990, Teixeira da Silva, 2004). The use of other plant growth regulators, such as urea-type citokinins can improve shoot multiplication of chrysanthemum cultivars, but there can be some side effects like hyperhidricity (Jevremović and Radojević, 2004).

We cultured apical shoots on medium without plant growth regulators at the beginning of May for root initiation (Fig. 1D) and shoots were successively rooted (100%) after three weeks of culture (Fig. 1E). Plantlets height, average number of developed roots varied 7.3-10 root/shoot and the length of regenerated roots varied depending on cultivars (Table 4). *In vitro* produced chrysanthemum shoots can easily rooted on medium without growth regulators and produced plantlets are with good quality (Fig. 1F). This fact is very important in regard of the final cost of *in vitro*-derived plantlets (George and Manuel, 2013).

Table 4. Morphological characteristics of *in vitro*-derived chrysanthemum plantlets.

Cultivar code	SHOOT	ROOT	
	Height (mm)	Nº	Length (mm)
RH2	$62.6 \pm 2.5^{a*}$	9.8 ± 0.5^{c}	58.8 ± 2.7^{c}
RH3	84.6 ± 2.8^{b}	$7.3 \pm 0.3^{a,b}$	37.9 ± 1.2^{b}
KH1	96.8 ± 4.5^{c}	10.0 ± 0.6^{c}	100.5 ± 4.6^{d}
KH2	85.8 ± 4.0^{b}	7.9 ± 0.4^{b}	40.0 ± 3.4^{b}
KH4	114.7 ± 4.7^{d}	6.4 ± 0.3^{a}	32.3 ± 2.8^{a}

* Mean ± standard error. Values signed in columns with different letter are significantly different according to LSD test.

Regenerated plantlets were planted and acclimatized in field conditions (Fig 1 G). In total 173 (RH2), 960 (RH3), 920 (KH1), 360 (KH2 and 1000 (KH4) plantlets were planted at the end of May. In total, from 102 initial explants, 3413 in vitro plantlets were regenerated from the period of five months. All plantlets flowered from September to November depending on cultivar (Fig. 1H).

Figure 1. Micropropagation of selected chrysanthemum cultivars. A Aseptic inernodal stem segments grown 7 days on induction medium. B Indirect shoot induction after 4 weeks of culture. C Shoot culture during multiplication. D Apical shoots before rooting. E Rooted shoot cultures. F *In vitro*-derived plantlets ready for planting. G Plantlets grown in field conditions during August. H Flowered plantlets in late September, cultivar RH3, 'Cremist' flower type.

The true-to-typeness is especially important for ornamental plants. All *in vitro*-derived chrysanthemum plants showed inflorescence colour and shape typical for the cultivars. In our previous study, we find that spider type of chrysanthemum can be very stable during long term micropropagation. Shoots of this cultivar can be propagated more than ten years in culture *in vitro* without any change in flower morphology (Jevremović *et al*. 2012). On the other hand, *in vitro* propagation of some cultivars can be very useful for chrysanthemum breeding as a source of new varieties (Zalewska *at al*., 2007).

Conclusions

We implemented efficient, fast and true-to-typeness protocol for chrysanthemum production by micropropagation in culture *in vitro*. It is possible to regenerate up to 1000 plantlets ready for planting from one donor shoot in a period of five months. The best regeneration potential showed cultivar with yellow spider type of flowers. From initial 9 aseptic stem segments cultured in January more than 920 fully developed plantlets ready for planting were obtained in May. The final cost of chrysanthemum plantlets depended on cultivar. But, if we know that for production of 3413 plants in period of five months from six initial shoots we used 40L of culture media and know that the cost of 1L of culture media is approximately 1 euro, it is not easy to conclude that micropropagation is the method of choice for mass production of chrysanthemum cultivars.

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THE RESPONSE OF ALFALFA TO INOCULATION WITH INDIVIDUAL AND COMBINED CULTURES OF MICROORGANISMS

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Abstract

From the point of view of agricultural production, alfalfa is one of the most important forage legumes in Serbia and the world. Considering the significant area on which this plant species is grown and production potential of alfalfa, average yields in practice are not high enough. One way to improve the productive performance of alfalfa seed is inoculation of the seed with effective strains of microorganisms, which would allow the better use of potential for the development of plants and microorganisms.In this study the influence of pre-sowing inoculation with individual and combined cultures of rhizobia (*Sinorhizobium meliloti*), azotobacter (*Azotobacter chroococcum*) and actinomycetes (*Streptomyces sp*.) on the weight of alfalfa plants was examined. Analyses were carried out in the beginning of blooming stage. In the experiment the influence of seven variants of microbial inoculation on plant weight was examined on two alfalfa cultivars. After inoculation seed from each inoculum variant were planted in the pots filled with soil. For tested parameter, interaction between cultivar and inoculation resulted in positive effect. Only the application with combined cultures *A. chroococcum* and *Streptomyces* sp.(6) in the variety K-28 did not result in a statistically significant increase in green mass per plant compared to the control.

Key words: *alfalfa, inoculation, cultivar, weight.*

Introduction

Alfalfa is one of the most widely cultivated forage legumes because of its high nutritional quality for livestock, high biomass production and broad adaptability to a wide range of ecological conditions (Herrmann *et al.,* 2010; Bouton, 2012). Its importance as a forage crop lies in its high protein, vitamin and mineral contents, as well as its nitrogen-fixing performance through *Sinorhizobium meliloti* symbiosis (Barnes *et al.,* 1988). The potential for nitrogen fixation via symbiosis is supposed to be, in average, 450 kg ha^{-1} $year^{-1}$ (Fishbeck *et al.,* 1987). Symbiotic nitrogen fixation is a renewable source of nitrogen and an environmentally friendly way of supplying the alfalfa plant and soil with nitrogen (Delić *et al.,* 2016). The bacteria enzyme system supplies a constant source of reduce nitrogen to the host plant and the plant furnishes nutrients and energy for the activities of the bacteria (Shetta *et al.,* 2011). Rhizobia synthesize polysaccharides, vitamins B group, indole-3-acetic acid and cytokine, siderophore production, effect on phosphorus availability, antagonistic activity of rhizobia against plant pathogenic micro-organisms (Denison and Kiers 2004; Baset Mia and Shamsuddin, 2010).

Azotobacter is free-living soil microbe which fixes nitrogen from the atmosphere, aerobic, oval or spherical bacteria that form thick-walled cysts, and may produce large quantities of capsular slime. The beneficial effects of azotobacter are not only due to its ability to fix atmospheric nitrogen, but also to secrete biologically active substances: auxin, gibberellins, pyridoxine, biotin and nicotinic acid (Dobbelaere *et al.,* 2003; Hayat *et al.,* 2010), produces antifungal compounds, antibiotics (Dey *et al.,* 2017). Actinomycetes enhance plant growth producing biologically active substances such as indole-3-acetic acid (IAA) to help growth of roots or produce siderophores to improve nutrient uptake (Suzuki *et al.,* 2000).

These microorganisms are present, in higher or lesser abundance, in the soil and after harvest they can end up on the seed and plant roots. But application of selected microorganisms for seed inoculation leads to better results in crop production. The effect of inoculation depends on host plant activity, species, strain and cell concentration of microorganisms in the inoculum, the structure of indigenous populations, soil type, nutrient pool and toxic metal concentrations, soil health, microbial diversity, and soil disturbances caused by management practices. (Walker *et al*. 2003; de Souza *et al.*, 2015). The use of efficient inoculants can be considered an important strategy for sustainable management and for reducing environmental problems by decreasing the use of chemical fertilizers (Alves *et al.*, 2004).
The aim of this study was to examine the influence of rhizobium, actinomycetes and azotobacter (especially and in combinations) on the green mass of alfalfa.

Material and Methods

The experiment was carried out in 10 l volume vegetation pots in semi-controlled conditions at the Institute for Forage Crops in Kruševac. The soil chemical characteristics were the following: pH/KCl 5.90; pH/H_2O 6.44; total nitrogen 0.138%; humus 2.62%; P_2O_5 6.6 mg 100 g^{-1}; K_2O 24.05 mg 100 g^{-1}.
The experiment was a two-factorial, random block design with 5 replicates, where the first factor was alfalfa cultivar and the second was the variant of microbial inoculation. Research was conducted on two alfalfa cultivars: K-28 (bred in the Institute for forage crops in Kruševac) and Syntéza 1 (bred in Plant Production Research Center (PPRC) in Piešťany.

The inoculation was done using the following inocula:
1. *Sinorhizobium meliloti* ($10.8x10^{11}$ per ml);
2. *Azotobacter chroococcum* ($28x10^{11}$ per ml);
3. *Streptomyces* sp. (actinomycetes), ($7.6x10^{10}$ per ml);
4. *Sinorhizobium meliloti* + *Azotobacter chroococcum*;
5. *Sinorhizobium meliloti* + *Streptomyces* sp.;
6. *Sinorhizobium meliloti* + *Streptomyces* sp.;
7. *Sinorhizobium meliloti* + *Azotobacter chroococcum* + *Streptomyces* sp.;
8. Control – no inoculation.

The *Sinorhizobium meliloti* cultures were grown on YM substrate by Vincent (1970), *A. chroococcum* cultures were grown on the liquid substrate by Feodorov (1949), and *Streptomyces* sp. were grown on the substrate by Krasiljnikov (1965). These microorganisms were obtained from the collections of the Department of Microbiology of the Faculty of Agriculture in Novi Sad. Alfalfa seed was sterilized with 0.2% solution of $HgCl_2$ and 70% ethanol, rinsed several times with sterile tap water and then immersed in the corresponding inocula. Ten seeds from each inoculum variant were planted in pots filled with soil. In each pot, 10 ml of the appropriate inoculum was added.
Green mass per plant (g).was measured in first cut at the beginning of flowering.
The results were processed using the statistical package STATISTICA 8.0. The significance of the difference between the investigated treatments was determined by analysis of variance, and LSD tests.

Results and Discussion

The results obtained showed that the application of microorganisms and combinations thereof can affect on the green mass of alfalfa. For the successful alfalfa cultivation, the microbial inoculants are more and more applied for the better utilization of potential for the development of plant and microorganisms N fixation efficiency (Delić *et al.*, 2007). The

application of microbial inoculants may reduce the use of expensive nitrogen fertilizers, speeds up the decomposition of pesticides and increases the biological activity of soil (Djukić et al., 2006).

The plants of both examined cultivars of alfalfa in treatments with inoculation had significantly greater weight than the control in almost all variants (Table 1). Only the application with combined cultures A. *chroococcum* and *Streptomyces* sp.(6) in the variety K-28 did not result in a statistically significant increase in green mass per plant compared to the control.

Table 1. The effect of inoculation and cultivar on the green mass per plant (g)

Cultivar	Variants	Green mass per plant (g)
K-28	1	4,87 ^{j}
	2	5,02 ^{i}
	3	4,72 ^{k}
	4	4,09 ^{n}
	5	4,60 ^{l}
	6	4,05 $^{n, o}$
	7	5,31 ^{h}
	8	4,00 ^{o}
Syntéza 1	1	6,58 ^{b}
	2	6,78 ^{a}
	3	5,51 ^{g}
	4	6,08 ^{d}
	5	5,63 ^{f}
	6	6,00 ^{e}
	7	6,25 ^{c}
	8	4,37 ^{m}

Note: Mean values with the same superscript(s) are not significantly different according to Fisher's LSD test ($p < 0.05$)

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By using mixed inoculum of combined cultures microorganisms - *Sinorhizobium meliloti*, *Azotobacter chroococcum* and *Streptomyces* sp., Jarak *et al*. (2007) achieved the fastest germination and maximum height and weight of alfalfa. Similar results were reported by Andjelković *et al.* (2014) in the research of the effect inoculation of these microorganisms in the production of alfalfa in acid soil. In our research better results were determined in treatments inoculated with individual cultures of microorganisms or a combination of all three species of bacteria than using combination of two cultures microorganisms.

In comparison with the control, the best effect on the green mass of alfalfa was recorded with monovalent inoculum *Azotobacter chroococcum* in cultivar Syntéza 1 (Table 1). Numerous investigations into the influence of inoculation on crops have proved its positive effects (Biswas *et al*., 1994; Kizikaya, 2008; Stamenov *et al*., 2012). The plants of cultivar of alfalfa Syntéza 1 had a bigger green mass in the variants of inoculation, and control in comparison to cultivar K-28. The effect of inoculation on the plant can be direct, that is through plant growth promotion, or indirect, that is through improving plant nutrition via the better development of the roots, and it is difficult to distinguish between them (Nihorimbere *et al*., 2011).

Conclusions

The results obtained in our research indicate that the plant response to the application of appropriate microorganisms and their combination was generally positive. Microorganisms used for the inoculation of alfalfa seeds can, also, be found in the soil, though their number may vary. However, the indigenous strains were not always effective, so the inoculation with selected microorganism strains should be used for the better results in the alfalfa production.

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ANALYSIS OF VARIABILITY OF MEADOW FESCUE (*FESTUCA PRATENSIS* HUDS.) POPULATIONS AND CULTIVARS

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Abstract

Meadow fescue (*Festuca pratensis* Huds.) is one of the important perennial fodder grasses for animal feed production on grasslands and pastures, with high yield potential and biomass quality. As in other species, basic requirement for successful breeding of meadow fescue is variability of the initial material in breeding programme. The collection examined consisted of five populations and six meadow fescue cultivars. The study was conducted in an experimental field of the Institute for Forage Crops, on degraded alluvium soil type. The trial was set up in a space plant nursery with plant to plant distance 60x60cm as a randomized block design with 30 plants per genotype in two years. The aim of these study was to determine and compare variability of the most important phenological (heading date) and morphological traits (plant height, sward diameter, length, width and leaf number, terminal internodes length and the number of tillers per plant), seed yield components, herbage quality, green mass and dry matter yield of populations and cultivars. The data were analysed by ANOVA. Populations have demonstrated higher variability compared to cultivars for most studied parameters. The highest genetic coefficient of variability in populations was determined for seed yield per plant and number of tillers per plant (CV_G 66.05% and 63.35% in second year of investigation). In cultivars, the highest genetic coefficient of variability determined for green mass yield and number of tillers per plant (CV_G 50.47% and 46.65% in second year). The lowest variability in both studied groups in both investigated year, was determined for heading date. Broad sense heritability (h_b^2) was high, in both studied groups, in populations and cultivars, for all investigated traits. The highest heritability was determined for plant height (99.32%) and ash content (99.84%) in populations, and for green mass yield in the third cut (99.67%) in cultivars.

Keywords: *meadow fescue, variability, heritability, populations, cultivars*.

Introduction

Meadow fescue is forage grass species well adapted to continental climates where it is an alternative to *Lolium perenne.* Meadow fescue is diploid (2n = 14), with high level of self-incompatibility. It is a species of increasing importance, particularly in the northern hemisphere, where it dominates in the meadows and pastures (Casler and Santen, 2000). It has been usually used for intensive production of hay meadows. These species is highly productive, with high nutritive value (Babić et al., 2012). The herbage quality changes depending on the stage of maturity, but because it flowers late compared with other forage grasess, high quality of herbage is retained for a long time. This is a very important characteristic for meadow fescue to be a component in grass-legumes mixtures. Meadow fescue is very compatible with many forage species, especially legumes like alfalfa, red and white clover and sainfoin. Of grasses, associates with *Phleum pratense* is in use in temporary cutting grassland in north areas, like Scandinavia. It is one of the most productive forage perennial grasses, especially in extreme environmental conditions, like summer drought or winter hardiness. By quality and digestibility, meadow fescue can be compared to species of

the genus *Lolium* (Kölliker, 1998). Also, meadow fescue is very tolerant to grazing; therefore pastures where it dominates are long lasting. It can be grown as a pure crop, but more often in mixtures with other grasses and legumes.

First step in grass breeding process is the determination of genetic variability of breeding material. The existence of genetic variability within available breeding material (autochthonous populations, breeding populations and cultivars) influences the choice of breeding material and ensures success of a plant breeding program. Autochthonous populations have natural variation and very often have satisfactory yielding performance in comparison with cultivars (Babić et al., 2014), and often can be used for direct phenotypic selection and for cultivars release (Posselt and Willner, 2007). Most of created cultivars contain autochthonous populations and represent great and important resource of genetic variability. Variability within populations and cultivars of meadow fescue were documented for important agronomical traits, forage yield, and forage quality (Kanapeckas et al., 2005; Babić, 2009).

The genetic variability of all traits important for breeding process represents a basic prerequisite for successful selection. The primary aim in meadow fescue forage breeding is obtaining cultivars with a high yield and improved quality for livestock feed. The aim of this investigation was to determine genetic variability of five breeding populations and six cultivars. The most important breeding criteria in meadow fescue breeding programs, like as other forage grasses, are increasing of forage yield and digestibility, disease resistance and drought (Sokolović et al., 2012) and frost tolerance and persistency (Sokolović et al., 2010). Since is meadow fescue use as a component of grass-legume mixtures in Serbia, dry matter yield and quality, persistency and rust resistance is most important breeding criteria of this species.

Material and methods

Investigated collection consisting of five breeding populations and six cultivars of meadow fescue was investigated during a two years period. Breeding populations have passed 2 cycles of selection. Four of them originated from the local populations, collected mainly in Eastern Serbia (OP-1, OP-2, OP-3 and OP-4), and the population OP-5 originated from Czech Republic. Cultivars included in this study were: Kruševački 21 (K-21) (Serbia), Jabeljska (Slovenia), Premil, Pradel, Preval (Switzerland) and Rožnovska (Czech Republic). The study was conducted in an experimental field of the Institute for Forage Crops, on degraded alluvium soil type. The trial was set up in a space plant nursery with plant to plant distance 60x60cm as a randomized block design with 30 plants per accession in three replications.

Variability of heading date (HD) and morphological traits (plant height (PH), sward diameter (SD), leaf length (LL), leaf width (LW), number of leaves per tiller (NLT), first internodes length (FIL), number of tillers per plant (NTP)), seed yield components (panicle length (PL), lower panicle branches length (LPBL), number of branches in panicle (NBP), seed yield per plant (SYP), 1000 seed weight (1000 SW), green matter yield (GMY) and dry matter yield per plant (DMY) were investigated in 2 years. Biomass quality (crude protein (CP), crude fibre (CF), crude fat (CF), BEM and ash) was determined by the Weende system, in the first cut. All data were analysed by mono-factorial analysis of variance for both years of research, due to large differences in agroecological conditions in studied years, which reflected largely on the achieved results.

Genetic (σ_g^2) and phenothypic (σ_f^2) component of variance were calculated according to the formulas:

$$\sigma_g^2 = \frac{MS_2 - MS_3}{r} \qquad \sigma_f^2 = \sigma_g^2 + \sigma_e^2$$

Broad sense heritability (h_b^2) was calculated for all traits according to the formula:

$$h_b{}^2 = \frac{\delta_g{}^2}{\delta_f{}^2} \cdot 100$$

Coefficients of genetic (CV_G) and phenotypic variation (CV_F) were calculated in the following manner:

$$CV_G = \frac{\sqrt{\sigma_g^2}}{\overline{X}} \times 100 \qquad CV_F = \frac{\sqrt{\sigma_f^2}}{\overline{X}} \times 100$$

Results and discussion

For successful breeding and creation of new cultivar of meadow fescue, as well as other species, one of the basic conditions is existence of variability in the starting breeding material. For successful breeding is necessary to know the structure of genetic and phenotypic variance and heritability of most important and functionally related traits.

Calculated values of genetic and phenotypic components of variance, heritability and coefficients of genetic and phenotypic variation of meadow fescue populations and cultivars are shown in Table 1 and 2.

Table 1. Components of variance (genetic-σ_g^2, phenotypic-σ_f^2), heritability (h_b^2) and coefficients of variation (genetic-CV_G, phenotypic-CV_F) of meadow fescue in first year

	σ^2_g		σ^2_f		h^2_b(%)		CV_G(%)		CV_F(%)	
Traits	P	C	P	C	P	C	P	C	P	C
HD	2.97	3.4	3.0	3.6	98.5	94.3	4.1	4.4	4.1	4.5
PH	20.5	27.3	20.5	32.3	99.3	84.3	15.3	4.9	15.3	5.4
SD	13.6	9.7	14.1	9.8	96.2	98.9	11.5	9.9	11.7	9.9
LL	4.6	4.6	4.9	5.9	95.1	77.2	9.0	8.1	9.3	9.3
LW	0.4	0.4	0.4	0.4	97.5	97.2	7.9	8.2	8.0	8.3
NLT	0.9	0.1	1.0	0.1	92.8	90.0	34.5	11.9	35.8	12.6
FIL	39.8	17.6	42.2	18.3	94.3	96.7	19.4	12.5	19.9	12.7
NTP	1420	345	1468	385	96.7	89.6	37.4	14.6	38.0	15.4
PL	2.6	0.7	3.1	1.1	82.8	65.7	6.9	3.5	7.7	4.3
LPBL	1.03	0.5	1.3	0.8	82.4	64.2	11.6	8.9	12.8	11.1
NBP	0.5	0.7	0.7	1.1	73.0	61.8	10.6	11.4	12.4	14.5
SYP	3.8	3.9	9.2	8.4	41.7	46.5	9.1	7.6	14.1	11.2
1000 SW	0.07	0.03	0.1	0.1	55.2	32.2	12.9	8.1	17.4	14.2
GMY	6368	5153	7248	5557	87.9	92.7	19.3	14.1	20.6	14.7
DMY	855	746	898	770	95.2	96.9	23.9	17.1	24.5	17.4
CP	1.4	1.1	1.6	1.2	89.0	91.1	12.3	11.1	13.0	11.6
CC	5.5	5.2	5.6	5.3	97.7	98.3	6.9	6.4	7.0	6.4
CF	0.2	0.2	0.2	0.2	97.8	98.0	20.0	25.5	20.2	25.7
Ash	0.8	0.1	0.8	0.1	99.8	94.5	10.1	4.0	10.1	4.1
BEM	6.2	1.7	6.7	3.1	88.7	55.5	5.5	2.9	5.8	3.9

P – Populations, C – Cultivars

HD - heading date, PH - plant height, SD - sward diameter, LL - leaf length, LW - leaf width, NLT - number of leaves per tiller, FIL - first internodes length, NTP - number of tillers per plant, PL - panicle length, LPBL - lower panicle branches length, NBP - number of branches in panicle, SYP - seed yield per plant, 1000 SW - 1000 seed weight, GMY - green matter yield, DMY - dry matter yield per plant, CP - crude protein, CF - crude fibre, CF - crude fat

Relationship of genetic and phenotypic variance for all pheno-morphological traits, GMY, DMY and quality traits (except BEM) indicate that the variability of these traits in this collection of breeding populations and cultivars largely derived from plant genotype. For these traits broad sense heritability was high, which means that these traits largely depend on genetic factors. The high heritability values determined for these traits, indicates the great proportion of genetic to total phenotypic variability of the traits and suitability for breeding.
For seed components traits and BEM ratio of genetic and phenotypic variance was significantly different, indicating the significant influence of ecological factors on variability of these traits in these collection. For these traits broad sense heritability was lowest. Based on the results can be concluded that these traits are quite variable, so significant variation in some individual genotypes can be expected depending on environmental factors and the year of investigation.

Table 2. Components of variance (genetic-σ_g^2, phenotypic-σ_f^2), heritability (h_b^2) and coefficients of variation (genetic-CV_G, phenotypic-CV_F) of meadow fescue in second year

	σ^2_g		σ^2_f		h^2_b(%)		CV_G(%)		CV_F (%)	
Traits	P	C	P	C	P	C	P	C	P	C
HD	2.9	1.3	3.4	1.9	85.8	71.6	4.3	2.9	4.7	3.4
PH	56.8	23.6	60.1	31.6	94.4	74.5	9.4	6.4	9.6	7.4
SD	6.1	4.4	7.9	5.4	76.9	82.1	9.5	8.0	10.9	8.9
LL	6.2	14.2	6.7	14.9	92.8	95.0	9.6	14.3	9.9	14.6
LW	0.8	0.3	0.9	0.6	82.0	51.2	11.8	7.7	13.0	10.7
NLT	0.06	0.07	0.07	0.08	90.4	92.2	10.4	11.3	10.6	11.8
FIL	5.3	3.8	7.3	5.1	72.6	75.4	6.3	5.1	7.4	5.8
NTP	659	452	733	469	89.1	96.5	63.4	46.6	67.1	47.5
PL	3.5	5.6	4.3	6.5	81.7	87.4	10.7	13.4	11.9	14.3
LPBL	0.8	0.3	0.9	0.6	90.3	55.9	17.6	11.8	18.5	15.8
NBP	0.2	0.1	0.3	0.2	72.7	52.2	6.6	5.6	7.5	7.8
SYP	28.8	3.1	29.8	4.7	96.7	65.3	66.0	35.6	67.2	44.0
1000 SW	0.03	0.02	0.07	0.06	41.4	39.6	7.9	7.2	12.4	11.4
GMY	2278	1727	2303	1755	98.9	98.4	39.4	39.6	39.7	39.9
DMY	275	134	288	142	95.6	94.4	40.6	32.6	41.6	33.5

P – Populations, C – Cultivars
HD - heading date, PH - plant height, SD - sward diameter, LL - leaf length, LW - leaf width, NLT - number of leaves per tiller, FIL - first internodes length, NTP - number of tillers per plant, PL - panicle length, LPBL - lower panicle branches length, NBP - number of branches in panicle, SYP - seed yield per plant, 1000 SW - 1000 seed weight, GMY - green matter yield, DMY - dry matter yield per plant

Genetic and phenotypic coefficients of variation are relative indicators of variability. They are used to compare the level of variability of traits expressed different units of measure. The most variable traits, as expected, are number of tillers per plant, green and dry matter yield per plant and seed yield per plant, in both year of investigation (Table 1 and 2). The lowest genetic and phenotypic coefficients of variation in both year determined for heading date in breeding populations and cultivars also. Similar results presented Kanapeckas et al., 2005. and Babić, 2009. Fang et al., 2004. reported that heading date and plant height had very low

genetic coefficient of variation, while the seed production traits like plant seed yield, seed weight per panicle and panicle fertility exhibiting the highest genetic coefficient of variation values. As expected, for most studied traits calculated higher values for genetic and phenotypic coefficients of variation in breeding population than in cultivars.

Conclusion

In two-year study of genetic variability of pheno-morphological traits, seed yield components and biomass quality five breeding populations and six cultivars of meadow fescue high genetic variability were determined. The relationship of genetic and phenotypic variance, broad sense heritability and coefficients of variation for the most important traits, especially for number of tillers per plant, green and dry matter yield, leaf length and seed yield per plant indicate a high breeding potential of investigated breeding populations and cultivars in meadow fescue breeding process. High values of broad sense heritability for most traits indicate that an improvement of these traits could be possible and predictable in these collections. The influence of genotype on all traits was highly significant. Share of genetic to phenotypic variance for all pheno-morphological traits, GMY, DMY and quality traits (except BEM), indicating that the variability of these traits is largely derived from plant genotype and consequently the heritability of these traits was high.

For most studied traits parameters of variability were similar for breeding populations and cultivars included in investigation except genetic and phenotypic coefficients of variation who are calculated highly values in breeding population than in cultivars.

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CHEMICAL PROPERTIES OF BLACK CURRANT (*RIBES NIGRUM* L.) BERRY AND LEAF EXTRACTS

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Abstract

The objective of this experiment was to identify and quantify individual invert sugars (glucose, fructose, sucrose) organic acids (citric acid, malic acid), total anthocyanins, anthocyanin glycosides (delphinidin 3-glucoside, delphinidin 3-rutinoside, cyanidin 3-glucoside, cyanidin 3-rutinoside), vitamins (C, A, B3) and minerals (K, Ca; Mg, Na, P, Cu, Zn, Fe) in berry and leaf extracts of black currant. The extracts prepared from black currant leaves and berries exhibited different characteristics. Berries had higher contents of the tested parameters compared to leaves. Fructose was the dominant invert sugar in all extracts, followed by glucose, while the amount of sucrose was very low. Berry extracts contained 4.35 times more fructose than leaf extracts, and glucose was 3.92 times higher in berries than in leaves. The main organic acid was citric acid, whereas twice as low quantities of malic acid were observed in all tested berries and leaves. The content of total anthocyanins ranged from 289.2 to 12.3 mg C3G 100 g^{-1} in berry and leaf extracts, respectively. In the present research, cyanidin-3-rutinoside was the most abundant anthocyanin in both berries and leaves. The major vitamin in the tested black currant was vitamin C (213.5 and 38.7 mg 100 g^{-1}, respectively), whereas the most abundant mineral was K (328.1 and 20.3 mg 100 g^{-1}, respectively). The results suggested that black currant berries and leaves can be used as valuable ingredients for functional foods.

Keywords*: black currant, berry, leaf, chemical properties*

Introduction

Black currant (*Ribes nigrum* L.) has been recognized as a good source of polyphenols, especially anthocyanins, phenolic acid derivatives, flavonols and proanthocyanidins, compared to other berries (*e.g.*, strawberries and raspberries) (Karjalainen *et al*., 2009; Mattila *et al*., 2011). The fruit of black currants is a rich source of sugars and organic acids as important primary metabolites. The contents of sugars and acids and the sugar/acid ratio are important compositional parameters influencing the sensory properties and consumer acceptance of berry products (Sandell *et al*., 2009; Laaksonen *et al*., 2012). Analyses of black currant constituents have revealed that the fruit has high levels of vitamins and minerals, especially potassium, calcium, magnesium, and iron (Hegedűs *et al*., 2008; Lefevre *et al*., 2011). Vitamins and minerals are very important in biological processes as they promote normal growth and development, and play a vital role in preventing some chronic diseases (Gorinstein *et al*., 2001). Also, leaves of black currants are an excellent source of total phenols, flavonoids and phenolic acids (Tabart *et al*., 2006; Raudsepp *et al*., 2010). Recent papers on epidemiological studies have summarized the positive contribution of black currant berries and leaves to human health, including their antimutagenic, antimicrobial, anti-inflammatory, anti-cancer and antihypertensive properties (Declume, 1989; Puupponen-Pimiä *et al*., 2005; Mazza, 2007; Tabart *et al*., 2012).

Considering that there are not many scientific studies on the chemical compounds present in berries and leaves of black currant, the objective of this research was to identify and quantify chemical properties in berry and leaf extracts of black currant.

Material and Methods

Aerial parts of black currant (*Ribes nigrum* L.) were collected in Čačak, Western Serbia, during 2012-2014. To obtain comparable samples, berries were sampled at full ripeness in June, while leaf samples were collected in July, at the stage of full development. Berries and leaves were taken from the inner and outer ranges of the bush.

The chemical analysis of the berries and leaves included the following parameters: 1. Invert sugar components (glucose, fructose, sucrose) in berry samples, as determined by high-performance liquid chromatography (HPLC; Waters Breeze, Milford, USA), with absorbance measured in the range of 210 to 327 nm. 2. Organic acids (citric acid, malic acid), as analyzed using a Hewlett-Packard HP1100 system equipped with a photo diode array detector (Palo, Alto, CA, USA), with absorbance measured in the range of 490 nm to 600 nm. 3. Total anthocyanins, as assessed by the single pH and pH differential method, with absorbance measured at $\lambda = 515$ nm and $\lambda = 700$ nm. 4. Anthocyanin glycosides (delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside and cyanidin-3-rutinoside), as determined using a Perkin Elmer Series 400 high performance liquid chromatograph equipped with a Hewlett-Packard 1040A photodiode array detector, with absorbance measured at 290, 350 and 520 nm. 5. Vitamin C, as assessed by a Perkin Elmer UV/VIS spectrometer (Lombda 25), with absorbance measured at 665 nm. 6. Vitamins A and B3, as determined by a high-performance liquid chromatograph (HPLC; Milford, MA, USA) fitted with a fluorescence detector, with absorption spectra recorded at 347 nm for vitamin B3 and 295-330 nm for vitamin A. 7. Mineral content of the fruit, as determined by flame atomic absorption spectrometry using a Varian Spectar AA 200 instrument equipped with a GTA 110 graphite furnace (Varian, USA). Phosphorus concentration was assessed by spectrophotometry - Spectrophotometer MA9523-SPEKOL 211 (ISKRA, Horjul, Slovenia), at 725 nm, using the molybdenum blue phosphorus complex.

The results are presented as mean ± standard deviations of three determinations. Statistical analyses were performed using Student's t-test and one way analysis of variance. Multiple comparisons of means were made by LSD (least significant difference) test. A probability value of 0.05 was considered significant. All computations were made by employing the statistical software (SPSS, version 11.0).

Results and Discussion

Glucose, fructose and sucrose are the major sugars, citric acid is a major organic acid, and malic acid is present in minor concentrations in the fruit of black currant (Hummer and Barney, 2002; Rubinskiene *et al*., 2006; Milivojević *et al*., 2009). Individual invert sugars and organic acids in berry and leaf extracts were identified by the HPLC-DAD analysis, with the corresponding results presented in Figures 1 and 2. The results show significant differences between berries and leaves. Berries had a higher content of invert sugars and organic acids compared to leaves. Fructose was the dominant invert sugar in all extracts, followed by glucose, while the amount of sucrose was very low. Berry extracts (127.1 mg g^{-1}) contained 4.35 times more fructose than leaf extracts (29.2mg g^{-1}), and glucose (83.2mg g^{-1}) was 3.92 times higher in berries than in leaves (21.2 mg g^{-1}). The amount of sucrose was very low in both extracts. In this study, the main organic acid was citric acid, while twice as low quantities of malic acid were observed in all tested berries and leaves. The content of citric acid in berry extracts was 56.1 times higher than that in leaf extract. Heiberg *et al.* (1992) reported that the sugar content in black currant berries was 9.0%, of which fructose made up 45%, glucose 40% and sucrose 15%. The ratio of sugars to organic acids is an important indicator of perceived taste, maturity/ripeness and general quality, which may serve as an index of consumer acceptance (Bordonaba and Terry, 2008). Fruits with pleasant sensory characteristics are often considered to have a high content of sugars and a relatively low amount of acids (Zheng *et al*., 2009).

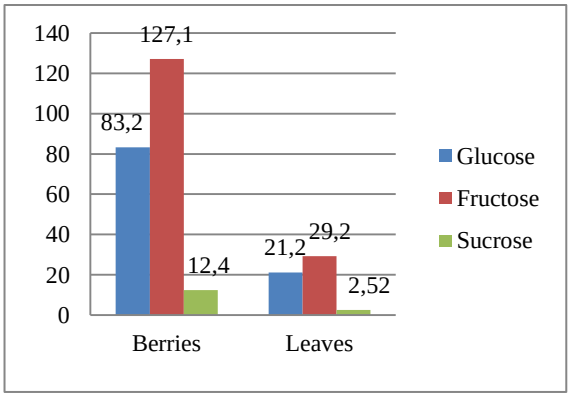

Fig. 1. Invert sugars in berry and leaf extracts

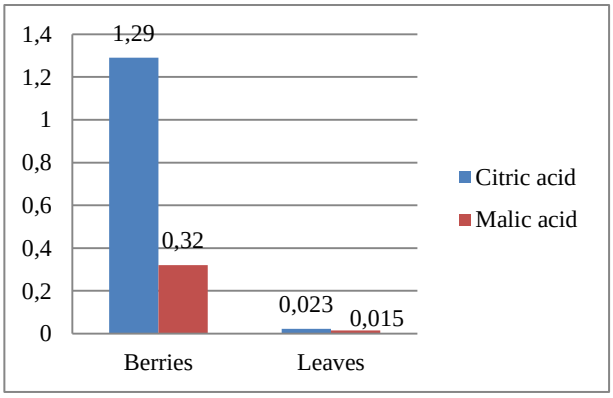

Fig. 2. Organic acids in berry and leaf extracts

Black currant berries are an important source of anthocyanins with concentrations up to four-fold greater than that of other common fruits. Anthocyanins are the major group of phenolics in black currants, accounting for approximately 80% of total phenolics. The four main pigments delphinidin 3-O-glucoside, delphinidin 3-O rutinoside, cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside contribute up to 97% of total anthocyanins in black currants (Anttonen and Karjalainen, 2006). Many studies have reported high concentrations of phenolic compounds in black currant berries and, to a lesser extent, in buds and leaves (Maatta *et al*., 2003; Tabart *et al*., 2006; Raudsepp *et al*., 2010). Total anthocyanins and anthocyanin glycosides in berry and leaf extracts are presented in Table 1.

Table 1. - Total anthocyanins and anthocyanin glycosides in berry and leaf extracts of black currant

	Total anthocyanins mg C3G 100 g^{-1}	Cyanidin -3-rutinoside mg 100 g^{-1}	Cyanidin -3-glucoside mg 100 g^{-1}	Delphinidin -3-rutinoside mg 100 g^{-1}	Delphinidin -3-glucoside mg 100 g^{-1}
Berries	289.2±4.50	44.7±0.38	21.6±0.27	2.61±0.11	3.74±0.11
Leaves	2.07±0.29	1.23±0.02	1.15±0.01	0.06±0.0001	0.16±0.003

The levels of total anthocyanins in berry extracts (289.2 mg C3G 100 g^{-1}) were higher than in leaf extracts (12.3 mg C3G 100g^{-1}). Berries had 23.5 times more anthocyanins than leaves. Compared with the present results, Tabart *et al*. (2011) and Vagiri *et al*. (2013) recorded a higher content of total anthocyanins, while Moyer *et al*. (2002) and Lister *et al*. (2002) reported similar contents. Delphinidin-3-rutinoside was the most dominant anthocyanin in studies by Wu *et al*. (2004) and Szajdek and Borowska (2008), whereas cyanidin-3-rutinoside predominated elsewhere (Rubinskiene *et al*., 2006; Oszmianski and Wojdylo, 2009). In the present research, cyanidin-3-rutinoside was the most abundant anthocyanin in both berries and leaves. Black currants are a major source of vitamins, which in turn provide black currants with powerful antioxidant effects (Walker *et al*., 2010; Raudsepp *et al*., 2010; Nour *et al*., 2011).

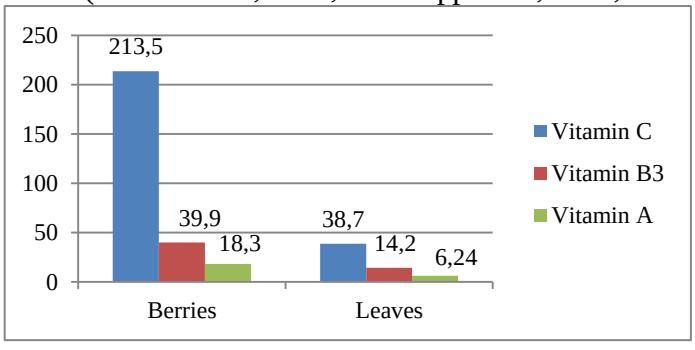

Fig. 3. Vitamins in berry and leaf extracts

In comparison with other fruits, black currants are low in calories, and they have significant levels of vitamin A, vitamin B1, vitamin B3, vitamin E, iron, potassium and calcium (Hummer and Barney 2002). Ascorbic acid and anthocyanins are the most beneficial compounds contributing to the antioxidant capacity of the black currant fruit (Lister *et al*., 2002). The results on the contents of vitamins in black currant berries and leaves are given in Figure 3. Berry extracts were found to have higher contents of vitamins in comparison to leaf extracts. The major vitamins in the tested black currant berries and leaves were vitamin C (213.5 and 38.7 mg 100 g^{-1}, respectively), followed by vitamin B3 (39.9 and 14.2 mg 100 g^{-1}, respectively) and vitamin A (18.3 and 6.24 mg 100 g^{-1}, respectively). Zurawicz *et al*. (2000), Siksnianas *et al*. (2006) and Nes *et al.* (2012) reported a higher content of vitamin C in berries than the value reported in the present study. Tabart *et al*. (2006) found a higher content of vitamin C in the berry than in the leaf. Moyer *et al*. (2002) emphasized that the significant antioxidant capacity of black currant fruits and their processed products is accounted for by a very high content of vitamin C. High levels of vitamin C and polyphenols contribute to the antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic activities of the fruit (Mazza, 2007). Black currants are rich in minerals which contribute to their health benefits. Borges *et al*. (2010) and Lugasi *et al*. (2011) reported that currant fruits are a good source of minerals and natural antioxidant substances. In the present experiment, eight essential minerals were determined, and the results are given in Table 2. High levels of macro and microminerals were present in the berries compared to leaves.

Table 2. - Mineral contents in berry and leaf extracts of black currant

	K mg 100 g^{-1}	Na mg 100 g^{-1}	Ca mg 100 g^{-1}	Mg mg 100 g^{-1}	P mg 100 g^{-1}	Cu mg 100 g^{-1}	Zn mg 100 g^{-1}	Fe mg 100 g^{-1}
Berries	328.1±0.84	25.4±0.27	30.9±0.21	24.2±0.31	188.9±0.26	0.46±0.007	0.30±0.003	5.47±0.11
Leaves	20.3±0.02	0.14±0.003	2.56±0.01	0.67±0.02	23.6±0.07	0.012±0.0001	0.028±0.0001	0.52±0.006

Mineral levels in berry extracts were higher by 8.00 (P) to 181.4 (Na) times than in leaf extracts. The main elements in berry and leaf extracts included K, P, Ca, Mg and Na, whereas Fe, Cu and Zn were present in minor amounts. The most abundant mineral was K, with levels of 328.1 and 20.3 mg 100 g^{-1} in berry and leaf extracts, respectively, whereas Zn had the lowest levels (0.30 and 0.028 mg 100 g^{-1}, respectively). The macro- and microelements followed the order: K > P >Ca> Mg > Na > Fe > Cu > Zn. Compared to the present experiment, Perkins-Veazie and Collins (2001), Hummer and Barney (2002) and Cosmulescu *et al*. (2015) reported higher values for K and Ca, but significantly lower levels for P, Fe and Na, whereas Nour *et al*. (2011) obtained comparable levels of Ca and Zn, a higher content of Mg, and lower amounts of K, Na and Fe. Lefevre *et al*. (2011) determined high levels of minerals, especially P, Ca, Mg, and Fe, in the fruits, whereas Tahvonen *et al*. (1993) obtained significant amounts of Ca, Mg, Fe, Mn and Zn. As found by Hegedűs *et al*. (2008), black currants, which offer dietary benefits and produce numerous physiological effects in humans, have high levels of macroelements (Ca, K, Na, Mg and P), but relatively low concentrations of microelements (Fe, Cu and Zn).

Conclusions

Berry and leaf extracts of black currant are exceptionally rich sources of sugars, acids, anthocyanins, vitamins and minerals, and they provide an interesting nutritional alternative. Berries had higher contents of the tested parameters compared to leaves. Generally, the results on chemical properties indicated that berries and leaves can be used in primary agricultural production, as well as in the pharmaceutical and food industries.

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GENETIC POTENTIAL AND YIELD COMPONENTS OF WINTER BARLEY

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Abstract

The experiment was established in the experimental field at the experimental field of Secondary Agricultural-chemical school "Dr Djordje Radic" in Kraljevo (Serbia) during the 2009/10 and 2010/11 growing seasons. The objective of this study was to investigate the influence of genotype and environment on the yield of winter barley cultivars (Rekord, Grand, NS 565 and Atlas). The following characteristics were analysed: grain yield, 1000 grain weight and test weight. The grain yield of the studied genotypes of winter barley ranged from 4.340 t/ha (2009/10) to 5.217 t/ha (2010/01), while the average grain yield was 4.779 t/ha. The highest two-year average of 1000 grain weight (50.22 g) were obtained by NS 565 cultivar. The largest two-year average value of test weight was found in the cultivar Rekord (69.25 kg/hl). The average value of all genotypes was 66.78 kg/hl. Highly significant influence of the year on grain yields and 1000 grain weight were established at investigated winter barley cultivars by variance analysis, while genotype influence on 1000-grain weight and test weight was significant. Significant differences in 1000-grain weight at investigated barley cultivars were found relative to the interaction of environmental factors and cultivars. In view of the ever-changing climate change and the global warming trend, barley production is becoming increasingly risky and unstable. In such circumstances, there is a need for the creation of varieties, which in addition to good agronomic qualities, will be characterized by high and stable yields.

Key words: *cultivar, grain yield, winter barley*

Introduction

Barley cultivars that were in production until the end of the eighties were characterized by the lower yields, good technological quality and higher stem sensitive on lodging. Barley is globally cultivated on an area of about 47.5 million hectares, an average yield of 2.6 t/ha being realized making it the fifth grown crops (maize, rice, wheat and soybeans). In Serbia, barley is grown on 84.166 ha with an average yield of 2.9 t/ha and ranks third with 244.081 tons (FAO, 2010). The largest areas under barley are in Russia, Australia, Ukraine and Canada **(**FAO, 2010).

The interaction between the genotype and environment greatly limits the effectiveness of selection, if it is performed only on the basis of the average yield. Varieties that have a smaller contribution to interaction are less sensitive to changing environmental conditions, such varieties are stable (Bratković et al., 2014; Đekić et al., 2012; Jelić et al., 2014). New varieties are characterized by good technological quality, better resistance to lodging and diseases, shorter stem and more efficient assimilates usage (Pržulj et al., 2004).

The need for a variety of short or long growing season depends on the environmental conditions of a particular area. In winter barley often variations in yield can be detected, also between the years and between locations (Đekić et al., 2011; 2015; Popović et al., 2011). The 1000 grain weight and test weight are direct components of grain yield and change under the

influence of environmental factors, but primarily varietal characteristics. They indicate the grain size and are important criterions in breeding barley.
The aim of this study was the determination of the cultivars and ecological environmental factors influence on differences in stability and adaptability of cultivars regard the grain yield, 1000 grains weight and test weight of tested winter barley cultivars, as well as specificity cultivars exploring regard growing seasons conditions.

Materials and methods

Experimental design and statistical analysis

During the 2009/10 and 2010/11 growing seasons, three cultivars of winter barley (Rekord, Grand, NS 565 and Atlas), cultivated at the experimental field of Secondary Agricultural-chemical school "Dr Djordje Radic" in Kraljevo (Serbia) were investigated. The studied soil is extremely acid pseudogley. Experiments have been conducted in randomized block systems, with a plot size of 50 m^2 (10 m x 5 m) in five replicates. The usual techniques for barley production were applied, and it was done in the optimum sowing time in late October. Fertilizers have used in the 300 kg/ha of form of complex NPK fertilizer (8:24:16, superphosphate (17% P_2O_5) and ammonium nitrate (AN) 34.4% N). The following properties were analyzed: grain yield (t/ha), 1000 grain weight (g) and test weight (kg/hl).
On the basis of achieved research results the usual variational statistical indicators were calculated: average values, standard error and standard deviation. Statistical analysis was made in the module Analyst Program SAS/STAT (SAS Institute, 2000).

Meteorological conditions

Kraljevo area is characterized by a moderate continental climate, which general feature is the uneven distribution of rainfall by months. Data in Table 1 for the investigated period (2009-2011) clearly indicate that the years in which the researches were conducted differed from the typical multi-year average for Kraljevo region, regarding the meteorological conditions. The average air temperature in 2009/10 was higher by 0.9°C and 2010/11 was higher by 0.4°C than the average of many years.

Table 1. Middle monthly air temperature and precipitation amount (Kraljevo)

Interval	Months									Average
	X	XI	XII	I	II	III	IV	V	VI	
Mean monthly air temperature (°C)										
2009/10	11.3	8.2	3.8	1.1	2.7	7.2	12.1	16.6	20.2	9.2
2010/11	9.2	11.1	2.7	0.3	0.6	6.6	12.2	15.6	20.4	8.7
Average	11.5	6.2	1.4	-0.5	2.3	6.5	11.7	16.2	19.1	8.3
Monthly sums of precipitation (mm)										Sums
2009/10	137.9	63.1	97.7	34.4	81.6	38.6	100.2	84.0	136.4	773.9
2010/11	93.6	34.1	64.9	28.1	59.2	48.9	37.1	82.9	71.7	520.5
Sums	53.1	55.7	54.8	44.0	42.8	44.7	65.1	74.9	86.5	521.6

The sum of rainfall precipitation in 2009/10 was higher by 226.4 mm, where the sum of rainfall in 2010/11 was 27.0 mm lower than the average of many years and with a very uneven distribution of precipitation per months. During the April in 2009/10 it was 100.2 mm of rainfall, what was 46.2 mm more compared with the perennial average. During the June in 2009/10 it was 136.4 mm of rainfall, what was 52.8 mm more compared with the perennial average. Regard the high importance of sufficient rainfall amounts during the spring months, particularly May for small grains production, the distribution and amount of rainfall over the growing season 2010/11 were considerably more favorable, what resulted with the increment of yields during that year. Based on the fact that sufficient amounts of rainfall in

these months are very important for the successful production of cereal crops it can be concluded that the years in which the researches were conducted were favorable for the barley growing.

Soil conditions

Agrochemical properties of the treated pseudogley are very unfavorable. Soil analyzes have been performed by standard chemical methods, as follows: pH in H_2O and KCl, electrometrically with a glass electrode in a 1:2.5 suspension; content humus by Kotzmann's method; total nitrogen has been determined by the Kjeldahl's method, available phosphorus and potassium by Egner-Riehm's Al method. The humus content in the surface layer of soil was low (2.18), and a substitution and total hydrolytic acidity were quite high (pH H_2O=5.24, KCl=4.48). The examined soil is characterized by very unfavorable composition of the adsorptive complex. The soil was medium provided with total nitrogen (0.14% N), while the available phosphorus content was low (under 7-8 mg/100 g of soil P_2O_5). When it comes to the content of easily available potassium the treated soil is well supplied, and it belongs to an average supplied soil class (13.8 mg/100 g of soil K_2O).

Results and discussion

Average values of yield (t/ha), test weight (kg/hl) and 1000 grain weight (g) at investigated winter barley cultivars grown at the experimental field of Secondary Agricultural-chemical school "Dr Djordje Radic" in Kraljevo (Serbia) during two growing seasons, 2009/10 and 2010/11, are presented in Table 2.

Table 2. Average values of investigated barley cultivars characteristics

Traits	Cultivar	2009/10			2010/11			Average		
		x	S	Sx	x	S	Sx	x	S	Sx
Grain yield (t/ha)	Rekord	4.238	0.444	0.199	5.118	0.630	0.282	4.678	0.692	0.219
	Grand	4.384	0.595	0.266	5.189	0.665	0.298	4.786	0.731	0.231
	NS 565	4.646	0.663	0.297	5.670	0.083	0.037	5.158	0.700	0.221
	Atlas	4.093	0.205	0.092	4.891	0.500	0.223	4.492	0.554	0.175
	Average	4.340	0.512	0.114	5.217	0.562	0.126	4.779	0.692	0.109
1000 grain weight (g)	Rekord	43.16	1.538	0.688	51.12	0.847	0.379	47.14	4.356	1.377
	Grand	41.55	1.980	0.886	50.09	0.660	0.295	45.82	4.711	1.490
	NS 565	46.24	1.228	0.549	54.21	0.619	0.277	50.22	4.299	1.360
	Atlas	36.90	2.216	0.991	42.04	2.086	0.933	39.47	3.384	1.070
	Average	41.96	3.828	0.856	49.36	4.742	1.060	45.66	5.670	0.896
Test weight (kg/hl)	Rekord	69.84	1.164	0.520	68.66	0.896	0.401	69.25	1.159	0.366
	Grand	64.26	1.910	0.854	61.42	0.952	0.426	62.84	2.065	0.653
	NS 565	69.52	2.349	1.050	68.31	0.370	0.165	68.91	1.709	0.540
	Atlas	66.64	3.155	1.411	65.62	0.396	0.177	66.13	2.187	0.692
	Average	67.56	3.126	0.699	66.00	3.041	0.680	66.78	3.145	0.497

The grain yield of winter barley significantly varied across years, from 4.340 t/ha in 2009/10 to 5.217 t/ha in 2010/11 (Table 2). During the first year of investigations, cultivar NS 565 achieved the highest grains yield (4.646 t/ha), followed by Grand (4.384 t/ha), while the lowest yield was at Atlas cultivar (4.093 t/ha). During the second year of investigations (2010/11), the yield of NS 565 cultivar was the highest with 5.670 t/ha. Average grains yield observed in the two-year period was the highest at NS 565 and Grand varietes (5.158 t/ha and 4.786 t/ha), while the lowest yield was obtained by Atlas cultivar (4.492 t/ha). Spring month April in 2009/10 were the surplus of precipitation, what affected unfavorable on the crops. Larger precipitation amounts during the spring months the growing season 2009/10 (March-May) caused rising groundwater levels and

flooding of agricultural land. The excessive amount of moisture influenced the poorer heading and filling of grain, lodging of crops, the abundance of the weeds and the intense occurrence of the disease in the examined triticale and other winter grains. Regard the high importance of sufficient rainfall amounts during the spring months, particularly May for small grains production, the distribution and amount of rainfall over the growing season 2010/11 were considerably more favorable, what resulted with the increment of yields during that year. Considerable variation in yield depending on years of research have established Bratković et al. (2014), Đekić et al. (2011), Jelić et al. (2014), Madić et al. (2009) and Popović et al. (2011).

During the first and second years of investigation, NS 565 cultivar achieved the highest average 1000 grain weight (46.24 g and 54.21 g) compared with other tested barley cultivars. Average 1000 grain weight observed in the two-year period was the highest at NS 565 variety (50.22 g), while the lowest was obtained by Atlas cultivar (39.47 g). The 1000 grain weight of winter barley significantly varied across years, from 41.96 g in 2009/10 to 49.36 g in 2010/11 (Table 2). Malting barley is expected to have 1000 grain weight of 40 and 46 g and test weight of 68 to 75 kg/hl (Paunović et al., 2006).

The test weight of winter barley significantly varied across years, from 67.56 kg/hl in 2009/10 to 66.00 kg/hl in 2010/11 (Table 2). Average test weight observed in the two-year period was the highest at Rekord variety (69.25 kg/hl), while the lowest was obtained by Grand cultivar (62.84 kg/hl). Generally, the test weight of barleys ranged from 52 to 72 kg/hl (Pržulj et al., 2004).

Table 3. Analysis of variance of the tested parameters (ANOVA)

Effect of year on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 1, 38	p-level
Grain yield (t/ha)	7.6852	0.28862	26.627**	0.000008
1000-grain weight (g)	547.9701	18.57118	29.506**	0.000003
Test weight (kg/hl)	24.3828	9.50939	2.564	0.117598
Effect of cultivar on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 3, 36	p-level
Grain yield (t/ha)	0.78790	0.45247	1.741	0.175910
1000-grain weight (g)	204.5709	17.77673	11.508**	0.000019
Test weight (kg/hl)	88.6510	3.32741	26.643**	0.000000
Effect of the year x cultivar interaction				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 3, 32	p-level
Grain yield (t/ha)	0.027561	0.266287	0.103	0.957419
1000-grain weight (g)	5.871396	2.324312	2.526*	0.075007
Test weight (kg/hl)	1.833289	2.809498	0.652	0.587227

**Statisticaly significant difference (P<0.05)* ***Statisticaly high significant difference (P<0.01)*

Table 3 shows the impact of the year, cultivar and interaction of year x cultivar on yield, 1000-grain weight and test weight. Analysis of variance was found highly significant effect of year on the yield (F_{exp}=26.627**) and 1000-grain weight (F_{exp}=29.506**). Analysis of variance in barley cultivars tested showed statistically highly significant differences in 1000-grain weight (F_{exp}=11.508**) and test weight (F_{exp}=29.506**) in relation to the growing season (P<0.01). Statistically significant difference in 1000-grain weight is determined under the influence of the interaction year x cultivar. Grain quality is a qualitative property influenced by the genetic factors and environmental factors, as well as their interaction, so the variety can behave in some years as beer or fodder/forage crop, which can be determined by the testing and

directed for a particular purpose (Pržulj et al., 2004; Paunovic et al. 2006; Madić et al. 2009; Popović et al. 2011; Bratković et al., 2014; Đekić et al., 2015).

Table 4. Correlation coefficients by studied environments in barley

Correlations between the traits analyzed in the 2009/10			
	Grain yield (t/ha)	1000-grain weight (g)	Test weight (kg/hl)
Grain yield (t/ha)	1.00	0.36^{ns}	-0.02^{ns}
1000-grain weight (g)		1.00	0.39^{ns}
Test weight (kg/hl)			1.00
Correlations between the traits analyzed in the 2010/11			
Grain yield (t/ha)	1.00	0.40^{ns}	0.18^{ns}
1000-grain weight (g)		1.00	0.30^{ns}
Test weight (kg/hl)			1.00

Correlation coefficients by studied environments in barley in the two growing season, are shown in Table 4. Positive and non significant correlations were found between the grain yield and 1000-grain weight in all growing season. Barley yield in 2009/10 was negatively correlated with test weight (Table 4). Negative correlations of yield and 1000-grain weight, seeds that are not significant, are confirmed in their study of barley by Dodig (2000).

Conclusion

Based on the gain results during the two-year investigation on four winter barley cultivars, it can be concluded that the grain yield of barley ranged from 4.492 t/ha (Atlas) to 5.158 t/ha (NS 565). More favourable conditions in 2010/11 resulted in a higher average the grain yield value (5.217 t/ha) compared to 2009/10 (4.340 t/ha). The average 1000-grain weight in the study was 45.66 g, with a variation of 41.96 g in the vegetation year 2009/10 to 49.36 g in the vegetation year 2010/11. During the 2010/11, statistically significantly higher grain yield per area unit, as well as 1000 grain weight was achieved, compared with 2009/10 year.

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THE STABILITY PROPERTIES OF TRITICALE PRODUCTION ON ACID SOIL

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Abstract

The study was carried out in a stationary field trial involving fertilization over a two (2010/11 and 2011/12) growing seasons, three varieties of winter triticale were investigated (Favorit, Trijumf and KG Tempo) at the Small Grains Research Centre in Kragujevac (in central Serbia). The following properties were analyzed: grain yield (t/ha), 1000 kernel weight (g), test weight (kg/hl) and chemical quality of grain. The grain yield of triticale significantly varied across years, from 3.773 t/ha in 2010/11 to 4.815 t/ha in 2011/12. The highest two-year average of grain yield (4.499 t/ha) and 1000-kernel weight (49.80 g) were obtained by KG Tempo cultivar. The highest two-year average of test weight (71.23 kg/hl) were obtained by Trijumf variety. The highest two-year average value of protein content was found in the variety Favorit (11.170%). Highly significant influence of the year on grain yield, 1000-kernel weight, test weight and protein content was established for investigated winter triticale cultivars by variance analysis. Triticale yield in 2010/11 and 2011/12 years was positively correlated with test weight and 1000-grain weight, but negatively correlated with protein content. Environmental conditions have had a significant effect on grain yield and quality in triticale. Grain yield shows a tendency to increase in the years having a higher total amount and better distribution of rainfall during critical plant development stages.

Keywords: *grain yield, protein, test weight, triticale*

Introduction

Triticale is cereal species gained by cross-breeding of wheat and rye. The yield per unit area is the result of the action of factors of variety fertility in interaction with environmental factors. Therefore, the yield is relative term and is determined by the variety, environmental conditions and the level of applied technology. Yield is largely dependent on the genetic potential, which could be defined as yield of variety which was grown in conditions on which it had adapted, with adequate amounts of water and nutrients and efficient control of pests, diseases, weeds and other stresses (Đekić et al., 2014; Milovanovic et al., 1998). Yields vary considerably primarily as a result of agro-ecological conditions during the growing season (Biberdžić et al., 2012; Đekić et al., 2010; Đurić et al., 2016; Janušauskaitė, 2013; Kendal et al., 2014; Kondić et al., 2012; Milovanović et al., 2012).

Triticale presented high tolerance regard the acid soils, as well as good productive results on sandy soils (Đekić et al., 2010; 2014). Soil acidity frequently affects agricultural production in Serbia. Triticale was planting where corn did not prosper, as well in the areas with moderate climate (Djekic et al., 2011). It could be said that it inherited very good up to excellent tolerance regard the most important pathogens and small grains pests. Triticale presented high adaptability on local agro-ecological conditions, and it was influenced on stabile yield reaching.

New perspective triticale lines and varieties has more and better filled grain, higher yield, grain mass and farinaceous content, while proteins and lysine was smaller compared with older varieties (Kendal et al., 2014; Milovanović et al., 2014).
The aim of this study was the determination of the cultivars and ecological environmental factors influence on differences in stability and adaptability of cultivars regard the grain yield, 1000 grains weight, test weight and protein content of tested winter triticale cultivars.

Materials and methods

Materials and field trials

The study was carried out in a stationary field trial involving fertilization over a two years. During the 2010/11 and 2011/12 growing seasons, three varieties of winter triticale were investigated (Favorit, Trijumf and KG Tempo) at the Small Grains Research Centre in Kragujevac (in central Serbia). The experiments were conducted in randomized block systems, with a plot size of 50 m^2 (5 m x 10 m) in five replications. The sowing was carried out using a machine with row spacing of 12.5 cm. The soil on which the trial was conducted was uniform and well prepared. The amount of seed per square meter amounted to 400-450 viable seeds, depending on the characteristics of varieties. It was sown in the third decade of October, with 400 kg/ha of fertilizer NPK 15:15:15, which was added in the fall, while during the spring fertilization soil was supplemented with 300 kg/ha (KAN 27%N).
The following properties were analyzed: grain yield (t/ha), 1000 kernel weight (g), test weight (kg/hl) and chemical quality of grain. Grain yields were measured for each plot and converted to yield tons per hectare on the basis of 14% grain moisture. The total protein content in the grain was determined by infrared spectroscopy technique on the apparatus Perten DA 7000 (NIR/VIS Spectro-photometer) employing non-destructive method.

Meteorological and soil conditions

This study was conducted over a two growin seasons in the Šumadija region, Central Serbia, on a Vertisol soil, at Kragujevac location, 173-220 m a. s. l. (44° 22′ N, 20° 56′ E), in a temperate continental climate having an average annual temperature of 11.5°C, typical of Šumadija districts in Serbia and a rainfall amount of about 550 mm. Kragujevac area is characterized by a moderate continental climate, which in general is characterized by uneven distribution of rainfall by month.

Table 1. Middle monthly air temperature and precipitation amount (Kragujevac)

	Months									
Interval	X	XI	XII	I	II	III	IV	V	VI	Average
	Mean monthly air temperature (^oC)									
2010/11	10.2	11.4	2.4	0.9	0.5	7.2	12.0	15.8	20.9	9.0
2011/12	10.4	3.1	4.6	0.7	-3.7	8.1	12.9	16.1	23.0	8.4
Average	12.5	6.9	1.9	0.5	2.4	7.1	11.6	16.9	20.0	10.5
The amount of precipitation (mm)										
2010/11	86.9	27.9	50.1	29.1	48.5	20.4	20.8	65.8	32.3	381.8
2011/12	33.3	1.3	43.3	117.2	60.1	5.7	74.5	87.3	57.8	480.5
Average	45.4	48.9	56.6	58.2	46.6	32.4	51.9	57.6	70.4	468.0

The data presented in Table 1 for the analyzed triticale growing season (2010-2012) clearly suggest differences in weather conditions between the years of the study and the long-term mean for the region. The average air temperatures were by 1.5^{o}C and 2.1^{o}C lower in 2010/11 and 2011/12, respectively, as compared to the long-term mean, whereas the sums of rainfall were by 12.5 mm higher in 2011/12 years and lower by 86.2 in 2010/11 years as compared to the long-term mean. Compared to the long-term mean, total rainfall values, especially in the

first year, second and third year, were considerably higher in February, April and May, whereas total rainfall in April 2010/11 decreased by 31.1 mm. Given the high importance of sufficient rainfall amount during the spring months, particularly April and May, for triticale production, the distribution and amount of rainfall over the growing season 2010/11 were considerably more favorable, resulting in increased yields in this year. Apart from the rainfall deficiency during the spring months and the non-uniform distribution of rainfall across months, an increase in average air temperatures was also observed.

The trial at the Small Grains Research Centre in Kragujevac (in central Serbia), was performed on soil that is characterized as smonitsa in process of degradation. The physical properties of the soil are very unfavorable and it belongs to the type of heavy clays. According to the analysis, this is a soil of medium acidity, poor in humus (2.65%), with a substitution and total hydrolytic acidity that were quite high (pH in H_2O=5.99, in KCl=4.91). The soil was moderately provided with total nitrogen (0.14% of N), it is poor in easily accessible phosphorus (P_2O_5=<12 mg/100 g of soil), a medium level of easily accessible potassium (K_2O=14-23 mg/100 g of soil) was recorded.

Statistical Analysis

On the basis of achieved research results the usual variational statistical indicators were calculated: average values. Experimental data were analyzed by descriptive and analytical statistics using the statistics module Analyst Program SAS/STAT (SAS Institute, 2000) for Windows. All evaluations of significance were made on the basis of the ANOVA test at 5% and 1% significance levels.

Results and discussion

Average values of grain yield, 1000 kernel weight, test weight and protein content at investigated Kragujevac's winter triticale varieties are presented in the Table 2.

The grain yield of triticale significantly varied across years, from 3.773 t/ha in 2010/11 to 4.815 t/ha in 2011/12. During the first year (2010/11), the grain yield of the variety KG Tempo was the highest with 4.125 t/ha, while the slightly lower yield was realized by the varieties Trijumf (3.676 t/ha) and Favorit (3.518 t/ha). During the second growing season is better (2011/12), the variety Trujumf achieved the highest yield of grain (5.042 t/ha), while the lowest yield was obtained by the varieties Favorit and KG Tempo (4.529 t/ha and 4.873 t/ha). Achieved statistically significantly higher yields in 2011/12 were, primarily, the result of heavy rainfalls and their good distribution as well as favorable air temperatures during the vegetation period (Table 1). Đekić et al. (2016), states that the air temperatures and the rainfall amount and distribution during the triticale growing season have the greatest impact on high yields and grain quality. Milovanovic et al. (2001), states that in the domestic production conditions, higher yields are achieved by varieties with shorter growing season because they manage to form the largest part of the yield before the advent of high temperatures.

The thousand kernel weight of winter triticale grain was variable, depending on environmental conditions. The second investigation year was significantly more favorable so that the average thousand kernel weight were higher (51.17 g) compared to the first year (46.24 g). Average thousand kernel weight observed in the two-years period were higher at KG Tempo variety (49.80 g), compared by Favorit and Trijumf cultivars (48.90 g and 47.41 g). Previously, many researchers have reported that the thousand kernel weight values of triticale genotypes have ranged from 23.9 g to 54.9 g (Kendal et al., 2014; Janušauskaitė, 2013; Đekić et al., 2014; Madić et al., 2018).

Table 2. Average values of investigated triticale varieties characteristics

Varieties	2010/11			2011/12			Average		
	$\bar{x}$	S	Sx	$\bar{x}$	S	Sx	$\bar{x}$	S	Sx
Grain yield (t/ha)									
Favorit	3.518	0.566	0.253	4.529	0.442	0.197	4.024	0.716	0.226
Trijumf	3.676	0.691	0.309	5.042	0.756	0.338	4.359	0.992	0.314
KG Tempo	4.125	0.528	0.236	4.873	0.560	0.250	4.499	0.647	0.205
Average	3.773	0.615	0.159	4.815	0.598	0.154	4.294	0.797	0.146
1000 kernel weight (g)									
Favorit	46.72	0.432	0.193	51.08	1.228	0.549	48.90	2.456	0.777
Trijumf	45.28	0.476	0.213	49.54	0.594	0.266	47.41	2.302	0.728
KG Tempo	46.72	0.844	0.377	52.88	0.192	0.086	49.80	3.297	1.043
Average	46.24	0.903	0.233	51.17	1.593	0.411	48.70	2.810	0.513
Test weight (kg/hl)									
Favorit	68.08	0.524	0.234	73.12	0.595	0.266	70.60	2.708	0.856
Trijumf	69.01	2.128	0.952	73.45	1.183	0.529	71.23	2.848	0.901
KG Tempo	70.03	1.319	0.590	71.77	1.339	0.599	70.90	1.553	0.491
Average	69.04	1.597	0.412	72.78	1.257	0.324	70.91	2.369	0.432
Proteins content (%)									
Favorit	12.120	0.669	0.299	10.221	0.895	0.400	11.170	1.248	0.395
Trijumf	11.684	0.617	0.276	10.100	0.333	0.149	10.892	0.957	0.302
KG Tempo	11.328	0.817	0.365	10.040	0.432	0.193	10.684	0.917	0.290
Average	11.711	0.735	0.190	10.120	0.566	0.146	10.915	1.034	0.189

Table 2 presents average values for grain test weight across years and varieties. The average values for test weight in all cultivars were as follows: 72.78 kg/hl in 2011/12 and 69.04 kg/hl in 2010/11, suggesting significant variations in test weight during the two years of the study. Average test weight observed in the two-years period was the highest at Trijumf variety (71.23 kg/hl), while the lowest yield was obtained by Favorit and KG Tempo cultivars (70.60 kg/hl and 70.90 kg/hl). Generally, the test weight of triticale ranged from 63 to 80 kg/hl (Barnett et al., 2006; Đekić et al., 2014; Janušauskaitė, 2013; Kendal et al., 2014).

The average crude protein content of triticale significantly varied across years, from 10.120% in 2011/12 to 11.711% in 2010/11 (Table 2). During the all years of investigation (2010/11 and 2011/12), the variety Favorit achieved the highest average protein content (12.120% and 10.221%) compared with other tested triticale varieties. Kendal et al. (2014) have recorded 13.3% up to 14.7% and Perišić et al. (2008), have recorded 14.6% of crude proteins in winter triticale. Djekic et al. (2011), in different winter triticale genotypes, have recorded values ranging from 11.23 to 13.87% of proteins.

Analysis of variance between observed traits of triticale

The analysis of yield variance, 1000-kernel weight, test weight and proteins content of tested winter triticale varieties grown at investigated Kragujevac's during two growing seasons 2010/11 and 2011/12, are shown in Table 3.

Based on the analysis of variance, it can be concluded that there are highly significant differences in grain yield in regard to the year of investigation (F_{exp}=22,1087**), while among the investigated triticale varieties the differences were not significant. Analysis of variance was found highly significant effect of year on the 1000-kernel weight (F_{exp}=108.5666**), test weight (F_{exp}=50.8244**) and protein content (F_{exp}=44.1446**).

Table 3. Analysis of variance of the tested parameters (ANOVA)

Effect of year on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F (1, 28)	p-level
Grain yield (t/ha)	8.1370	0.368045	22.1087**	0.000063
1000-kernel weight (g)	182.0403	1.676762	108.5666**	0.000000
Test weight (kg/hl)	104.9070	2.064107	50.8244**	0.000000
Protein content (%)	18.9767	0.429875	44.1446**	0.000000
Effect of cultivar on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F (2, 27)	p-level
Grain yield (t/ha)	0.59682	0.638837	0.934231	0.405221
1000-kernel weight (g)	14.57033	7.401815	1.968481	0.159207
Test weight (kg/hl)	0.99300	5.952445	0.166822	0.847216
Protein content (%)	0.59457	1.104593	0.538274	0.589890
Effect of the year x cultivar interaction				
Traits	Mean sqr Effect	Mean sqr Error	F (2, 24)	p-level
Grain yield (t/ha)	0.240172	0.359636	0.667820	0.522098
1000-kernel weight (g)	2.858333	0.503833	5.673172**	0.009603
Test weight (kg/hl)	7.725000	1.681625	4.593771*	0.020457
Protein content (%)	0.234173	0.432458	0.541493	0.588822

Based on the analysis of variance, it can be concluded that there are not significant differences in grain yield, 1000-kernel weight, test weight and protein content at investigated triticale varieties were found relative to the cultivar of investigation (Table 3). The interaction of the investigated factors (Y x G) exhibits are not statistically significant affect in grain yield and protein content ($p>0.05$), but statistically significant affect in 1000-kernel weight and test weight. The present results confirm the opinion of many authors that the traits analyzed are genetically determined but are strongly modified by the environment and weather conditions (Đekić et al., 2014, 2016; Jelić et al., 2013).

Table 4. Correlations between the traits analyzed

Correlations between the traits analyzed in 2010/2011				
Traits	Grain yield	1000-grain weight	Test weight	Protein content
Grain yield (t/ha)	1.00	0.05	0.17	-0.03
1000-grain weight (g)		1.00	-0.48*	0.09
Test weight (kg/hl)			1.00	-0.03
Protein content (%)				1.00
Correlations between the traits analyzed in 2011/2012				
Grain yield (t/ha)	1.00	0.12	0.23	-0.06
1000-grain weight (g)		1.00	0.06	-0.11
Test weight (kg/hl)			1.00	0.14
Protein content (%)				1.00

Correlations between the analysed traits

The correlations of yield, 1000-kernel weight, test weight and proteins content of tested winter triticale varieties grown at investigated Kragujevac's during two growing seasons 2010/11 and 2011/12, are shown in Table 4. Triticale yield in 2010/11 and 2011/12 years was positively correlated with test weight and 1000-grain weight, but negatively correlated with protein content. Nagatively but significantly correlated with 1000-grain weight and test weight (-0.48*) as shown in Table 4.

Conclusion

The highest yield of grain, weight of 1000 grains and test weight in all triticale varieties were in the vegetation period with moderate temperatures at the time of grain filling and large amount of precipitation in the first vegetation period. Grain yields in triticale cultivars ranged from 4.024 t/ha (Favorit) to 4.499 t/ha (KG Tempo). The average 1000 kernel weight in the study was 48.70 g, with a variation of 46.24 g in the vegetation year 2010/11 to 51.17 g in the vegetation year 2011/12.

Based on the results of the research it can be concluded that a greater number of traits have a decisive role in the formation of grain yield. The contribution of each individual trait may be different in various genotypes and in various environmental conditions so that the correlation between two quantitative traits is not fixed.

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EFFECT OF LIGHT CONDITIONS ON THE TERRESTRIAL MICROALGAE GROWTH RATE DYNAMICS

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Abstract

The possibilities of using algae biomass are numerous, from production bioactive compounds for medical and pharmaceutical industries to their use in agriculture as a biopesticides, biostimulants and biofertilizers. Algal cell growth is significantly affected by light, temperature, aeration, medium and carbon source but also habitat area. The aim of this research was to investigate the effects of different light conditions on algal growth stimulation. Natural day/night (D/N) cycle and continuous artificial lighting (CAL) below 950 lux were tested. Microalgal strains were isolated from two acid soils in Vojvodina, dystric cambisol (pH 4.31) and vertisol (pH 5.50) using BG11 medium. Six selected monoalgal cultures of the genus *Chlorella* (strains C63, C71 and C72), *Dictyosphaerium* (strains D36 and D37), and *Tribonema* (strain T50) were chosen for testing. The growth curve was observed for 24 days. Determination of growth kinetics was monitored spectrophotometrically by measuring the optical density (OD) on the spectrophotometer (Unicam SP600 Series 2, Cambridge England) on 750 nm. The results showed that there was no significant difference in terms of growth rate. Algal strain T50 showed significantly better growing dynamics under CAL relative to D/N cycle. On the other side, under CAL, two *Chlorella* strains (C63 and C71) showed faster initial growth and entered the exponential phase sooner, compared to other examined strains. Faster initial growth indicated better physiological adaptation of the cell metabolism to growth in induction (lag) phase. Grown in their natural environment, photoautotrophic microorganisms showed stable increase in the levels of enzymes and metabolites involved in cell division and carbon fixation.

Keywords: *Terrestrial microalgae, light conditions, spectrophotometry, optical density.*

Introduction

Microalgae biopesticides have a lot of advantages in comparison to the chemical ones. Biologically active products containing beneficial microorganisms can improve soil fertility as well as control plant diseases. Technology for the commercial production of microalgae is economically feasible but there are a lot of potential risks throughout the production process. In order to use microalgae in an industrial process, it must comply to some basic characteristics: a) it must be able to grow in a simple and cheap medium, mineral if possible, preferably with growth factors only (vitamins, nucleotides, micro-elements and acids), b) microalgae should be able to grow rapidly in the medium. c) the products should not include toxic or undesirable materials, especially if they are destined for human consumption and should be suitable for product harvest at the end of the fermentation, and d) with physiological requirements that protect them against contaminants. Different algaes require certain environmental factors for growth. Changes in growth conditions (light and temperature) or nutrient media characteristics can impact on quantity and quality of cells (Liu et al., 2008). The environmental factors affect photosynthesis and growth rate of the algae, cell size, biochemical composition and nutrient requirements. Due to their wide range of potential uses, there is a need to conduct a controlled study in order to establish positive conditions for efficient growth of algal biomass. Direct measurement methods of algal cells are problematic for small volume systems and it is time consuming. Optical density is one of

the simplest methods of measuring cell growth. Optical density at 750nm (OD_{750}) is widely used to monitor algal growth, being inexpensive and reliable (Chioccioli et al., 2014). The energy for photosynthesis is delivered in the form of light. Photosynthesis is a process of sunlight energy conversion in which inorganic compounds and light energy are converted to organic matter by photoautotrophs. Photosynthetic microalgae are directly or indirectly dependent on light as a source of energy necessary for their growth (Masojidek et al., 2013). The most common artificial light sources used in microalgae biotechnology are fluorescent and white-LED with average luminous efficacy of 50-100 and 25-64 lm·W-1, respectively (Blanken et al., 2013). In this experiment the effect of light was evaluated, as one of the main environmental factor, on the growth of microalgae in small scale cultivation system under controled laboratory conditions.

Material and Methods

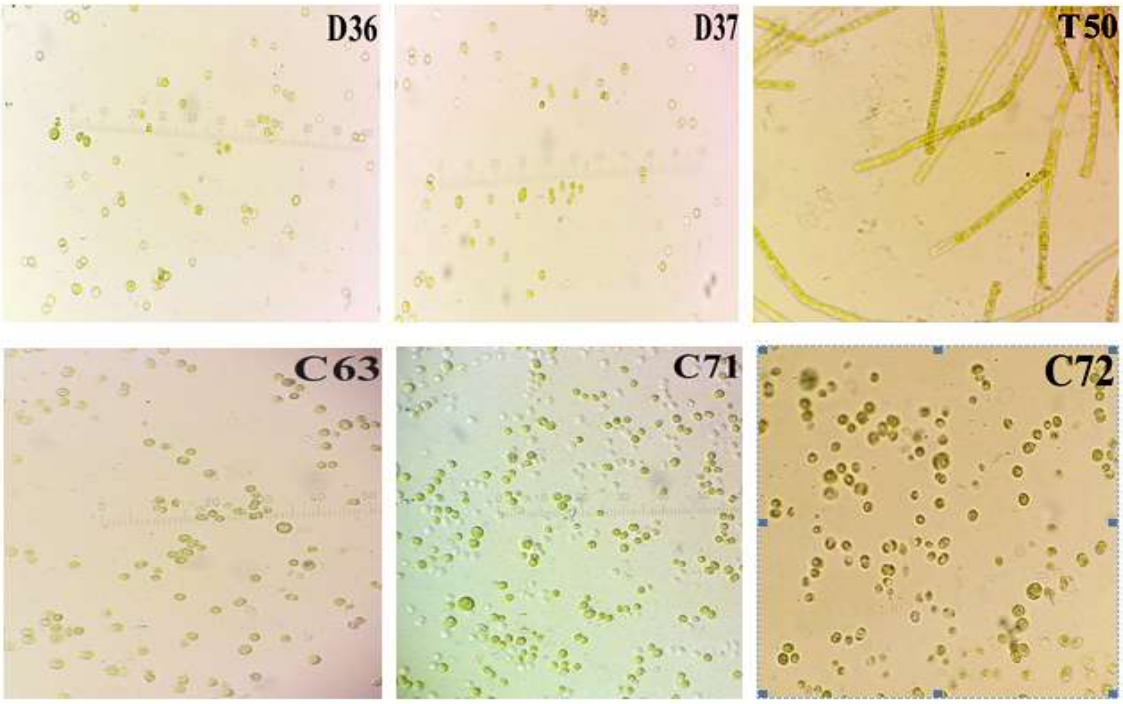
The microalgae species were isolated from acidic arable soils in Vojvodina, Serbia, dystric cambisol (pH 4.31) and vertisol (pH 5.50) using BG11 medium. BG11 medium per one liter of the distilled water contains: 1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.036 g $CaCl_2 \cdot 2H_2O$, 0.006 g Citric acid, 0.006 g Ferric ammonium citrate, 0.001 g EDTA, 0.02 g Na_2CO_3 and 1ml Trace metal solution A5. The composition of the trace metal mix solution is: 2.86 g H_3BO_3, 1.86 g $MnCl_2 \cdot 4H_2O$, 0.22 g $ZnSO_4 \cdot 7H_2O$, 0.39 g $Na_2MoO_4 \cdot 2H_2O$, 0.08 g $CuSO_4 \cdot 5H_2O$, 0.05 g $Co(NO_3)_2 \cdot 6H_2O$. The pH of BG11 medium was adjusted to 7.1 and autoclaved at 121ºC. To demonstrate the effect of light on algal growth two different light regimes were chosen, natural day/night (D/N) cycle and continuous artificial illumination (CAL) of 950 lm. BG11 medium ($150cm^3$) were inoculated with six selected monoalgal cultures (1 ml). Three microalgae species are from the genus *Chlorella* (strains C63, C71 and C72), two of the genus *Dictyosphaerium* (strains D36 and D37), and strain T50 of the genus *Tribonema*. Taxonomic identification was performed on the basis of morphology and cell size. The experiment was carried out at room temperature (about 24 ºC). Growth curve was observed for 24 days, daily for ten days and later every third day to the end of the experiment. Determination of growth kinetics was monitored spectrophotometrically by measuring the optical density (OD) on the spectrophotometer (Unicam SP600 Series 2, Cambridge England) on 750 nm which is above chlorophyll absorption and outside photosynthetically active radiation (PAR).

Results and Discussion

Microalga *Dictyosphaerium sp.* (Picture 1, D36 and D37) was identified as promising microorganism for biotechnological production of exopolysaccharides (Kumar et al., 2017). Thallus colonial or singlecelled, planktonic, adult cells oval or broadly ellipsoid to spherical, 2–8 μm. The genus *Dictyosphaerium* formerly consisted of 16 species (Comas, 1996; Bock et al., 2011).

Tribonema species (Picture 1, T50) are reported from all over the world in all kinds of freshwater, marine and terrestrial habitats (e.g. Europe, North America, South America, Japan, India, Africa (Lokhorst, 2003), but also in Serbia. Cell wall shape varies from cylindrical with indistinct H-shaped segments (more usual in the smaller species) to slightly inflated with distinct H-shaped segments more apparent in the bigger *Tribonema* species.

Chlorella is a small green microalga (Picture 1, C63, C71 and C72). Cell size is 2-10 μm in diameter and has a high content of about 51-58% protein, 12-17% carbohydrates and 14-22% lipid (Spolaore et al., 2006). More than 100 *Chlorella* species have been named since the description of the type species *Chlorella vulgaris* Beijerinck in 1890. These taxa have been described from freshwater, marine, and edaphic habitats or as endosymbionts (Bock et al., 2011).



Picture 1. Microscopic view of six microalgae strains used in the experiment, genus *Dictyosphaerium* (D36 and D37), genus *Tribonema* (T50) and genus *Chlorella* (C63, C71 and C72), respectively (magnification 40x).

A response to D/N cycle and CAL of six microalgae strains isolated from terrestrial habitats was compared. Outside the visible spectrum in the infrared area on wavelength of 750 nm the density of the algal cell growth was measured. The results showed significant differences in terms of algal strains growth rate between the two light sources. Genus *Tribonema* (Fig. 1) showed significantly better growing dynamics under CAL, relative to D/N cycle. It could be due to its shape, because it was the only filamentous strain involved in the assay, therefore reaction to the different light exposure regimes varied in relation to other strains. The specific growth rate is mainly dependent on algal species, light intensity and temperature (Carlsson et al., 2007).

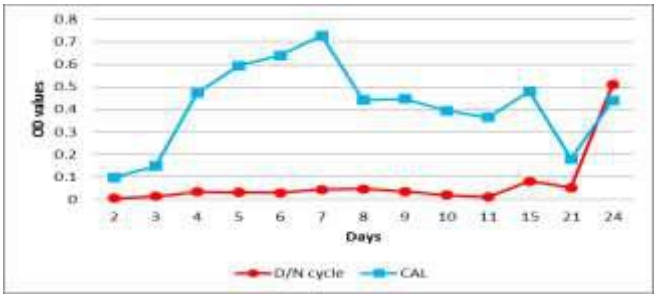


Fig. 1. Growth diagram of T50 monitored for 24 days under D/N cycle and CAL.

Terrestrial microalgae are accustomed to natural lighting with a day and night shift. Grown in their natural environment, photoautotrophic microorganisms show stable increase of the levels of enzymes and metabolites involved in cell division and carbon fixation. Under the CAL, all of three *Chlorella* strains (C63, C71 and C72) showed faster initial growth and entered the exponential phase sooner, compared to other investigated strains (Fig. 2, 3 and 4). Faster initial growth indicates better physiological adaptation of the cell metabolism to growth in induction (lag) phase. These strains proved to be the most resistant, although it is a terrestrial species that naturally develops in the scarcity of light. Light is the source of energy and light

intensity plays an important role that requirement to light substantially dependent on the culture depth and the density of the algal culture (Barsanti and Gualtieri, 2014). The optical density increases with higher light intensity. According to Fatemeh and Mohsen (2016), the best growth of *C. vulgaris* was obtained in lux 650 as well as OD values indicated rapid growth with addition amount of lux.

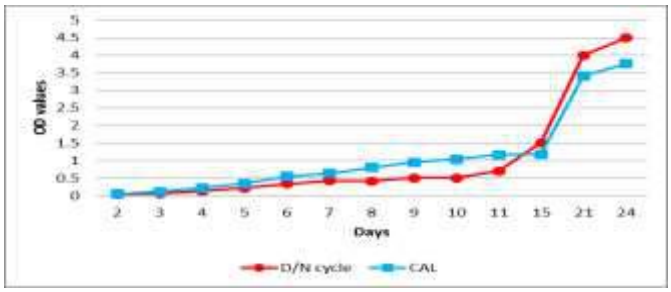


Fig. 2. Growth diagram of C63 monitored for 24 days under D/N cycle and CAL.

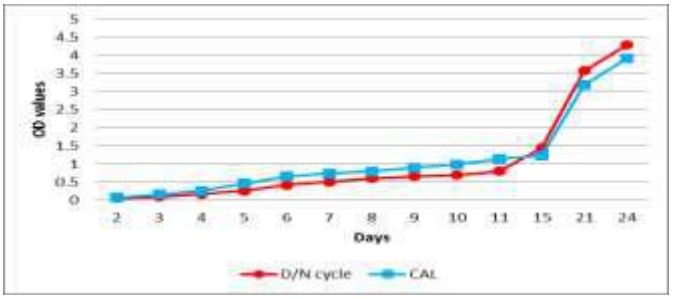


Fig. 3. Growth diagram of C71 monitored for 24 days under D/N cycle and CAL.

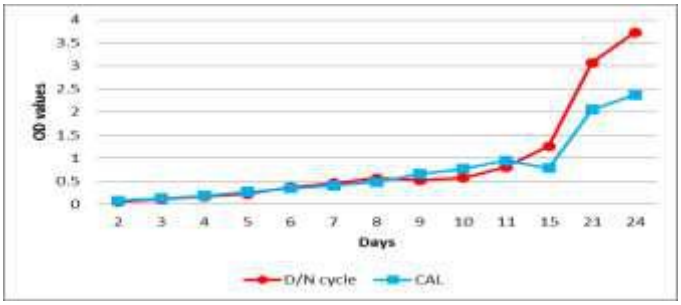


Fig. 4. Growth diagram of C72 monitored for 24 days under D/N cycle and CAL.

Round and coccoid shape strains showed slower but uniformous entry into the exponential phase and greater total OD values at the end of the examined period under natural lighting. As well as in the previous case, strains of the genus *Dictyosphaerium* showed uniform growth in both cases (under D/N cycle and CAL) but at the end of examined period Fig. 5 and 6 shows greater OD values strains cultivated under the D/N cycle. Light intensity and photoperiod are the most important factors for growth rate and biomass composition (Khoeyi et al., 2012). In phase of declining growth rate, many factors such as; nutrients, light, pH, carbon dioxide or other physical and chemical factors limited the microalgae growth and the cell division to be down slow (Carlsson et al., 2007).

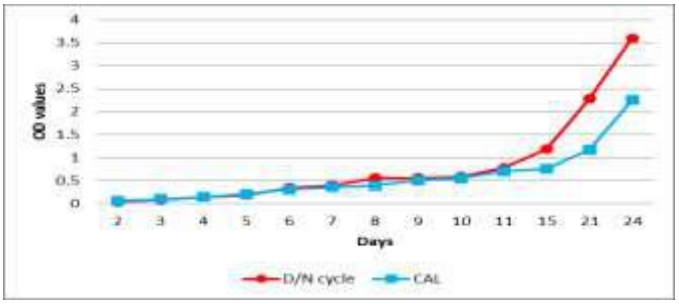


Fig. 5. Growth diagram of D36 monitored for 24 days under D/N cycle and CAL.

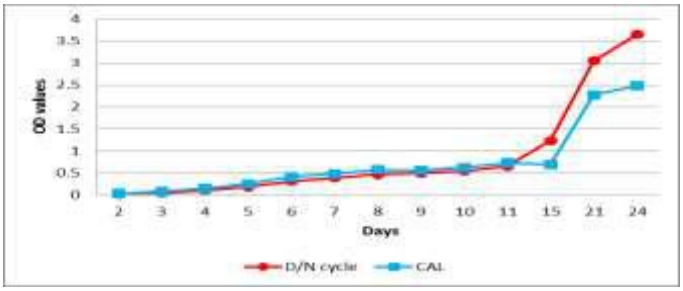


Fig. 6. Growth diagram of D37 monitored for 24 days under D/N cycle and CAL.

Conclusions

A microalgae with optimum growth and productivity at moderate light intensity has great advantage over others. In general, artificial light positively influences algal growth, but in order to achieve economically optimal growth efficiency, rather than maximum efficiency, it is advisable to use natural light conditions in algal biomass production, both in small and large scale cultivation systems.

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EFFECTS OF LOW TEMPERATURES ON CABERNET SAUVIGNON AND SAUVIGNONE BLANC CV. RESISTANCE GROWN IN CONDITIONS OF OPLENAC (TOPOLA MUNICIPALITY IN SERBIA)

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Abstract

In vineyard of King Peter I Karadjordjevic-Royal Winery at Oplenac-Topola municipality in Serbia and laboratories of the Faculty of Agriculture at the University of Belgrade, varieties Cabernet sauvignone and Sauvignone blanc were examined on winter degree bud freezing. The aim of research was to test Cabernet Sauvignon and Sauvignon blanc cultivars during two years on degree resistance to low negative temperatures. Test carried out with one-year-old shoots in chamber, in three terms on december 15th, january 15th and february 15th at three different temperatures -15, -25 and -20°C. Conditions in chamber were identical to the external conditions. The highest resistance was shown for both varieties in the second testing term. Sauvignone blanc had the highest percent of partially frozen buds in first term, while for Cabernet sauvignone the highest percent of partially frozen buds was recorded in the third term. Statistical significance founded for percent of partially frozen buds were it was significant influence of interaction of variety*treatment.

Key words: *resistance, buds, freezing, Cabernet Sauvignon, Sauvignone blanc*

Introduction

Grapevine is one of the most important agricultural crops, with 56% of world's largest area is the most spread in Europe (Casstelluci, 2013). In overall assortment Cabernet Sauvignon is one of the most widespread red wine varieties. Due to expressed ecological adaptability were cultivated variety in 43 world countries, while Sauvignon blanc is cultivated in 31 countries. By statistical data percent of areas which is covered by Cabernet Sauvignon is the biggest overall cultivated assortment in the world and occupies the first place, and Sauvignone blanc is on fourth place (Fregoni, 2010).

Frost is limiting abiotic factor for grapevines growing. The greatest damage vines had from winter frosts, while minor damage is caused by early autumn and late spring frosts. Temperature decreasing during autumn and optimum shoot preparation affect good frost resistance (Jackson, 2008; Nakalamić and Marković, 2009).

Vine resistance to low winter temperatures is required with genetic basis and variety origin, growing condition, selection and terrain exposure, choice of variety, applied growing tecnology, etc. In order to increase variety resistance to low winter temperatures, it is important late budbreake, high fertility and regeneration buds capacity in case of freezing (Marković, 2012).

By Žunić et al. (2010) the highest resistance to low winter temperatures is characteristic for varieties from *Proles occidentalis,* which also belong varieties Cabernet sauvignon and Sauvignon blanc. Clones of these varieties had special resistance to low winter temperatures. On the other hand, long-term frosts can cause significant buds damage, which can influence grape (Ribereau-Gayon et al., 2006) and wine quality, but also growing in the current year and especially the young shoots (Lisek, 2009).

Material and methods

Experiment carried out (2009/10 and 2010/11 year) in vineyard of King Peter I Karađorđević foundation at Oplenac (Royal Winery at Oplenca). Vineyards with Cabernet sauvignon and Sauvignon blanc are situated at altitude of 250 m above sea level, belonging to Sumadijsko-velikomoravski vine region in Serbia. Vineyard planted with Sauvignon blanc (clone R 3) covers area of 3.7 ha, located on cadastral parcel KP 730/2 which belongs to cadastral municipality of Bozurnja. Geographically is positioned on GPS coordinates N 44° 14' 4" and E 20° 41' 15". Research on Cabernet sauvignon (clone ISV-FV 5) was carried out on cadastral parcel KP 629, cover area of 0.55 ha geographically is positioned on GPS coordinates N 44° 14' 35" and E 20° 41' 22". In both cases was formed training system with Guyot pruning system, spacing between rows is 2.70 m and 1 m in row.

Test of buds resistance to low temperatures was carried out exposing one-year-old shoots to low temperatures in chamber. On selecting of freezing regime aim was that conditions for cooling in chamber must be similar to external conditions.

For testing from vineyard was sampled representative sample of 10 shots with 10 buds. Shoots were transferred to chamber where they were subjected to temperature of -5°C for 24 hours. After 24 h temperature in chamber was reduced every hour for 3°C to final temperature. At that temperature samples were kept for 12 hours, after which they taken out of chamber and left at room temperature 5 days. After five days by making a cross-section of buds was examined level of freezing of central and second bud. Number and percent of non frozen, partly frozen and completely frozen buds was determined.

Resistance of grapevine buds is not same during whole winter period, test was carried out in three terms during 2009/10 and 2010/11: December 15th, January 15th and February 15th at three different temperatures -15, -25 and - 20°C whereby to determine resistance degree of Sauvignon blanc and Cabernet Sauvignon at the beginning, mid and late winter period.

The IBM SPSS Statistics 2.0 (statistical package for social sciences), Chicago, IL, USA was used for data analysis. For factor influence was used analyze of variance with fixed factors level. In tests a significance level of 0.05 was used. For post-hoc comparisons, Tukey HSD and LSD test were used.

Results and discussion

In 2009/2010 research period both varieties had the highest resistance to low temperatures in the second test period (15.1.). In this term the highest was recorded the bigest percentage non froze buds. After this term Cabernet sauvignon had the highest resistance to frost in the third term-15.2. and finally in the first term-15.12. Sauvignon blanc had opposite variation trend of resistance. After the second term, the first term-15.12 was followed and finally was the third term 15.2. (table 1). During 2010/2011 period, both varieties had same variation trend of resistance to low temperatures, with difference that Sauvignon blanc had a higher percentage of non frozen buds in first term-84%, then in the second-73% and finally in the third term of 43%. Markovic (2001) states that in resistance examination of Cabernet sauvignon buds to freezing, percent of non-frozen buds increasing from december to mid-January. Resistance degree, i.e. percent of non-frozen buds was in a positive correlation with dry matter content in shoots, while percent of frozen buds was in correlation with free water content in shoots.

Wolf and Cook (1994) emphasize that, with adequate temperature regime and monitoring of temperature decreasing in chamber effect of froz as well as in environment can be simulated, and in this way determine resistance degree of variety to low temperatures. By two-factor analysis of variance was founded that in both research year was influence of treatment and interaction of variety*treatment. Therefore, there are further specially compared varieties especially for every treatment and treatments for each variety. This comparison (table 2-3) was carried out by a single-factor analysis of variance (in the comparison of varieties).

Table 1. Average value of percent of non frozen buds

Year	Variety	Term of treatment			Average
		15.12.	15.1.	15.2.	
2009/10.	Cabernet sauvignon	48.00	70.00	64.00	60.67
	Sauvignon blanc	60.00	77.00	39.00	58.67
	Average	54.00	73.50	51.50	59.67
2010/11.	Cabernet sauvignon	35.00	74.00	58.00	55.67
	Sauvignon blanc	84.00	73.00	43.00	66.67
	Average	59.50	73.50	50.50	61.17

Table 2. Two-factor variance analysis for 2009/2010. and 2010/2011. year

Year	Sours of variance	Variance	F	*p* value
2009/10.	Variety	60.000	0.201	0.656
	Treatment	2901.667	9.732	0.000
	Variety*Treatment	2015.000	6.758	0.002
2010/11.	Variety	1815.000	6.849	0.011
	Treatment	2686.667	10.138	0.000
	Variety*Treatment	5660.000	21.358	0.000

p=0.05

Table 3. Two-factor variance analysis for treatments for 2009/2010. and 2010/2011. godinu

Year	Term of tretament	Differences	Variance	F	*p* value
2009/10.	15.12.	Between groups	720.000	3.273	0.087
		Inside group	220.000		
	15.1.	Between groups	245.000	0.512	0.483
		Inside group	478.000		
	15.2.	Between groups	3125.000	15.935	0.001
		Inside group	196.111		
2010/11.	15.12.	Between groups	12005.000	65.681	0.000
		Inside group	182.778		
	15.1.	Between groups	5.000	0.015	0.904
		Inside group	336.111		
	15.2.	Between groups	1125.000	4.074	0.059
		Inside group	276.111		

p=0.05

Statistically significant difference between Cabernet sauvignon and Sauvignon blanc was at 15.2. 2009/2010. and 15.12. 2010/2011. years. In both treatments, Sauvignon blanc had higher percent of non-frozen buds compared to Cabernet sauvignon for same period.

In period 2009/10. at Sauvignon blanc was statistically significant difference between following terms: 15.12. and 15.1, while 15.2. does not differ from these two dates. In same period at Cabernet sauvignon was statistically significant difference for term of 15.2. in relation to two remaining dates (which do not differ).

In 2010/11. for Sauvignon blanc was founded statistically significant difference between 15.12. dates and two remaining dates. For Cabernet sauvignon 15.2 is different. from two remaining dates.

During 2009/10 Cabernet sauvignon had the highest percentage of partly frozen buds (36%) in period from 15.12., while Sauvignon blanc had in the third term (15.2), where was determined 45% partly frozen buds (table 4). In 2010/11 was recorded same variation trend.

Using two-factor variance analysis for 2009/2010. and 2010/2011. was faunded same trend variety*treatment interaction (table 5-6).

Table 4. Average percent of partially frozen buds

Year	Variety	Term of treatment			Average
		15.12.	15.1.	15.2.	
2009/10.	Cabernet sauvignon	36.00	17.00	16.00	23.00
	Sauvignon blanc	29.00	15.00	45.00	29.67
	Average	32.50	16.00	30.50	26.33
2010/11.	Cabernet sauvignon	37.00	17.00	16.00	23.33
	Sauvignon blanc	9.00	20.00	32.00	20.33
	Average	23.00	18.50	24.00	21.83

Table 5. Two-factor variance analysis for 2009/2010. and 2010/2011. year

Year	Sours of variance	Variance	F	*p* value
2009/10.	Variety	666.667	3.571	0.064
	Treatment	1621.667	8.688	0.001
	Variety*Treatment	1901.667	10.187	0.000
2010/11.	Variety	135.000	135.000	0.409
	Treatment	171.667	0.882	0.420
	Variety*Treatment	2555.000	13.127	0.000

p=0,05

Table 6. Two-factor variance analysis for treatments for 2009/2010. and 2010/2011.

Year	Term of tretament	Differences	Variance	F	*p* value
2009/10.	15.12.	Between groups	245.000	1.182	0.291
		Inside group	207.222		
	15.1.	Between groups	20.000	0.077	0.784
		Inside group	258.889		
	15.2.	Between groups	4205.000	44.787	0.000
		Inside group	93.889		
2010/11.	15.12.	Between groups	3920.000	30.678	0.000
		Inside group	127.778		
	15.1.	Between groups	45.000	0.162	0.692
		Inside group	278.333		
	15.2.	Between groups	1280.000	7.200	0.015
		Inside group	177.778		

p=0,05

During 2009/10 both varieties had the highest percent of total frozen buds in period from 15.2., to Sauvignon blanc determined 20% and at Cabernet sauvignon 16% (table 7). Sauvignon blanc showed in all three research periods a low tolerancy to winter temperatures, with a higher percent of frozen buds compared to Cabernet sauvignon.

In 2010/11, percent of total frozen buds to Cabernet sauvignon was significantly lower than Sauvignon blanc. The highest percent of freezing was recorded at 15.2, while in Sauvignon blanc significant freezing was recorded in period from 15.12. and 15.2.

By Lisek (2009; 2012) Sauvignon blanc in conditions of Poland is very sensitive to frost. High frequency of long-lasting frosts causes to Sauvignon blanc up to 96% of total frozen buds, which makes this variety less suitable for growing under long and frequent winter frosts. Cabernet sauvignon shows possibility of adaptation to different climatic conditions as evidenced by fact that it is cultivated in most important world areas, where average annual temperature varies by average of 3.5°C, which for most wine varieties is not adequate (Jones, 2010; Ruml et al., 2012; Markovic et al., 2016 and Vuković et al., 2016).

According to Maletic et al. (2012) variation of agrobiological and technological characteristics of Cabernet sauvignon and Sauvignon blanc is consequence of large varietal

variation and result large number of isolated clones. Variety divergence is expressed to Cabernet sauvignon with more than thirty clones of this variety separated. One part is introduced and cultivated in Serbian vineyards (Žunić et al, 2012).

Table 7. Average percentage of total frozen buds

Year	Variety	Term of treatment			Average
		15.12.	15.1.	15.2.	
2009/10.	Cabernet sauvignon	16.00	13.00	20.00	16.33
	Sauvignon blanc	11.00	8.00	16.00	11.67
	Average	13.50	10.50	18.00	14.00
2010/11.	Cabernet sauvignon	28.00	9.00	26.00	21.00
	Sauvignon blanc	7.00	8.00	25.00	13.33
	Average	17.50	8.50	25.50	17.17

By dual factor analysis of variance did not faunded interaction between variety and treatment, only effect of treatment existed (table 8). In period 2010/11 (15.12) was interaction of variety*treatment.

Table 8. Two-factor variance analysis for 2009/2010. and 2010/2011. year

Year	Sours of variance	Variance	F	*p* value
2009/10.	Variety	326,667	3,885	0,054
	Treatment	285,000	3,390	0,041
	Variety*Treatment	1,667	0,020	0,980
2010/11.	Variety	881,667	6,340	0,015
	Treatment	1446,667	10,402	0,000
	Variety*Treatment	666,667	4,794	0,012

p=0,05

Table 9. Two-factor variance analysis for treatments for 2009/2010. and 2010/2011.

Godina	Merenje	Variance	F	*p* value
2010/11. godina	15.12.	2205,000 109,444	20,147	0,000
	15.1.	5,000 69,444	0,072	0,791
	15.2.	5,000 238,333	0,021	0,886

p=0,05

Conclusion

In research period 2009/2010 and 2010/2011, both varieties had the highest resistance to low temperatures in the second test period (15.1.). In this term was recorded the highest percent of non frozen buds. Sauvignon blanc had the highest percent of frozen buds in period from 15.12, while at Cabernet sauvignon the largest percent of partially frozen buds was recorded in the third test period (15.2.). The highest percent of frozen buds for both varieties was determined at 15.2. In both years of research there was treatment interaction.

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BANANA FIBER FROM CANARY ISLANDS: SCIENCE AND EXTRACTION

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Abstract

Natural fiber composites are nowadays used in various engineering applications to increase the strength of a product as well as optimize its weight and cost. One such natural fiber which can be used as a reinforcement material is banana fiber. Banana fiber is produced from the leaves that make up the pseudo-stem of the plant, that is, its stalk or trunk. In the same way as other natural fibers, they contain five basic chemical components, namely cellulose, hemicellulose, lignin, pectin, and certain extractable compounds (vegetable fat, proteins and inorganic salts). Although the Canary Islands is a significant producer and consumer of bananas, the fiber which the plant produces has not been put to much use at all, and, thus, there has been little research into it. The main sectors in which the fiber does have a potential use are those of textiles, paper and plastic. This paper will briefly describe the science of banana fibers and review various methods of banana fiber extraction which are already in existence. The first method is a manual one. There are two chemical methods that can be used to make banana bast fiber. The first comprises an acid treatment of the banana stalk, and the second consists in a basic treatment with lime. Another method use steam explosion-based degumming. Another way of obtaining banana fibers is by employing the biological process of retting. Finally, there is also a variety of machines that can be used to extract banana fibers.

Keywords: *Waste, banana, fiber, extraction, Spain.*

Introduction

Banana fiber is produced from the leaves of the banana tree. It is a bast fibre and based on its dry weight, it is composed of cellulose (40-90), semicellulose (1-30), lignin (1-35), pectin (1-2) and water soluble matter, namely lipids, proteins and inorganic salts) (2-3 weight %) (Wu *et al*., 2005; Albinante *et al*., 2012). Lignin is one of the most abundant natural polymers and acts as a cementing agent to bind the matrix of cellulose and semicellulose fibers together into a rigid structure. Due to its chemical structure, it being a complex non-carbohydrate aromatic polyphenol (Figure 1), it is highly resistant to decomposition and oxidation (Albinante *et al*., 2012). In their study, Ganan *et al*. (2004a) describe the hierarchical microstructural ordering present in banana fiber bundles in both the bundle surface and the inner region. The chemical composition of the banana fiber bundles was investigated using a FTIR spectroscopy. It was observed that the main structures, which form the outer walls of the bundle, are composed of hemicellulose and pectins. In addition, hierarchical helicoidal ordering on the bundle surface as well as orientation on the longitudinal axis of the bundle were observed using Optical Microscopy (OM) and Scanning Electron Microscopy (SEM), for 3-4 mm surface fibers and 10-15 mm inner elementary fibers, respectively (Ganan *et al*., 2004a).

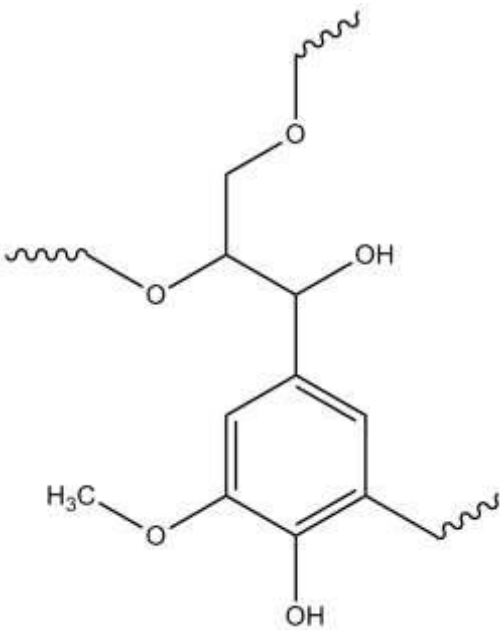

Figure 1. Phenyl propane units of lignin.

The following studies demonstrate the wide range of possibilities that already exist for exploiting banana fiber. Yan and Lin (2005) describe a process for producing paper and paper products, which possess high tensile strength, high bending strength, good water resistance and high density while Burton (2008) refers to a technique which involves laying cross-fibers across the grain of a paper sheet, to increase tear resistance. Nachane and Shanmugam, (2014a) talk about a carding machine which is capable of efficiently carding previously unspinnable banana pseudostem fibers into fine spinnable textile grade fibers as well as a machine in which raw fibres can be straightened out and converted into a fiber of a thickness suitable for yarn formation (Nachane and Shanmugam, 2014b). Wu (2014) points out that it is possible to combine banana fiber with other natural fibers, including jute and cotton fiber, to make a kind of multi-functional fiber and, finally, **Datta** *et al*., (2012) discuss the use of raw materials, such as banana fiber, to produce cheap membranes for wastewater treatment.

Material and Methods

The data in this paper was compiled from a number of authentic online data bases, including Chemical Abstracts, now part of Scifinder. A comprehensive bibliographic search was carried out using the keywords "fiber and banana", refined with the word "extraction". Once the results were obtained, the duplicates were excluded.

Results and Discussion

Banana bast fibers are firm in structure and wear-resistant. Their moisture absorption and air permeability is good, inhibiting bacteria and preventing mildew. They can be spun into yarn, and used to make material which is environmentally friendly and a non-irritant to the skin. This means it can be used for clothing or, alternatively, made into products such as ropes, handbags, and doormats. A number of different ways of preparing the banana fibers will now be described in detail.

The first method is a manual one, involving banana fibers with an average fiber length of 10-50 mm, and an average fineness of 0.5-80 dtex. Firstly, the epidermis and bark are removed from a harvested banana stem to leave a bundle of fibers. These are dried, and then treated with 3% caustic soda at 130°C at high pressure. They are then washed again and the soda

neutralized. After drying out, they are cut to give a staple fiber with an average length of 35 mm (Takamori and Kawaharazaki, 2010).

There are two chemical methods that can be used to make banana bast fiber. The first comprises an acid pretreatment of the banana stalk with 0.1-0.4 % aqueous H_2SO_4, at a ratio of 30-50:1 and 20-50 °C for one to two hours. After this, the stem is boiled in a mixed aqueous solution of sodium hydroxide, sodium sulfite and sodium pyrophosphate for one to three hours and then stewed in a mixed aqueous solution of sodium hydroxide and industrial soap at 90-98 °C for a further one to three hours. It is then bleached in 2-5 wt % sodium hypochlorite at 5-25 °C for 5 to 12 minutes, acid washed in 0.1-0.2 % aqueous H_2SO_4 solution at 5-25 °C for 5 to 10 minutes, and then drained and dried (Wu *et al*., 2005). Another chemical method for extracting fibers from banana stalks consists of dissolving quick lime in 20-25 °C water to obtain a 3 % lime solution, which the banana stalks are placed into. When the pH value reaches 3.5-4.5, the liquid is drained and the banana stalks are washed to remove the impurities, and sun-dried. Then, the product is soaked in a 3% sodium hypochlorite solution, bleached, and sun-dried. It is then treated with a 1% N-octadecyl N'-ethylene urea solution at 80°C, cooled to room temperature, and then sun-dried. The fiber hemp which is produced weighs little and gives high water-absorption performance (Huang, 2008).

Another method for preparing fibers from the banana stalks and leaves using steam explosion-based degumming was described by Chen and Peng (2011). The advantages of this method are that it is inexpensive, causes no pollution, has a high yield, and offers a high quality product. To begin with, the stalks and leaves are cut into 5-15 cm. long segments, and then subjected to steam explosion under 1.0-1.8 MPa for 1 to 8 minutes, after which they are rinsed with water 10 to 20 times to remove the degraded pectin, and hemicellulose. Finally, they are bleached twice at 50-60 °C and at a solid - liquid ratio of 1:(10-20) for 20 to 40 minutes each time. The first bleaching solution is composed of: 10 g L^{-1} Na_2SiO_3, 10 g L^{-1} H_2O_2, and 10 g L^{-1} NaOH whereas the second bleaching solution consists of: 5 g L^{-1} Na_2SiO_3, 5 g L^{-1} H_2O_2, and 5 g L^{-1} NaOH. The steam explosion treatment can degrade over 90% of the pectin and hemicellulose and degrade some of the lignin in the banana stalks and leaves as well as completely separate the fiber bundles from other components. In addition, the steam explosion treatment splits the banana fibers into loose structures, thus facilitating the bleaching and oiling. It also loosens the banana fibers and increases their quality (Chen and Peng, 2011).

A similar method for extracting and degumming banana fibers by steam explosion was described by Sheng *et al*. (2011), who cite the same advantages as above. It comprised the following steps: the freshly cut banana stalks are placed in a steam explosion tank, and a steam explosion is performed under 1.20-1.60 MPa for 1-2 minutes. The stalks are then decompressed, washed in water, and dehydrated to obtain coarse banana fibers, which are soaked in 10-20 g L^{-1} NaOH at room temperature for 24 to 48 hours. They are then washed in water to be neutralized, and dehydrated, after which they are placed in the steam explosion tank again, under 1.35-1.55 MPa for 1-2 minutes, with the water content at 20-40 %. Once again the coarse banana fibers are decompressed immediately, washed in water, and dehydrated before being bleached in a bleaching solution (1-1.6 % Na_2SiO_3, 2-2.8 % NaOH, and 2-2.9 % H_2O_2) at 55-62 °C and 1:(14-18) ratio for 1 to 2 hours, rinsed and then bleached again for the same amount of time in another bleaching solution (1-3 % Na_2SiO_3, 1 % NaOH, and 1-1.6 % H_2O_2) at the higher temperature of 60-68 °C and 1:(15-19). After then being washed in water, and oiled and dried, the white banana fibers are loosened, and carded (Sheng *et al*., 2011).

Another way of obtaining banana fibers is by employing the biological process of retting. The action of micro-organisms and moisture on plants can dissolve or rot away much of the cellular tissues and pectins surrounding bast-fiber bundles, thereby facilitating separation of

the fiber from the stem (Editors of Encyclopaedia Britannica, 2009). Depending on the exposure time, the fiber bundle configuration presents small variations in composition, the main changes being related to hemicellulose and pectin, as they are found in the outer walls of the bundle. With an increase in exposure time, the fiber bundle walls lose integrity, as reflected in their mechanical behavior (Ganan *et al*., 2004a,b). Banana bast fibers can also be prepared by biological degumming (Li *et al*., 2010). The preparation method comprises sterilizing, removing impurities, inoculating, fermenting, water washing, adding caustic soda, boiling for 20-30 minutes, water washing, adding hydrogen peroxide, and then boiling for 20-30 minutes, followed by inactivation, stamping, and post-treatment. The obtained banana bast fiber is soft, loose and odorless. It is 80-200 mm in length, with a fiber count of 300-500 Nm. The bundle fiber breakage strength is 3.5-5.0 g D^{-1}, and it has a residual gum rate of 1.5-3.2 %. There is also a variety of machines that can be used to extract banana fibers and some of these will be described below. The first one comprises a chassis assembly consisting of a chassis and a pair of pneumatic tires attached to an axle, which is secured to a pair of leaf springs located under the chassis. The chassis has a towing bar, and telescopic legs, and longitudinal angular bars. There are two engine bases, and two decorticating frames, each of which has an upper member, a vertical member, a slanted member, and a base member. These parts are fixed together, forming a trapezoidal shape, and there is a stabilizer plate welded on perpendicularly near the upper portion of the slanted member. An anvil and chute assembly, consisting of a pair of swing arms, is pivotally attached to the chassis. There is a retainer plate fixed to the upper portion of the swing arms, as well as a pair of retainer nuts welded to the retainer plate, with a pair of bolt stabilizers passing through the retainer nut and, thus, the retainer plate can be adjusted. There is also a pair of lock nuts on the bolt stabilizers. There is an anvil and a bracket connected to the swing arms, with a pair of adjuster bolts on the bracket. In addition, there is a circular spring plate fixed to the adjuster bolt, which has a compression spring coiling around it (Valenzuela *et al*., 2007).

Another machine, which was designed and developed in India for the commercial extraction of fiber from the previously unused waste portions of banana plants, such as pseudo-stems, peduncles, and leafstalks, and which is considered to be user friendly and inexpensive, is the Central Tobacco Research Institute (CTRI) Banana Fiber Extractor Machine. This machine consists of a rigid frame, on which a roller, consisting of twenty seven mild steel or stainless steel horizontal blades (6 mm size) with blunt edges, rotates on a free moving shaft. The roller can be driven by a standard one horse power single phase electric motor, using a belt and pulley arrangement, which is fixed over the rigid frame. The advantages of the machine are that it can reduce drudgery and increase fiber production by 20-fold as compared to the manual process. The extraction of fibers means that an additional net income of 12,000 Indian rupees per hectare is guaranteed to the banana cultivators (Sudhakar *et al*., 2010).

A further invention is an automated mechanical tool which can be used to separate fibers from the banana sheath in an efficient and non-cumbersome manner, according to Murugesan (2013). It has between 16 and 20 serrations along one edge, with dimensions of 2 x 1.5 cms. By striking the plant across the serrations, it is possible to extract the fibers.

Yang and Qian (2017) describe another recent invention for banana stalk fiber extraction, which consists of a rack, with a motor affixed to it. The apparatus also has a rotating shaft, which is connected to the rack, with two turntables and a blade. This apparatus uses the force of natural spring compression to extract the fibers. Zhang (2017) refers to yet another machine which works by feeding the banana stalks between an upper and lower rack. A transmission device allows the racks to be spun and the fibers are stripped from the stalk as the machine rotates. Not only does the impurity rate appear to be low, particularly since insects caught in the fiber fragments are shaken loose, but the fiber extraction is considered to be efficient and

the extraction of the fibers, which can then be sold in addition to the bananas themselves, enhances the income of the banana farmers.

Conclusion

Natural banana fibers can be extracted in a number of different ways, either manually, chemically, biologically, using steam explosion-based degumming, or using modern machines. Each method has its own benefits and drawbacks, depending on the amount of fiber produced or the quality and properties of the fiber bundles obtained. It is hoped that the application of the banana fiber extraction methodology in the Canaries could benefit the local agricultural economy as it is already doing in other parts of the world.

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ASSESSMENT OF GENETIC SIMILARITY AMONG THE PROGENIES OF THREE APPLE SEEDLING ROOTSTOCKS BY USING SSR MARKERS

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Abstract

This investigation was done at the General Commission for Scientific Agriculture Research (GCSAR) – Pome and Grapevine Division in Sweida province (Syrian Arab Republic) during 2012 and 2013, in order to study the genetic similarity among 10 seedlings and the mother plant of three different seedling rootstock genotypes (A, B and C), which have been included in the breeding programme for apple rootstocks, by using 17 SSR markers. The results revealed the ability of 16 primer pairs to amplify alleles in all studied progenies and mother plants. The highest polymorphism was observed in progeny raised from the genotype B (87.1 %), followed by the progenies of genotypes A and C (80% and 78.1%, respectively). Genetic similarity of both genotypes A and C with their progenies were more than 0.5 in each of them, while in genotype B with its progeny was more than 0.5 in 8 seedlings, whereas genetic similarity among the progeny of each genotype (A, B and C) were 0.53, 0.53 and 0.58, respectively. Cluster analysis divided the progeny of genotype A into two groups and progenies of B and C genotypes into three groups. The expected heterozygosity (He) was 0.39 in each studied genotype, and the observed heterozygosity (Ho) was 0.27, 0.3 and 0.5 in A, B and C, respectively. On the other hand, the results showed high efficiency of some markers to detect genetic differences within genotypes, such as CH01H02, Hi04g05, O2b1 and CH01f02. These results showed the degree of homogeneity among the seedlings produced by each genotype, and the importance of using SSR markers in apple rootstock breeding programme.

Keywords: *Malus, rootstock, genotyping, microsatellites.*

Introduction

Apple rootstock breeding programme consists of essential steps represent in determining the objectives related to actual problems such as the age of trees, propagation ability, compatibility between rootstock and scion, in addition to the productivity (Cummins and Aldwinckle, 1995). However, apple rootstock breeding programmes are worldwide in most countries producing apple depending on their local own needs such as winter hardness tolerance , dwarfing, pest resistance, and precocity, like as America (Johanson *et al*., 2001), UK (Webster and Wertheim, 2003), Germany (Fischer, 1997), Poland (Jakubowski and Zagaji, 2000), Latvia (Bite and Lepsis, 2004; Rubauskis and Skrivele, 2007), and Russia (Bite and Lepsis, 2007). In Syria apple rootstock breeding programme is focused on the selection of resistant rootstock to woolly apple aphid and establishing a source for seedling rootstocks (Al-Halabi *et al.,* 2012). Molecular markers are an important tools for discrimination of species and different plant genotypes (Kiraly *et al*., 2008), particularly in woody plants (Wunsch and Hormaza, 2002). Also, molecular markers are used in apple rootstock breeding programmes to get accurate characterization for produced rootstocks (Gupta *et al*., 2004). Simple sequence repeat (SSR) markers have many properties over the other markers, they are co-dominant markers, have many alleles per locus, very available in genome, and can be used for cultivar identification, pedigree analysis, genetic diversity and genetic mapping (Lu *et al*., 2010). The genetic information about the wild *Malus* species of apple rootstocks are not well defined, thus 86 SSR markers were used to identify commercial rootstocks with their wild parents (Fazio and Baldo, 2005). However, SSR markers are widely used for apple genetic studies

(Farrokhi *et al*., 2011; Muzher and Al Halabi, 2017). This study was carried out to determine the genetic similarity between seedlings with their mother plants using SSR markers in order to select the best genotype as a source for seeds in apple rootstock breeding programme in Syria.

Materials and methods

The present investigation was carried out at Pome and Grapevine Division – GCSAR in Sweida province, which is located at 1,525 m altitude in the south of Syrian Arab Republic, during 2012–2013.

Plant material

Three apple rootstock genotypes (A, B and C) which derived from apple rootstock breeding programme, and their progenies (10 seedlings from each genotype) were used in this study.

DNA extraction

Genomic DNA was extracted from young leaves of assessed genotypes, using CTAB method described in Porebski *et al*. (1997).

PCR amplification

PCR amplifications were carried out by the using 17 primer pairs developed from apple genome as follows: 10 primer pairs (Hi-SSR) were developed by Silverberg *et al*. (2006), 6 primer pairs (CH-SSR) by Gianfranschi *et al*. (1998), and one primer pairs (O2b1) by Guilford *et al.* (1997).

The PCR reaction was performed in a 10 µl volume with 1 µl 10 X buffer + 1 µl dNTPs + 1 µl forward primer + 1 µl reverse primer + 3 µl DNA + 0.1 µl taq + 2.9 µl dH_2O. The cycling profile consisted of an initial denaturation step of 3 min at 94ºC, followed by 35 cycles of denaturation of 30 s at 94ºC, annealing of 30 s at an optimal temperature for each primer combination (55–58ºC) and extension of 1min at 72ºC, with a final 5-min extension step at 72ºC.

Visualization of the PCR products

The PCR products were detected by electrophoresis on 2% agarose gel in 1X TBE buffer, stained with ethidium bromide and visualized by gel documentation. The molecular weight of obtained alleles was estimated by Bioprofil-Bio-1D software using 100 bp DNA ladder (Gene direX, USA)

Data analysis

All alleles were scored as present alleles (1) and the absent one (0), then polymorphism calculated as % of polymorphic alleles. Genetic similarity (GS) was estimated according to Jaccard coefficient (Jaccard, 1908). Dendrogram was clustered by cluster analysis using UPGMA method (unweighted pair-group method using arithmetic averages) by Past software. Expected Heterozygosity (He) was calculated according to Lorenzo *et al*. (2007). While the observed Heterozygosity (Ho) was calculated according to Wunsch and Hormaza (2007), and marker index (MI) according to Powell *et al*. (1996).

Results and discussion

SSR primer pairs (16 pairs) revealed 30 alleles in genotype A with its progenies, of which 24 alleles were polymorphic (80%), the number of alleles ranged between 1 and 4 in different loci, with an average 1.88 alleles per locus; concerning genotype B with its progenies, 31 alleles were revealed, of which 27 alleles were polymorphic (87.1%), the number of alleles ranged between 1 and 3 in different loci, with an average 1.93 alleles per locus; while 32 alleles in genotype C with its progenies were revealed, of which 25 alleles were polymorphic (78.1%), the number of alleles ranged between 1 and 3 in different loci, with an average 2 alleles per locus (Table 1). In addition, the most efficient SSR markers were CH01H02, Hi04g05, Hi09a01, O2b1 and CH01f02 (Figure 1). On the other hand, the primer pair O2b1 informatively showed co-dominant alleles in the mother plant of each studied genotype.

Obtained results confirmed that these genotypes are hybrids, and the progeny of genotype B is the most varied among studied genotypes. The produced alleles size was between 92 bp and 265 bp (Table 1), and the size of all alleles was in agreement with the findings of Guilford *et al*. (1997), Gianfranschi *et al*. (1998) and Silfverberg *et al.* (2006). Zhang *et al*. (2012) evaluated genetic diversity between wild apple species and commercial cultivars using 19 SSR primer pairs, and found that the number of alleles was between 2 and 11 alleles in different loci. Also, the results of this investigation were consistent with results from Farrokhi *et al*. (2011), who studied the genetic variation among Iranian apple cultivars by using 16 SSRs and found that the number of alleles ranged from 2 to 5 alleles in different loci, with an average 2.8 alleles per locus.

Table 1. Number of alleles and polymorphic alleles, polymorphism (%), and allele size (bp) produced by SSR primer pairs in studied genotypes with their progenies.

Locus	**Alleles number**			**Polymorphic alleles**			**Polymorphism %**			**Allele size (bp)**		
	A	B	C	A	B	C	A	B	C	A	B	C
Hio4a05	2	3	2	2	3	2	100	100	100	211/220	186/198/220	198/211
Hi04g05	2	3	2	2	3	1	100	100	50	208/243	190/219/260	219/255
Hi07h02	2	2	2	2	2	2	100	100	100	246/258	246/265	250/265
Hi08c05	1	2	2	0	2	2	0	100	100	225	225/231	189/200
Hi08d09	1	2	1	0	2	0	0	100	0	194	165/173	180
Hi08e06	1	2	1	0	2	0	0	100	0	127	135/140	135
Hi09a01	2	2	2	2	2	2	100	100	100	190/200	176/185	190/200
Hi12a02	1	2	2	0	2	2	0	100	100	235	240/260	223/235
Hi08g06	1	1	1	0	0	0	0	0	0	198	198	198
CH01F02	2	2	3	2	2	2	100	100	67	162/170	183/192	170/183/ 211
CH01H01	2	1	3	2	0	3	100	0	100	92/100	128	113/128/ 140
CH02B03 b	2	1	2	2	0	2	100	0	100	94/105	94	86/105
CH02B12	2	2	3	2	2	2	100	100	67	120/128	120/140	110/120/ 134
CH01H02	3	2	2	3	1	2	100	50	100	208/245/ 259	185/226	235/245
CH01E01	2	2	1	1	2	0	50	100	0	108/122	127/140	108
O2b1	4	2	3	4	2	3	100	100	100	216/234/ 246/258	210/234	226/239/ 258
Total	**30**	**31**	**32**	**24**	**27**	**25**	**80**	**87.1**	**78.1**			
Average	**1.88**	**1.93**	**2**	**1.5**	**1.69**	**1.56**						

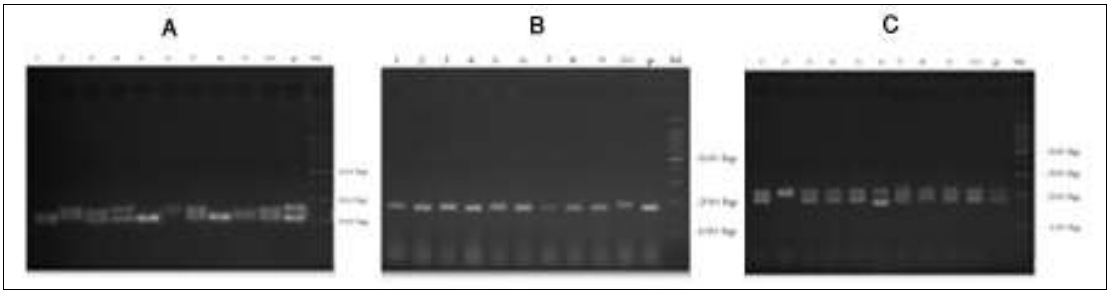

Figure 1. Alleles profile of SSR primer pairs O2b1, Hi09a01 and CH01F02 in A, B and C genotypes, respectively; M: Marker 100 bp ladder; the numbers from 1 to 10 represents seedlings of each genotype; P: the mother plant.

The highest genetic similarity between genotype A and its progenies was 0.84, while the lowest one was 0.52, on the other hand, the average genetic similarity among the progenies of genotype A was (0.53), which reflected sufficient genetic similarity between genotype A and its progenies; and among the progenies according to Zaied *et al*. (2006). The genetic similarity of 8 out of 10 seedlings derived from genotype B with their mother plant was more than 0.50, however the highest genetic similarity between genotype B and its progenies was 0.68, and the lowest one was 0.40, on the other hand the average genetic similarity among the progenies of genotype B was 0.53. Seedlings of genotype C distinguished by high genetic similarity with their mother plant (the highest 0.81; the lowest 0.61), as well as the average genetic similarity among the progenies was 0.58. These results showed the degree of homogeneity among the progenies with their mother plants especially in genotypes A and C, which is considered as essential step in selecting seedling rootstocks. These results were in agreement with Al-Halabi and Muzher (2015), who reported that the highest genetic similarity between Sukari 2 genotype and its progenies was 0.81.

Cluster analysis divided seedlings of genotype A and the mother plant into two clusters (I cluster – six seedlings and the mother plant; II cluster – four seedlings; Figure 2). Cluster analysis grouped seedlings of genotype B and the mother plant into three clusters, i.e. the first cluster with four seedlings and the mother plant, the second cluster with five seedlings and the third cluster with one seedling (Figure 2). Regarding seedlings of genotype C and the mother plant, three clusters were obtained (I cluster – five seedlings and the mother plant; II cluster – four seedlings; III cluster – one seedling; Figure 2).

The expected heterozygosity (He) was the same (0.39) in all studied genotypes with their progenies, while the genotype C revealed with its progeny the highest observed heterozygosity (Ho) which was 0.5, followed by 0.3 and 0.27 in genotypes B and A with their progenies, respectively. He and Ho values indicated to the low degree of genetic variation within the studied genotypes, which were lower than the literature reviews using SSR markers on apple (Wunsch and Hormaza, 2007; Sikorskaite *et al*., 2012). The highest MI value was in genotype B with its progenies (10.53), followed by genotypes C and A with their progenies (9.75 and 9.36, respectively). This index was correlated with the number of polymorphic alleles and He value reported by Muzher (2004).

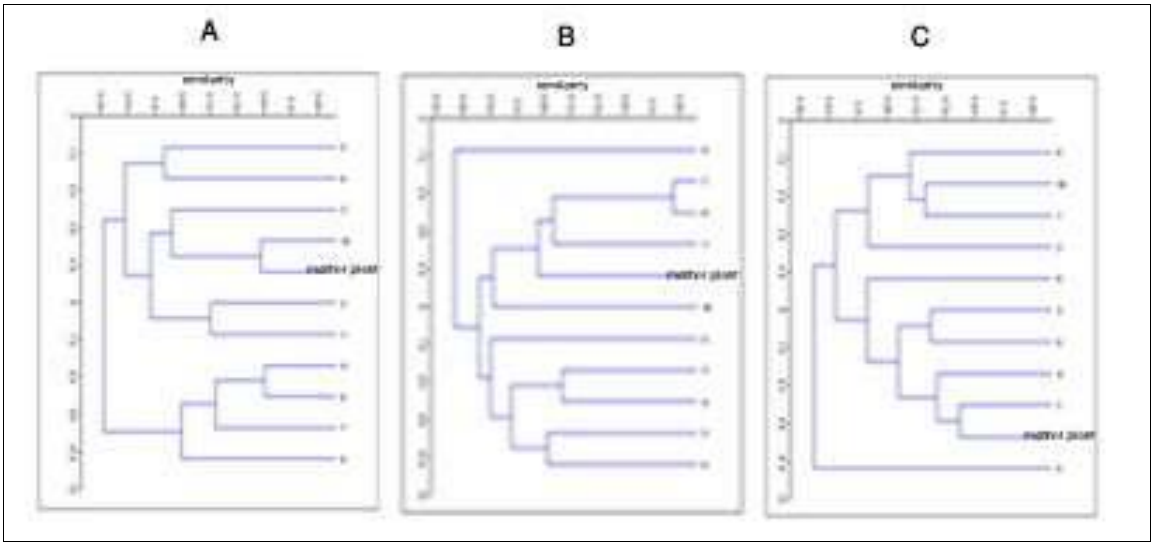

Figure 2. Cluster analysis as derived from SSR marker, for the seedlings and their mother plants (A, B and C genotypes).

Conclusion

The results of studied genotypes and their progenies using SSR markers showed that the genotypes A and C gave more homogeneous seedlings in comparison to genotype B. Therefore, genotypes A and C can be recommended as a source for seed production. In addition, the obtained results illustrated the importance of using SSR markers as an efficient tool for discrimination relative individuals in breeding programmes.

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INFLUENCE OF STRATIFICATION PERIODS AND CULTURE MEDIUMS ON THE GERMINATION OF APPLE ROOTSTOCK SEEDS

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Abstract

This investigation was conducted at General Commission for Scientific Agriculture Research – Pome and Grapevine Division in Sweida Province (Syria) during 2014 – 2015, to study the ability of apple seedswhich derivedfromthe hybrid genotype (HS) and seedling rootstock (B) in apple rootstock breeding program in addition to 'Golden Delicious' variety (GD) to germinate at alternative periods 45, 60 and 90 days of cold stratification at 4°C in different mediums Murashige-Skoog, Murashige-Skoog + 3% sucrose, sand, and sand + 3% sucrose. The results showed that the highest seed germination percentage in HS(87.5% and 80%)was obtained after 60 days of stratification in Murashige-Skoog + 3% sucrose and sand respectively. In B rootstock the highest seed germination percentage was 88.7% after 90 days' stratification in Murashige-Skoog + 3% sucrose, followed by 85% in sand + 3% sucrose after 60 days. However, the highest seed germination percentage in GD cultivar was 87.5% after 60 and 90 days' stratification in Murashige-Skoog and sand + 3% sucrose respectively. The mean time for germination was between 2.7 and 9 days depending on the studied treatments. These results illustrated the importance of determining the best cold stratification period and stratification mediumfor each genotype seeds to produce high apple seedlings in breeding programs and nurseries.

Keywords: *apple, cold stratification, seed germination.*

Introduction

Many seeds of plant species need to incubate at low temperature after moistening to achieve germination and this procedure is called stratification. The period of stratification varies between plant species either between cultivars as well (Daskalyuk and Toma, 1994).On the other hand, the length of the stratification period depends on the environmental conditions during seed formation and ripening (Lewandowski and Zurawicz, 2009; Militaru*et al*.,2009).

Germination consists of all processes which start from absorbing water by the dormant dry seed, and the germination is complete when the radicle penetrates the structures surrounding the embryo (Bewley, 1997). By definition, dormancy of apple seeds is caused by many inhibitors preventing germination process, which considered as compound dormancy (physical and physiological). Physiological dormancy is the most common type of dormancy, and it can be divided into deep, intermediate and non-deep levels, of which the non-deep level accounts for a substantial proportion of all dormant seeds (Graeber *et al.* 2012 ; Baskin and Baskin 2014).Seed coat and embryo coat are responsible of physical dormancy, while physiological dormancy is related to physiological state of embryo (Lewak, 1981; Daskalyuk, 2002).

Embryo dormancy can be removed completely by low temperature (5° C is optimal),According to Bakke *et al*. (1926), embryo hydration is essential to eliminate seed dormancy. However, no factor can affect the removal of dormancy without partial hydration at least for embryo. The depth of seed dormancy is determined by the cooled period needed to germinate the seeds soaked in water, and the optimal temperature for germination is 18° C(Lewak, 2011).

The levels of abscisic acid (ABA) and gibberellin (GA) were determined in apple seeds for hybridizations between different cultivars, and all seeds showed similar changes in ABA and

GA levels during 80 days of stratification (Lee and Looney, 1978).BartheandBulard (1983) stated that ABA plays an important role in embryo dormancy of apple seeds, and there is a relation between dormancy depth and the accumulated amount of ABA.
Locules serve as protectors for seeds as long as the seeds remain in the fruits, due to unknown inhibitor (s) preventing germination(WanandDennis,1992). Pauwels*etal.* (1999) studied seed germination characters useful in apple breeding program, and they found that the cultivar has an obvious influence in the time of seed germination. In hybrids the female parent effected on the time of seed germination whilst the male parent had a little influence, and there was insignificant association between bud break and seed germination. In a study of plant seeds which were extracted from ripen stored fruits of 12 apple hybrids at 2-3°C for 59 days, then transferred to an unheated plastic tunnel, germination percentage was 86.7%, time for germination 2.5-4.2 days, and this provides a high production of seedlings in a breeding process during a short time after stratification (Pitera and Odziemkowski, 2006).
In Syria, the dependence on seedling rootstocks isthe commonapproach in most regions where apple trees are grown, and apple rootstock breeding program is aimed to select the seedling rootstocks most suitable for Syrian conditions (Al-Halabi and Muzher, 2015). This research was conducted to study the effect of stratification periods and culture mediums on germination of apple seeds for some genotypes from apple rootstock breeding program in addition to 'Golden Delicious' variety.

Materials and methods

This investigation was achieved during 2014-2015at Pome and Grapevine Division- GCSAR in Sweida province which is located in the south of Syria at 1525 m altitude.
Apple seeds from hybrid genotype (HS) between 'MM106' rootstock and the local apple cultivar 'Sukari', and seedling rootstock (B) from apple rootstock breeding program in addition to 'Golden Delicious' variety (GD).
Seeds for each genotype were put in different mediums after surface sterilized: (I)25ml of Murashige-Skoog with 0.8 agar without hormones and vitamins, (II)25 ml of Murashige-Skoog+ 3% sucrose, (III) sand, and (IV) sand + 3% sucrose. For treatments (I) and (II) the seeds were sown in 10 × 10 cm vessels then covered with Parafilm and aluminum foil.While treatments (III) and (IV) were sown in 20 ×40×10 cm plastic pots between two layers of sand, and keep themmoist during stratification. Then they were incubated at 4°C (cold stratification) for 45, 60 and 90 days. After each stratification period seeds were removed and washed with distilled water. In each replicate from each treatment and for each genotype 10 seeds were put on moistened filter paper in Petri dish (with 4 replicates), covered with aluminum foil, and incubated at 24℃ for ten days with observing their moist and score thegermination daily. The seeds were considered germinated when 5mm root penetrated seed coat.After 10 days of incubation the germination percentage was calculated for each treatment. Mean time for germination was calculated according to Daskalyuk(2002) using the formula:

$$T = (\sum_{i=1}^{L} \text{ni ti}) / \sum_{i=1}^{L} ni$$

where: L: germination period (10 days).
n_i: number of germinated seeds on the $_i$th day.
t_i: number of days.
The variance was analyzed by two way ANOVA, and LSD test (significance level 1%)was calculated to compare means using the statistical program SPSS17.

Results and Discussion

Germination percentage

The results showed that the stratification period of 45 days was not sufficient to germinate the seeds of studied apple rootstock genotypes in all studied mediums (Table, 1). However, seeds of B rootstockdid not germinate in mediums I (Murashige-Skoog) and II (Murashige-Skoog + 3% sucrose), while the highest germination percentage after 45 days stratification among studied genotypes was 35% in seeds of 'Golden Delicious' varietyin medium I. This result is in agreement with literature findings that the ability of apple seeds to germinate is tightly related to stratification period (Daskalyuk, 2002; Lewak, 2011).In genotype HS seedsgermination percentage in mediums I and IIafter 60 and 90 days of stratification, in addition to medium III (sand) after 60 days were insignificantly different (Table 1). The highest percentage was 87.5% after 60 days in medium II, and the same percentage was after 90 days in medium I. This results indicated that the addition of sucroseto Murashige-Skoog medium fasten the germination of seeds after 60 days' stratification. This is due to the necessity to energy and metabolites which sucrose provided to the embryo to start germination (Daskalyuk, 2002). On the other hand, in medium IV (sand + 3% sucrose) the germination percentage was lower in comparison with other mediums (Table 1).In B rootstock the results showed that medium IV (sand +3% sucrose)played an important role in shorten stratification period, which obtained high germination percentage (85%) after 60 days' stratification. This was insignificant with germination percentage of medium I and II after 90 days' stratification (83.3% and 88.8% respectively). The highest germination percentage of 'Golden Delicious' (GD) seeds was 87.5% in the mediums I and IV after 60 and 90 days respectively. On the other hand, the mediums II and III obtained low germination percentage of 'Golden Delicious' seeds in contrast with the other two mediums (Table 1).The results showed that the differences in seed germination percentage among studied genotypes, mediums, and after 60 and 90 days of stratification were insignificant. As well, these results revealed that 60 days' stratification is enough to have high germination percentage in all studied genotypes, but each genotype has a favorable medium. These results are in agreement with result the obtained by Daskalyuk (2002), that almost all 'Golden Delicious' seeds germinated after 60 days' stratification.

Mean time for seed germination

Table (1) showed that the mean time for seed germination decreased with the increase of stratification period in all studied mediums and genotypes. The mean time for seed germination insignificantly differed in the genotypes HS and Bin studied mediums and stratification periods. The mean time for seed germination in HS genotype was 2.7 days in medium III after 90 days' stratification, while it was 7.8 days in medium I after 45 days stratification. As well, the mean time for seed germination in B genotype was 3.1 days in medium II after 90 days' stratification,and reached 7.6 days in medium IV after 45 days' stratification. However, in mediums I and II after 45 days' stratification there was no germination. On the other hand, the mean time for seed germination of 'Golden Delicious' showed significant differences among studied mediums and stratification periods. It was between 3.5 days in medium I after 90 days' stratification, and 9 days in medium II after 45 days' stratification (Table 1). These results illustrated clearly the relationship between the stratification period and the speed of seed germination. This is in agreement with the results obtained by Daskalyuk (2002), Lepse*et al*. (2011), and Suleiman and Omran (2011)that seeds germination percentage and time for germination of apple seeds correlated to stratification period.When we compared the germination percentage between genotypes on the same medium for

different periods, we found that each genotype showed differentbehavior than the other studied genotypes, especially 'Golden Delicious' variety, because the length of stratification period depends on species and cultivars, which was in agreement withLewandowski and Zurawicz (2009).

Table 1: Germination (G) percentage (%) and mean time (T) for germination (days) in each medium, stratification period and genotype.

Medium	Stratification period	Genotype					
		HS		B		GD	
		G (%)	T(days)	G (%)	T(days)	G (%)	T (days)
I (Murashige-Skoog)	45	22.5 c	7.8	0 h	-	35 d	6.6 b
	60	80 a	5.1	38.7 f	6.3	**87.5 a**	4.1de
	90	**87.5 a**	3	83.3 ab	3.9	69 b	3.5 e
II (Murashige-Skoog + 3% sucrose)	45	10 d	7.5	0 h	-	2.5 e	9 a
	60	**87.5 a**	5.1	50 e	7.1	62.5 b	5.9bcd
	90	82.5 a	3.7	**88.8 a**	3.1	*	-
III (sand)	45	30 c	7.2	2.5 h	7	30 d	5.1bcde
	60	80 a	3.9	75 c	6.3	51.3 c	6.5bc
	90	28.8 c	2.7	62.5 d	5.2	67.5 b	4.6cde
IV (sand + 3% sucrose)	45	7.5 d	7	12.5 g	7.6	10 e	5.9bcd
	60	50 b	5	**85 ab**	6	45 c	8.2a
	90	57.5 b	4.2	77.5 bc	5.3	**87.5 a**	4.1de
LSD 1%		9.4		7.7		9.6	1.9

*the treatment is lost.

Same letters (a,b,c ….) in each column indicated that the difference between mean values is insignificant.

Conclusion

The results showed that 60 days' stratification period was efficient to get high seed germination in all studied genotypes, in the mediums Murashige-Skoog + 3% sucrose for HS genotype, Murashige-Skoog for 'Golden Delicious' variety, and sand + 3% sucrose for B rootstock. On the other hand,90 days' stratification period was the better for shortened the time needed for germination. Hence, it is important for breedersand nurserymen which use seedling apple rootstocks to test the best medium and stratification period to improve their production.

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THE EFFECTS ON FLOWER QUALITY AND FLOWERING TIME OF SOME APPLICATION IN FONDANT HYACINTH (*HYACINTHUS*) BULB CULTIVAR

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Abstract

This study was conducted to determine the optimal planting time and the effect of humic substance in the Fondant *Hyacinthus* variety bulbs in Selcuklu district in Konya Province. We investigated the effects on flower quality and flowering period of applications: 1) I. planting time (November 7) (unfertilized), 2) II. planting time (November 28) (unfertilized), 3) III. planting time (December 19) (unfertilized), 4) I. planting time (November 7) (Tki-Humas), 5) II. planting time (November 28) (Tki-Humas), 6) III. planting time (December 19) (Tki-Humas). According to findings, the longest flower stems we had at the III. planting time with 12.02 cm (unfertilized) and III. planting time with 12.17 cm (Tki-Humas); the longest flower stalks (Tki-Humas) we had at I. planting time with 1.15 cm (Tki-Humas), at II. planting time with 1.16 cm (Tki-Humas), at III. planting time with 1.17 cm (Tki-Humas) and at III. planting time with 1.19 cm (unfertilized); the maximum number of flowers per stem with 37.00 number had II. planting time (Tki-Humas); the longest flower diameter with 6.98 cm had III. planting time (Tki-Humas); the longest leaf with 16.72 cm had I. planting time (Tki-Humas); the widest leaf with 3.33 cm had III. planting time (Tki-Humas); the opening of the first flowers began on March 26 in all applications; the duration of flowering is determined to be 22 days in all applications; the effect of the applications on the number of leaves was not statistically significant.

Keywords: *Fondant Variety (Hyacint), Planting Time, Humic Substance, Quality, Flowering Period.*

Introduction

Garden hyacinth (*Hyacinthus orientalis* L.) belong to the *Hyacinthaceae* family, *Hyacinthus* genus. Turkey is one of the world's richest countries in terms of the multitude of plant species. There are approximately 10.150 plant species in Turkey. About 3.000 of these plants are endemic. The plants growing in our country carry commercial value both in the country and abroad. The number of species collected from the nature and exported is 347 of them are bulbous tubers (Özhatay *et al*., 1997). In Turkey can be considered paradise geofit, dismantling the nature of more accelerated reduction and agreed on the complete ban would be a great benefit in the next 10 years. Sectors engaged in geofitler also emphasized that they should continue their production activities they have started in recent years, not to be dependent on nature. They have proven that geofitler can be produced easily within the framework of sustainability rules as long as the choice of the place, especially the Western Taurus, is benefited from the studies made (Karagüzel *et al*., 2011). Reducing the K + / Na + and Ca2 + / Na + ratios under salt stress of *Hyacinthus orientalis* L. leaves have been found to be a major contributor to the hyacinth salt tolerance (Köksal *et al*., 2014).

Onion plants have an important export potential with its different usage areas, and the need for these plants is increased by the increase of usage areas. Initially, the natural disasters meet the needs of these plants, but recent dismantling has failed to meet this need, and even extreme dismantling from nature has stressed that some of these endemic plants threaten their generation. Natural production methods could not fully meet the needs of these plants. Rapid propagation can be done by tissue culture methods and that an alternative production method

to natural production methods can be developed by using these methods. Thanks to the rapid propagation, tissue culture methods should be regarded as an alternative production method in order to protect the rich biodiversity of our country and that these works need to be supported by these works, in order to be able to respond to increasing needs and to prevent the generations of these plants from being destroyed (Karaoğlu, 2010). Considering the increase in number of onions, environmental length and environmental length, the study predicted that if the cultures were taken from geophytes, they would be able to obtain marketable quantities and quality, meet market demand for flower bulb exports, and have potential to become an alternative product (Özel and Erden, 2010).

Materials and methods

In this study, Fondant hyacinth variety was used as research material (Figure 1). The variety, Asia Lale Construction Agriculture, Animal Husbandry Trading Timited Company. It is operating in Konya. Onions are Health Certified, ISO-9001 Quality Standards and TSE 2547 Number Flower Bulbs Standards. The effects of three different planting times and humic substance (Tki-Humas) on the flower quality and flowering period were tried to be determined in the Fondant hyacinth variety in Selçuklu district in Konya province. Experimental design; 1) I. planting time (November 7) (unfertilized), 2) II. planting time (November 28) (unfertilized), 3) III. planting time (December 19) (unfertilized), 4) I. planting time (November 7) (Tki-Humas), 5) II. planting time (November 28) (Tki-Humas), 6) III. planting time (December 19) (Tki-Humas). The first planting of the hyacinth was on November 7th. Other plantings were made with 20 day intervals. In each planting, 180 pieces and 3 replications total 540 pieces of onion were used. The experiment was set up on openly field. The parcel was set at 60 x 75 cm, between rows and 15 cm x 15 cm at the planting distance. All bulbs were planted at an average depth of 12 cm.

Figure 1. Fondant hyacinth variety

Composition of tki-humas

Tki-humas; leonardite and a liquid natural organic soil conditioner produced from low quality lignite containing 12% humic and fulvic acid (Gezgin, 2013). Total Organic Matter: 5%, Humic Acid + Fulvic Acid: 12%, Water Soluble Potassium Oxide (K_2O- 3%), PH: 11-13.

Application of tki-humas

The hyacinth bulbs were sprayed onto the soil surface at a rate of 20 l/da just before planting, ensuring homogeneous mixing of the humic material with 20 cm soil depth. The application was made at cool time near the evening hours.

Examined parameters

Flower stem length (cm): The flowering of 10 plants until the end of flowering with the deviation of flowers begins to bloom from the bottom of the flower deviations. The arithmetic averages of the figures obtained are taken into account.

Flower stalk thickness (cm): The flower stem thicknesses of 10 plants selected randomly for each replicate were measured using digital calipers. The arithmetic averages of the figures obtained are taken into account.

Number of flowers per stem: The number of flowers per stem was determined by counting method for 10 randomly selected plants for each replicate. The arithmetic averages of the figures obtained are taken into account.

Flower diameter (cm): The diameters of 10 flowers collected randomly according to the varieties from the parcels; the distance between the two opposite points of the opposite points was determined with the help of the ruler in the period when the flowers fully opened. The arithmetic averages of the figures obtained are taken into account.

Leaf number (number/plant): During the time until the end of flowering, the leaves of 10 plants selected randomly per parcel were counted and averages were calculated. The arithmetic averages of the figures obtained are taken into account.

Leaf length (cm): The leaves of 10 plants randomly selected per plot during the time until the end of flowering were measured with the aid of a length ruler at the distance from the leaf exit point on the neck portion of the onion to the extreme point. The arithmetic averages of the figures obtained are taken into account.

Leaf width (cm): The diameter of the best growing leaves of 10 plants randomly selected per plot was measured with the aid of a ruler until the end of flowering. Measurements were made with reference to the middle, which is the largest of the leaf. The arithmetic averages of the figures obtained are taken into account.

Determination of flowering time: All the hyacinth plants in the control group and the fertilizer application were taken as the flowering time of the first flowers opened.

Determination of flower life: The number of days between the appearance of the first flower and the fading of the flower was taken into account.

Evaluation of data: Results obtained were analyzed in JMP (version 7.0, SAS Institute, Cary, NC, USA) statistical program.

Results and discussion

The measurements and findings obtained in the fondant hyacinth variety were interpreted as 3 repetition averages (Table 1).

Table 1. The Effects on Flower Quality and Flowering Time of Some Application in Fondant Hyacinth (*Hyacinthus*) Bulb Variety

APPLICATIONS	Flower Stem Length (cm)	Flower Stalk Thickness (cm)	Number of Flower Per Stem (number)	Flower Diameter (cm)	Leaf Number (number)	Leaf Length (cm)	Leaf Width (cm)
I. Planting Time (unfertilized)	11.03 b	0.96 c	22.00 f	5.97 d	6.67	15.83 b	3.13 bc
II. Planting Time (unfertilized)	10.73 b	1.05 b	27.61 d	6.50 c	6.58	15.11 c	3.03 c
III. Planting Time (unfertilized)	12.02 a	1.19 a	33.00 b	6.82 ab	6.58	15.77 b	3.17 bc
I. Planting Time (Tki-Humas)	11.69 ab	1.15 a	23.56 e	6.45 c	6.70	16.72 a	3.09 bc
II. Planting Time (Tki-Humas)	11.51 ab	1.16 a	37.00 a	6.71 b	6.72	16.07 ab	3.19 ab
III. Planting Time (Tki-Humas)	12.17 a	1.17 a	31.00 c	6.98 a	6.72	16.12 ab	3.33 a
LSD %5	0.97	0.08	1.52	0.16	N.S.	0.65	0.16

a, f: The difference between averages with different lowercase letters in the same column is important (P<0.05), N.S.: No significant, I. Planting Time (07 November), II. Planting Time (28 November), III. Planting Time (19 December).

Effect on flower stem length of applications

The longest flower stems had III. planting time with 12.02 cm (unfertilized) and III. planting time with 12.17 cm (Tki-Humas); The shortest flower stem had II. planting time with 10.73 cm (unfertilized) and I. planting time with 11.03 cm (unfertilized) was determined (Table 1).

Effect on flower stalk thickness of applications

The longest flower stalks thickness had I. planting time with 1.15 cm (Tki-Humas), II. planting time with 1.16 cm (Tki-Humas), III. planting time with 1.17 cm (Tki-Humas) and III. planting time with 1.19 cm (unfertilized); The shortest flower stalk thickness had I. planting time with 0.96 cm (unfertilized) (Table 1).

Effect on number of flower per stem of applications

The maximum number of flowers per stem with 37.00 number had II. planting time (Tki-Humas); The least number of flower per stem had I. planting time with 22.00 number (unfertilized) (Table 1).

Effect on flower diameter of applications

The longest flower diameter with 6.98 cm had III. planting time (Tki-Humas); The shortest flower diameter had I. planting time with 5.97 cm (unfertilized) (Table 1).

Effect on leaf number of applications

The effect of the applications on the number of leaves was not statistically significant (Table 1).

Effect on leaf length of applications

The longest leaf with 16.72 cm had I. planting time (Tki-Humas); The shortest leaf had II. planting time with 15.11 cm (unfertilized) (Table 1).

Effect on leaf width of applications

The widest leaf with 3.33 cm had III. planting time (Tki-Humas); The shortest leaf width has II. planting time with 3.03 cm (unfertilized) (Table 1).

Effect on flowering time

The opening of the first flowers began on March 26 in all applications (Table 1).

Effect on flower life

The duration of flowering was 22 days in all applications.

Conclusion

The longest flower stems had III. planting time with 12.02 cm (unfertilized) and III. planting time with 12.17 cm (Tki-Humas); The longest flower stalks thickness had I. planting time with 1.15 cm (Tki-Humas), II. planting time with 1.16 cm (Tki-Humas), III. planting time with 1.17 cm (Tki-Humas) and III. planting time with 1.19 cm (unfertilized); The maximum number of flowers per stem with 37.00 number had II. planting time (Tki-Humas); The longest flower diameter with 6.98 cm had III. planting time (Tki-Humas); The longest leaf with 16.72 cm had I. planting time (Tki-Humas); The widest leaf with 3.33 cm had III. planting time (Tki-Humas); The opening of the first flowers began on March 26 in all applications. The duration of flowering was 22 days (March 26–April 17) in all applications. As a result, number of flowers per stem with II. planting time (November 28) (Tki-Humas) and flower diameter with III. planting time (December 19) (Tki-Humas) can be increase in Fondant hyacinth variety.

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DETERMINATION OF YIELD AND FACTORS AFFECTING YIELD IN SOME SESAME GENOTYPES

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Abstract

This study was carried out to determine yield and the factors affecting yield of some sesame varieties and lines in the experimental field of Siirt University Faculty of Agriculture at the sesame growing seasons of 2016 and 2017 as second crops. In the experiment, ten different sesame genotypes were used as material, that consisting of three registered sesame varities (Arslanbey, Hatipoğlu, Boydak) and seven different lines. The study was designed with 3 replications according to randomized block trial design. According to the results obtained for two years study, the plant length is 104.13-152.87 cm, the first branch height is 2.67-11.87 cm, the number of plant branches is 1.87-10.33, the number of capsules per plant is 90. 93-200.33 units, 1000 grain weights were found to be between 3.25 and 4.83 g and the hectare yield varied between 479.29-1579.88 kg. The highest grain yield was obtained from the Arslanbey variety with 1579.88 kg / ha in 2016 and from line-8 with 1502.41 kg in 2017. The lowest grain yield was obtained from line-7 with 479.29 kg in the first year and from line-3 with 68.69 kg in the second. It has been determined that genotypes are significantly different from one another in terms of yield and other characteristics, and it turns out that these varieties show different responses.

Keywords: *Sesame, Genotype, Yield, Factors affecting yield*

Introduction

Sesame (*Sesamum indicum* L.) was cultivated and domesticated on the Indian subcontinent during Harrapan and Anatolian eras (Bedigian and Van der Maesen, 2003). This is evidenced by the presence of archaeological remnants of the crop dating back to 5500 BC in the Harappa Valley in the Indian subcontinent (Weiss, 2000; Ashri, 2007). The Assyrian tablets depict how the gods ate bread and drank sesame wine together prior to battling and restore order to the universe. Sesame was a major oilseed crop in the ancient world due to its easiness of extraction, great stability, and resistance to drought. It is also known as benniseed, gingelly, simsim, ajonjoli, sesamo and til. The genus *Sesamum* is a member of Pedaliaceae family, which contains 16 genera and 60 species. The number of sesame species is not clear; however, about 40 species have been described, and 36 are mentioned in the Index Kewensis. Many occur in Africa (18 exclusively), 8 occur in the Indian – Ceylon region (5 exclusively). Almost all of the wild species are prevalent in Africa. *Sesamum indicum*, as well as *S. capense* Burm. (*S. alatum* Thonn.) and *S. Schenkii* Aschers, has a somatic number 2n = 26. For *S. laciniatumthis* is 2n = 28. For *S. angolens* and *S. prostratum* it is 2n = 32. For *S. occidenale* and *S. radiatum* Schm & Thonn. it is 2n = 64. *Ceratotheca sesamoides*, related to *Sesamum*, has 2n = 32. Only *Sesamum indicum* is cultivated; however, a few other species: *S. angustifolium, S. calycinum,* ssp. *Baumii, S. malabaricum,* and *S. radiatum* are harvested and eaten occasionally, particularly during famine or food shortage (Ashri, 2007).

Oil extracted from sesame is honoured as a rich food because of its high nutritive quality and stability (Nayar et al., 2002). It has many uses and it is markedly different from other vegetable oils due to its high nutritional and therapeutic values. Potential health benefits of

sesame include anti oxidative, anticancer, anti-hypersensitive and anti-immunoregulatory actions (Reshma et al., 2010). The seeds are used for the production of oil, paste, salads and in various food formulations. From the chemical composition of sesame, it is known that the seeds contain 50-60% oil, 18-25% protein, 13.5% carbohydrate and 5% ash (Elleuch et al., 2007). The noteworthy stability to oxidation can be attributed to its endogenous anti-oxidant lignans along with tocopherols (Reshma et al., 2010). Sesamin (0.4-1.1%), sesamolin (0.3-0.6%) and traces of sesamol contribute to the unique properties of sesame oil (Shahidi et al., 1997). In the eastern parts of the world, sesame has long been considered as a 'health food' that provides high energy and prevents ageing (Yoshida et al., 2007). The oil is rich in unsaturated fatty acids (85%) and has a mild taste. It is said to be plant breeder's dream crop because of its great genetic diversity (Banerjee et al., 2009). Sesame is a rich source of calcium (approx 1%) and phosphorous (approx 0.7%) (Nayar et al., 2002). Sesame contains ample amounts of oleic (43%), linoleic (35%), palmitic (11%) and stearic acid (7%) which together comprise 96% of the total fatty acids (Saydut et al., 2008).

Sesame seeds are tiny, flat, oval with a nutty taste and delicate with almost invisible crunch. They vary in size from small to large and come in a host of different colours, depending upon the variety, including white, yellow, black, grey, brown and red. The seeds from northeast region of India are black; those from eastern region are brown to black and from south are predominantly red or reddish brown.

Taking into consideration the use of the existing oil shortage and sesame in different areas of our country, it is of great importance to increase the yield to be obtained from the unit area in sesame seeds. In fact sesame agriculture offers a very profitable production, with little input from the other side except labor input. If progress is made in the harvest mechanization of sesame, sesame agriculture in the Southeastern Anatolia region is thought to be more prevalent as a second crop in irrigated areas.

Material and Methods

This study was carried out to determinate of appropriate sesame genotype in the experimental field of Siirt University Faculty of Agriculture at the sesame growing seasons of 2016 and 2017 as second crops. In this study, it was aimed to investigate the possibilities of breeding of different sesame varieties foreseen under the conditions of Siirt Province. In the experiment, ten different sesame genotypes were used as material, that consisting of three registered sesame varities (Arslanbey, Hatipoğlu, Boydak) and seven different lines. The study was designed with 3 replications according to randomized block trial design. In the experiment, the distance between the rows was 70 cm and the distance from the rows was 15-20 cm.

Test site soil properties; Siirt University Kezer Campus Agricultural Faculty is the land of the field trial field trial, terraced, with an east-facing, flat and steeply sloping, alluvial construction, with deep profiled soil. According to the result of soil analysis at the depth of 0-30 cm before planting in the experimental area, the whole area of the study site was roughly textured (47,99% sand, 8,49%, 43,51% clay), little salty (463 mS / organic matter (1.02%), with respect to the amount of phosphorus that can be absorbed, and organic (organic matter) There is insufficient substance, sufficient capacity for potassium.

The experiment was carried out every two years after the harvest of the lentil as the second crop. 1 year; 15 June was planted in 2016, 2nd year; July 5, 2017 sowing was done. The trials were irrigated with drip irrigation system that was done 6 times in both growing season. Plants were harvested from middle two rows of 4 rows per plot treatment for **seed yield.** Harvest was done by hand. The harvesting and blending process began at the middle of October and finished towards the end of November.

The meteorological data were recorded from planting date to the harvest time in Table 1. Below are temperature and humidity tables for 2016 and 2017 of Siirt province (Anonymous 2018).

Table 1. Some meteorological data related to the research area

		Mounths						
Meteorological data	**Years**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Monthly Maximum Temperature (° C)	2016	32.9	40.5	43.1	43.2	37.9	32.5	23.8
	2017	31.9	39.8	41.2	42.9	39.5	28.4	23.8
	Long Term	36,2	39,9	44,4	46,0	39,9	36,6	25,6
Monthly Average Temperature (° C)	2016	19.4	26.2	31.3	30.9	23.8	18.1	8.6
	2017	19.6	31.4	40.8	37.2	32.8	23.3	14.2
	Long Term	19,3	26,0	30,6	30,1	25,1	17,9	10,4
Monthly Minimum Temperature (° C)	2016	11.7	16.5	21.2	19.8	14.2	9.5	1.5
	2017	10.1	12.8	22.1	21.5	17.2	9.8	0.8
	Long Term	2,0	8,2	13,1	14,4	8,6	0,3	-4,1
Monthly Total Precipitation (mm=kg÷m²) OMGİ	2016	56.7	22.0	0.0	0.0	6.0	16.4	0.6
	2017	3.0	0.0	0.0	0.4	0.0	5.2	97.0
	Long Term	62,0	8,7	1,6	0,9	4,9	49,1	81,4
Monthly Relative Humidity (%)	2016	61.3	40.2	27.7	26.4	34.7	42.0	62.5
	2017	63.0	25.8	11.3	17.2	17.9	28.0	63.3

Source: General Directorate of Meteorology

Results and Discussion

In the study; seed yields of sesame cultivars and lines, plant size, number of capsules per plant, number of side branches in the plant and 1000 seed weight were given in the table 2, According to the results obtained for two years study, the plant length is 104.13-152.87 cm, the first branch height is 2.67-11.87 cm, the number of plant branches is 1.87-10.33, the number of capsules per plant is 90. 93- 200.33 units, 1000 grain weights were found to be between 3.25 and 4.83 g and the hectare yield varied between 479.29-1579.88 kg, The highest grain yield was obtained from the Arslanbey variety with 1579.88 kg / ha in 2016 and from line-8 with 1502.41 kg in 2017, The lowest grain yield was obtained from line-7 with 479.29 kg in the first year and from line-3 with 68.69 kg in the second. It has been determined that genotypes are significantly different from one another in terms of yield and other characteristics, and it turns out that these varieties show different responses.

Table 2: Yield and some factors affecting yield of sesame genotypes

Year	Sesame genotypes	Yield (kg/ ha)		Plant height (cm)		First branch height (cm)		Number of branches		Number of plant capsules		1000 seed weight (gr)	
2016	**Arslanbey**	1579,88	a	104,97	cd	8,97	ab	3,57	d	155,50	a	4,17	ab
	Hatipoğlu	1489,88	a	104,13	d	5,00	b	7,20	a	125,70	abc	4,33	a
	Boydak	822,38	bcd	106,63	cd	12,83	a	7,60	a	113,53	bc	4,42	a
	4	1005,83	b	109,60	bcd	9,03	ab	5,77	bc	131,43	abc	4,33	a
	6	1051,55	b	113,63	bc	4,67	b	7,50	a	138,03	ab	3,58	cd
	7	479,29	d	106,95	cd	11,65	a	5,85	bc	101,65	bc	3,25	d
	8	579,88	cd	106,63	cd	4,37	b	6,40	ab	135,33	abc	4,42	a
	10	941,79	bc	113,63	bc	10,37	a	4,70	cd	110,97	bc	4,08	ab
	26	809,52	bcd	117,33	b	11,13	a	6,60	ab	108,67	bc	3,67	c
	27	1074,05	b	105,33	cd	11,87	a	6,93	ab	97,00	c	3,83	bc
	Average	**983,40**		**111,97**		**8,99**		**6,21**		**121,78**		**4,01**	
	CV	22,74		4,55		33,55		12,25		19,37		5,33	
	LSD	383,61		8,73		5,17		1,31		40,47		0,37	
	Sesame genotypes	**Yield (kg/ ha)**		**Plant height (cm)**		**First branch height (cm)**		**Number of branches**		**Number of plant capsules**		**1000 seed weight (gr)**	
2017	**Arslanbey**	1258,87	abc	126,60	cd	9,33	a	1,87	c	140,40	ab	3,92	e
	Hatipoğlu	1188,36	abc	136,87	abcd	3,13	b	9,00	ab	137,60	ab	4,83	a
	Boydak	686,93	d	139,00	abc	10,27	a	8,13	ab	99,07	b	4,58	abc
	4	1356,19	abc	147,73	ab	6,13	ab	6,67	b	110,67	ab	4,25	cde
	6	1162,65	abc	121,13	cd	4,07	b	7,33	ab	93,87	b	4,08	de
	7	1037,14	cd	152,87	a	3,53	b	10,33	a	200,33	a	4,17	de
	8	1502,41	a	119,93	d	2,67	b	7,73	ab	132,60	ab	4,92	a
	10	1436,34	ab	136,00	abcd	4,80	b	5,93	b	108,60	b	4,33	bcd
	26	1154,11	abc	133,87	bcd	4,07	b	7,67	ab	110,60	b	4,67	ab
	27	1055,80	bcd	124,47	cd	4,93	b	7,27	ab	90,93	b	4,17	de
	Average	**1183,88**		**133,85**		**5,29**		**7,19**		**122,47**		**4,39**	
	CV	19,05		7,79		45,57		27,98		42,70		5,49	
	LSD	386,81		17,88		4,14		3,45		89,71		0,41	

Conclusions

The study was carried out for 2 years. In terms of the factors affecting yield and yield in different sesame genotypes, different responses were seen in every two years. Such as, in 2016, it is seen that the production of Arslan bey is higher than the yield in 2017 (Table 2), but from Chart 1 it is seen that the sesame growing season of 2016 is warmer than the season of 2017. This can be expressed as the fact that the genotypes of different genotypes are different from each other and thus react differently to changing climatic conditions.

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THE BUDDING SUCCESS IN LOQUAT (*ERIOBOTRYA JAPONICA* LINDL.) ON DIFFERENT QUINCE ROOTSTOCK

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Abstract

The purpose of the work was to find out the success of budding of loquat on different Quince rootstocks and the effect of these rootstocks on the growth of the nursery plants. For this purpose, Hafif Çukurgöbek loquat cultivar was budded on Quince-A, Quince-C and BA-29 rootstocks with chip budding on 25 April (spring), and 21 October (dormant), 2016. The experiment was laid out in a split-plot experimental design with five replications and fifteen plants per replication. The percentage values were transformed by the angle transformation before submitting the data to the analysis of variance. The means were separated by Tukey's Honestly Significant Difference (HSD) method at p=0.01 and p=0.05. The average success rate of budding on Quince rootstocks was better in October (89.2%) than in April (79.3%). In the both budding periods, it was found that buddings on BA-29 (respectively, 85.5% and 93.1%) were more successful than the ones on Quince-A (respectively, 69.8% and 88.5%) and Quince-C (respectively 83.2% and 86.0%). However, the highest bud sprout rate (86.1 %) was taken from the buddings which were done on Quince-C rootstock in October. Budding performed in autumn gave the higher values (81.4%) of bud sprout rate than the budding done in spring (28.4 %). In the measurements made, the highest values of stock and scion diameter and annual shoot length of the nurseries were obtained from Quince-C rootstock. As result, preliminary data obtained from this research show those quince rootstocks as dwarf rootstock in intensive loquat cultivation can be used.

Keywords: *dwarf rootstock, loquat, budding success, high density.*

Introduction

In all Mediterranean countries, the rootstocks used are loquat seedlings; they are very well adapted to calcareous soils which are very abundant in the Mediterranean basin. Trails of quince trees (A, C, BA-29) have often been studied in some countries. This species allows smaller and more compact trees, a shorter juvenile period and bigger fruits, with high sugar content and nice color. However, quince trees are very sensitive to calcareous soils and they show graft union incompatibility with many varieties (Llácer *et al*. 2003). On the other hand, in China which is the main loquat producing country, the most used rootstock is loquat seedlings while in some areas, quince is used as dwarfing rootstock (Lin, 2007).

The use of loquat seedling rootstock usually results in a comparatively large tree with a high canopy. Cultivars grown on quince rootstock produce a dwarfed tree of early bearing character. The smaller tree has no effect on fruit size and gives adequate fruit production with the advantage of easier picking (Crane and Caldeira, 2006). Commonly, trees are grafted onto loquat seedlings, occasionally on *Eriobotrya deflexa*, and *Photinia serrulata*, and may be grafted onto dwarfing rootstocks of quince (*Cydonia oblonga* Mill.) and pyracantha (*Pyracantha* spp.).

At present, the rootstocks used are loquat seedlings in Turkey. However, the dwarfing rootstocks are very important in loquat culture. The utilization of the dwarfing rootstocks therefore higher yields, but also will have other advantages such as easier and more effective cultural applications (Polat and Kaska 1992a, 1992b). We suggest dwarfing rootstocks such as Quince-A and BA-29 for loquat production. This species allows growers to obtain smaller and

more compact trees. In some of our studies (Polat and Kaska, 1992a; Polat 1995), Quince-A rootstock had a 25% dwarfing effect compared to loquat seedlings. Polat and Calıskan (2007) recommended that nurseries should initiate grafting loquat onto quince rootstock. The purpose of the work was to find out the success of budding of loquat on different Quince rootstocks and the effect of these rootstocks on the growth of the nursery plants.

Material and Method

This research was carried out during two growing sessions 2016 and 2017 at experimental nursery of Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University, Antakya, Hatay, Turkey. Hafif Çukurgöbek loquat cultivar was budded on Quince-A, Quince-C and BA-29 rootstocks with chip budding on 25 April (spring), and 21 October (dormant), 2016. The plants were irrigated by drip irrigation. The experiment was laid out in a split-plot experimental design with five replications and fifteen plants per replication. In the spring period, the percents of bud-take successes were recorded after two months of the budding operations, whereas the observations on bud sprout rates were determined after four months of the budding. Bud take and bud sprout rates were determined respectively, 22 February 2017 and 31 March 2017, in the dormant buddings. The annual shoot length, plant height and trunk diameter (5 cm below and above of bud union) of all nurseries were determined interval one month during the growth period. The percentage values were transformed by the angle transformation before submitting the data to the analysis of variance. The means were separated by Tukey's Honestly Significant Difference (HSD) method at p = 0.01 and p=0.05.

Result and Discussion

Budding Success

The success rates of budding made in different periods are presented in Table 1. In all rootstocks, the budding success rates were found higher in fall (dormant) buddings than in spring ones. The average budding success rate was higher in October (89.2%) than in April (79.3%). The differences between the budding periods were statistically significant at $p<0.01$. In the both budding periods, it was found that buddings on BA-29 (respectively, 85.5% and 93.1%) were more successful than the ones on Quince-A (respectively, 69.5% and 88.4%) and Quince-C (respectively 83.2% and 86.0%). The differences between the rootstocks were statistically significant at $p<0.01$.

Table 1. The success rates of budding made in different times

Rootstocks	Time of budding		Average (%)
	24 Ap, 2016	21 Oct. 2016	
Quince-A	69.51 d$^{(y)}$	88.45 ab	78.98 B$^{(x)}$
Quince-C	83.24 c	86.00 bc	84.62 AB
BA- 29	85.22 bc	93.13 a	89.17 A
Average (%)	79.32 B$^{(x)}$	89.19 A	

$^{(x)}$Means followed by different capital letters are for rootstocks and budding dates and indicate significant difference by Tukey's test at 0.01.

$^{(y)}$Means followed by different lowercase letters are for rootstock x budding dates interaction and indicate significant difference by Tukey's test at 0.01.

However, the highest bud sprout rate (86.1 %) was taken from the buddings which were done on Quince-C rootstock in October, 2016. The bud sprout rates were found higher in autumn (81.4%) buddings than in spring (28.4 %) ones (Table 2).

Table 2. The bud sprout rates of budding made in different times

Rootstocks	Time of budding		Average (%)
	24 Apr.2016	21 Oct. 2016	
Quince-A	31.43 c[(y)]	76.99 b	54.21 AB[(x)]
Quince-C	30.33 cd	86.11 a	58.22 A
BA- 29	23.31 d	81.05 ab	52.18 B
Average (%)	28.36 B	81.38 A	

[(x)]Means followed by different capital letters are for rootstocks and budding dates and indicate significant difference by Tukey's test at 0.01.
[(y)]Means followed by different lowercase letters are for rootstock x budding dates interaction and indicate significant difference by Tukey's test at 0.01.

Rootstock × budding period interaction was statistically significant at p=0.01 indicating that rootstocks are affected differently with budding time. In both time of budding, BA-29 had the highest bud-take rates while the lowest bud-take rates was observed in Quince-A on April, 2016, Quince C on Oct., 2016. In previous studies with Quince-A (Polat and Kaşka, 1992a), indicate 54.8% budding success in spring budding and 36.0% in dormant buddings made in fall. The budding made in April gave more successful those than in May. In loquat budded onto Quince-C rootstock, 56.7% budding success has been achieved (Polat and Kaşka, 1992b) and a budding success rate of 66.5% was achieve by Yinanç (2000) onto Quince-A.

Vegetative trait

The annual length of shoot, height of plant and scion-stock trunk diameter of nurseries are presented in Table 3. In the measurements made, the highest values of stock and scion diameter, plant height and annual shoot length of the nurseries were obtained from Quince-C rootstock and this was followed by BA-29. The lowest values were observed on Quince –A rootstock for all parameter. The differences between the rootstocks were found statistically significant at $p<0.01$ for the scion diameter while found statistically significant at $p<0.05$ for the stock diameter, plant height and annual shoot length.

Table 3**.** The effects of quince rootstocks on the characteristics vegetative growth of Hafif Çukurgöbek cultivar.

Rootstock	Scion diameter (mm)	Stock diameter (mm)	Plant height (cm)	Lenght of shoot (cm)
Quince-A	13.73 b[(x)]	17.73 b	53.96 b	30.90 b
Quince-C	20.57 a	23.94 a	69.66 a	44.88 a
BA- 29	17.31 ab	21.63 ab	64.46 ab	38.76 ab
HSD	p<0.01	p<0.05	p<0.05	p<0.05

[(x)]Means within a column followed by different letter are significantly at the 5% level by Tukey's HSD test.

Conclusion

Budding success depends on a lot of factor in fruit trees. Ecology, rootstock and variety features, budding or grafting techniques, and care after budding or grafting can be counted among these factors. When the results compared to the other research, it shows that our results

are quite successful. The average success rate of budding on Quince rootstocks was better in October (89.2%) than in April (79.3%). In the both budding periods, it was found that buddings on BA-29 were more successful than the ones on Quince-A and Quince-C. However, the highest bud sprout rate (86.1 %) was taken from the buddings which were done on Quince-C rootstock in October. In the measurements made, the highest values of stock and scion diameter and annual shoot length of the nurseries were obtained from Quince-C rootstock. As result, preliminary data obtained from this research have shown that can use of quince rootstocks as dwarf rootstock in intensive loquat cultivation.

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EFFECTS OF SOME QUINCE ROOTSTOCKS ON PHENOLOGICAL PROPERTIES AND FRUIT SET RATES IN HAFIF CUKURGÖBEK LOQUAT CULTIVAR

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Abstract

In the research, it was aimed to determine the effects of different quince rootstocks on phenological stages and inflorescence properties besides to the success of obtaining dwarf loquat nursery, which was a requirement of intensive loquat cultivation. For this purpose, one year old nursery of Hafif Çukurgöbek loquat cultivar budded on BA-29, Quince-A and Quince-C quince rootstocks was used. The experimental plants were planted at high density with planting spaces of 1.0 m x 0.5 m in January 2017. The plants were irrigated by drip irrigation since their transplantation. The experiment was arranged according to a completely randomized designed with 5 replications and 6 plants were used in each replicate. In the study, effects of the rootstocks on the flowering periods, the inflorescence characteristics, flowering and fruit set rates of the cultivar were evaluated. In terms of the number of flower buds in the inflorescence (bud/per cluster), plants with Quince-A rootstock (114.91) were given higher values than the BA-29 (106.13) and Quince-C (104.06) rootstocks. However, the highest blossoming rate was observed on BA-29 (99.22%), followed by Quince-C (87.36%). The lowest blossoming rate was measured in plants with Quince-A rootstock (86.27%). Plants with BA-29 rootstock reached full bloom 7-15 days before the other two rootstocks. The fruit set ratios were observed similar in plants with Quince-A and Quince-C rootstocks (14.68 %, 14.11, respectively) whereas the lowest fruit set ratio was observed in and BA-29 rootstock (10.29%).

Key words: *BA-29, Quince-A, Quince-C, rootstrock, phenological properties.*

Introduction

Loquat seedlings, quince, and hawthorn can be used as rootstocks for loquat (Polat, 1995). Loquat seedlings are preferred over quince or pyracantha rootstocks under most conditions. Quince and pyracantha may cause extreme dwarfing–to less than 8 ft (2.5 in). Quince rootstock tolerates heavier and wetter soils than loquat but is apt to put out numerous suckers (Crane and Caldeira, 2006).

Loquat is a large tree which restricts the number of trees that can be planted per unit area and makes it necessary to harvest fruit with ladders. Furthermore many of the large trees show considerable juvenility, delaying the onset of commercial harvest. The use of dwarfing rootstocks using quince (*Cydonia oblonga*) is one method to reduce tree size, facilitate harvest, and increase early yield, but compatibility of loquat and quince is low(Janick, 2011).

The use of dwarfing rootstocks in loquat production reduces tree size making it possible to plant more trees per unit area and thereby increase early yield (Polat et al., 2004), facilitate harvest, and reduce costs (Polat et al., 2003). Quince rootstocks slow scion growth reducing tree size by 20 to 25% as compared to loquat seedlings, increase earliness, and increase fruit quality and size (Demir, 1987; Polat and Kaşka, 1992a, b; Polat, 1995).

At present, the rootstocks used are loquat seedlings in Turkey. However, the dwarfing clonal rootstocks are very important in loquat culture. The utilization of the dwarfing therefore higher yields, but also will have other advantages such as easier and more effective cultural applications (Polat and Kaska, 1992c, 1992d, 1992e). We suggest dwarfing, clonal rootstocks such as Quince-A and BA-29 for loquat production. This species allows growers to obtain

smaller and more compact trees. In some of our studies (Polat and Kaska, 1992c; Polat, 1995), Quince-A rootstock had a 25% dwarfing effect compared to loquat seedlings. Polat and Calıskan (2007) recommended that nurseries should initiate grafting loquat onto quince rootstock.

In the research, it was aimed to determine the effects of different quince rootstocks on phenological stages, and inflorescence properties besides to the success of obtaining dwarf loquat nursery, which is a requirement of intensive loquat cultivation.

Material and Method

This research was carried out during two growing sessions 2016-2017 and 2017-2018 at experimental nursery of Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University, Antakya, Hatay, Turkey. In the study, one year old nursery of Hafif Çukurgöbek loquat cultivar budded on BA-29, Quince-A and Quince-C quince rootstocks was used. The experimental plants were planted at high density with planting spaces of 1.0 m x 0.5 m in January 2017. The plants were irrigated by drip irrigation since their transplantation. The experiment was arranged according to a completely randomized designed with 5 replications and 6 plants were used in each replicate. In the study, effects of the rootstocks on the flowering periods, the inflorescence characteristics, flowering and fruit set rates of the cultivar were evaluated. Flower buds were observed from full rest until fruit set. Data were taken on date of first blossoming, full bloom, end of blossoming and harvest time. We considered the beginning of flowering as the date when 5% of the flowers were open; 70% as full bloom and 90% petal drop as the end of blossoming (Durgac et al., 2006). To determine the flowering periods and the fruit set rate of the cultivar according to rootstocks, a branch was selected randomly from the four sides of the canopy and they were tagged accordingly. All flower buds were counted at the pre-blossom phase at each of these selected branches to carry out phenological observations. The flowers of each tagged branch were counted and % blossom rate was calculated by taking the proportion of the flower amount to bud amount during the observations made between the phase when blossoming starts and ends. 10 days after the end of blossoming, the number of initial fruits was found and by dividing this number by flower amount, % initial fruit set was determined. The fruit set was determined one month after the initial fruit set. Percentage final fruit set was determined by taking the proportion of the amount of fruit during the maturing period to the amount of flowers. The percentage values were transformed by the angle transformation before submitting the data to the analysis of variance. The means were separated by Tukey's Honestly Significant Difference (HSD) method at $p = 0.01$.

Result and Discussion

Cluster Characteristics and Fruit Set

The effects of the Quince rootstocks used in the experiment on blossoming rate and inflorescence characteristics of the Hafif Çukurgöbek cultivar are given in Table 1.

As shown in Table 1, in all three rootstocks, the number of cluster per branch of the Hafif Çukurgöbek cultivar was the same and did not show any difference according to the rootstocks. Although there is no significant difference in terms of the average number of secondary axis on the main panicle axis, the number of secondary axis on Quince-A was found to be somewhat higher. On the other hand, in terms of the number of flower buds in the inflorescence (bud/per cluster), plants with Quince-A rootstock (114.91) were given higher values than the Quince-C (104.06) and BA-29 (106.13) rootstocks (Table 1).

Table 1**.** The effects of some Quince rootstocks on blossoming and fruit set rates and inflorescence characteristics of the Hafif Çukurgöbek cultivar.

Rootstocks	No. of secondary axis per main panicle axis	No.of flower bud per cluster	No.of opened flower per cluster	Blossoming Rate (%)	Inital fruit set (%)	Fruit set (%)	Final fruit set (%)
Quince-A	12.74 a[(x)]	114.91 a	98.57 ab	86.27 b	22.60 a	14.68 a	1.80 c
Quince-C	12.57 a	104.06 b	89.55 b	87.36 b	20.34 a	14.11 a	6.59 a
BA-29	12.58 a	106.13 b	105.23 a	99.22 a	14.49 b	10.29 b	4.97 b
Significance	NS[(y)]	**	**	**	**	**	**

[(x)]Means within a column followed by different letter are significantly at the 1% level by Tukey's HSD test.
[(y)]Not significance

In terms of the number of flowers opened in the cluster, it was determined that the Hafif Çukurgöbek/BA-29 combination (105.23) bloomed higher than the Hafif Çukurgöbek/Quince-A(98.57) and Quince-C(89.55) combinations. Blossoming percentages were significantly affected by rootstocks. The highest blossoming rate was observed on BA-29 (99.22%), followed by Quince-C (87.36%). The lowest blossoming rate was measured in plants with Quince-A rootstock (86.27%). Initial fruit set rates of the rootstocks showed significant differences. The highest initial fruit set rate was in plants with Quince-A rootstock (22.60 %) and this was followed by Quince-C(20.34 %) and BA-29(14.49) rootstocks. The highest fruit set rate was observed on Quince-C (6.59%), followed by BA-29 (4.97 %). The lowest fruit set rate was measured in plants with Quince-A rootstock (1.80 %). The differences between the rootstocks for all these parameters were statistically significant at $p<0.01$ (Table 1).

We did not have a chance to compare our findings because we did not find a similar study in the literature for quince rootstocks. There are some studies on flowering and fruit set rates of different loquat cultivars. Polat (2007) observed that fruit set rates for loquats depend upon different genotypes, years, and environmental conditions. Our initial fruit set rates (14.49%–22.60%) are higher than those of Erdoğdu (1987) who tested 30 loquat cultivars under Adana conditions. The 5.0% fruit set rate which was found by Ateyyeh and Qrunfeh (1998) for 'Tanaka' was lower than our range. However, our results were similar those by Demir (1989) who studied the six cultivars with the range of 16.4–17.4% under Antalya (Turkey) conditions. The finding of Mann and Sagar (1987) of 15% is lower than our finding. It is obvious that fruit set rates for loquats depend upon different genotypes, and canopy facing side.

Phenological Data

The results related to the effects of the Quince rootstocks used in the experiment on the phenological stage of the Hafif Çukurgöbek cultivar are given in Table 2.

Flowering period of the Hafif Çukurgöbek/Quince rootstock combination were completed between 15 Dec. and 08 Feb. (Table 2). The first flowering was observed on the plants with BA-29 rootstock, plants with Quince-C rootstock began flowering later. Plants with BA-29 rootstock reached full bloom 7-15 days before the other two rootstocks. In terms of the other phenological phases, BA-29 rootstock was found earlier than the Quince-A and Quince-C rootstocks. The earliest fruit ripening was observed on plants with BA-29 rootstock (Table 2).

Table 2**.** The effects of the Quince rootstocks on the phenological stage of the Hafif Çukurgöbek cultivar

Rootstocks	The phenological properties					
	Bud swelling	Beginning of blossoming	Full bloom	End of blossoming	Fruit set	Maturity period
Quince-A	01.12.2017	28.12.2017	30.01.2018	05.02.2018	17.02.2018	16.05.2018
Quince-C	01.12.2017	30.12.2017	23.01.2018	08.02.2018	24.02.2018	16.05.2018
BA-29	29.11.2017	15.12.2017	15.01.2018	02.02.2018	15.02.2018	9.05.2018

Conclusion

As result, preliminary data obtained from this research show those quince rootstocks as dwarf rootstock in intensive loquat cultivation can be used. In terms of the all phenological phases, BA-29 rootstock was found earlier than the Quince-A and Quince-C rootstocks. The earliest fruit ripening was observed on plants with BA-29 rootstock.

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EVOLUTIONARY INSIGHTS INTO MICRORNAS OF KIWIFRUIT *ACTINIDIA CHINENSIS* AND ITS CLOSE RELATIVES

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Abstract

The advent of next-generation sequencing technologies and newly developed bioinformatics tools have provided us complete sequence information of organisms. Plant microRNAs (miRNAs), small non-coding RNAs about 21-24 nucleotides, and their regulatory roles in biological processes have been uncovered since the identification of the first miRNA. MicroRNA biogenesis and modes of actions have also been elucidated in previous studies.

In the presented study, we identified putative microRNAs from *Actinidia chinensis*, *Arabidopsis thaliana*, *Solanum lycopersicum*, *Solanum tuberosum* and *Vitis vinifera* to compare their miRNA repertoire. According to the results, the highest synteny was found between *V.vinifera-A.chinensis* and the least synteny was found between *A.thaliana-A.chinensis*. The highest number of putative miRNAs were identified from *A.thaliana* whereas the least amount of putative miRNAs were identified from *V.vinifera*. This may be depended on the size of the genomes. We also analyzed the targets of putatively identified miRNAs for each organism. Expectedly, the target pathways of the predicted putative miRNAs were similar between the closest organisms. Expressed miRNA families and copy number of miRNA genes were compared between all organisms. In *A.thaliana*, the number of expressed putative miRNAs are more than the other organisms. For all the organisms, different miRNA families had the high copy number of genes. Therefore, highly represented miRNA families on each genome may have specific functional roles. The findings in this study will help the research community to identify the roles of miRNA players on critical biological pathways.

Keywords: MicroRNAs*, Actinidia chinensis, Solanum tuberosum, Solanum lycopersicum, Vitis vinifera*

Introduction

Increasing world population, the drastic climate changes, the threat of biotic stress and abiotic stress factors on the plants and the scarcity of arable lands have created a big concern about the future sufficiency of food demands. To prevent this unwanted scenario in the next centuries, some new agricultural technologies should have been developed to ensure food security (Liu and Chen, 2010; Akpinar et al., 2012; Avsar, 2011). Recent studies show that revolutionary advances in next-generation sequencing techniques became popular in plant biology area. The advent of this new technologies has increased our knowledge from genomics to plant breeding and evolutionary studies. An increasing number of sequenced plant genomes will provide us the greater understanding of their genome, growth and developmental mechanism and evolutionary processes (Egan et al., 2012).

Non-coding RNAs or ncRNAs, such as transfer RNA (tRNA), ribosomal RNA (rRNA) and small nuclear RNA (snRNA) have been identified for a long time ago. Among ncRNAs, microRNAs (miRNAs) and small interfering RNA (siRNA) have gained more attention since they have been found as essential regulators of gene expression (Choudhuri, 2009). MicroRNAs have short lengths about 21-24 nucleotides emerged from their longer precursor sequences that are variable sizes between the plant species contrary to the animal

counterparts. After the discovery of plant miRNAs and their great regulatory roles in the cell, both computational and experimental strategies have been studied for identification of the related genes and microRNAs' targets. Experimental methods are based on direct cloning and genetic screening whereas computational methods are dependent on new generation sequencing technologies because this powerful technology is time-saving and cost-efficient. With the help of the bioinformatics and computational tools, miRNA studies have also been increased enormously for plant genomes. (Zhang and Wang, 2015; Avsar and Aliabadi, 2017a; Avsar and Aliabadi, 2017b; Avsar and Aliabadi, 2018).

The kiwifruit or Chinese gooseberry is the edible berry member in the Actinidia genus and recent genomic studies reveals that the kiwifruit species often have polyploidy structure with a chromosome number as x=29 results from hexaploidization and two more recent whole genome duplication events (McNeilage and Considine, 1989; Huang et al., 2013) and it completes the divergence from Solanaceae species such as tomato and potato. These duplication events have provided neo-functionalization of important genes including in vitamin C, flavonoid, and carotenoid metabolic activities. It also has one of the well-known fleshy fruit since it is an excellent source of several vitamins, minerals, dietary fibers and other related health benefit dietary nutrients (Skinner et al., 2011). In addition to this, recent studies show that the consumption of kiwifruit has positive effects on cardiovascular health through antioxidant activity and by promoting gut microflora. It has also found that the kiwifruit support immune system either by up-regulating some defense-related genes or activating 'DNA-repair' mechanism in the cells (Skinner et al., 2011).

Taken together, we predicted some putative miRNAs in silico between the kiwifruit *A. chinensis* and its close relatives by using genomic sequence data and computational tools to gain a better understanding of their relations and to provide a valuable resource for the evolutionary processes in the Asterid lineage. We also showed that clustering of miRNAs repertoire between chosen organisms does not show the same pattern as the relationship between their whole genome.

Material and Methods

Reference miRNAs and Datasets: miRBase corresponds to 4,802 unique mature miRNA sequences, and these mature miRNAs were used as a query in homology-based in silico miRNA identification. For *A.chinensis*, genome sequence was retrieved from the website. *A.thaliana*, S. lycopersicum, *S.tuberosum* and *V.vinifera* masked Ensemble Plants website provided genomic sequences.

In silico miRNA Identification based on Sequence Homology and Secondary Structure Conservation: A two-step strategy was adopted based on the preliminary selection of database sequences with homology to a previously known plant mature miRNA and their subsequent retention assessing the consistency of their secondary structure with pre-established pre-miRNA features (Zhang et al., 2006). The prediction was employed using two previously developed, in-house Perl scripts: SUmirFind and SUmirFold, described in detail in the publications. In the first step of homology-based miRNA prediction, SUmirFind script, which utilizes BLAST+ stand-alone toolkit, version 2.2.31 (Camacho et al., 2009) was used for detection of database sequences with homology (mismatch cutoff parameter set to <=3) to previously known plant mature miRNAs (Zhang et al., 2006).In the second step, SUmirFold, a script that generates secondary structures through UNAFold version 3.8 was used with parameters optimized to include all possible stem-loops generated for each miRNA query. SUmirFold output was further processed to eliminate redundant hits, resulting from cases where identical miRNAs were predicted from two similar query mature miRNA sequences. Moreover, hairpins with multi-branched loops, with inappropriate DICER cut sites at the ends of the miRNA-miRNA* duplex, or with mature miRNA sequence portions at the head of the

pre-miRNA stem-loop were also manually removed. This process was done for all genomic datasets of plant species.

Clustering of plant species based on the variety of their cumulative miRNAs: Mature miRNA sequences from each plant species were separately listed and the binary matrix showing cumulative miRNA datasets in all five plant species was formed. For hierarchical clustering, euclidian distance based centroid clustering was adopted. Distance matrix construction, clustering, and dendrogram generation were performed in the MINITAB program.

Target annotation of predicted miRNAs: Mature sequences were collected from each species and duplicates were removed. By using an online web tool, psRNA, an analysis was performed, and hit sequences were retrieved. These retrieved sequences were used as input data for the Blast2Go online web tool (Conesa and Götz, 2009). Target annotation charts were created for all plant species. Experimentally validated targets of predicted putative miRNAs were also detected in miRBase (Kozamara and Jones, 2013).

The copy number of miRNA genes and expression analysis of predicted miRNAs: Repeated same miRNAs that resulted from the similar query miRNA stem-loop sequences were eliminated to avoid over-representation. The EST-database was formed separately as a specific to each plant species, and the restricted criteria were used for the analysis as the only miRNA families who had hits above the threshold as 98% identity, and 99% query coverage were retrieved.

Results and Discussions

Putative predicted miRNAs and their distribution across plant species: We identified putative miRNAs from each organism (Figure 1).

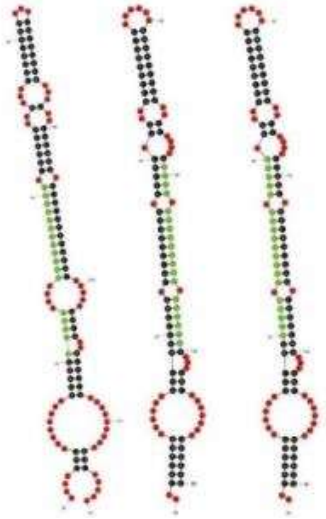


Figure 1. Predicted pre-miRNA stem-loop structures of selected miRNAs from *A.thaliana*. Mature miRNAs start and end points are shown by green color.

For each organism, average sequence length with a median for identified pre-miRNAs, mature miRNAs, average GC % content for pre-miRNAs with median and min-max values were calculated, and they are shown in Figure 2. Minimal folding free energy index (MFEI), which is calculated from the minimum folding free energy (MFE), sequence length and %GC content of the pre-miRNA, differentiates miRNAs with typically higher MFEIs (0.67) from other types of cellular ssRNAs for which MFEIs were previously characterized; transfer RNAs (0.64), ribosomal RNAs (0.59), and mRNAs (0.62–0.66)(Schwab et al., 2005). The low negative MFE values show the higher stability of the predicted miRNA (Zhang et al., 2007; Jin et al., 2008).

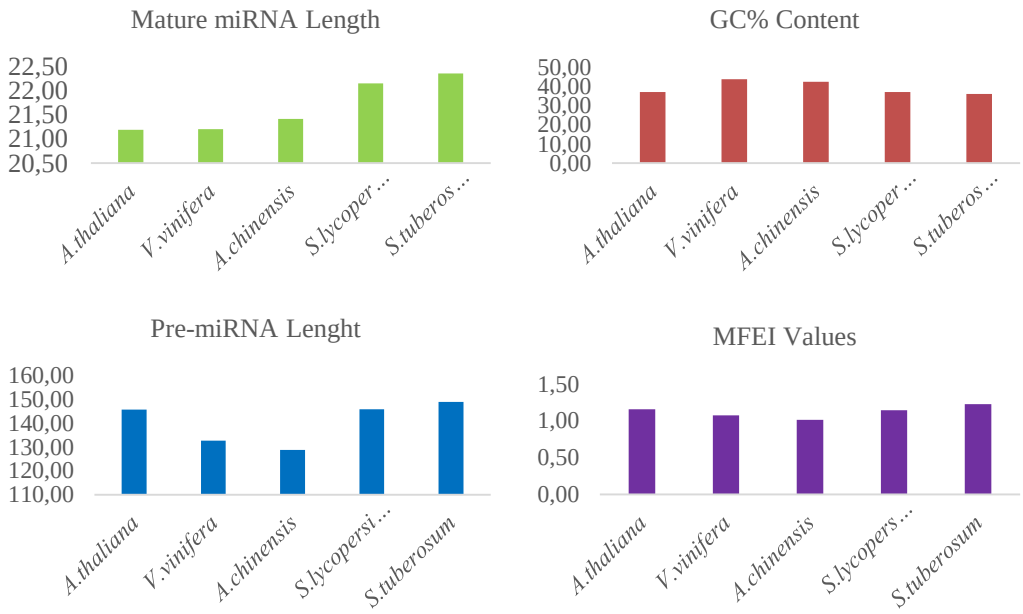


Figure 2. MFE and MFEI tables with minimum, maximum and median values for unique pre-miRNA sequences.

Prediction of putative miRNAs from genomes of different plant species enabled us to compare the variety of miRNAs across species. In the *A.thaliana* genome, we predicted as 93 putative miRNAs; in *S.lycopersicum* genome 57 putative miRNAs were predicted and analyzed. *S.tuberosum* had 61 putative miRNAs to be analyzed whereas, in *V.vinifera* genome, we predicted 43 putative miRNAs (Figure 3). In our previous study, we found 52 putative miRNAs in *A.chinensis* genome during the last study (Avsar and Esmaeili, 2015). According to our results, *A.thaliana* had the highest number of putative miRNAs in its genome whereas *V.vinifera* had the lowest number of miRNAs. Although the genome size of V. vinifera (~500Mbp) is larger than the *A.thaliana* (~135Mbp), the number of predicted putative miRNAs did not show the similar correlation. This may be caused by different evolutionary biogenesis characteristics of miRNAs across plant species.

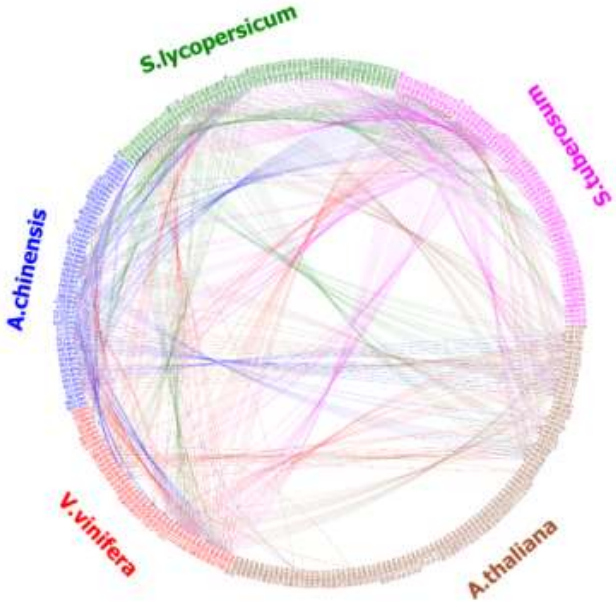


Figure 3. All miRNAs predicted from five organisms (Avsar et. al., 2016).
Link:http://APresenter.com/view.faces?id=2055757656

Between all those five plant species, some miRNA families were found commonly: miR156, miR157, miR160, miR162, miR167, miR169, miR170, miR171, miR172, miR319, miR390, miR396, miR398, miR399. Those common miRNAs are probably not "species-specific" type of miRNAs but have some critical regulatory roles including development, growth, modulation of auxin-response. All common miRNAs between organisms are shown in Figure 4.

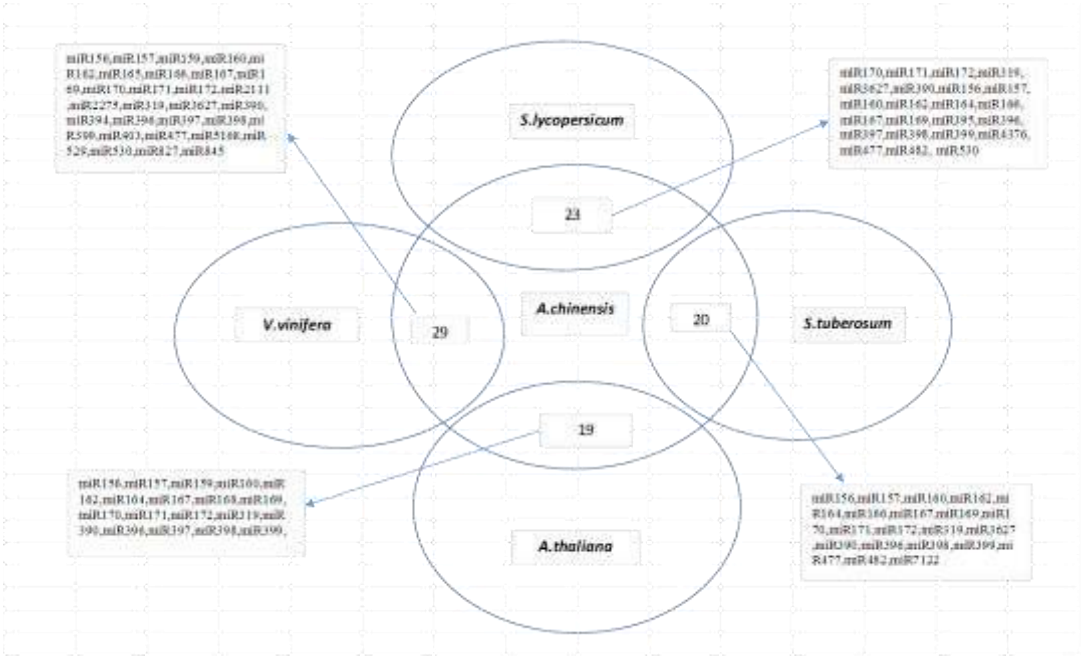


Figure 4. Common miRNAs between all organisms.

Clustering of putative miRNAs: The clustering of miRNA families of plant species showed that the higher similarity between *S.lycopersicum-S.tuberosum* than *V.vinifera-A.chinensis* (Figure 5). Huang et al. analyzed some other sequenced plant genomes to compare with the kiwifruit and they showed that the tomato had the closest evolutionary relationship (Huang et al., 2013) however regarding miRNA families clustering, grapevine showed more similarity to kiwifruit genome. These findings might be used for further evolutionary studies.

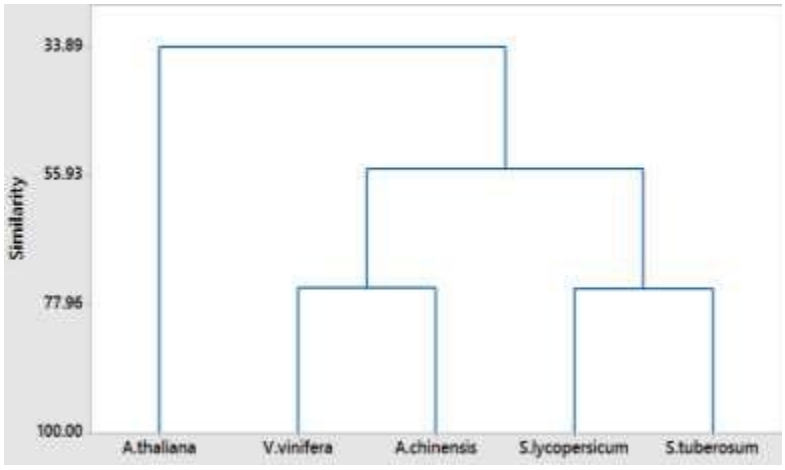


Figure 5. Clustering analysis for all species.

Targets of putative miRNAs and their annotations: Duplicate mature miRNA sequences were removed and the remaining mature miRNA sequenced were used for target annotations. These annotations were conducted to identify the miRNA targets in biological processes, molecular pathways, and cellular components.Based on the biological processes results, targets of *S.tuberosum* and *S.lycopersicum* putative miRNAs showed similarity as they have roles in metabolic processes, single-organism processes, response to stimulus and cellular processes. However, *A.chinensis* and *V.vinifera* had the similar functions mentioned above in addition to the cellular component organization and localization. *A.thaliana* miRNA targets were not identical to any organism since they had roles in different pathways (Figure 6).

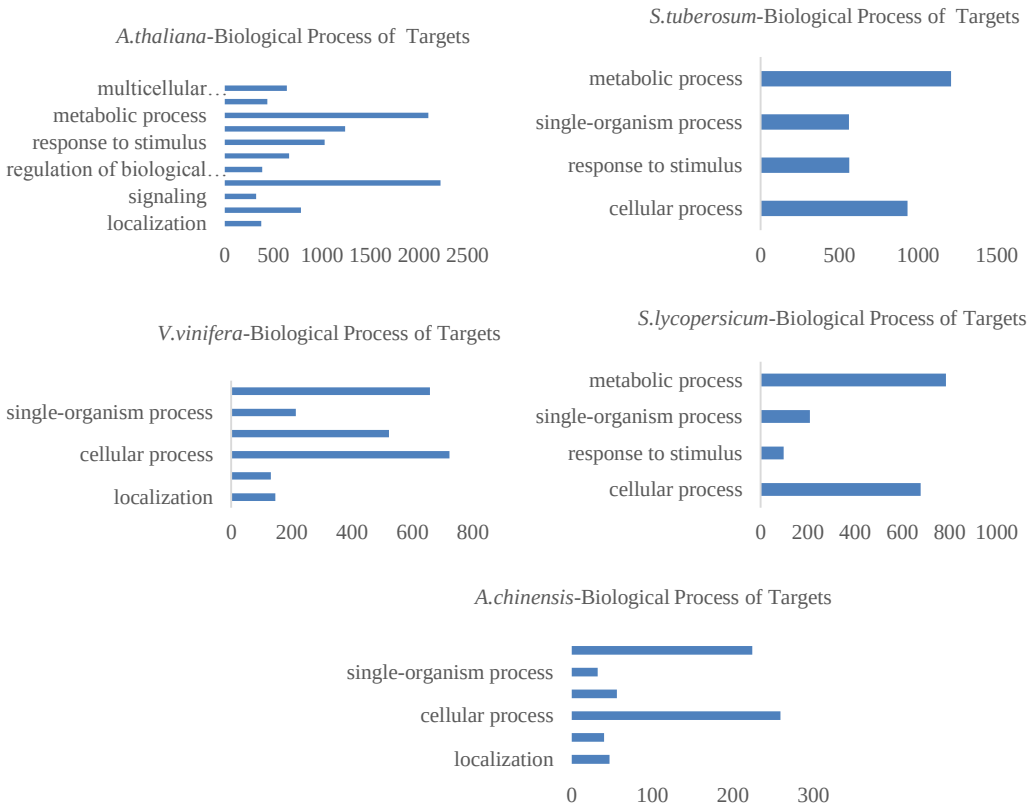
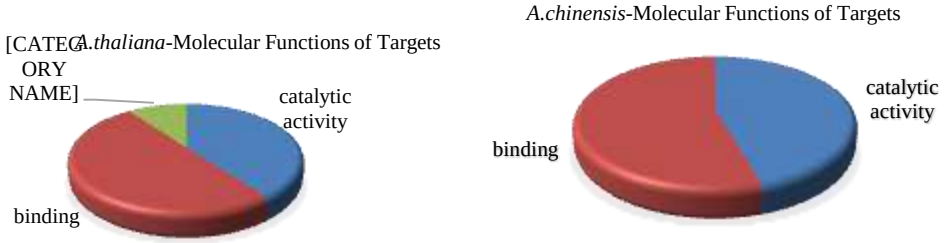


Figure 6. Blast2Go target annotations chart based on biological processes.

For the molecular functions of targets, almost all the organisms showed the similar results except for *A.thalina*. Putative miRNA targets are mostly found in catalytic activity and binding processes. In *A.thaliana*, additionally, nucleic acid binding transcription factor was found. In *A.chinensis*, putative miRNA targets were primarily found in binding activities (Figure 7).



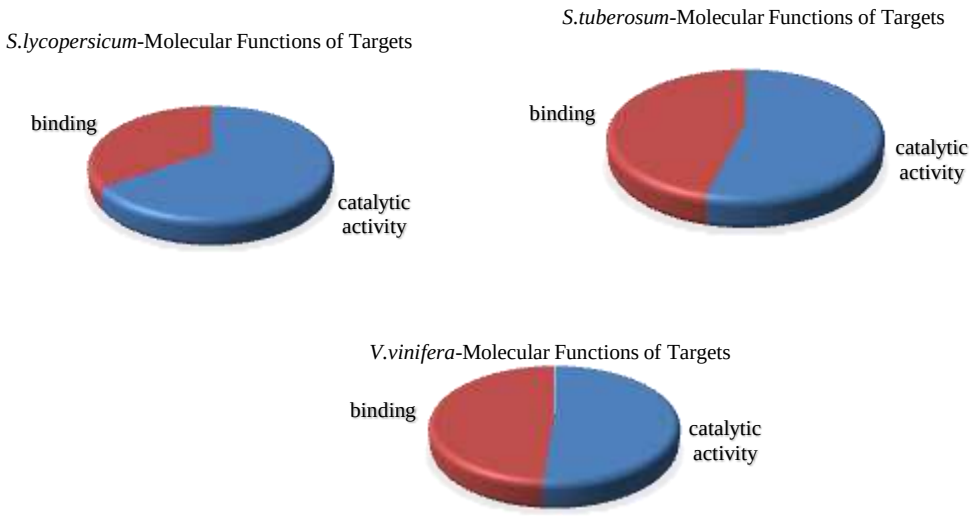


Figure 7. Blast2Go target annotations chart based on their molecular functions.

Representative miRNA families and the expression analysis of predicted miRNAs: We cannot determine the absolute copy number of each miRNA families by indeed since some miRNA genes may be covered by more than one while others may not be covered at all. However, the representation of each miRNA within the dataset provides us to estimate its prevalence on the genome. According to those results, miR169 and miR156 families were highly represented in the *A.thaliana* genome. In *S.lycopersicum*, miR5303 families had the highest representative whereas in *S.tuberosum* miR7984 families were represented mostly. miR3631 families were the most represented in *V.vinifera* genome however in *A.chinensis*, miR156 families and miR171 families were highly represented.

1,527,298 and 300,445 EST sequences were retrieved from NCBI database for *A.thaliana* and *S.lycopersicum*, respectively. 250,136 and 460,107 EST sequences were retrieved for *S.tuberosum* and *V.vinifera*, sequentially. Finally, 132,583 *A.chinensis* EST sequences were obtained from NCBI. All EST databases were built separately and analyzed in silico. According to those results, the highest number of expressed miRNA families (miR160, miR167, miR171, miR398, miR5654, miR5656, miR8170, miR826, miR830, miR840, miR860, miR869) was found in *A.thaliana* genome whereas the least number of expressed miRNA families was found in *A.chinensis* (miR535). This may result from the number of EST sequences in the database that we used since *A.thaliana* had the highest EST sequences and *A.chinensis* had the lowest EST sequences in their databases. All other expressed miRNA families for all organisms are shown in Table 1.

Table 1. *In silico* expression analysis on all plant species.

A.thaliana	miR160, miR167, miR171, miR398, miR5654, miR5656, miR8170, miR826, miR830, miR840, miR860, miR869
A.chinensis	miR535
S.lycopersicum	miR157, miR156, miR172, miR482, miR5303, miR5304, miR7983, miR7997
S.tuberosum	miR6027, miR156, miR172, miR396
V.vinifera	miR3623, miR172, miR3633, miR3634, miR396, miR397, miR827

Conclusions

In this study, *A.thalinana*, *V.vinifera*, *S.lycopersicum* and *S.tuberosum* were used for the comparison of miRNA repertoire since they were also performed for better understanding the genome of *A.chinensis* in draft genome sequencing article (Huang et al., 2013). *A.chinensis* is the first sequenced genome in the order of Ericales, so our study will be a good source for comparative genomics and evolutionary studies in the asterid lineage. It has also substantial duplication events after the divergence from tomato and potato and the genome structure might be affected by gaining some additional properties. In our study, we predicted that *A.chinensis* harbored some putative miRNAs that were not found in *S.tuberosum* or *S.lycopersicum* and these different putative miRNAs might result from the divergence events. Kiwifruit also has a rich source of vitamin C and other nutritional compounds so those identified miRNAs might have some regulatory roles on the pathways. In other words, kiwifruit genome will be an invaluable source of better understanding genome evolution and improvement of some agronomical properties in other plants including nutrient metabolism, disease resistant, sex determination and polyploidy events and the findings in the presented study will help to research community to elucidate the roles of miRNAs in these critical pathways.

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THE EFFECTS OF HUMIC SUBSTANCE APPLICATION ON CLUSTER AND SHOOT CHARACTERISTICS OF "TRAKYA ILKEREN" GRAPE VARIETY

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Abstract

Heavy clay soils having firmly structure make difficult to growth of plant roots. Humic substances contribute to plant growth and development by improving the structure of the soil. This research was carried out in order to determine the effects of humic substance application on grape cluster and shoot characteristics of "Trakya Ilkeren" variety. In heavy clay soil conditions, humic substance (total humic + fulvic acid 15%, total organic matter 10%, water soluble K_2O 2%, pH 8-10) was applied to soil and foliar in 2014-2015. In the study, humic substance was applied in pre-flowering and post-flowering stages on grapevines. The effects of humic substance application on cluster weight, cluster width, berry width, berry length and berry weight was found insignificant except for cluster length. The effect of humic substance on titratable acidity, pH, and maturity index was found to be significant except TSS. The highest TSS, and cluster weight was found on soil application while the highest berry weight, pH and titratable acidity obtained in foliar application of humic substance. The highest cluster weight, cluster width and berry length was found in soil application while cluster length, berry width and berry weight measured in foliar application of humic substance. The effect of humic substance application on leaf area and shoot length was found sıgnificant, but there was no effect on shoot diameter. In the study TSS, cluster weight, berry weight, pH and titratable acidity was affected positively on humic applications. According to results, the both humic substance application to the soil and foliar will be more effectively.

Keywords: *humic substance, grape, cluster, vegetative development, quality*

Introduction

In organic farming, the use of organic origin fertilizers such as compost and animal manure is supported. However, animal manure has only healed a certain part of the soil, there is no effect deeply. In addition, the intensive use of manure in farming increases the emergence of weeds. However, humic substances contribute to plant growth and development by improving soil structure. It also plays an important role in soil fertility and plant nutrition (Asgharzade and Babaeian, 2012). Natural sources "lignite" and "leonardite" are the most common sources of humic substances (Kutluca, 2007). Humic acid have also important roles in agriculture. They are the most active components of soils and organic composts that act directly on the plant (Chen *et al.,* 2004). There are some plants hormone-like structures in humic substances. Thus, they have a stimulating effect on growth in plants (Pizzeghello *et al.,* 2002). In particular, it has been noted that humic acid reduce the adverse effects of chemical fertilizers in viticulture (Akıncı, 2011).

Humic acid is a complex macro-organic molecule found in dissociated organic matter, peat, coal deposits and soils. When the humic acid applied to soil, It increase the germination capacity of the seed and multiplies the vitamin content of plants (Oğuz *et al.,* 2012). They are also a reserve for microorganisms thanks to elements such as C, N, S and P in their structure. Because of this features, humic acid enrich soil microflora (Yılmaz, 2007; Larcher, 2003). Ferrara and Brunetti (2007) stated that when the humic acid obtained from soil and compost applied to foliar in grapevine, it was significantly affected the quantity and quality of the product compared to control. It has been reported that application of both types of humic acid

to grapes causes a significant increase in berry length, diameter and weight. In the study of Flame Seedless grape variety, the best result was obtained by applying 0.1% humic acid in addition to a 1/3 cluster reduction after berry set (Mohamed, 2016). It has been reported that humic acid application increases berry weight, titratable acidity and maturity index at full bloom period in "Italia" grape variety (Ferrara and Brunetti, 2010; Akın, 2011). Ferrara and Brunetti (2010) reported that application of humic acid at a dose of 100 mg l^{-1} four times to soil during the full flowering period in the "Italia" grape variety increased the berry width, berry weight, titratable acidity and maturity index considerably. It has been reported that humic acid stimulate plant growth by increasing the uptake of macro elements by the plants (De Kock, 1955). Humic acid increasing microbial activity in the soil indirectly promote plant growth by stimutating hormones (Vaughan and Mc Donald 1976). Hafidi et al. (1995) reported that application of humic acid to soil increased the favorable phosphorus. However, Ferrara et al. (2008) has been stated that the reaction of humic acid to foliar application in table grape varieties is still limited and cannot be fully determined.

Akın and Alagöz (2016) obtained the highest yields from humic acid application in "Kabarcık" grape variety. Popescu and Popescu (2018) reported that the humic acids had a positive effect on grape growth and fruit quality when applied at the pre-bloom and fruit set stages. Researchers proposed that the humic acid concentration of 40 mgL^{-1} optimal application rate for grapevines.

In the study, the effects of humic substance application to the soil and foliar on shoot and cluster characteristics in "Trakya Ilkeren" grape variety, which was growing in heavy clay soil structure were investigated.

Materials and Methods

The research was conducted in the experimental vineyard of Ondokuz Mayıs University, Turkey in 2014-2015. The vineyard located at 41°21'52 N latitude and 36°11'29 E longitude with an altitude of 195 m and a distance of about 2.8 km from the Black Sea coast of Turkey. The research vineyard has 62.2% clay soil, pH 7.1 and 4.33% organic matter content. In the study; 10-year-old "Trakya Ilkeren" grape variety grafted on 5C rootstock was used. Spur pruned grapevines were trained onto double cordon with 3x1.5 m spacing. Grapevines were not irrigated, not fertilized and the water only received from rainfall. In the study, 22.5 mg/L humic substance per vine (total humic acid + fulvic acid 15%, total organic matter 10%, water soluble K_2O 2%, pH 8-10) was applied to the soil and foliar at pre-flowering and post-flowering periods. Each treatments were done within 5 L water. While the foliar sprays using a hand pressure sprayer, soil application was applied to the root region of each vines. Sample fruits were harvested at maturity stage from each replicates. In the study, the effect of the humic substance application on cluster weight (g), cluster width and length (cm), berry weight (g), berry width and length (mm), pH, titratable acidity (TA %) TSS (Brix %), maturity index (TSS / acidity), shoot length (cm), shoot diameter (mm), leaf area per shoot and total leaf area (cm^2) was investigated. Leaf areas were calculated non-destructively [$-1.41 + 0.527x(W^2) + 0.254x(L^2)$] according to Elsner and Jubb (1988). Leaf width (W) and leaf length (L) were used to calculate leaf areas.

Statistical Analysis

Complete randomized block design with three replications were used and the mean of the measurements was evaluated in the study. Vegetative growth and cluster characteristics were measured in four grafted vines of each replication. The data were analyzed by two-way analysis of variance (ANOVA) to test main effects and interactions between for application and year. Data analysis was performed using SPSS 16.0 for Windows. Results studied were presented as means and a pooled standard error of mean (SEM). Differences among means were detected using Duncan's multiple range tests at significance levels of ($p<0.05$).

Results and Discussion

The effects of the humic substance application to the soil and foliar on cluster and berry weight, cluster width and length, berry width and length in "Trakya Ilkeren" grape variety are given in Table 1. In the study, while the effects of humic substance application on cluster and berry weight, cluster width, berry width and length was found to be insignificant, it was found significant in cluster length. However, it has been found that the application of humic substance from the soil and foliar increased the cluster and berry weight, and cluster width and cluster length, compared to control vines. The cluster weight was found to be 613.97 g on control grapevines; 620.44 g on foliar application and 621.63 g on soil application. In the research, the highest cluster width (16.52 cm) and cluster length (23.86 cm) was obtained in the foliar application. Berry weight was determined as 4.91 g. on control grapes; 5.07 g. on foliar application and 5.19 g. on soil application. The highest berry width (20.49 mm) was found on foliar application, while the highest berry length was obtained on soil application (19.51 mm). Humic substance had a significant effect on cluster length and the highest cluster length was obtained in foliar application (23.86 cm). Ferrara and Brunetti (2008) stated that when the humic substance was applied at 5 and 20 mg L^{-1}, the average yield was 32.2 and 29.9 kg, respectively, whereas in the control group this was only 28.2 kg. Asgharzade and Babaeian (2012); reported that the humic acid had a significant effect on cluster length and the highest cluster length was obtained in 300 mgkg-^{1} humic acid.

Table 1. The effects of humic substance applications on cluster characteristics in "Trakya Ilkeren" grape variety

Applications	Cluster weight (g)	Cluster width (cm)	Cluster length (cm)	Berry weight (g)	Berry width (mm)	Berry length (mm)
Control	613.97	16.21	21.74 b	4.96	20.35	19.46
Foliar	620.44	16.72	23.86 a	5.19	20.49	19.22
Soil	621.63	16.84	22.94 ab	5.07	20.12	19.51
Pooled SEM	18.202	0.409	0.354	0.058	0.160	0.123
Year	**	**	**	**	**	**
Application	ns	ns	*	ns	ns	ns
Year* Applications	ns	ns	ns	ns	ns	ns

* Pooled SEM: Standart error of the means (NS. $p>0.05$; * .$P\leq0.05$; **. $P<0.05$)

The effects of humic substance application to the soil and foliar in "Trakya Ilkeren" grape variety on the maturity parameters such as TSS, pH, titratable acidity and maturity index are given in Table 2. While the effect of humic substance application to the soil and foliar on TSS in "Trakya Ilkeren" grape variety is not significant, the effect on pH, titratable acidity and maturity index was found to be more important than control. The amount of TSS in the soil application was 18.01%, 17.42% in foliar application and 17.85% in control grapes. The maturity index is statistically significant among applications. Maturity index was determined as 46.97 in control grapes, 39.15 in soil application and 38. 55 in foliar application. Titratable acidity was obtained as 0.49% in foliar application, 0.46% in soil application and 0.36% in control grapes. The pH level was measured as 4.16 in the foliar application, 4.06 from the soil application and 3.57 in the control grapes. In our study, the effect of humic substance application to the soil and foliar on the TSS was insignificant, but it was slightly higher than the others in soil application. According to Köse et al. (2016), humic substance application to the soil significantly increased TSS in "Narince" and "Alphonse Lavallée" grape varieties

compared to control. It was reported that humic acid application in "Erciş" grape variety no significant effect on yield, cluster weight, berry weight and amount of juice but it was affected on TSS and titratable acidity (Yaşar, 2005). Mostafa and Akın (2017) was recommended the application of 333 ml of humic acid/5 liters of water from the soil to increase grape yield, cluster weight and 100-berry weight in "Italia" grape variety. In this regard, Ferrara and Brunetti (2010) reported that application of humic acid at 100 mg/L four times in full bloom period in grape variety significantly increased berry width and weight, titratable acidity and maturity index values. Akın (2011) reported that humic acid application increased yield, berry weight, and red and blue color density values in the "Horoz Karası" grape variety.

Table 2. The effects of humic substance applications on maturity parameters in "Trakya Ilkeren" grape variety

Applications	TSS (Brix%)	Maturity Index	pH	Titretable Acidity (TA %)
Control	17.85	48.07 a	3.57 b	0.38 c
Foliar	17.42	36.07 b	4.16 a	0.49 a
Soil	18.01	39.91 b	4.06 a	0.46 b
Pooled SEM	0.198	0.793	0.043	0.006
Year	ns	**	ns	**
Application	ns	**	**	**
Year* Applications	ns	**	**	*

* Pooled SEM: Standart error of the means (NS. $p>0.05$; * .$P\leq0.05$; **. $P<0.05$)

The effects of humic substance application to the soil and foliar on leaf area, shoot length and shoot diameter of "Trakya İlkeren" grape varieties are given in Table 3. While the leaf area and shoot length are significantly influenced by humic substance application, the effect on shoot diameter is not significant. The leaf area was measured as 2796.6 cm^2 in soil application. The shoot length (261.2 cm) and shoot diameter (9.97 mm) were found to be the highest in foliar application.

Table 3. The effects of humic substance applications on vegetative characterictics in "Trakya Ilkeren" grape variety

	Leaf area (cm^2)	Shoot length (cm)	Shoot Diameter (mm)
Control	2328.0 b	184.81 b	9.69
Foliar	2323.2 b	261.62 a	9.97
Soil	2796.6 a	231.11 b	9.36
Pooled SEM	53.135	7.322	0.190
Year	**	**	ns
Application	ns	**	ns
Year* Applications	ns	ns	ns

* Pooled SEM: Standart error of the means (NS. $p>0.05$; * .$P\leq0.05$; **. $P<0.05$)

It has been reported that humic acid directly affect the ion exchange, convert plant nutrients into useful form, increase microbial activity and induce plant growth indirectly with the resulting hormones. In our study, the effect of humic substance application on vegetative development was significant but did not affect shoot diameter. According to Köse et al. (2016), although the effect of humic substance application to the soil application on vegetative development in "Narince" and "Alphonse Lavallée" grape varieties was not

significant but it was determined that the growth of shoots was reduced slightly. On the other hand, Ferrara and Brunetti (2008) stated that foliar application of humic substance effected positively on qualitative and quantitative parameters of "Italia" grape.

Conclusion

In the study, both applications of humic substance to soil and foliar were found beneficial even if there is no difference between applications, statistically. The positive effect was found in terms of soil humic substance application on cluster weight and TSS. It was seen that the positive effect of humic substance application on TSS will contribute to the increase of quality, especially for organic grape production, early ripening varieties and under greenhouse cultivation. At the same time, the obtained results show that it would be very useful to carry out in different grape varieties and different application doses. The combined applications both soil and foliar will be give more positive results.

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CULTIVATING FORAGE RAPE WITH FORAGE PEA FOR FEED PRODUCTION IN WINTER PERIOD

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Abstract

Most of the researchers say that mixed growing has lots of advantages and it can produce more balanced feed compared to sole production. Prominent advantage of forage rape and pea is that they can be grown during the winter period in our region. Thus, there is no irrigation demand and this mixture reach the harvesting stage before the sowing time of main warm season crops such as corn, tobacco, sunflower, sugar beet, watermelon, melon and other vegetables. There is a big roughage gap in our region, as well as in Turkey. Aim of this study was to determine suitable forage rape and forage pea cultivars, their binary mixture and harvesting time without causing any restriction to warm season crops. The research was designed as split plot in randomized blocks with four replications and carried out in coastal Central Black Sea Region. Forage rape (Cv. Lenox) was grown as binary mixtures with Gölyazı, Kirazlı, Ulubatlı, Tamkoc (Turkey), Letin, Adam (Croatia) and Kosmaj (Serbia) cultivars of forage pea. All the cultivars were grown solely, also. The harvests were made at budding and blossoming periods of forage rape. Letin, Kirazlı and Gölyazı forage pea cultivars started blossoming one week earlier than forage rape. Average blossoming duration of these was 138 days after sowing. Blossoming time of Ulubatlı cultivar was the same with forage rape, but Adam, Tamkoç and Kosmaj blossomed later. Plant height of sole forage rapes (63.5 cm) were higher than mixed (55.9 cm) ones. While the highest forage pea was Kirazlı cultivar (78.2 cm), the shortest one was Adam cultivar (47.0 cm). Forage yield and quality parameters of mixed plots were generally higher than sole forage rape plots.

Keywords: *Forage rape, Forage pea, Mixture, Harvesting time.*

Introduction

There is scarcely less opportunity to increase agricultural lands in Turkey as well as in the world. However, on the other hand, population is growing day by day. In this case, we should use more effectively available agricultural areas. One of these ways is to apply mixed growing system. Light cannot be deposited by green plants for using later. If there was not a limit to supply water and nutrients, dry matter production depends on light holding capacity of green plants. In successful mixed growing systems, light holding capacity of plants increase. Actually, plants grow up mixed in grassland areas. We should establish forage crops as mixof a few suitable species similar to natural grassland areas (Acikgoz, 2001).

Rape plants have a rapid growing ability and they can reach seed ripening period in 4-5 months. Even though, rape is mainly used as oil crop (Acikgoz, 2001 and Gizlenci *et al.*, 2003), recently forage rape farming start to increase in the aim of to meet forage gap. Forage rape can be used as fresh herbage, hay or silage to feed the ruminants. Nevertheless, there are some anti-nutrients such as erucic acid, glucocids and nitrate in rape tissues, for this reason it is necessary to take some precautions when feeding the livestock with forage rape.

In order to decrease detrimental effects of anti-nutritional compounds, forage rape can be grown mixed with other annual forage crops. Especially forage pea is very suitable to grow mixed with forage rape, because of its rapid growing ability, high yield and nutritional values and very tall plant high (Balakhial *et al.*, 2008; Khodaparast *et al.*, 2011 and Neely *et al.*,

2009). In our region forage rape has some advantages like rapid growing ability, higher forage yield and it can be grown rainfed conditions (because it grows vegetatively during the winter and spring periods). The aim of this study is to determine the best suitable forage pea cultivar in terms of forage yield, quality and growing stage when harvested in budding and blossoming stage of forage rape.

Material and Methods

The experiment was established according to randomized block design with 4 replicates. In the study, forage rape (*Brassica napus* L.) cv. Lenox, forage pea (*Pisum sativum* L.) cv. Gölyazı, cv. Tamkoç, cv. Ulubatlı, cv Kirazlı (Turkey), cv Kosmaj (Serbia), cv. Letin and cv. Adam (Croatia) were used. The study was carried out in experimental area of Ondokuz Mayis University, Agricultural Faculty during 2017-18 growing season. Consider the soil sample analysis, 8 kg da^{-1} phosphorus applied the whole experiment. N fertilizer was separated in 2 parts, one part in sowing time, the other part in spring was distributed by hand. The amounts of N were 10 kg da-1 for solely forage rape plots 6 kg da^{-1} for mixed plots and 3 kg da^{-1} for solely pea plots. There was no irrigation. The first harvest was performed at budding stage of forage rape (17.04.2018) and second harvest was at blossoming stage of forage rape (25.04.2018). Forage samples were analyzed by using NIRS device. The data were statistically analyzed with SPSS program.

Results and Discussion

Blossoming dates of cultivars and their differences from forage rape cv Lenox blossoming date one given Table 1. Forage rape cv Lenox started blossoming at 27.03.2018. Two forage pea cultivars Letin and Kirazlı were earlier than cv Lenox 12 and 7 days, respectively. Forage pea cultivars Ulubatlı and Gölyazı blossomed same date with Lenox. Compare to Lenox, Tamkoç and Adam 29 days, Kosmaj 41 day later blossomed.

Table 1. Blossoming dates of cultivar and differences

Cultivars	Blossoming date	Difference
Lenox	27.3.2018	-
Letin	15.3.2018	12 days earlier
Kirazlı	20.3.2018	7 days earlier
Ulubatlı	27.3.2018	0
Gölyazı	27.3.2018	0
Tamkoç	25.4.2018	29 days later
Adam	25.4.2018	29 days later
Kosmaj	07.5.2018	41 days later

There were significant differences among the cultivars grown in both as solely and as binary mixture in the point of plant height (Table 2). In general, plant height of forage pea cultivars decreased when they were grown as binary mixture with forage rape, except for Ulubatlı and Letin. The highest group was consists of Gölyazı, Kirazlı and Kosmaj in solely plots, while all of the cultivars took place the top group in binary mixture plots, except Ulubatlı and Adam.
In terms of hay yield significant differences amongst the cultivars in solely plots. Except for the earliest cultivar Letin, all of the cultivars were in the highest group. Hay yield was increased in all binary mixture plots. In binary mixture plots hay yield was determined as total of pea and rape. Probably high yielding forage rape might be prevented statistical differences amongst the forage pea cultivars. In mixture growing systems, land equivalent ratio (LER) value is generally increase, thus yield of mixture plots also is increase, compared to solely plots (Zeybek, 2017).

Table 2. Plant height and hay yields of cultivars grown as solely and binary mixture*

Treatments**	Plant height (cm)		Hay yield (kg/da)	
	Sole	Mix	Sole	Mix
Tamkoç	96.50bd	90.75ab	368.01a	780.33
Kirazlı	106.75ac	106.00a	260.46ab	607.19
Gölyazı	118.75a	104.75ab	387.17a	694.80
Ulubatlı	83.50d	87.66bc	327.40ab	559.28
Adam	82.25d	72.00c	282.01ab	599.88
Kosmaj	104.50ac	100.75ab	293.93ab	664.45
Letin	91.5cd	97.66ab	166.16b	571.80
Lenox	112.75ab	-	360.19a	-

*There is no significant difference ($p \leq 0.05$) amongst the figures indicated in the same letter within the same column

Crude protein, ADF and NDF ratios of cultivars can be seen in Table 3. Consider the crude protein ratios, there are significant ($p \leq 0.05$) differences amongst the cultivars in both solely and mixture plots. All the cultivars, except cv Kosmaj took place in the highest group in solely plots while the top group was consisted of Ulubatlı, Adam and Tamkoç cultivars in mixture plots. Crude protein content of forage rape was significantly lower than the forage pea cultivars. Even though, there were significant differences amongst the cultivars in the point of ADF and NDF contents, the values of forage pea cultivars close to each other. Higher values of forage rape cultivar Lenox might be caused statistical differences. ADF ratios varied between 28% and 34% while NDF contents ranged from 43% to 48% of forage pea cultivars (Table 3).

Table 3. Crude protein, ADF and NDF values of cultivars grown as solely and binary mixture*

Treatments**	Protein (%)		ADF (%)		NDF (%)	
	Sole	Mix	Sole	Mix	Sole	Mix
Tamkoç	21.67a	20.41ab	31.54b	28.69ab	46.9775	43.25ab
Kirazlı	20.95ab	18.52b	28.57b	30.40ab	43.5325	45.41ab
Gölyazı	21.30ab	19.66b	29.13b	31.25ab	43.0800	46.06ab
Ulubatlı	21.77a	24.86a	29.96b	27.27b	45.1700	40.40b
Adam	18.96ab	20.80ab	32.71ab	31.09ab	46.3425	45.01ab
Kosmaj	17.09b	16.42b	32.82ab	34.31a	47.5425	48.88a
Letin	17.91ab	19.28b	32.52ab	29.24ab	46.1025	43.64ab
Lenox	12.25c	-	38.55a	-	50.1200	-

*There is no significant difference ($p \leq 0.05$) amongst the figures indicated in the same letter within the same column

Some mineral contents of cultivars were given in Table 4. In terms of Ca, K, Mg and P ratios of hay, there were significant differences ($p \leq 0.05$) amongst the cultivars in both solely and mixture plots. Ca, K, Mg and P contents of forage pea cultivars were ranged as follows: Ca: 1.04%-1.21%; K: 2.67%-3.90%; Mg: 0.22%-0.25% and P: 0.35%-0.51%. Mineral contents of forage pea cultivars generally higher than forage rape (Table 4).

Table 4. Ca, K, Mg and P contents of cultivars grown as solely and binary mixture*

Treatments**	Ca (%)		K (%)		Mg (%)		P (%)	
	Sole	Mix	Sole	Mix	Sole	Mix	Sole	Mix
Tamkoç	1.16ab	1.13ab	3.51a	3.40ab	0.25a	0.22ab	0.46a	0.42b
Kirazlı	1.16ab	1.09ab	3.30a	3.13b	0.24a	0.22b	0.43ab	0.39b
Gölyazı	1.17a	1.12ab	3.32a	3.33ab	0.25a	0.22b	0.43ab	0.42b
Ulubatlı	1.15ab	1.21a	3.59a	3.90a	0.24ab	0.25a	0.45ab	0.51a
Adam	1.12ab	1.16ab	3.29a	3.45ab	0.22ab	0.23ab	0.40ac	0.43b
Kosmaj	1.08ab	1.04ab	2.67b	2.79b	0.25a	0.25a	0.35c	0.36b
Letin	1.16ab	1.15ab	3.04ab	3.07b	0.23ab	0.23ab	0.38bc	0.39b
Lenox	1.07b	-	2.65b	-	0.21b	-	0.34c	-

*There is no significant difference ($p \leq 0.05$)amongst the numbers indicated the same letter within the same column

Conclusion

Ulubatlı and Gölyazı forage pea cultivars blossomed exactly same date with forage rape cultivar Lenox. In the point of high yield, quality parameters and growth stage conformity we can recommend Gölyazı cultivar to grow with forage rape cv Lenox in coastal area of Central Black Sea Region.

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DETERMINATION OF SUITABLE MIXING RATIOS OF CHICORY WITH ORCHARDGRASS AND RED CLOVER

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Abstract

Researchers and farmers have paid more attention to chicory recently, because of its palatability, high yield, nutritive value, digestibility, kept greenery throughout the summer period, deep and strong root system and drought resistant ability. In addition, it has some positive effects on animal health. Chicory is very common in natural areas of Turkey. This study was conducted according to randomized block design with three replicates in Samsun conditions in 2017. In the study, chicory (C), orchardgrass (OG) and red clover (RC) were grown as solely and binary mixture in rain fed conditions. Binary mixtures were 80% C + 20% OG, 60% C +40% OG, 40% C + 60% OG, 20% C + 80% OG, 80% C + 20% RC, 60% C + 40% RC, 40% C + 60% RC, 20% C + 80% RC. Harvests were made when chicory plants reached 25 cm average plant height. Three cuts were performed (twice in June and once in July) in 2017. Only chicory plants could reach the harvest stage for the third cut. The highest fresh yields was 55.7 and 17.7 t ha^{-1} at the 20% C + 80% RC mixture for the first and second cuts, respectively. Yield was getting decreased with each cutting sequence and it declined to 5.7 t ha^{-1} for the third cut. In totally, 20 % C + 80% RC binary mixture gave the highest yield. Total fresh yield was determined between 65.1 – 8.8 ha^{-1}. The highest hay yield was obtained from the treatments contain red clover as follows; 40 % C + 60 % RC, 100 % RC, 20 %C + 80 % RC, 80 % C + 20 % RC and 60 % C + 40 % RC (respectively; 12.30, 12.00 , 11.50 , 10.40 and 9.90 t ha^{-1}) . Crude protein ratios were 24.1 –16.1 % in the first, 26.9 – 17.5 % in the second and 22.9 – 20.3 % in the third cut. In the first, second and third cuts ADF ratios were 32.4 – 24.1 % , 33.5 – 20.4 % and 23.6 – 20.1 % ; NDF contents were 54.6 – 34.3 % , 58.2 – 34.0 % and 35.0 – 31.7 % , respectively.

Keywords: *Chichory, Orchardgrass, Red Clover, Mixture Rates.*

Introduction

In Turkey, in order to increase quality and amount of forage new species and cultivars were had to joined the agricultural system. In addition to alfalfa, sainfoin and vetch we need new alternative forage species. New cultivars were improved from alternative species and they were served to farmers in lots of developed countries. Other reasons to develop these cultivars are obtained varieties winter-hardly, drought-hardly and resist to other marginal lands. For example, there was a severe drought conditions in New Zealand in 1978, chicory genotypes survived these conditions and gave nutritious forage yield (Barry, 1998). Chicory has been used for different aims for a long time. But, recently it was focused on to use for forage. Chicory was eaten voluntarily by livestocks, its digestibility and nonstructural carbonhydrate levels are high, and has rich mineral content, resist to drought conditions. Chicory has some secondary compounds that prevent bloating and endoparasite in ruminants, it provides high forage especially during the summer period (Barry, 1998; Athanasiadou et al, 2007). It expands grazing period in pastures because it grows in summer period that lots of cool season perennial grass were dormant (Kemp et al, 2002). In study, suitable mixture forage yield and quality, of chicory, orchardgrass and red clover grown alone and binary mixture of chicory with the other species were investigated at grazing stage.

Material and Methods

This study was carried out in experimental area of Ondokuz Mayis University Agricultural Faculty in 2017. Average temperature and total rainfall were 13.4 ^{0}C and 386.2 mm during the study period of 2017 (between January and July). The study was established according to Randomized Block Design with 3 replicates. Chicory *(Cichorium intybus L.)* (C) with orchardgrass *(Dactylis glomerata L.)* (OG) and red clover *(Trifolium pratense L.)* (RC) were used as plant materials. These species were sown as solely and binary mixtures as follows: 80 % C + 20 % OG, 60 % C + 40 % OG, 40 % C + 60 % OG, 20 % C + 80 % OG and 80 % C + 20 % RC, 60 % C + 40 % RC, 40 % C + 60 % RC, 20 % C + 80 % RC. Species were sown alternate rows with 20 cm row spacing. There was no irrigation. Harvests were made at grazing stage (imitating the grazing) when chicory plants reach to 25 cm plant heights during the growing season. Three cuts were performed (twice in June and once in July) in 2017. Only chicory plants could reach the harvest stage for the third cut. After harvest, green herbage weighed in solely plots, but in binary mixture plots firstly sorted species and weighed separately. During harvest 500 g sample was taken for each plot and dried in an oven at 60 ^{o} C temperature until constant weight. Then these samples grounded and analysed protein, ADF and NDF Analyses of grinded samples were performed in Foss NIRSystems (Hoy et al., 2002) Model 6500 Win ISI II v1.5 device by using IC-0904FE calibration program. Resultant data were subjected to statistical analyses according to randomized block design. All data obtained from this study was analyzed by using SPSS 17.0 program. The differences amongst the mean values were calculated according to DUNCAN test.

Result and Discussion

The results about plant height fresh and hay yield obtained from solely and binary of chicory with orchardgrass and red clover plots are given Table 1. In terms of plant height there were significant differences ($P \leq 0.05$) amongst the treatments in all cuttings. The highest plant height was determined as 44.4 cm for alone red clover but values of some of the other treatments were same highest statistical group. The lowest value was observed from orchardgrass with 29.9 cm. Average plant height of alone chicory plots was 31.2 cm. Majority of the plant height values were same statistical group in the second harvest and values ranged from 29.5 to 24.4 cm. The highest values were measured for alone chicory plots in the second harvest. Only chicory plants could reached the harvest stage for the third cut. The highest plant height was 20.2 cm for 60 % C + 40 % OG plot while the lowest was 13.2 cm for 40 % C+60 % RC plot in the third cut (Table 1). Plant height values were generally low because of the first year of the experiment.

Table 1.Plant height, fresh and hay yield values obtained from the treatments

Treatments	Plant height (cm)			Fresh yields (t ha^{-1})				Hay yields (t ha^{-1})			
	1. cut	2. cut	3. cut	1. cut	2. cut	3. cut	Total	1. cut	2. cut	3. cut	Total
Chicory	31.2bc	29.5a	18.6ac	7.6d	5.3cd	2.8bc	15.8cd	2.4bc	0.7c	0.5bc	3.7b
Orchardgrass	29.9c	24.4b	-	6.0d	2.8d	-	8.8d	3.1bc	0.7c	-	3.8b
Red clover	44.4a	25.4b	-	25.9c	7.4bc	-	33.3b	10.2a	1.7b	-	12.0a
80C+20OG	32.3bc	24.4b	18.4ac	13.4d	8.0bc	4.9a	26.4bc	2.2c	1.4b	1.0a	4.8b
60C+40OG	32.0bc	26.8ab	20.2a	16.3cd	8.9b	3.5ab	28.9bc	2.9bc	1.6b	0.7ab	5.1b
40C+60OG	30.3c	25.1b	19.8ab	13.1d	7.3bc	2.8bc	23.2bc	2.4bc	1.4b	0.6bc	4.5b
20C+80OG	34.8ac	27.2ab	17.3ac	11.1d	6.9bc	1.3c	19.4cd	2.2c	1.5b	0.2c	4.0b
80C+20RC	39.0ac	27.4ab	17.8ac	41.6ab	14.6a	3.0ac	59.3a	7.0ab	2.7a	0.6bc	10.4a
60C+40RC	36.3ac	25.6b	17.2ab	38.1b	13.6a	2.6bc	54.4a	6.4ac	2.8a	0.6bc	9.9a
40C+60RC	41.5ab	25.6b	13.2c	50.5a	13.6a	0.9c	65.1a	9.1a	2.9a	0.2c	12.3a
20C+80RC	39.6ac	27.2ab	13.8bc	46.8ab	14.9a	1.1c	62.9a	8.0a	3.2a	0.2c	11.5a
Average	**35.6**	**26.2**	**17.4**	**28.5**	**10.6**	**2.6**	**36.2**	**5.1**	**1.9**	**0.5**	**7.5**

* There was not significant differences amongst to figures shown the same letter in the same column ($P<0.05$)

In consider the fresh yields there were significant differences amongst the treatments in all three cuts. The highest fresh forage yields were obtained from 40 % C + 60 % RC (50.5 t ha^{-1}) for 1. cut, from 20 % C + 80 % RC (14.9 t ha^{-1}) for 2. cut and from 80 % C + 20 % OG (4.92 t ha^{-1}) for 3. cut. Regard the total fresh forage yields, the highest values determined for 40 % C + 60 % RC, 20 % C + 80 % RC , 80 % C + 20 % RC and 60 % C + 40 % RC (Respectively 65.1 , 62.9 , 59.3 and 54.4 t ha^{-1}) fresh forage yields were decreased as cutting sequence advance. In general, the plots contain RC gave higher fresh forage yield (Table 1).

Significant differences were determined amongst the treatments in the point of hay yield in 3 cuts and total. Hay yields were varied between 10.2 and 2.2 t ha^{-1} in the first cut and the treatments contains RC generally gave higher hay yield. Hay yield were ranged from 3.2 to 0.7 t ha^{-1} in the second cut. Only chicory plants could reached the harvest stage for the third cut. The highest chicory yield was obtained from 80 % C + 20 % OG treatment as 1.0 t ha^{-1}, but 60 % C + 40 % OG treatment was also same statistical group in the third cut. Total hay yield was varied between 3.70 and 12.30 t ha^{-1}. The highest hay yields were obtained from 40 % C + 60 % RC, 100 % RC, 20 %C + 80 % RC, 80 % C + 20 % RC and 60 % C + 40 % RC treatments (respectively; 12.30 , 12.00 , 11.50 , 10.40 and 9.90 t ha^{-1}). The treatments contain RC, generally, gave higher hay yield (Table1). Frame (2008), indicated that red clover well adapted to humid and cool climates and heavy, clay soil and red clover mixtures gave higher yield in the first year of establishment. An experiment was conducted by Ayan et al., (1997) with alfalfa, sainfoin, red clover, timothy, smoot brome, orchardgrass and red fescue in rainfed conditions of Samsun. Species were grown as alone and binary mixtures. The highest hay yield was obtained from alone red clover (14.7 t ha^{-1}) and red clover + orchardgrass mixture (12.6 t ha^{-1}).

Crude protein, ADF and NDF contents of the hay obtained from the treatments are given at Table 2. In terms of crude protein ratio, there were significant differences amongst the treatment in all cuts, except for third cut. Crude protein contents were ranged from 16.1 % to 24.1 % in the first and from 17.5 % to 26.9 % in the second harvest and crude protein ratios of the treatments that contain red clover have higher values compared to the others. Crude protein ratios of chicory were between 20.3 % - 22.9 % in the third cut (Table 2).

Table 2. Crude protein, ADF and NDF ratios determined to the treatments *

Treatments	Crude protein ratio			ADF ratio			NDF ratio		
	1. cut	2. cut	3. cut	1. cut	2. cut	3. cut	1. cut	2. cut	3. cut
Chicory	18.6b	21.5b	22.3	24.1d	20.4e	20.9	34.3e	34.0e	32.0
Orchardgrass	16.8bc	17.5cd	-	32.4a	33.5a	-	54.6a	58.2a	-
Red clover	22.9a	26.5a	-	28.1c	24.2d	-	41.5cd	38.1d	-
80C+20OG	18.4b	21.4b	21.5	28.0c	25.2cd	22.2	42.7cd	43.7c	33.4
60C+40OG	17.9bc	20.3bc	21.1	28.7bc	25.3cd	22.3	44.7c	43.8c	33.5
40C+60OG	16.7bc	19.3cd	20.6	30.4ab	27.3bc	23.1	48.4b	46.7c	34.4
20C+80OG	16.1c	19.1cd	21.8	32.0a	29.5b	21.9	52.7a	51.1b	33.1
80C+20RC	22.1a	26.3a	21.2	28.4c	23.0d	22.0	41.6cd	37.0de	32.7
60C+40RC	22.7a	25.9a	21.3	28.3c	23.8d	22.1	41.2d	37.8d	32.9
40C+60RC	24.1a	26.9a	20.29	27.6c	23.8d	23.6	41.3d	38.1d	35.0
20C+80RC	24.0a	26.1a	22.9	27.7c	24.3d	20.1	42.4cd	38.4d	31.7
Average	**20.1**	**22.8**	**21.4**	**28.7**	**25.5**	**22**	**45.5**	**44**	**33.1**

*There was not significant differences amongst to figures shown the same letter in the same column ($P<0.05$)

There were significant differences amongst the treatment in the first and second cut, in the point of both ADF and NDF ratios (Table 2). ADF ratios were changed between 24.1 – 32.4 % and 20.4 – 33.5 %, respectively in the first and second harvests. The lowest ADF content was determined in the alone chicory. According to American Forage and Grassland

Council (AFGC, 2009) standards, all forages obtained from this study were in prime and premium classes when regard the ADF contents.

The highest NDF ratio was 54.6 % for 100 % OG and 52.7 % for 20 % C + 80 % OG treatments in the first cut. The lowest value was obtained from alone chicory as 34.3 %. In the second cut, the highest ratio was 58.2 % for 100 % OG and the lowest was 34.0 % for 100 % chicory. NDF ratios of chicory was ranged from 31.7 % to 35.0 %, in the third cut (Table2). When we evaluate the forages based on AFGC standards, in consider to NDF contents, all samples, except for 100 % OG plots were in prime and premium classes.

Conclusion

In the light of the first year results, the highest forage yield was obtained from 40 % C + 60 % RC, 20 % C + 80 % RC and 80 % C + 20 % RC binary mixture treatments. In consider forage yield, crude protein, ADF and NDF contents, 40 % C + 60 % RC and 60 % C + 40 % RC binary mixtures might be recommended to Samsun and similar ecological conditions. But, to clear the recommendations for perennial forage crops, the study should carry out at least two year.

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EFFECT OF INFUSION AND DECOCTIONS ON ANTIOXIDANT ACTIVITY, TOTAL PHENOL, FLAVONOID CONTENT AND PHENOLIC COMPOUNDS OF OLIVE LEAVES

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Abstract

Total phenol, antioxidant activity and flavonoid contents of olive leaves extract were determined as 12.13 mg GAE/g, 89.47 % and 32.65 mg CE/g catechin, respectively. While total phenol contents of olive leaves changed between 0.89 and 8.81 mg GAE/g depending on infusion time, antioxidant activity values ranged from 8.21 to 28.11%. Flavonoid contents of infusion varied between 2.71 and 14.81 mg CE/g catechin. While total phenol contents of decoction were determined between 1.81 and 8.41 mg GAE/g, antioxidant activity values changed between 10.41 and 25.33%. Also, flavonoid contents of olive leave decoction ranged from 8.67 to 37.44 mgCE/g. The most abundant compounds in olive leaves were oleuropein, hydroxytyrosol, caffeic, the flavone-7-glucosides, luteolin, apigenin and verbascoside. While luteolin contents of infusion changed between 0.09 and 0.91 µg/kg, this compound ranged from 0.14 to 0.89 µg/kg in decoction. While oleuropein content of infusion changed between 0.10 and 0.97 µg/kg, oleuropein contents of decoction samples varied between 0.13 and 0.87 µg/kg. In addition, rutin contents of decoction varied between 0.19 and 0.99 µg/kg. Also, caffeic acid contents of decoctions ranged from 0.09 to 0.83 µg/kg. Generally, phenolic compounds of decoction (except 10.min) were found higher than those of phenolic compounds of infusion. olive leaves can be received much attention as antioxidant sources due to its phenolic compounds. Because, there are considerably higher ration of by products arising olive leaves.

Keywords: Olive leaves, *Ayvalık* cultivar, antioxidant, phenol, flavonoid, phenolic compounds, infusion, decoction.

Introduction

Polyphenols are very important due to the natural antioxidant nactivity of these constituents. Plant extracts like infusion and decoction are natural alternatives to synthetic antioxidants as they posses similar or even higher antioxidant activity. Olive leaves have many useful pharmaceutical effects (Sabry, 2014). Several studies were conducted on the antioxidant activity, hypoglycemic and antimicrobial effect of olive leaves (Soni *et al*., 2006; Wang *et al*.,2008). Sabry (2014) reported that decoctions of the dried olive fruit and leaves taken orally for diarrhea and to treat urinary tract infections, and also hot water extract of the fresh leaves treated high blood pressure and bronchial asthma. The olive (*Olea europaea* L.) leaves are composed of 10% of the dry weight arriving at olive mills, and they are an agricultural waste of the olive oil industry (Goldsmith *et al*., 2014). The olive leaves refer to a mixture of leaves and branches from both the pruning of olive trees and the harvesting and cleaning of olives (Molina-Alcaide and Yanez-Ruiz, 2008; Vogel *et al*., 2015). In recent years, there has been great interest in the health effects of various herbal teas as infusion and/or decoctions. Olive leaf tea is one of the most common, traditional herbal teas used among Mediterranean people to treat several diseases. Oleuropein is the most abundant polyphenol of olive leaves prevented cardiac disease by protecting membrane from lipid oxidation (Somova *et al*., 2003; Molina-Alcaide and Yanez-Ruiz, 2008; El and Karakaya, 2009). Pereira *et al*., (2007)

suggested that olive leaves may be used in infusions, allowing a considerable intake of bioactive compounds. The aim of present study was to determine antioxidant activity, total phenol and flavonoid contents and phenolic compounds of olive leave infusion and decoctions prepared at different heating times in hot water at 90 °C.

Material and Methods

Olive leaves (the cultivar *ayvalık*) were randomly collected from all round of each tree at the same time and height from ground in Mersin (Mut) province in South of Turkey in October 2016. The olive trees have ten years old. They grow in dry ground. After leaves were collected, they were transferred to Laboratory in cool bag, and kept at 4 °C.

Sample extraction of olive leaves

Phenolic compounds were extracted according to Singleton, *et al*., (1999) with some modifications. 4 g of each sample was added to 10 ml of acetone (80% v/v). The mixture was sonicated for 15 min, followed by centrifugation at 6000 rpm for 15 min and the supernatants were collected. These steps were repeated twice. The extract was concentrated at 37°C in a rotary evaporator under vacuum. Prior to injection, the extract was filtered through a 0.45 µm nylon filter. All analyses were performed in triplicate.

Extraction of phenolic compounds with infusion and decoction

For infusion, ground olive leaves (about 6 g) were added into boiled water (200 ml) at 90 °C, and separately infused for 1., 3., 5., 7., and 10. min. Then mix was filteres by a Büchner funnel. After extract was lyophilised, they were analysed by HPLC by re-dissolving in water. For decoctions, each ground olive leave sample (about 6 g) was bolied in 200 ml water for 1., 3., 5., 7., and 10. min at 90 °C. Following processing is the same such as in infusion processing.

Total phenol content

Total phenol contents of olive leave extracts were determined with the Folin-Ciacueltau (FC) reagent according to Yoo *et al*.,(2004). 1 ml of Folin–Ciacueltau was added into samples, and mixed for five minutes. After 10 mL of Na_2CO_3 was added into mix, the final volume was completed to 25 ml with distilled water. At the end of 1 hours, sample was measured in 750 nm in spectrophotometer. The results were given as mg GAE/100 g.

Antioxidant activity

The antioxidant activity was determined with DPPH (1,1-diphenyl-2-picrylhydrazyl) (Lee *et al*., 1998). the extract was mixed with 2 mL methanolic DPPH, and the mixture was shaken, and kept at room temperature for 30 min. The absorbance was measured at 517 nm.

Total flavonoid

Total flavonoid content was estimated according to Dewanto *et al*., (2002) and used Catechol as standart.

Determination of phenolic compounds

Total flavonoid content was estimated according to Juhaimi et al.,(2018) and used Shimadzu-HPLC equipped with DAD detector and Inertsil ODS-3 (5µm; 4.6 x 250mm) column.

Statistical analyses

All results were stated as mean±standard deviation (MSTAT C) of fruit samples (Püskülcü and İkiz 1989)

Results and Discussion

Total phenol, antioxidant activity, and flavonoid contents of olive leaves infused and decoctioned in boiled water at 90 °C are given in Table 1. While total phenol contents of olive leaves infused change between 0.89 and 8.81 mg GAE/g depending on infusion time, antioxidant activity values ranged from 8.21 to 28.11%. Flavonoid contents of infusion varied between 2.71 and 14.81 mg CE/g catechin. All properties of olive leave infusion

increased together with infusion times. According to infusion results, it is considered that optimum infusion time can be 7. min due to the highest bioactive substance increasing. The total phenol, antioxidant activity and flavonoid contents of olive leave decoction increased till 7. min of decoction processing (except flavonoid). This reduction may be probably due to the reduction of bioactive substances exposed to higher temperatures at about 90 °C. While total phenol contents of decoction are determined between 1.81 and 8.41 mg GAE/g, antioxidant activity values changed between 10.41 and 25.33%. Also, flavonoid contents of olive leave decoction ranged from 8.67 to 37.44 mg CE/g. Total phenol, antioxidant activity value and flavonoid contents of infusion were found lower when compared with results of decoction during heating. But, antioxidant values of decoction were found low after from 7. min of infusion. This situation can be probably due to affected of phenolic compounds from continuously boling at 90 °C. The total flavonoid contents of fresh and dried olive leaves changed between 3.4 and 11.78 to 6.23 to 21.47 mg CE/g, respectively (Abaza *et al*., 2011). The total phenol contents of olive leaf extracts ranged from 9.07 to 13.68 and 16.52 to 24.93 mg GAE/g, respectively (Abaza *et al*., 2011). Also, solvent polarity will play a key role in increasing phenolic solubility. The increase in heating time resulted in an increase of phenolic and flavonoid contents in all samples. Lafka *et al*., (2013) reported that olive lşeave extracts exhibited 18.8% (n-propanol extract) to 55.0% (ethanol extract) antioxidant activity. Olive leaves could be a low-cost renewable and abundant source of phenolic antioxidants, with potent use in fatty foods and medicinal herbal teas (Wissam *et al*., 2016).

Table 1. Bioactive properties of infusion and decoctions of olive leaves

Time	Infusion			Decoction		
	Total Phenol mg GAE/g	Antioxidant activity (%)	Flavonoid mg CE/g	Total Phenol mg GAE/g	Antioxidant activity (%)	Flavonoid mg CE/g
1	0.89±0.09*e	8.21±1.17d	2.71±0.24d	1.81±0.42d	10.41±1.36d	8.67±0.59e
3	3.21±0.23d**	13.57±0.78c	4.68±0.49c	2.17±0.58d	21.17±1.42c	14.41±0.76d
5	5.18±0.17c	27.69±2.36b	8.44±1.36b	4.75±1.45c	37.81±0.96a	21.18±0.49c
7	7.32±0.58b	32.47±1.67ab	9.17±2.29b	7.01±1.28a	25.33±0.24b	28.81±0.41b
10	8.81±0.93a	35.11±2.35a	14.81±1.25a	6.74±0.69b	23.71±0.59bc	37.44±0.84a

*mean±standard deviation; **Values within each column followed by different letters are significantly different ($p<0.05$)

The phenolic compounds of leave infusion and decoctions are shown in Table 2. The most abundant compounds in olive leaves were oleuropein, hydroxytyrosol, caffeic, the flavone-7-glucosides, luteolin, apigenin and verbascoside. While phenolic compounds of infusion increase depending on infusion times, phenolic compounds of decoction decreased after from 7. min of decoction processing. Generally, luteolin, catechin, hydroxytyrosol, caffeic, oleuropein and rutin contents were found high in both infusion and decoction during heating.

Table 2. Phenolic compounds of infusion and decoctions of olive leaves in hot water at 90 °C (µg/g)

Phenolics	Infusion					Decoction				
	1.	3.	5.	7.	10. min	1.	3.	5.	7.	10. min
Luteolin	0.09 ±0.01 *e	0.58 ±0.03d	0.71 ±0.07c	0.83 ±0.11b	0.91 ±0.17a	0.14 ±0.03d	0.67 ±0.11c	0.82 ±0.09b	0.89 ±0.07a	0.81 ±0.13b
Catechin	0.13 ±0.01e**	0.64 ±0.03d	0.86 ±0.11c	0.91 ±0.07b	0.95 ±0.21a	0.18 ±0.09e	0.74 ±0.07d	0.91 ±0.03c	0.97 ±0.19a	0.93 ±0.13b
Hydroxytyrosol	0.07 ±0.01d	0.63 ±0.09c	0.89 ±0.11b	0.91 ±0.17b	0.97 ±0.21a	0.17 ±0.01e	0.84 ±0.15c	0.93 ±0.09b	0.98 ±0.13a	0.71 ±0.05d
Caffeic	0.05 ±0.01e	0.41 ±0.011 d	0.74 ±0.09c	0.81 ±0.09b	0.95 ±0.08a	0.09 ±0.01d	0.76 ±0.09c	0.79 ±0.11c	0.83 ±0.09a	0.80 ±0.07b
Verbascoside	0.06 ±0.01e	0.24 ±0.03d	0.47 ±0.03c	0.59 ±0.03b	0.64 ±0.03a	0.11 ±0.03d	0.28 ±0.01c	0.51 ±0.03b	0.57 ±0.01a	0.50 ±0.03b
Oleuropein	0.10 ±0.03e	0.44 ±0.07d	0.83 ±0.07c	0.91 ±0.11b	0.97 ±0.16a	0.13 ±0.01d	0.49 ±0.03c	0.83 ±0.17b	0.87 ±0.09a	0.81 ±0.09b
Luteolin-7-glucoside	0.04 ±0.01e	0.11 ±0.01d	0.17 ±0.01c	0.24 ±0.01b	0.48 ±0.09a	0.06 ±0.01e	0.14 ±0.01d	0.19 ±0.01c	0.36 ±0.05a	0.31 ±0.01b
Rutin	0.17 ±0.03e	0.64 ±0.09d	0.83 ±0.13c	0.91 ±0.07b	1.09 ±0.13a	0.19 ±0.03d	0.68 ±0.13c	0.97 ±0.09a	0.99 ±0.11a	0.75 ±0.07b
Apigenin-7-glucoside	0.05 ±0.01e	0.09 ±0.01d	0.14 ±0.03c	0.21 ±0.03b	0.28 ±0.01a	0.08 ±0.01d	0.11 ±0.03c	0.17 ±0.01a	0.14 ±0.01b	0.11 ±0.03c
Vanillin	0.03 ±0.01c	0.04 ±0.01c	0.07 ±0.01b	0.09 ±0.01a	0.10 ±0.01a	0.11 ±0.01d	0.13 ±0.01c	0.24 ±0.03a	0.19 ±0.03b	0.15 ±0.01c
Tyrosol	0.07 ±0.01d	0.11 ±0.01c	0.18 ±0.03b	0.21 ±0.08ab	0.23 ±0.03a	0.14 ±0.01e	0.21 ±0.01d	0.27 ±0.03b	0.35 ±0.01 a	0.25 ±0.03c

*mean±standard deviation; **Values within each row followed by different letters are significantly different ($p<0.05$)

While luteolin contents of infusion change between 0.09 and 0.91 µg/kg, this compound ranged from 0.14 to 0.89 µg/kg in decoction. While oleuropein content of infusion changed between 0.10 and 0.97 µg/kg, oleuropein contents of decoction samples varied between 0.13 and 0.87 µg/kg. In addition, rutin contents of decoction varied between 0.19 and 0.99 µg/kg. Also, caffeic acid contents of decoctions ranged from 0.09 to 0.83 µg/kg. Generally, phenolic compounds of decoction (except 10. min) were found higher than those of phenolic compounds of infusion. These differences can be probably due to more boiled of olive leaves at 90 °C. In previous study, Pereira *et al*., (2007) reported that caffeic acid was found as 1% of total phenolics. The amount of the phenolic present in the aqueous extract revealed a high amount of these compounds. The amount of phenols extracted at 90 °C increased with increasing extraction time up to 7. min of decoction processing. The differences detected in

olive leave extraction could be related with the times of infusion and decoction. Briante *et al*., (2002) reported that the phenolic compounds of olive leaves vary also at the time of fruits maturation. The chromatographed results of infusion and decoction of olive leaves were found different to the corresponding extracts. The extraction of dried leaves with hot water increased the extraction of certain phenolic compounds, when compared with methanol:water extraction (Silva, 2004). Silva *et al*., (2006) reported that fresh and dried olive leaves contained 11.6-17.4 and 11.7-40.1 g/kg total phenol, respectively. As far as known, there are no reports concerning the study of total phenol contents, antioxidant activity and phenolic compounds of both infusion and decoctions of the cultivar *Ayvalık* olive leaves.

Conclusion

So, olive leaves can be received much attention as antioxidant sources due to its phenolic compounds. Because, there are considerably higher ration of by products arising olive leaves.

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PERFORMANCE OF THE PLUM (*PRUNUS DIVARICATA*) UNDER DIFFERENT PROLIFERATION MEDIA

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Abstract

The study was performed with shoot tips that were selected from plum genotype displaying remarkable rootstock potential. The aim of this study was to determine the most appropriate protocol for the propagation of different plum genotype in vitro. The shoot tips of the selected plum belonging to Prunus divaricate species were surface-sterilized with sodium hypochlorite (15%) for 10 minutes. Aseptic culture was established on hormone free (HF) Murashige and Skoog (MS) and shoot tips were cultivated for four weeks at initiation medium. After establishment of aseptic culture, proliferation capacity of shoots was evaluated on HF MS medium containing with 30% sucrose and 8% agar and on HF MS media supplemented with different concentration of thidiazuron (TDZ), naphthalene acetic acid (NAA), gibberellic acid (GA3) and Indole-3-butyric acid (IBA). The lowest proliferation capacity (0.75) was found in HF medium and shoots did not exhibit signs of hyperhydricity (vitrification). At the same time, the highest incidence of vitrification (33%) was observed in the medium containing 0.75 mg/l TDZ, 0.5 mg/l NAA, 0.5 mg/l GA3 and 0.5 mg/l IBA with proliferation rate being 1.21. The highest proliferation rate (2.26) was achieved in the medium with similar hormonal composition as previous one with exception of TDZ concentration (1.5 mg/l). The results of the study suggest that TDZ exhibits great effect on proliferation rate and incidence of vitrification in this plum genotype.

Key words: *In vitro, rootstock, proliferation, vitrification.*

Introduction

Due to the special ecological conditions in Turkey, many species and cultivars of fruits are grown commercially. Stone fruits have taken substantial position in fruit cultivation both in Turkey and in the world. In Turkey, stone fruits production is accounted of approximately 15% in total fruit production. According to Turkish Statistical Institute (TUIK, 2018) data, production amount of stone fruits are arranged in order; apricot, nectarine, cherry, plum and sour cherry, respectively. Turkey has ranked first in apricot production with 730.000 t in 2016 and 985.000 t in 2017. Between the mentioned years, production increased by 34.9% (TUIK, 2018). Size-controlling rootstocks are needed for cultivation of table apricots especially in Mediterranean and Aegean regions. In both regions, apricots are mostly grown on plum rootstocks. The most important reasons for choosing plum rootstocks are that these rootstocks perform well in the heavy soils in the Mediterranean region and can reduce the tree size. Plums belong to the species of genus Prunus (family Rosacea) which have edible fruits. The plum which grows naturally in Anatolia has been moved from another part of the world due to immigration. Out of two thousand plum varieties in the world, more than 200 varieties are grown in Turkey. In spite of rich genetic source, selection of suitable rootstock is one of the most important problem. There are two types of plant propagation production methods: sexual and vegetative. Grafting, one of the vegetative production method, is widely used in horticultural fruit production. In this method there are two parts, the scion and the rootstock. Rootstocks constitute the subsurface system and influence the phenological, physiological and

economic life of the above ground part of plant. Also, they influence the degree of adaptation of the plant to ecological conditions, such as salinity, pH, drainage, etc. Because of this reason, the use of proper rootstock is a very important issue in fruit farming. The rootstocks obtained by different breeding programs in fruit cultivation are widely used for different purposes. Unfortunately, rootstock breeding research started late in our country. In other countries, the use of plum in rootstock breeding for apricot cultivation is quite common. These rootstocks have been bred for ease of vegetative propagation, dwarfing characteristics and resistance to different climatic and soil conditions. In most rootstock breeding and production studies, cuttings and tissue culture have been the main methods used in vegetative production. Both production methods have advantages and disadvantages. Cuttings which are the classic production practice, are limited to seasonal production periods. In vitro production, on the other hand, can be carried out continuously with appropriate explants and subculture applications. Also, homogenous plant material is obtained by this method. The other advantages of this method are that a large numbers of material can be produced in a short period of time and in a small area.

Bassi and Cossio (1991) reported that genotype plays a significant role in tissue culture regeneration studies, and they emphasized that a successful regeneration protocol in one genotype, may not be successful in another genotype. The most commonly used plum rootstocks in breeding studies are. cerasifera, P. persica, P. insititia and P. domestica. Mariana obtained from breeding by P. cerasifera × P. munsoniana, Myrobolan (P. cerasifera) and Pollizo ve Pixy (P. insititia) and all have different regeneration protocols (Moreno, 2009).

The aims of this study are to determine the best proliferation protocol for the KLDİ-47 plum genotype which belongs to the Prunus divaricataspecie which has the potential to be a rootstock for many stone fruit species.

Materials and methods

This study was carried out at tissue culture laboratory of Horticulture Department of Çukurova University and research field with tissue culture laboratory of East Mediterranean Transitional Zone Agricultural Research of Institute (DAGTEM). The CLDi-47 (*Prunus divaricata*) genotype was used, which is good for vegetative propagation that considering cutting experiment results. Single nodes are taken from fresh shoots of CLDi-47 genotype as initial plant material (Figure 1). The single nodes were kept under the tap water for 30 minutes to remove the phenolic compounds. After keeping at 70 % ethyl alcohol at 20 seconds for surface sterilization, single nodes allowed to stand for 20 minutes in 10 % commercial bleach (0.525 % NaOCl) containing one or two drops of Tween 20. Surface sterilization of single nodes was completed by washing with sterile distilled water 3 times for 5 minutes. All these plant materials were inducted in Murashige and Skoog (MS) as initiation medium for four weeks (Figure 2). After this period of time, they were divided into four groups for evaluating the performance of propagation and vitrification under the different medium. Medium information was given at Table 1. A study carried out by Palaz and Uğur (2018) and reported positive results of another rootstock candidate (SP-2) were used as reference. Three different media containing different combinations of four plant growth regulators were tested.

Figure 1. (A) plant material of CLDi-47 genotype used in this study (B) cutting of single nodes

Figure 2. *In vitro* plants obtained from CLDi-47 genotype

Table 1. Hormonal composition of media used in the proliferation stage

Medium designation	TDZ (mg/l)	NAA (mg/l)	GA_3 (mg/l)	IBA (mg/l)
Medium 1	0.00	0.0	0.0	0.0
Medium 2	0.75	0.5	0.5	0.5
Medium 3	1.5	0.5	0.5	0.5
Medium 4	3.00	1.5	0.5	0.5

1-) Thidiazuron (TDZ), Naphthalene acetic acid (NAA), Gibberellic acid (GA3), Indole-3-butyric acid (IBA).

To determine the performance of the media in terms of regeneration and vitrification, twenty explants were used. Mean of these was taken and evaluated.

Results and discussion

The effect of the different plant growth regulator combinations and concentrations on the propagation and vitrification were given in Table 2. While the first medium was hormone-free MS (30 % sucrose and 8 % agar), the other ones contained different ratios of TDZ, NAA, GA_3 and IBA. The lowest propagation was found in hormone-free medium (Medium 1) with 0.75 value and vitrification was not observed. The highest vitrification (33 %) was recorded in the Medium 2 with 1.21 proliferation rate. The maximum shoot number was obtained from the Medium 3 with 2.26 proliferation rate. This medium was similar to the Medium 2 except the TDZ ratio. Our data showed similarity to results reported by Escalettes and Dosba (1993) and Nowak et al. (2004). Suitable level of TDZ was ranged between 3 μM and 7.5 μM depend on genotypes in their studies.

Table 2. Effect of tested hormonal composition of medium on proliferation capacity and incidence of vitrification

	Medium 1	Medium 2	Medium 3	Medium 4
Proliferation rate	0.75 ± 0.58	1.21 ± 1.58	2.26 ± 2.16	1 ± 0.58
Vitrification (%)	0 ± 0	33 ± 33.68	0 ± 0	18 ± 28.86

Petri and Burgos (2005) reported that there was difficulties in vitro shoot regeneration of the fruit trees. Low success rate was observed using different applications high such as level of auxin, root inducers, subculture and application times performed to increase rooting in commercially important cultivars (Gonzales Padilla et al., 2003; Tian et al., 2007). In our study, it was found that regeneration rate of explants increased in all of medium containing the plant growth regulators when compared with the control medium (Medium 1). Also, the vitrification level was influenced from the concentrations of TDZ and NAA. In an in vitro study carried out by Canlı and Tian (2009) in Japanese plums (Prunus salicina Lind.), a significantly positive correlation was found between TDZ content and shoot regeneration capacity. More number of shoots was obtained in the medium supplemented 9-15 μM TDZ than the medium containing 4.5 μM TDZ.

In a study carried out by Zou (2010), 'Gulf-ruby' plum variety were evaluated in terms of shoot number performance and vitrification rate in the woody plant medium (WPM) containing different combinations of plant growth regulators. The medium supplemented 0.2 mg/l IBA and 2 mg/l BA showed vitrification and severe abnormalities together with slow growth of shoots. The in vitro plants obtained could survive with 67 % success rate after transferring to the greenhouse. In our study, while the vitrification was promoted by low level of TDZ (0.75 mg/l), it was completely inhibited using higher level of TDZ (two fold than first medium). However, vitrification was again observed in Medium 4 containing the highest dose of TZD (3 mg/l). This conflicting can be rooted by highest level of NAA.

Conclusion

As a result of this study, it was clearly observed the effect of the type and concentration of plant growth regulators on the regeneration and vitrification rate on the plum genotype. Especially, TDZ showed great importance in this study. The Medium 3 was the best in terms of the highest regeneration rate and any vitrification symptom. This result is very important for stone fruit cultivation and also for CLDi-47 genotype having rootstock potential.

Acknowledgments

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TOXICITY OF SOME ALUMINUM DOSES ON CAB-6P (*Prunus cerasus* L.) CLONAL ROOTSTOCK

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Abstract

Abiotic and biotic plant stress factors are the major causes of yield limitations. Aluminum toxicity is one of the most important stressors for plant growth and development after salinity. The most common effect of Al toxicity on the plant is the reduction of root growth. Reduced root growth decreases yield and fruit quality. Thus, high-quality rootstocks, that can cope with elevated toxic mineral levels, are important resources for fruit growers and breeders. The CAB-6P (*Prunus cerasus* L.) seedlings were evaluated to determine aluminum toxicity and its effect on biomass production. Seedlings reproduced in tissue culture and grown for 2 months in growth room were used for the study. Plants with similar sizes were transplanted into 9-liter pots filled with turf and perlite medium (2:1). Four doses of aluminum (200-400-600-800 µM) were treated every 30 days one month after transplanting into pots. Above and below ground parts, and leaves were separated after four months of aluminum treatment. Data for root, shoot and leaf biomass (fresh and dry), trunk and root collar diameters, plant height and root length were collected. According to data analysis, there were significant differences between control and elevated doses of Al. Plant height, root length, root and shoot fresh and dry biomass were significantly reduced with increased Al doses. However, trunk (10.88 mm) and root collar diameters (14.74 mm) were larger on 200 µM Al treatment than control (9.90 mm and 12.96 mm, respectively) and other doses. Aluminum doses had a significant negative effect on CAB-6P rootstock growth.

Keywords: *Aluminum toxicity, CAB6-P, Root biomass, Shoot biomass.*

Introduction

Aluminum (Al) is the third most abundant element and the first most abundant metallic element in the earth's crust. A large part of Al is found as complex alumino-silicates, while a small part is found in soluble forms (May and Nordstrom, 1991; Moffat, 1999). Al toxicity is related to pH and is mostly observed on acidic soils (Kochian et al., 2005). Alumino-silicates and aluminum hydroxide minerals dissolve in soil with low pH (under 5.5). Subsequently, Al^{3+} is converted to mononuclear species such as $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$ and $Al(OH)_4$ (Kochian, 1995; Ryan et al., 2001; Panda and Matsumoto, 2007). Mononuclear species of Al^{3+} and Al_{13} are the most toxic forms of Al (Kinraide, 1991; Kochian, 1995). However, some other monomeric Al species (like sulphate, phosphate, and silicate Al species) are less harmful. In addition, organic Al-complexes have low toxicity (Schecher and Driscoll, 1987; Schecher and McAvoy, 1992).

It is estimated that 30-50% of arable land in the world is acidic (Jardim, 2007; Panda et al., 2009) and Al toxicity in such soil is one of the main factors limiting the yield (Maron et al., 2008). Al toxicity poses risks mainly for aquatic plants living in acidic fresh waters (Gensemer and Playle, 1999), plants living in forests with acidification risk (Driscoll et al., 2001; de Vries et al., 2007), and crops grown in acidic tropical soils (Baligar and Fageria, 1997). Even though Al toxicity is a serious issue worldwide, our knowledge of Al chemistry

is still limited because of its extremely complex nature. A large spectrum of polynuclear inorganic and organometallic complexes of strongly varying stability in soil and water is the reason for this complexity (Kinraide, 2003; Poschenrieder et al., 2008).

Although the primary target of Al toxicity on the plant is not yet known (apoplastic or symplastic) (Taylor, 1995; Matsumoto, 2000), the literature suggests that Aluminum leads to primary damage in the apoplasts of peripheral stem cells. These structures participate in important processes such as cell wall assembly, ion flow, and structural properties of the plasma membrane (Hecht-Buchholz and Foy, 1981; Horst, 1995; Kochian, 1995; Rengel, 1996). Additionally, a possible symplastic target of Al, such as the root cytoskeletal structures, (Barlow and Baluska, 2000) is the direct binding to the meristematic stem cell nucleus (Foy et al., 1978; Silva et al., 2000). Al binds directly to the microtubules found in the nuclei of these cells, resulting in dysfunction (Blancaflor et al., 1998; Sivaguru et al., 1999a, 1999b). Growth inhibition is widely used in the assessment of Al toxicity. Root/shoot ratio, total root and shoot lengths and surface area reduction are commonly used parameters (Clarkson, 1969; Alam, 1981; Fagria, 1982; Pavan and Bingham, 1982a; Kochian, 1995; Moustakas et al., 1996; Blancaflor et al., 1998; Ramachandran et al., 2000; Barcelo and Poschenrieder, 2002; Zobel et al., 2007; Azmat and Hasan, 2008). At the same time, the parts of the plant which are affected by Al are mostly roots. The most sensitive region of the root against Al toxicity is the transition zone. This zone is located between active cell division and fast cell elongation zones (Sivaguru and Horst, 1998). The reason for this sensitivity is that it is the place where the elements necessary for rapid elongation are prepared (Verbelen et al., 2006). The Al damages the active cell division zone and neutralizes other factors that cause root growth (Doncheva et al., 2005).

Al toxicity creates a root system with many short and fragile lateral roots (Ryan et al., 1993; Doncheva et al., 2005). As these roots are reduced in length, their thickness increases (Blancaflor et al., 1998; Ramachandran et al., 2000; Zobel et al., 2007). The shoots and leaves of plants start to become damaged due to imbalances in water and nutrient uptakes resulting from root damage (Moustakas et al., 1996). According to some studies, Al reduces biomass in the above-ground parts (Azmat and Hasan, 2008), increases thickness in cell wall (Konarska, 2010), increases lipid peroxidation (Zhang et al., 2007), causes nutritional imbalances (Guo et al., 2007), changes in the photosynthetic performance (Jiang et al., 2008), and changes in the amount of chlorophyll (Chen et al., 2005; Zhang et al., 2007; Azmat and Hasan, 2008; Jiang et al., 2008). It has also been reported that Al toxicity reduces CO_2 assimilation (Moustakas et al., 1996; Lidon et al., 1999) and causes photosystem damage (Zhang et al., 2007; Reyes-Diaz et al., 2010) in several species.

The aim of this study was to examine the effects of 4 different Al doses on some morphological characteristics of CAB-6P (*Prunus cerasus* L.) clonal rootstock.

Material and Methods

The study was carried out in the growth room and greenhouses of the Department of Horticulture, Faculty of Agriculture of the Selçuk University (Turkey) in 2016. Aluminum has been treated to each plant once a month for 4 months starting by June 25th, 2016. The study was planned according to randomized complete blocks design with 3 replications with one plant per replication. The treatments were; 0 µM, 200 µM, 400 µM, 600 µM, and 800 µM doses of Al. Plants produced with seeds were kept in a 2-liter pot at a temperature of 27,5^{o}C / 22,5^{o}C (day/night) and 14/10 h (day/night) day length in a growth chamber until a certain plant height (50 cm) was reached. A mixture of peat/perlite (2/1 v/v) was used as growing medium. When plants reached 50 cm in height, they were transferred to the greenhouse in 9-liter pots (same growth medium) and all necessary cultural processes were carried out during the study. As of November, the plants removed, plant trunk diameter (5 cm above ground),

root collar diameter (just above first lateral root), plant height (from ground to top), root length (from root collar to tip of root), stem fresh and dry weights, root fresh and dry weights (from root collar), and leaf fresh and dry weights were investigated. The data obtained were assessed at $p \leq 0.05$ significance level with the IBM SPSS v.20 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY) statistical software with one-way variance analysis (ANOVA) and subjected to Duncan Multiple Comparison Test.

Results and Discussion

Trunk diameters (TD) of the plants were measured with digital caliper 50 days after the last treatment of Al. Statistically significant differences were found ($p \leq 0.05$) and Al treatments thickened the plant trunk. In particular, 200 µM (10.88 mm) and 600 µM (10.80 mm) Al treatments provided the formation of thicker tree trunks than the control (9.90 mm). While TD was same in 400 µM Al treatment and control, 800 µM Al treatment (9.61 mm) decreased TD compared to control. The reason for this seems to be the limited plant growth because of high Al toxicity. Some previous studies reported that Al has positive effects on plant growth. According to Ghanati et al. (2005), root lengths of tea plants grown in nutrient solution with 500 µM Al (pH 4.3) were 2.5 times longer than the control (). Similarly, Al treatments had positive effects on root activity, stem cell lengths and relative dry weight in *Melastoma malabathricum*. However, in the same study, Al treatments caused toxic effects on barley (*Hordeum vulgare*) (Watanabe et al., 2005). In another study, *Quercus serrata* seedling traits such as shoot dry weight, root dry weight, length of newly grown roots, relative height, and number of branched roots were found to be higher than control and low pH (-Al) treatments in 2500 µM Al treated plants (Tomioka et al., 2005). When the results of the above studies are compared with this study, the TD value is expected to increase with low Al doses. However, while other characteristics are negatively affected by increased Al doses, the elevation of TD in the 600 µM Al dose cannot be explained by this approach. This may be due to the positive response of plants growing during the first few months under low Al doses. The growth of plants may have slowed down as they were exposed to more Al in 3rd and 4th month.

Table 1. Effect of aluminum treatments on trunk diameter (TD), root collar diameter (RCD), plant height (PH) and root length (RL).

Al Doses	TD (mm)	RCD (mm)	PH (cm)	RL (cm)
0 µM	9.90^{bc}	12.96^{b}	80.67^{a}	35.50^{a}
200 µM	10.88^{a}	14.74^{a}	77.00^{b}	33.33^{b}
400 µM	9.90^{bc}	12.25^{bc}	74.67^{c}	31.00^{c}
600 µM	10.80^{ab}	11.64^{c}	72.67^{d}	29.00^{d}
800 µM	9.61^{c}	11.60^{c}	68.33^{e}	28.50^{d}
LSD	2.31	0.77	1.35	1.32
P value	0,042	0,000	0,000	0,000

The RCD results determined by measuring with caliper, immediately after the plants were removed, were statistically significant ($p \leq 0.05$). The RCD increased with 200 µM Al treatment (14.74 mm) compared to control plants (12.96 mm), while RCD began to decrease from 400 µM Al treatment (12.25 mm) (Table 1). The RCD was significantly reduced in 600 µM (11.64 mm) and 800 µM (11.60 mm) Al treatments. The RCD values obtained from this study were similar to previous studies in which Al effects plant growth. It has been reported in several studies that low Al doses affected plant growth positively and Al has contributed to the growth of plants (Schier, 1985; Ghanati et al., 2005; Tomioka et al., 2005; Watanabe et al., 2005). However, the studies that indicate root damage as plants exposed to higher Al concentrations are quite extensive (Foy, 1983; Schier, 1985; Ryan et al., 1993; Mossor-

Pietraszewska et al., 1997; Blancaflor et al., 1998; Ramachandran et al., 2000; Doncheva et al., 2005; Zobet et al., 2007). High Al concentrations used in this study resulted in similar results with previous studies, and the RCD values decreased as the treated dose increased.

The plant heights were measured from the ground level to apical meristem with a metric measure before the plants removed. The plant height (PH) results obtained from the current study was statistically significant ($p \leq 0.05$). The longest plants were obtained from the control (80.67 cm) (Table 1). Plants began to shorten in length (200 µM, 77.00 cm) and the plant height continued to decrease (800 µM, 68.33 cm) as the Al concentration increased and the shortest plants were obtained from 800 µM Al. These results do not coincide with studies reporting that low Al doses affect plant growth positively (Ghanati et al., 2005; Tomioka et al., 2005; Watanabe et al., 2005). However, the difference between control and the lowest Al dose (200 µM) was not very large. On the other hand, there have not been many studies investigating the effect of high Al doses on PH. In a study conducted with Boron (B) and Al by Riaz et al. (2018), Al (400 µM) treated plants were shorter compared to the control. In this study, the plant length was shortened with the increased Al doses. However, plants treated with B (10 µM) and Al (400 µM) were taller compared to the control. In addition, studies on *Oryza sativa* (Fagria, 1982), *Coffea arabica* (Pavan and Bingham, 1982a, 1982b), *Manihot esculenta* (De Carvalho and Cesar, 1984), and Gleditsia species (Thornton et al., 1986b, 1986c) have shown that the height of shoots is reduced with Al treatments.

After the plants removed, the length from the root collar to the tip of the main root was measured with a metering device. The results obtained were evaluated and found statistically significant ($p \leq 0.05$). According to the results, the effect of Al doses on the root length was negative. It can be seen that root length starts to decrease with the 200 µM Al (33.33 cm) treatment and the root length decrease with higher Al doses are continued (Table 1). In plants treated with 800 µM Al (28.50 cm), root length was considerably shorter than control (35.50 cm). In many studies, roots of plants exposed to toxic Al doses are shortened (Foy et al., 1978; Ryan et al., 1993; Mossor-Pietraszewska et al., 1997; Ramachandran et al., 2000; Doncheva et al., 2005; Zobel et al., 2007). Similar results were obtained from this study, and as Al doses increased, plant root lengths were reduced. Some researchers reported that roots extend to soil zones where Al is lower by orienting lateral roots (Foy, 1984; Barcelo and Poschenrieder, 1990; Ryan et al., 1993; Doncheva et al., 2005). However, since this study was carried out in pots, there was no soil part with low Al dose where the roots could be oriented, and therefore the root weight decreased with the dose increase, as can be seen in table 2.

Table 2. Effect of aluminum treatments on above ground fresh mass (AGFM), above ground dry mass (AGDM), root fresh mass (RFM) and root dry mass (RDM).

Al Doses	AGFM (g)	AGDM (g)	RFM (g)	RDM (g)
0 µM	84.22^{a}	30.90^{a}	153.61^{a}	48.45^{a}
200 µM	60.18^{b}	28.87^{b}	111.54^{b}	37.54^{b}
400 µM	56.98^{b}	27.12^{c}	105.78^{c}	37.11^{b}
600 µM	51.22^{c}	24.16^{d}	102.13^{cd}	36.70^{b}
800 µM	45.75^{d}	23.58^{d}	98.67^{d}	36.43^{b}
LSD	4.33	1.22	4.60	1.98
P value	0.000	0.000	0.000	0.000

After the treatments were completed and the plants were removed, the fresh and dry weights of the above ground parts were determined by weighing. The results were evaluated and statistically significant differences were found ($p \leq 0.05$). The AGFM (84.22 g) and AGDM (30.90 g) of control were higher than all Al treatments. However, while AGFM of plants treated with 200 µM Al (60.18 g) decreased more than 25% compared to the control, AGDM

(28.87 g) decreased at a lower rate. Similarly, the reduction rates in AGFM (45.75 g) and AGDM (23.58 g) were different in 800 µM Al treatment. It is understood that Al treatments affect AGFM more than AGDM (Table 2). Earlier studies suggested different views on the effect of Al on shoots and leaves. There are studies show that shoots and leaves are as sensitive as roots against Al toxicity, but that damage starts from the roots first (Thornton et al., 1986b; Thornton, 1986c). However, Foy (1983) states that Al toxicity does not lead to any considerable damage on shoots when visible damage was observed in the roots. Schier (1985) reports that serious damage to the roots of *Picea rubes* and *Abies balsamea* species occurs without any symptoms except slow growth in shoots. However, majority of the studies reports that Al toxicity damages shoots and leaves at various levels (Rees and Sidrak, 1961; Awad et al., 1976; Alam, 1981; Hutchinson et al., 1986; Pegtel, 1986; Foy, 1996; Azmat and Hasan, 2008). Unlike above studies, Krizek et al. (1997) reported that shoots react more rapidly to elevated Al doses than roots and begins to be affected by lower Al doses. In the current study, , it was understood that Al toxicity damages the shoots as well as roots (Tables 1, 2 and 3). Even though short-term and low-dose treatments may not affect shoots as mentioned, plants in this study were exposed to different doses of Al for 4 months, and the height, fresh and dry weights of shoots were affected as much as roots from Al toxicity.

After the plants were removed, fresh and dry weights of the roots which cut from the root collar were weighed with a precision scale. The differences between the treatments were found to be statistically significant ($p \leq 0.05$). Fresh and dry weights of roots decreased from 200 µM Al treatment due to increased Al dose. 200 µM Al treatment reduced RFM (111.54 g) compared to control (153.61 g) by approximately 25%, the RDW (37.54 g) was reduced by approximately 22% compared to the control (48.45 g) (Table 2). At 800 µM Al treatment, RDM (36.43 g) decreased by about 25% while RFM (98.67 g) decreased by approximately 36%. The proportion of the decrease in RFM was higher, which can be explained by the decrease in water uptake due to damaged roots. While well-developed roots of control plants can take up high amounts of water and water-soluble minerals, the weak roots of Al-treated plants cannot achieve this. The results obtained from the present study are similar to previous studies on Al toxicity. In some studies, Al toxicity reported reducing root length (Foy, 1983; Mossor-Pietraszewska et al., 1997), fresh (Watanabe and Osaki, 2002) and dry (Fagria, 1982; Foy, 1996) weights by damaging the roots. Al reduced root fresh and dry weights in *Oryza sativa* (Fagria, 1982), *Picea abies, Betula pendula* and *Pinus sylvestris* (Eldhuset et al., 1985), *Picea rubens* and *Abies balsamea* (Schier, 1985), *Gleditsia triacanthos* (Thornton et al., 1986b) and *Secale cereale* (Silva et al., 2012), depending on the dose. The decrease in the root fresh and dry weights of plants as the treated Al doses increased indicates that this study is similar to previous studies.

Table 3. Effect of Aluminum treatments on leaf fresh mass (LFM) and leaf dry mass (LDM).

Al Doses	LFM (g)	LDM (g)
0 µM	21.76[a]	7.15[a]
200 µM	17.00[b]	6.09[b]
400 µM	15.20[c]	5.97[b]
600 µM	11.84[d]	4.36[c]
800 µM	11.39[d]	4.42[c]
LSD	1.80	0.23
P value	0.000	0.000

After the plants were removed, leaves were separated and weighed as fresh and dry. Results were evaluated and found statistically significant ($p \leq 0.05$). Al treatments reduced leaf fresh mass (LFM) and leaf dry mass (LDM) (Table 3). The LFM and LDM continued to decrease

as the Al dose increased. However, LDM (4.42 g) of plants treated with 800 µM Al was higher than LDM (4.36 g) of plants treated with 600 µM Al dose. However, both results were still in the same statistical group. The LFM of the same doses (600 µM and 800 µM) are in the same group and their values were 11.84 g and 11.39 g, respectively. In addition, LDM decreased at a relatively low rate (control: 7.15 g, 200 µM Al: 6.09 g) while 200 µM Al treatment (17.00 g) decreased LFM at a higher rate compared to control (21.76 g). These results in above ground mass (AGM) and root mass (RM) may be related to water and nutrients uptake. According to previous studies, leaves are affected by Al toxicity and the leaf weight is decreased (by dose increase). Al toxicity reduced leaf initiation in *Gleditsia triacanthos* (Thornton et al., 1986a), leaf number, surface area and leaf dry weight in *Medicago sativa* (Dessureaux, 1969), and reduced leaf initiation due to the decreased shoot extension in *Picea abies*, *Betula pendula* and *Pinus sylvestris* (Eldhuset et al., 1985). Accordingly, the current study is consistent with previous studies and Al toxicity has adverse effects on the leaves.

Conclusion

Al toxicity is a potential threat for plants in acidic soils. Agricultural production can be restricted as a result of excessive Al in soils with low pH values. However, as noted above, lower doses of Al may positively affect the growth and development of some plant species. Increased Al concentration and decreased pH causes damage and decreased crop production and profit.

Researchers seem to agree that there are two methods of control on Al toxicity. These methods are tolerance to Al toxicity (tolerance mechanisms may differ according to species) and removal of Al from the root zone. However, the most emphasized subject seems to be to determine the tolerant species and cultivars. In future studies, fruit species and cultivars with tolerance to Al and can grow in acidic soil should be determined and acidic agricultural soils with high Al content should be opened to economic production.

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DETERMINING THE RELATIONSHIPS BETWEEN SEED YIELD AND LEAF CHARACTERISTICS IN COWPEA

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Abstract

Cowpea is an important legume that is resistant to arid conditions and rich biomass. This study was carried out to determine the relationship between the seed yield, biologic yield and leaf properties. The experiments were conducted at two locations where one of them was warm (Samsun), other was with continental climate (Amasya). Sixteen cowpea line/cultivars were used in this study. The experiments were set up in the randomized complete block design with three replications. In the both locations, sowing on seeds was made with 60 cm row space to plots 12 m^2. The leaf samples were collected for both locations in a period at the beginning of pod setting period. All of leaf properties were determined by LICOR LI 3000A Portable Leaf Area Meter. Collected data were subjected to variance analysis in MSTATC package program by being combined over the location. According of variance analyses, total leaf area of plant, number of leaves, leaflet length, dry matter rate of leaf, seed yield per plant and biological yield showed statistical difference among genotypes. The significant differences between two locations were found for biological yield, seed yield and number of leaves. Plant seed yield and biological yield positively and significantly correlated with leaves number, leaf area per plant, leaflet number, leaflet area of per plant.

Key words: *cowpea, leaf properties, seed yield.*

Introduction

Water demand globally is projected to increase by 55% between 2000 and 2050. Much of the demand is used by agriculture, which accounts for 70% of global freshwater (URL-1). And in many places where large-scale irrigated agriculture takes place, demand is already outstripping supply. The regions at highest risk for future water stress are clustered around these areas: the Mediterranean, the Middle East, Western North America, Eastern Australia, Western Asia, Northern China, and Chile (URL-2).

Located in the Mediterranean basin, Turkey is a country where many cool and warm season legumes are grown. Beans grown in almost every part of Turkey and is a plant lovingly consumed. Our region is one of the major bean growing areas of Turkey. The seasonal water consumption of bean was measured as 732 mm (Şehirali ve ark.,2005). One of the factors limiting the growth of bean is the high water consumption while the other high temperatures. The cowpea is similar to bean plant as a morphological features and usage shape. It has been found that in many parts of the world the cowpea is more tolerant to warmth and constancy than other legume plants (Sing et al. 1997; Hall et al. 2003, 2004). Due to these characteristics, cowpea is a remarkable plant in terms of the areas where beans are grown in our country (Çulha ve Bozoğlu, 2016). Cowpea is mostly grown in the Aegean, Mediterranean and South-Eastern Anatolia in our country and in restricted areas in our region. (Çulha ve Bozoğlu, 2017).

Cowpea is consumed in the form of food similar to beans. And also it is a very high plant with a potential for feed (Genckan 2003). There is potential in tropical regions to include cowpea (*Vigna unguiculata* (L.) Walp.) in rice (*Oryza sativa*) based cropping systems, especially

during the post-monsoonal dry season (Timsina et al. 1994). Acoording to Umar and et al.(2010) the leaves, stems and seeds of cowpea have antimicrobial properties that frequent consumers had lower blood pressures and total serum cholesterol levels. Consequently, consumers were less likely to be diagnosed with high Blood Pressure, diabetes and other heart diseases. Based on this information, studies are being carried out for the spread a long time in our region. Up to now, studies have shown that the cowpea is adapted to the conditions of the region. In experiments conducted under Samsun conditions, seed yield of cowpea is about 129-169 kg in genotypes and the results shown that it is possible to grow without irrigation except extremes years. (Gülümser ve ark., 1989; Bozoğlu ve Gülümser, 1995). It has been seen that the genotypes used in the adaptation studies for the development of the suitable varieties for the region brought plenty of vegetative biomass. Accordingly, this study was carried out in order to determine whether these properties could be the criterion of choice for the varieties to be developed for dry seed by establishing the relationship between leaf properties and seed yield.

Material and Method

Materials of this trial were 14 lines selected with invidual plant selection from population were collected different regions of Turkey and 2 registered varieties (Karagöz, Akkız). The testa color of B_7 and B_{11} are red, other genotyes were white. Hilum color of white color seed at B_9, B_{10}, B_{13} are brown, B_{14} is white and other are black.

The experiments were carried out two locations (Samsun (S), Amasya (A)) that were selected for representation of Middle Black Sea Region. First location (S) represents costal area of region, second (A) the interior of region. Some climate data of these locations can be sown at the figure 1.

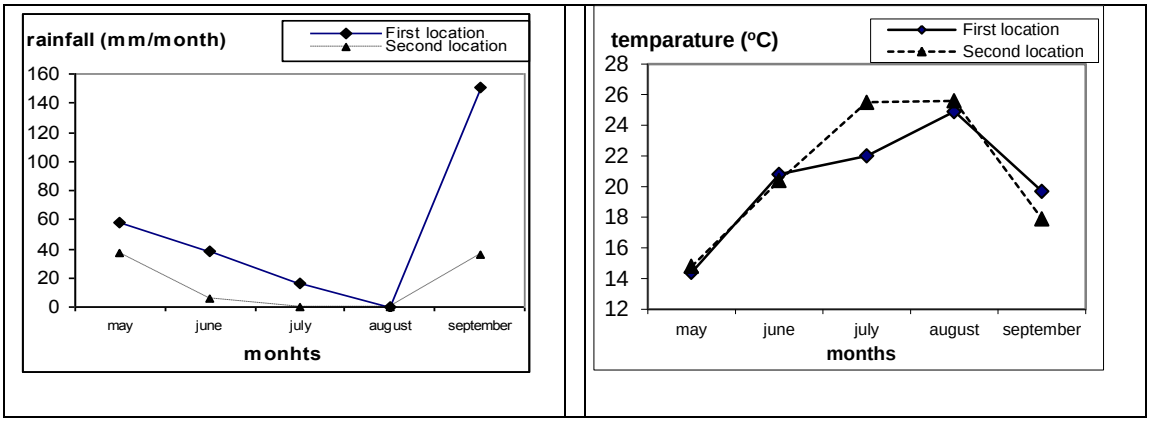

Figure 1. Some climate data of experiment areas

The trial soil of Samsun (first location) has clay, neutral ph, unsalty, middle organic material and unlime soil type, Amasya (second location) area has an average altitude of 450 m from the sea, has clay loam, alkaline ph, lightly salty, middle organic material, calcic soil type. Sowing were done on the last week of May both locations. Row spacing was 60 cm, with 15 cm between plants and five rows per plot. The trial was fertilized at the emergence time of plant with nitrogen fertilizer that was set total 4 kg per decare. Irrigation were three times in both locations. The leaf samples have been collected for both locations in a period which are beginning of pod filling period. Number of leaves, total leaf area per plant, leaflet length, leaflet with, dry leaf weight per plant, dry matter of leaf rate were determined. Leaf measurements were done with LICOR LI 3000A Portable Area Meter. Jesus et al.(2001) reported that, the LAI-2000 can be used to estimate the LAI of common bean.

All of data were transformed with square root. After than analysis of variance using the MSTATC program was performed after data was combined over the locations. Mean separation was done Duncan's multiple range test. Correlation analysis was applied in order to determine the relationships between the properties

Result and Discussion

In the study carried out with 16 cowpea genotypes at two different locations, the variance analysis showed that number of leaves, leaf area, leaf length, dry leaf weight, seed yield and biological yield statistically effected by genotypes (Table 1); leaf number and leaf dry weight by the locations (Figure 2).

The number of leaves in different genotypes varied between 11.8-37.3. The genotype with the highest number of leaves and the seeds with the lowest number of leaves were found to have the same seed and hilum colors (black). The number of leaves was found to be positively and significantly related to leaf area and dry leaf weight and leaf dry matter ratio (Table 2). Jose et al., (2003) founded that LAI reached its maximum value at the flowering and pod-filling stages. The leaf area determined in the pod filling cycle varied between 8.64-20.04 dm^2 in the genotypes used. Akinyomi et al., (2003) determined for different the tillage systems ranged between 34 and 45, with a mean of 38 leaves and 23 and 27 with a mean of 25 cm^2 leaf area. It was determined that the genotypes in our study were more variable in leaf count and leaf area was higher.

The statistical effect of locations on leaf number and area is not affected. However, less leaf area was obtained in Amasya (Figure 2). While the Samsun location received a precipitation of approximately 262 mm during the growing season, the amount of precipitation in Amasya was 79 mm in the same period. There was no significant difference in temperatures except for the August average (Figure 1). In addition to the irrigation, the vegetation period was extended in Samsun, which is more than one month. As the period elapsed, both the biological yield and the seed yield became higher due to this. The correlation analysis showed no significant relationship between leaf yield and other leaf characteristics except leaf dry matter ratio and biological yield (Figure 2).San Jose et al (2003), reported that the leaf area index was similar in varieties (2.13 and 2.15m^2) in the maximum growth period when they compared the energy changes during growth on the two cowpea varieties.

The cowpea leaf consists of 3 leaves. In the measurements, each leaf was measured and the width and length were determined by taking the average of all of them. In general, the terminal leaves are longer and the lateral leaves are wider. It has been determined that the leaf length of the genotypes used is statistically influenced by varieties. Leaf length ranged from 7.2 to 12.5 cm and width varied from 2.3 to 4.6 cm. The shortest leaf was seen in Karagöz variety which has black hilum coloring. Genotypes differing in seed and hilum color were found to be in the same statistical group in terms of length.

Table 1. Mean of cowpea genotyes that grown at two locations for some leaf characteristic and seed and biological yield per plant

*P<0.05, **P<0.01, hiulm color: red (R), black (B)

Varieties	Leaves number/plant			Leaf area (dm^2/plant)			Leaflet lenght (cm)			Leaflet width (cm)			Dry leaf weight (g/plant)			Dry matter of leaf (%)			Seed yield (g/plant)			Biological yield (g/plant)		
	S	A	Mean **	S	A	Mean *	S	A	Mean *	S	A	Mean	S	A	Mean **	S	A	Mean	S	A	Mean **	S	A	Mean **
B1	30.8	16.3	23.6 b	18.47	9.30	13.89ab	7.7	12.9	10.4abc	2.6	6.5	4.6	9.1	6.8	7.9bc	25.3	16.1	20.7	17.4	9.8	13.6abc	68.4	54.6	61.5 ab
B2	17.7	11.7	14.7bc	17.40	13.78	15.59ab	9.3	14.1	11.7ab	4.3	2.8	3.5	7.2	5.8	6.4bcd	20.3	16.3	18.3	13.1	11.6	12.4bc	74.0	59.9	66.9a
B3	50.9	23.7	37.3a	26.38	13.70	20.04a	7.4	7.4	7.4 c	2.6	2.8	2.7	12.2	8.3	10.2a	26.9	15.1	21.0	13.8	9.1	11.5bc	66.5	43.5	54.9a-c
B4	13.2	10.4	11.8c	11.10	10.43	10.76b	9.1	16.0	12.5a	3.2	2.3	2.8	6.2	5.6	5.9d	22.2	18.0	20.1	15.4	10.3	12.9abc	73.0	36.3	54.7a-c
B5	14.9	10.8	12.9bc	11.04	13.84	12.44ab	8.3	8.8	8.6bc	3.0	3.5	3.2	6.6	5.7	6.2bcd	18.3	15.6	17.0	16.2	10.8	13.5abc	72.3	44.8	58.6a-c
B6	14.8	10.6	12.7bc	12.62	8.50	10.56b	9.4	10.1	9.8abc	3.1	2.6	2.9	6.5	5.6	6.1cd	18.5	15.4	17.0	17.2	13.2	15.2ab	48.6	49.3	48.9a-d
B7 (R)	14.9	15.3	15.1bc	11.49	11.65	11.57b	8.8	12.3	10.5abc	3.0	2.4	2.7	6.7	6.6	6.7bcd	18.0	17.7	17.9	6.3	10.8	8.6c	25.8	46.5	36.1cd
B8	24.3	12.1	18.2bc	19.92	11.14	15.53ab	8.1	10.4	9.2abc	2.9	3.0	3.0	8.2	5.8	6.9bcd	22.3	17.1	19.7	15.6	13.2	14.4abc	71.2	53.2	62.2ab
B9 (B)	14.8	9.4	12.1bc	9.55	7.73	8.64b	8.3	15.9	12.1ab	2.4	2.9	2.7	6.7	5.3	5.9cd	20.6	16.1	18.3	18.5	11.9	15.2ab	57.3	50.2	53.8a-c
B10 (B)	18.3	12.8	15.5bc	14.58	13.33	13.96ab	8.2	9.4	8.8abc	3.2	3.7	3.4	7.3	6.2	6.7bcd	21.5	16.6	19.0	7.5	11.8	9.7bc	65.1	62.0	63.5a
B11 (R)	13.1	13.8	13.5bc	10.56	11.72	11.14b	8.5	9.0	8.7abc	3.0	2.8	2.9	6.2	6.4	6.3bcd	24.6	14.8	19.7	10.4	10.7	10.5bc	15.7	39.6	27.7d
B12	14.2	10.4	12.3bc	10.57	8.70	9.63b	8.5	8.0	8.3bc	2.9	3.3	3.1	6.5	5.5	6.0cd	23.4	17.3	20.3	13.6	10.0	11.8bc	37.5	37.3	37.4bcd
B13 (B)	17.4	13.1	15.3bc	21.26	10.63	15.94ab	8.5	8.6	8.6bc	3.5	3.0	3.3	7.2	6.2	6.7bcd	24.2	15.6	19.9	15.2	12.8	14.0abc	79.2	49.7	64.5a
B14	25.7	15.8	20.8bc	14.83	12.84	13.84ab	7.2	11.9	9.6abc	2.5	8.5	5.5	8.8	6.8	7.7bcd	19.7	18.0	18.8	11.1	10.6	10.8bc	27.4	44.1	35.7cd
Karagöz	23.6	16.9	20.3bc	16.69	15.60	16.14ab	7.7	6.8	7.2c	2.9	2.7	2.8	8.4	7.1	7.7bcd	25.1	19.3	22.2	21.8	15.7	18.8a	67.0	57.3	62.2ab
Akkız	20.6	23.5	22.0b	14.90	14.60	14.75ab	8.2	10.4	9.3abc	2.7	1.8	2.3	7.7	8.4	8.1b	24.5	16.0	20.2	11.8	9.8	10.8bc	44.0	42.6	43.3a-d

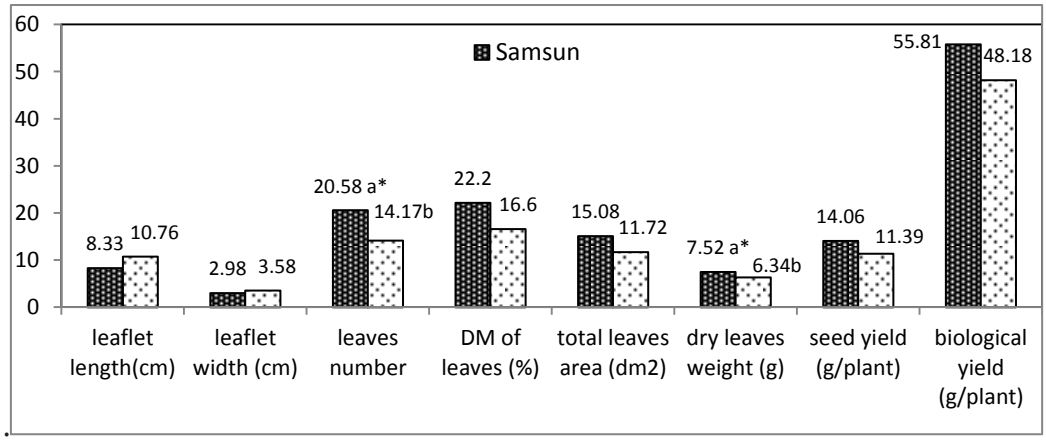

Figure 2 : Means for two locations some leaf characters, seed and biological yield of cowpea

Significant and negative relationships were determined by leaf length, dry leaf weight, dry matter content and seed yield (Figure 2). The seedlings have fallen as the leaves grow longer. Anyia and Herzog (2004) reported that some species of genotypes could be protected from drought by shrinking the leaf area and reducing the leaf area and gas permeability, resulting in a decrease of 11-40% in biomass across the genotypes. Reductions in biomass were due to decline in leaf gas exchange and leaf area during water deficit. Cowpea is a plant that can grow vegetative parts in excess amount and prolong the vegetative development when the temperature is not too high like our region. For these reasons, the vegetative cycle is prolonged and the pod setting is delayed, and the vegetation period of the region is not long enough, so there is not enough time for pod filling and the yield is low. Bozoğlu et.al.,(2016) reported that in these varieties, the harvest period was different statistically in the genotypes in the studies conducted at 2 years and 2 locations.

Table 2. Simple linear correlations among some leaf characteristics and seed and biological yield per plant

	Leaves number	Leaf area	Leaflet lenght	Leaflet width	Dry leaves weight	Dry matter of leaves	Seed yield
Leaf area	0.812**	1.000					
Leaflet lenght	-0.428*	-0.422*	1.000				
Leaflet width	-0.135	-0.082	0.211	1.000			
Dry leaves weight	0.988**	0.815**	-0.478**	-0.129	1.000		
Dry matter of leaves	0.571**	0.605**	-0.439*	-0.129	0.574**	1.000	
Seed yield	0.201	0.277	-0.290	-0.171	0.206	0.466**	1.000
Biological yield	0.261	0.497**	-0.122	0.065	0.251	0.293	0.575**

Conclusion

Turkey is not a cowpea plant origin center. It is cultivated in small areas in our country and mostly fresh fruit and dry seeds are used as food. However, in recent years, the value of the feed has also been understood, and it is especially regarded as silage cultivated with maize. As the researches increase, the different uses of the plant are also diversified. Especially, the fact that bringing vegetative parts to the ladies in a large amount reveals the idea that these evenings can be evaluated in different forms. There are some research about the inhibitory effect of cowpea leaf extracts on the growth of bacterial and fungal pathogens. In recent

years, some studies are being carried out for the widespread cultivation in our region. For this reason, we are trying to learn every properties want to learn very components of cowpea.
In this study, it was aimed to reveal the relations between leaf and leaf properties, which are the most important organs. There were no significant relationships between leaf yields (number of leaves, leaf area, leaflet length, leaf width, dry leaf weight) and seed yields examined in the study conducted with 16 different genotypes at two locations. It was concluded that there is a positive relationship between seed yield and biological yield and leaf dry matter content. This suggests that it is important for us to select the appropriate genotypes for the planting period and the growing period, especially considering the regional conditions. Cowpea is one of the brightest plants for the future in some regions where climate change and risk of drought.

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FORAGE YIELD AND SOME AGRICULTURAL TRAITS OF COWPEA GROWN AS DOUBLE CROP IN ECOLOGICAL CONDITIONS OF SAMSUN (TURKEY)

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Abstract

In addition to the good adaptation of high temperature and low nutrient soil, cowpea (*Vigna unguiculata* L.) can be a good source of forage for livestock. This research was conducted to determine forage yield and some quality parameters of cowpea grown as double crop in Samsun ecological conditions. In this study, we examined hay and seed yields and related traits of cowpea grown as double crop with different row spaces. In the experiment, two types of forage cowpea genetics Registered Cultivar Ülkem and genotype black seed cowpea and four different row spaces were used (20cm, 35cm, 50cm, 65cm). Field trials were designed in randomized block design with three replications and 8 rows in each plot. Cowpea seeds were sown after wheat harvesting at July 13, 2017. The first cut was after 57 days (8 Sep) and the second cut was made after 90 days (10 October). Hay yield of the first cut was higher than the second one. Other than the hay yield, plant height, stem diameter, crude protein, ADF, and NDF were also determined. Field results showed that the hay yield ranged from 3.98 to 7.12 ton ha-1 for the first cut and 0.72 – 1.46 ton ha-1 for the second cut. Plant height in the first cut was between 111.2 – 137.3 cm were height of the second cut, they were 24.0 – 29.6 cm. Plant stem diameter was between 6.10 - 8.77 mm. At the laboratory work, it was clarified that crude protein ratios of hay were 18.87 – 21.14% for the first cut and 24.7 – 27.65% for the second cut. ADF ratios were 25.95% - 28.00% in first cut, while the second cut was 19.13 - 24.24%. NDF ratios of hay were ranged from 33.66 to 36.64% and from 24.48 to 31.15% for the first and second cuts respectively.

Keywords: *Cowpea, Forage yield, Row space, Second crop*

Introduction

The cowpea (*Vigna unguiculata* L.) is an important legume widely cultivated in tropics and subtropics for forage, green pods and grains (Ali et al., 2004). Cowpea is usually better adapted to drought, high temperatures and other biotic stresses compared with other crops (Kuykendall et al., 2000; Martins et al., 2003). However, growth and development of many cowpea cultivars are affected by drought and high temperatures, especially during floral development (Dadson et al., 2005).

The biggest technical constraint in livestock production in Turkey is forage deficiency especially during summer period when pasture vegetation is dry. So there is a need for new warm-season forages to fill forage gap in this period. In general, solutions to forage shortages during the summer months have included the use of perennial and annual warm-season species for pastures, hay or silage. Forage cowpea is generally grows between 30-120 cm (Davis et al., 1991; Ismail and Hall, 2000; Worku, 2018) row distance. Ülkem is first registered forage cowpea cultivar of Turkey That it has lots of leaf and high biomass yield.

Cowpea hay is a nutritious balanced fodder for animals (Singh et al., 2003) and has a great function in feeding animals. Having a high nutritive value of green forage cowpea, hay contains 14-21% crude protein, while the seeds have the rate of 18-26% crude protein. It was stated that cowpea is grown alone for forage yield. However, it could be grown mixed with maize, sorghum and millets for silage (Ismail and Hall, 2000; Saricicek et al., 2002; Bilgili,

2009; Basaran et al., 2011; Ayan et al. , 2012). Cowpea can be grown as the main crop or double crop for the aim of forage production in agricultural system in Turkey. Especially in coastal area of Turkey like Black Sea Region that has a very mild climatic conditions, cowpea can be easily grown as double crop after winter cool season cereals.
The aim of this study is to determine the forage yield and some agricultural traits of cowpea grown as double crop with different row spacings in ecological conditions of Samsun.

Materials and methods

The study was carried out in 2017 and was designed as factorial design at randomized blocks with 3 replicates in Samsun (Turkey) ecological conditions. In the experiment, it was used two types of forage cowpea genetics Registered Ülkem and genotype black seed cowpea with four different row space were used (20 cm, 35 cm, 50 cm, 65cm). The experimental plots consisted of 8 rows and 5 m length. Throughout the vegetation period (from July to October) of 2017 total rainfall were 77.5 mm, mean temperature was 22.1 0C, average relative humidity was 63.68 %. The texture experimental area was clay (56.3), organic matter was medium (2.68), nötr (6.89), P_2O_5 content good (22.89 kg/da) and without salt (0.52). At the experiment, it was sowed after harvesting wheat crop exactly 13 July while we have made first forage cut after 57 days (8 Sep) and the second cut was made after 100 days (20 October). In the study hay yield, plant height and stem diameter were measured, while crude protein, ADF, and NDF were examined in the laboratory. Crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF contents of samples were determined by using near infrared reflectance spectroscopy (NIRS) 13-15. With software program coded IC-0904FE.
The study was completed in irrigated conditions nine times. Irrigation process was continued until soil humidity comes to field capacity. All data obtained from this study was analyzed by using SPSS 17.0 program. The differences amongst the mean values were calculated according to DUNCAN test.

Results and discussion

Plant Height and Stem Diameter

The results about plant height and stem diameter obtained from cowpea genotypes grown as double crop with different row spacings are given in Table 1. Plant height values of cowpea was varied between 137.27 and 111.20 cm, but there was no statistically difference between genotypes and row spacings. Some researchers declared plant height of main crop cowpea as follows; Gülümser et al. (1989) as 74 - 136 cm in Samsun Conditions, Akdağ et al. (1998) as 50.33 – 75.49 cm, Ceylan and Sepetoğlu (1999) as 52.30 – 161.30 cm, Büyükkılıç (1995) as 116 -122 cm. The highest plant height was determined as 29.60 cm for Ülkem cultivar grown with 20 cm row spacing in the second cutting. But plant height of the treatments were the same statistical group except for Ülkem cultivar grown with 20 and 35 cm row spacings. There was no difference between the genotypes (Table 1).

Table 1. Plant height and stem diameter values of cowpea grown as double crop with different row spacings (cm)*

Cultivar/ Genotip	Row Spacing (cm)	Plant Height (cm)		Stem Diameter (mm)
		1. cutting	2. cutting	
Ülkem	**20**	131.80	23,96 **c**	6.59 **b**
	35	137.27	25,46 **bc**	7.58 **ab**
	50	133.07	26,13 **ac**	6.82 **ab**
	65	129.47	27,47 **ab**	7.84 **ab**
Ortalama		**132.90 A**	**25.76 B**	**7.20**
Genotip	**20**	132.87	29,60 **a**	6.10 **b**
	35	111.20	26,76 **ac**	7.33 **ab**
	50	119.00	27,03 **ac**	7.32 **ab**
	65	131.93	28,38 **ab**	8.77 **a**
		123.75 B	**27.94 A**	**7.38**

*There is no difference (P≤0.05) amongst the values indicated same letters within the same column.

Average stem diameter was 7.20 mm in Ülkem cultivar and 7.38 mm in Genotype. Increasing row spacing caused increasing stem diameter as well. The stem diameter of Ülkem was lower than the stem diameters of the genotype (Table 1). Stem diameter of cowpea was found as 8.80 – 8.58 mm by Ayan et al. (2011) in Samsun conditions and 5.9 – 10.8 mm by Ünlü (2014)in İsparta conditions.

Hay Yield

The results about hay yield obtained from cowpea genotypes grown as double crop with different row spacings can be seen in Table 2.

Table 2. Hay yield values of cowpea grown as double crop with different row spacing (kg ha^{-1})*

Cultivar/ Genotip	Row Spacing (cm)	Hay Yield (kg ha $^{-1}$)		Total Hay Yield (kg ha -1)
		1. cutting	2. cutting	
Ülkem	20	7123.8 **a**	720.4 **b**	7844.3 **a**
	35	4726.7 **c**	1384.6 **ab**	6111.3 **ac**
	50	3989.9 **c**	854.5 **ab**	4844.5 **c**
	65	4119.4 **c**	951.4 **ab**	5070.9 **bc**
		4990.0 B	**977.7 B**	**5967.7 B**
Genotip	20	6654.3 **ab**	1357.2 **ab**	8011.6 **a**
	35	5410.9 **bc**	1466.7 **a**	6877.7 **ab**
	50	5244.7 **bc**	1061.7 **ab**	6306.5 **ac**
	65	4510.3 **c**	1276.7 **ab**	5787.1 **bc**
		5455.1 A	**1290.6 A**	**6745.7 A**

*There is no difference (P≤0.05) amongst the values indicated same letters within the same column.

The highest hay yield was obtained from Ülkem cultivar with 20 cm row spacing (7123.8 kh ha^{-1}) but hay yield of genotype with 20 cm row spacing was in highest group. Hay yield was decreased as the row spacing increased. It can be attributed to that increased in parallel to increase of number of plant per square meter. The genotype gave higher hay yield compared to cultivar. In the second cut, hay yield of the whole treatments were in the highest group except for Ülkem cultivar with 20 cm row spacing. Regrowth of the cowpea plants was after 8th of September, thus growth and difference were limited.
When we look at the total hay yield, the highest values were obtained from both genotype and Ülkem cultivar grown with 20 cm row spacing (8011.6 and 7844.3 kg ha^{-1}, respectively).
The lowest hay yield was determined for Ülkem cultivar with 50 cm. Some previous researchers revealed the hay yield of cowpea as follows; Ayan et al. (2017) 9.78 – 15.87 t ha^{-1}, Ayan et al. (2011) 5860 – 8760 kg ha^{-1}, Atış and Yılmaz (2005) 4580 – 6390 kg ha^{-1}, Boz (2006) 1480 – 4760 kg ha^{-1}, Jatasra et al. (1989) 3980 – 4730 kg ha^{-1}.

Crude Protein, ADF and NDF ratios of Hay
The values about crude protein, ADF and NDF contents of cowpea hay are given in Table 3.

Table 3. Crude Protein, ADF and NDF contents of cowpea hay (%)*

Cultivar/ Genotip	Row Spacing (cm)	Crude Protein		ADF		NDF	
		1. cutting	2. cutting	1. cutting	2. cutting	1. cutting	2. cutting
Ülkem	20	18.87	26.13	27.97	22.90	35.16	29,08ab
	35	21.14	25.10	28.00	23.49	36.64	30,45ab
	50	20.24	26.25	27.87	24.24	36.29	31,15a
	65	20.34	26.78	26.74	21.86	34.93	27,40ab
		20.15	**26.07**	**27.65**	**23.12**	**35.76**	**29.52**
Genotip	20	19.86	26.32	25.95	21.48	33.66	26,24ab
	35	19.86	27.65	26.02	23.87	34.01	30,18ab
	50	19.62	27.27	26.78	19.13	35.05	24,48b
	65	19.94	24.70	26.55	20.05	35.04	25,48ab
		19.82	**26.49**	**26.33**	**21.13**	**34.44**	**26.60**

*There is no difference (P≤0.05) amongst the values indicated same letters within the same column.

There were no statistical differences between treatments in terms of crude protein content of cowpea hay in both cutting. Crude protein content of cowpea hay ranged from 21.14% to 18.87% in the first cutting. Crude protein ratio values varied between 27.65% and 24.70% in the second cutting. The second cut was made earlier development stage of cowpea plants. Thus crude protein ratios were higher compared to the first cutting values (Table 3).
Cowpea fodder is a rich source of crude protein up to 184 g kg-1 (Khan et al., 2010). Ayan et al. (2012) nine Cowpea (Vigna unguiculata L. Walp) genotypes were assessed for forage yield and quality features during 2005 and 2006 summer growing season at two locations (Samsun and Kavak) in Turkey. Researchers were found the crude protein content of cowpea hay in ranged from 193.4 to 147.0 g kg-1.
Ayan et al. (2017) were determined the crude protein ratios of hay between 18.61% and 20.00% in ecological condition of Suluova. There were no significant differences among genotypes for ADF contents in both cutting (Table 3).

ADF contents of hay were 25.95 – 28.00% in the first cutting and 19.24 – 24.24% in the second cutting. Even if there was not any statistical difference between the genotypes, ADF content of Ülkem cultivar was slightly higher than the genotype. In term of NDF content, though there was not any differences amongst the treatments and genotypes in the first cutting, the differences amongst the treatments were significant in the second cutting. NDF ratios ranged from 33.66% to 36.64% in the first cutting. While the lowest NDF content was determined as 24.48% at the genotype with 50 cm row spacing, the highest one was 31.15% at the Ülkem cultivar with 50 cm row spacing in the second cut. The other treatments were in the same statistical group. Fiber (ADF, NDF) accumulation was lower in the second cut, because the plants were harvested earlier growing stage. NDF and ADF ratios of cowpea hay were explained as 32.73 – 33.21% and 30.05 – 30.81% by Ayan et al. (2017).

The CP, ADF and NDF contents of the cowpea genotypes were within the high quality forage group according to standards developed by the Hay Marketing Task Force of American Forage and Grassland Council (Rohweder et al., 1978). As the growing stage increase, crude protein content of forages decreases but ADF and NDF content increase (Kaya, 2008).

Conclusion

In future farming system, the importance of cowpea, known as resistant to high temperature and drought, is likely to increase due to changing climatic conditions. Cowpea can play an important role to fill forage gap by cutting for hay or by grazing during summer-autumn period when pasture yield is very low. In the light of this study, we can say that forage cowpea can be grown as double crop after winter cereals in the coastal area of Turkey like Black Sea Region. Both cv.Ülkem and the genotype can be recommended for forage as double crop with 20 – 35 cm row spacings.

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GENETIC DIVERSITY OF BREAD WHEAT (*Triticum aestivum* L.) GENOTYPES BASED ON PRINCIPAL COMPONENT ANALYSIS AND CLUSTER FOR YIELD AND QUALITY TRAITS

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Abstract

Drought is the mainly abiotic stress factor and amount and distribution of the rainfall during grain filling period affect bread wheat yield and quality. The study was carried out in the experimental field of Trakya ARI, Edirne (Turkey), in 2008-2009 and 2009-2010 years and 15 genotypes were planted in RCBD in a split-plot with three replications. The main plots were assigned to five moisture regimes, which included 3 drought stress environments, one non-stress and one non-treatment environment. Grain yield, 1000-kernel weight, test weight, protein content, wet gluten, gluten index, hardness and sedimentation value were investigated. Principal component analysis (PCA) and cluster analysis was used to determining for genotypes environment interaction. Principal component analysis (PCA), indicated that the first (20.63%) and second (41.42%) components justified 62.04% of variations between the criteria. According to GGE biplot results Kate A-1 and Tekirdağ was determined as most stable cultivars for grain yield. Mean values of the genotypes varied between 29.7-43.5 g for TKW, 73.6-83.2 kg/hl for test weight, 11.1-13.3% for protein content, 28.5-37.0% for gluten value, 34.3-56.0% for sedimentation, 68.9-95.3% for gluten index and 383.0- 658.3 for grain yield. When all parameters were evaluated using the PCA analysis, protein ratio, gluten value and hardness were involved in the same group, while yield and gluten index and sedimentation value and test weight were grouped in two different groups. The cluster analysis was done and 15 genotypes divided into 3 clusters based on Ward's method. The cluster I and II were more clearly separated than cluster 3. The cluster analysis revealed that considerable variation existed among genotypes that could be implicated in selection of bread wheat for the development or improvement of cultivars. According to the results of the study, genotypes with appropriate combination in terms of examined traits were selected for national breeding program of bread wheat.

Keywords: *Bread wheat, biplot analysis, genotypes main effect, genotypes environment interaction, quality parameters*

Introduction

Trakya region is located in the North western part of Turkey and mostly winter and facultative bread wheat is cultivated. Due to various environment condition (rainfall and temperature) could cause some production problem related with lower yield and quality in production (Öztürk and Korkut, 2017). Performance trials have to be conducted in multiple environments because of the presence of GE. Variety trials provide essential information for selecting and recommending crop cultivars. However, variety trial data are rarely utilized to their full capacity. Although data may be collected for many traits, analysis may be limited to a single trait (usually yield) and information on other traits is often left unexplored. Furthermore, analysis of genotype by environment data is often limited to genotype evaluation based on genotype main effect (G) while genotype-by-environment interactions (GE) are treated or a confounding factor (Yan and Tinker, 2006). Almost all breeding programs in the world aim to improve varieties with stable yields. The yield stability is generally grouped as static or

dynamic stability (Pfeiffer and Braun, 1989). Therefore, selection of wheat genotypes should be adapted to drought stress. In addition, drought tolerance mechanism should be identified during the development of new cultivars in order to increase the productivity (Rajaram et al. 1996). Stable yield performance of genotypes under both favorable and drought stress conditions is vital for plant breeders to identify drought tolerant genotypes (Pirayvatlou, 2001). Moreover, high-yielding genotypes under optimum conditions may not be drought tolerant (Blum, 1996). Therefore, many studies preferred the selection under stress and non-stress conditions (Rajaram and Ginkel, 2001).

The objective of this study was to evaluate the performance of the advance genotypes and cultivars and to investigate their yield stability across various environment conditions over two years and five environments.

Material and Methods

The research was carried out in the experimental field of Trakya Agriculture Research Institute, Edirne (Turkey) (41 m above sea level, 41º64´ N, 26º59´ E), during two years (2008-2009 and 2009-2010) and 15 genotypes were evaluated in randomized completely blocks design (RCBD) in a split-plot with three replications. Plot size was 6 rows, 6 m long and 17 cm between the rows. The main plots were assigned to five moisture regimes, which included 3 drought stress environments, one non-stress and one non-treatment environment. Grain yield, 1000-kernel weight, test weight, protein content, gluten value and gluten index, hardness and sedimentation were investigated. Cultivars Kate A-1, Gelibolu, Pehlivan, Tekirdağ, Selimiye, Aldane, Bereket, Flamura-85, Golia and 6 advanced lines were investigated. Drought treatments are placed on main parcel and genotypes on the sub-plot. Droughts were created under mobile rain shelter at various plant growth stages from shooting up to maturity. A mobile rain shelter was used to exclude rain and induce drought stress. In this experiment, all parcels were covered only when raining. A drip irrigation set was used and each plot was irrigated separately by controlling dripping irrigation system. The Zadoks Decimal Code (GS) was used to describe plant growth stages. The experiment consisted of five treatment; DS1: Drought stress applied from GS31 (stem starts to elongate) to GS51 (10% of spikes visible), DS2: Drought stress applied from GS51 (10% of spikes visible) to GS94 (over-ripe, straw brittle), DS5: Drought stress applied from GS31 (stem starts to elongate) to GS94 (over-ripe, straw brittle), one main plot was non-treatment and one main plot non-stress treatment. The statistical analyses of measurements were performed by using statistics program for and the differences among the means were compared with L.S.D at a 5% significant level (Gomez and Gomez, 1984; Kalaycı 2005) and PCI analyses.

Results and Discussion

The analysis variance for the yield and quality components was performed and given in Table 1, and 2. In the study 15 genotypes performance trials were tested at two years five locations to investigate yield and quality components based on Principal Component Analysis. The results of variance analyses showed that there were significant differences ($p<0.01$) among genotypes. The highest grain yield with 658.3 kg da^{-1} was determined in cv. Bereket and followed by genotypes BBVD7 with 651.0 kg da^{-1} and Kate A-1 with 631.5 kg da^{-1} (Table 1). The mean yield of the genotypes was 583.0 and biological yield 2252.9 kg da^{-1}, respectively. Mean values of the genotypes varied between 29.7-43.5 g for 1000-kernel weight, 73.6-83.2 kg/hl for test weight, 11.1-13.3% for protein content, 28.5-37.0% for gluten value, 34.3-56.0% for sedimentation value, 68.9-95.3% for gluten index.

Table 1. Mean grain yield and quality parameters of 15 genotypes in two years and five different environments

No	Genotypes	GY	TKW	TW	PRT	HARD	SED	GLT	IND
1	Kate A-1	631.5 bc	34.49 g	81.20 cd	11.43 fgh	55.3 d	41.23 d	33.83 cd	72,88 d
2	Gelibolu	613.0 cde	37.67 e	80.85 de	11.11 h	47.0 j	41.30 d	24.61 ı	95,34 a
3	Pehlivan	587.7 fg	43.54 a	81.63 b	11.73 d-g	53.5 f	41.63 d	34.70 bc	72,55 d
4	Tekirdağ	594.5 efg	38.82 d	78.91 g	11.91 cde	53.7 f	45.33 c	32.76 de	83,28 c
5	Selimiye	608.9 def	41.47 c	83.19 a	11.57 efg	55.1 d	45.10 c	31.91 e	90,26 b
6	Aldane	551.1 ı	42.14 bc	81.34 bc	12.78 b	55.2 d	56.03 a	34.36 bc	94,05 a
7	Flamura-85	518.9 j	37.28 e	80.65 e	12.05 cd	53.3 fg	47.77 b	30.40 fg	94,40 a
8	Golia	610.4 cde	31.90 h	79.86 f	11.95 cde	57.8 b	35.03 g	28.74 h	94,16 a
9	BBVD7	651.0 ab	42.70 ab	78.89 g	12.32 c	51.1 h	37.23 ef	34.60 bc	64,82 f
10	Bereket	658.3 a	37.91 e	80.63 e	11.41 gh	52.8 g	44.73 c	28.67 h	94,52 a
11	ÖVD26-07	579.1 gh	35.61 f	81.21 cd	11.83 def	58.6 a	36.63 f	31.56 ef	88,21 b
12	ÖVD2/21-07	563.0 hı	37.23 e	76.95 h	11.49 fgh	50.4 ı	47.80 b	28.47 h	93,89 a
13	ÖVD2/27-07	617.8 cd	34.56 g	79.87 f	11.42 fgh	55.7 d	36.90 f	35.20 b	60,76 g
14	EBVD24-07	577.4 gh	31.21 h	77.08 h	11.70 d-g	54.3 e	38.67 e	30.21 g	83,63 c
15	BBVD21-07	383.0 k	29.75 ı	73.56 ı	13.32 a	57.0 c	34.27 g	37.01 a	68,88 e
Mean		583.0	37.08	79.72	11.86	54.1	41.98	31.8	83.44
LSD (0.05)		21.94**	0.88 **	0.40**	0.41**	0.58**	1.59	1.29	2.32

Note: **: P<0.01, *: P<0.05, GY: Grain yield (kg da^{-1}), TKW: Thousand kernel weight (g), TW: Test weight (kg), PRT: Protein content (%), HARD: Hardness, SED: Sedimentation value (ml), GLT: Wet Gluten (%), IND: Gluten index (%).

A genotype having stabile grain yield across the environment condition is very important in wheat. Genotype x environment interaction is a mainly issue for plant breeders in improving high yield across variable environments. Stability analysis showed that there was a wide variation among genotypes. Cultivar Bereket produced the highest yield in all environments averaged for two years and five environments. Genotypes Pehlivan, Bereket, and BBVD7 produced high biological yield over two years five environment.

Table 2. Mean grain yield and quality parameters across two years and five different environments

Environment	GY	TKW	TW	PRT	HARD	SED	GLT	IND
DS1	549.9 d	38.50 b	80.81 b	11.69 c	54.0 a	43.07 b	31.93 c	81.92 c
DS2	563.9 c	35.71 c	79.16 d	12.54 a	55.4 a	43.02 b	33.68 a	82.61 bc
DS3	763.8 a	40.85 a	81.18 a	11.63 c	53.9 b	40.93 c	30.87 d	85.74 a
DS4	579.7 b	35.78 c	79.87 c	12.18 b	53.2 c	43.83 a	32.70 b	83.59 b
DS5	457.8 e	34.59 d	77.59 e	11.31 d	53.7 b	39.03 d	29.83 e	83.37 b
Average	583.0	37.08	79.72	11.86	54.1	41.98	31.80	83.44
LSD (0.05)	12.69**	0.38**	0.16**	0.19**	0.36**	0.72**	0.65**	1.14**

Note: **: P<0.01, *: P<0.05, ns: not significant, GY: Yield (kg da^{-1}), TKW: Thousand kernel weight (g), TW: Test weight (kg), PRT: Protein (%), HARD: Hardness, SED: Sedimentation (ml), GLT: Gluten (%), IND: Gluten index. DS: Drought stress.

An ideal genotype should have both high mean performance and high stability across environments. Therefore, genotypes located closer to the ideal genotype are more desirable than others. Thus, Aldane and Pehlivan were more desirable than G15 and G14 even though Bereket had higher average grain yield. G15 was the poorest genotype because it was consistently the poorest. The angle between two parameters indicates their similarity in response to the environments. An acute angle (test weight and sedimentation, protein and hardness) means that the two parameters responded similarly and that the difference between them was proportional in all environments. An obtuse angle (gluten and gluten index) means that the two parameters responded inversely and wherever the first parameter performed well the other parameter performed poorly. A right angle indicates that the two parameters (TKW and gluten value) responded to the environments independently (Figure 1).

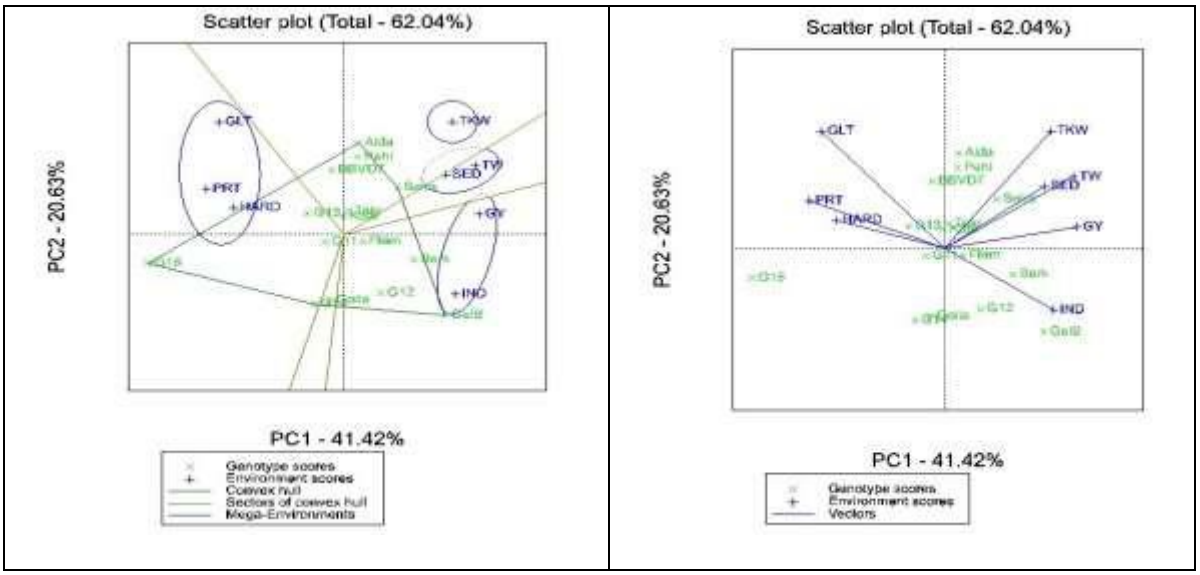

Figure 1. The GGE biplot to show which genotypes performed bets in which environments and a genotype by trait biplot representing 15 winter wheat genotypes measured for eight traits and polygon views of the GGE-biplot on symmetrical scaling for the which-won-where pattern for genotypes and environments

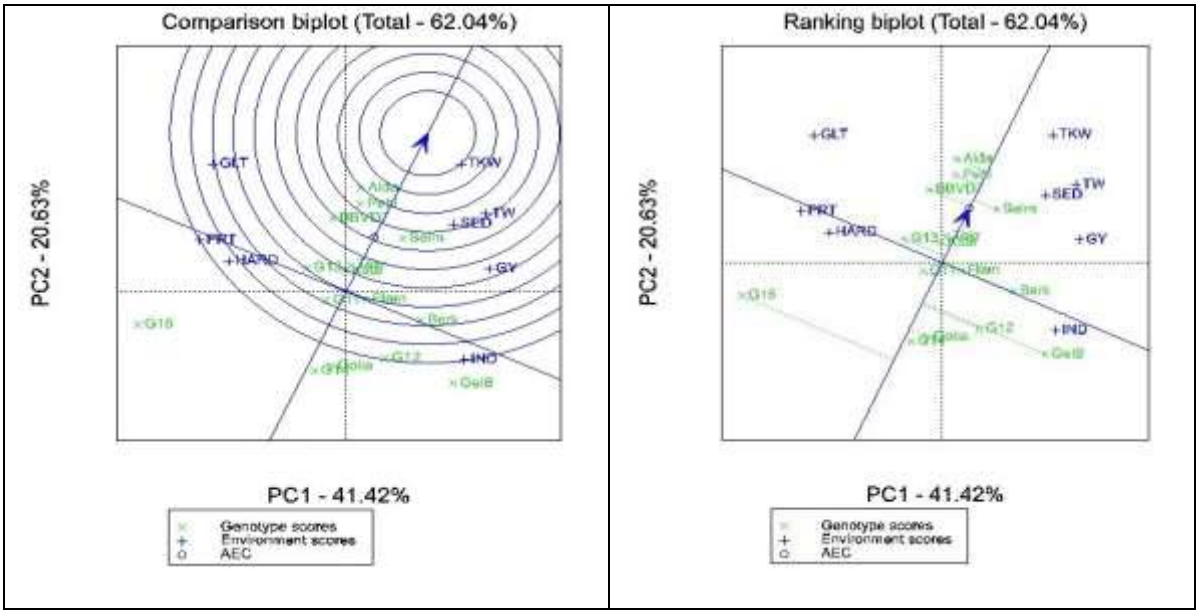

Figure 2. The average-environment coordination view to rank genotypes relative to an ideal genotype (the centre of the concentric circles) and the average-environment coordination view to show the mean performance and stability of the genotypes

Biplot analysis has evolved into an important technique in crop improvement and agricultural research. GGE biplot analysis provides an easy and comprehensive solution to genotype by environment data analysis, which has been a challenge to plant breeders, geneticists, and agronomists. It not only allows effective evaluation of the genotypes but also allows a comprehensive understanding of the target environment and the test environments (Yan and Tinker, 2006).

The distance between two genotypes approximates the Euclidean distance between them, which is a measure of the overall dissimilarity between them. In the study, Aldane and G15 are very different whereas cultivars Aldane and Pehlivan are quite similar. The dissimilarity can be due to difference in mean investigated parameters (G) or in interaction with the environments (GE).

The equality lines divide the biplot into sectors, and the winning genotype for each sector is the one located on the respective vertex. In this study, the five environments fall into two sectors. Aldane was the winner in first group environments, and Gelibolu was the winner for the other environments. This pattern suggests that the target environment may consist of two different mega-environments and that different cultivars should be selected and deployed for each. A genotype by trait biplot can help understand the relationships among traits (breeding objectives) and can help identify traits that are positively or negatively associated, traits that are redundantly measured, and traits that can be used in indirect selection for another trait. It also helps to visualize the trait profiles (strength and weakness) of genotypes, which is important for parent as well as variety selection (Yan and Tinker, 2006; Yan and Kang 2003). The biplot in Figure 1 presents data of 15 genotypes determined for eight traits. Across the 15 tested genotypes, sedimentation with test weight and protein ratio with hardness was positively associated. Protein ratio and hardness was negatively correlated with grain yield. Also, wet gluten value was negatively correlated with gluten index. 1000-kernel weight was independent of gluten value. These relationships suggest that it is possible to combine higher protein ratio and hardness, higher test weight and sedimentation in a single genotype.

The cluster analysis was done and 15 genotypes were divided into 3 clusters based on Ward's method. The cluster I and II were more clearly separated than cluster I. In the first group of cluster 6 genotypes and in second group of cluster 8 genotypes are located. The cluster analysis revealed that considerable variation existed among genotypes that could be implicated in selection of bread wheat for the development or improvement of cultivars.

Conclusions

Because of the various environment conditions yield and quality in wheat varies and GGE biplot analysis provides an easy and comprehensive solution to genotype by environment data analysis and it not only allows effective evaluation of the genotypes but also allows a comprehensive understanding of the target environment and the test environments. Drought is the mainly abiotic stress factor and low moisture during grain filling period affected bread wheat yield and quality. Based on mean value Pehlivan had higher 1000-kernel weight, and Selimiye had higher test weight. According to sedimentation Aldane had higher value and G15 had higher protein ratio and gluten value across two years five environment. Principal component analysis (PCA), indicated that the first and second components justified 62.04% of variations between the criteria. According to GGE biplot results Kate A-1 and Tekirdağ was determined as most stable cultivars for grain yield. Aldane and Pehlivan were more desirable than G15 and G14 even though Bereket had higher average yield. G15 was the poorest genotype. Test weight and sedimentation also protein and hardness responded similarly and that the difference between them was proportional in all environments. Aldane and G15 are very different whereas Aldane and Pehlivan are quite similar. Based on biplot analyses, sedimentation with test weight and protein ratio with hardness was positively associated. Protein ratio with hardness was negatively correlated with grain yield. Also, gluten value was negatively correlated with gluten index. When all parameters were evaluated using the PCA analysis, protein ratio, gluten value and hardness were involved in the same group, while yield and gluten index and sedimentation value and test weight were grouped in two different groups. The cluster analysis was done and 15 genotypes divided into 3 clusters based on Ward's method. The cluster analysis revealed that considerable variation existed among

genotypes that could be implicated in selection of bread wheat for the development or improvement of cultivars. According to the results of the study, genotypes with appropriate combination in terms of examined traits were selected for national breeding program of bread wheat.

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EVALUATION OF ADVANCED BREAD WHEAT (*Triticum aestivum* L.) MUTANT LINES FOR GRAIN YIELD AND SOME YIELD COMPONENTS

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Abstract

This research was conducted to assess yield and some yield components of 65 advanced bread wheat mutant lines of M_6 generation selected from M_4 populations of 4 different bread wheat genotypes. They were sown using augmented design consisting of five blocks of which every block had 13 mutant advanced lines and 4 standard varieties (parents) in the 2014-2015 growing season. According to the data obtained from the experiment, there was a great variation for plant height (69.15-119.56 cm), spike length (9.26-11.78 cm), number of grains per spike (30.59-59.84), grain weight per spike (1.43-3.13 g), harvest index (32.57-48.37%), thousand kernel weight (34.13-55.93 g) and grain yield (471.20-951.45 kg/da). The 33 lines for plant height, 22 lines for spike length, 21 lines for number of grains per spike, 31 lines for grain weight per spike, 35 lines for harvest index, 30 lines for 1000 grain weight and 33 lines for grain yield have been found to perform better than standard genotypes. According to the results of study, advanced mutant lines that were superior to the standard types in more than one trait were MT35 (IB-400), MT14 (B-100), MT27 (IB-300), MT19 (IB-200), MT20 (IB-200) and MT15 (IB-100). These mutant lines were selected for yield trials when compared to check cultivars, and they may prove useful for yield improvement in wheat breeding programme. Gamma irradiations with 100 and 200 Gy may be more beneficial for yield improvement in wheat mutation breeding.

Key words: *Bread wheat, mutant line, augmented, grain yield, yield components.*

Introduction

Mutagenesis and hybridization are generally the most commonly used breeding methods to develop new superior varieties. Mutation breeding has some advantages compared to cross breeding. When comparing mutation and hybridization; the occurrence of even a few desirable mutation in high yielding varieties has the great advantage of becoming homozygous and expressing its superiority within a couple of generations after induction in M_2 or M_3 as compared to F_6 or F_7 generations in case of hybridization (Chakraborty and Paul, 2013). Mutation breeding has become an appropriate option to improve plant characters when conventional breeding does not work, or the desired traits were recessive, or improving another character in an established plant variety, or improving one or two main character(s) (Ahloowalia and Maluszynski, 2001 and van Harten, 1998). In addition, mutagenesis is able to isolate mutant with multiple characters, as compared to transgenese where only line can be introduced, it's the major advantage of induce mutations **(**Louali *et al.,* 2015**).** Mutation breeding can be applied to improve a specific character without changing other characters and it is possible to improve a single line without causing an important disturbance in the genome. Furthermore, it may create a new character that was not belong to parental plants The mutants developed in wheat have a great potential for direct release and for inclusion in hybridization breeding programs (Sakin *et al.,* 2005). The released mutant cultivars in different crops had great economic impact on agriculture and food productions and added billion of dollars in the economy of many countries (Jain, 2006). More than 3000 varieties of different crops have been officially released by mutation breeding technique. Mutation induction with radiation has been the most frequently used method to develop direct mutant varieties (64% with

gamma-rays, 22% with X-rays), accounting for about 90% of obtained varieties (Ahloowalia, et al. 2004). Gamma ray mutagen was effective in broadening genetic variability and increasing means of wheat cultivars for grain yield and its components, helping plant breeders to practice an efficient selection in the M_2 and next mutated generations (Khanna *et al* 1986, Al-Naggar *et al* 2007). Mutant populations have now been created for many cereal crops, including rice (Suzuki *et al.*, 2008), durum wheat (Başer *et al.*, 1997, Sakin and Yildirim, 2004) and bread wheat (Slade *et al.*, 2005). The goal of a wheat breeding program is to develop superior genotypes as a result of many years of selection. Early generation selection is based on visual observations of yield components (ear length and size, number of ears, number of spikelets per ear), disease resistance, tillering potential, lodging resistance and seed quality. In the early stage of selection process, there could be insufficient seed of the new treatments' to undertake replicated experiments or the number of genotypes could be very large to manage in terms of resources. Efficient evaluation of such large numbers of lines in field experiments is laborious, expensive, and difficult to manage because of the confounding effects that genotype x environment interaction and soil heterogeneity. Therefore, augmented designs have been developed for the evaluation of genotypes in the early stages of a breeding program (Federer, 1956). Control treatments are used in these designs with replications in check plots, in order to evaluate the genetic material with no replications. More sophisticated augmented designs allow for the adjustment of test varieties by rows and columns (Federer and Raghavarao, 1975; Lin and Poushinsky, 1983). The efficiency of the analysis over another is usually measured in terms of reduced error variance, expected error mean square, or average standard error of the difference between genotype means (Binns, 1987; Cochran and Cox, 1957; Magnussen, 1990). The average standard error of the difference (SED) was reported to be more appropriate since it is used for comparison among genotypes using the same scale as the traits (Binns, 1987, Cullis and Gleeson, 1991; Gleeson, 1997).
In this study, it was aimed to evaluate the yield and yield components of M_6 advanced bread wheat mutant lines selected from M_4 populations induced different gamma rays of 4 different bread wheat genotypes using augmented design.

Material and Methods

Experimental site and growing conditions: This study was conducted out at the University of Tekirdağ Namık Kemal, Faculty of Agriculture, Department of Field Crops, Turkey in the 2014-2015 growing season. Tekirdağ district locates at latitude 40^o 36'-40^o 31' and longitude 26^o 43'-28^o 08'and altitude is 10 m. The total precipitation was 435.1 mm and the average temperature was 11.5 ^oC during the 2014-2015 wheat growing season (November, 2014-June, 2015) when the experiment was conducted. It is noted that the annual precipitation is about 30 mm lower than the long term average (466 mm) and the average temperature is similar to the long term average (11.5 ° C). According to soil analysis results, experimental area's soil was clay-loam, slightly acidic (pH 6.5), limeless, and poor (1.08%) in the organic matter.
Experimental materials, gamma irradiation and design: Four bread wheat (*Triticum aestivum* L.) genotypes, Avusturalya, Bezostaja 1, Kate A-I and IBWSN4 were used as the experimental material. The moisture contents of seeds of wheat genotypes used in the study were 12.1% for Avustralya, 11.4% for Bezostaja 1, 11.7% for Kate A-I and 12.0% for IBWSN4. Gamma treatment was obtained from ^{60}Cobalt, Ob-Servo Sanguis Co-60 Research Irradiator with isotope model, while the dose rate was 2.190 kGy h^{-1} in before the 2009-2010 growing season sowing at the Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Ankara, Turkey. Right after irradiation, the experiment was set up using a total of 20 M_0 (100, 200, 300, 400 and 500 Gy) combination seeds together with the un-irradiated (control) in the experimental field of the Field Crops Department of the Faculty of Agriculture of Tekirdağ Namık Kemal University during the growing season of 2009-2010.

The seeds obtained from the harvested plants in M_1 generation were sown in 2011-12 (M_2), 2012-13 (M_3) and 2013-14 (M_4) growing seasons as 20 cm row distance in 5 meters of 6-row plot with 4 replicates and as 400 seeds in each row. The experiment was carried out in augmented design with 5 blocks with 65 advanced bread wheat mutant lines of M_6 generation selected from M_4 populations of 4 different bread wheat genotypes and their parents (Table 1). Each block included 13 advanced bread wheat mutant lines and 4 standard bread wheat genotypes (A, IB, K and B).

Table 1. The experiment material of the study.

C1: Bezotaja 1 (B)	MT11: B400	MT25: B500	MT39: K100	MT53: K400
C2: IBWSN 4 (IB)	MT12: B400	MT26: B500	MT40: K100	MT54: A100
C3: Kate A-1 (K)	MT13: B400	MT27: IB300	MT41: K100	MT55: A100
C4: Avustralya (A)	MT14: IB100	MT28: IB300	MT42: K100	MT56: A100
MT1: B100	MT15: IB100	MT29: IB300	MT43: K200	MT57: A200
MT2: B100	MT16: IB100	MT30: IB300	MT44: K200	MT58: A200
MT3: B100	MT17: IB100	MT31: IB300	MT45: K200	MT59: A200
MT4: B100	MT18: IB200	MT32: IB400	MT46: K300	MT60: A300
MT5: B200	MT19: IB200	MT33: IB400	MT47: K300	MT61: A300
MT6: B200	MT20: IB200	MT34: IB400	MT48: K300	MT62: A300
MT7: B200	MT21: IB200	MT35: IB400	MT49: K400	MT63: A400
MT8: B300	MT22: IB300	MT36: IB400	MT50: K400	MT64: A400
MT9: B300	MT23: B500	MT37: IB400	MT51: K400	MT65: A400
MT10: B300	MT24: B500	MT38: IB400	MT52: K400	C: Standard genotype MT: advanced mutant line

Sown were made on Nov.7, 2014 by hand at the rate of 500 seeds per m^2 and were 5 m in length, with 2 rows 0.2 m apart. Nitrogen and P_20_5 at 140 and 70 kg ha^{-1}, respectively, were incorporated into the soil as compound fertilizer (20-20-0) before sowing, urea during tillering and ammonium nitrate before heading. The crop was kept free of weeds by hand hoeing when necessary. Morphological and yield characters were recorded on 15 random and guarded plants to study the effect of irradiation doses on the studied genotypes on plant height (PH-cm), spike length (SL-cm), the number of grain per spike (NGS-no), grain weight per spike (GWS-g), thousand grain weight (TGW-g), harvest index (HI-%), and grain yield (GY) was calculated as kg/da. The variance analysis on obtained data was performed according to the augmented design using the JUMP 5.0 statistical package program, and the differences between the averages were determined by Student's test.

Results and Discussion

As a result of the variance analysis, there were statistically significant differences in the level of 0.01 probability among mutant lines means for plant height, spike length, thousand kernel weight and grain yield; significances of them for number of grains per spike, grain weight per spike and harvest index were statistically significant at the 0.05 probability level. Mean performances of checks and adjusted performances of mutant bread wheat lines for investigated characters are given in Table 2.

Plant height (cm): One of the most obvious significant effects of mutagen treatments on plants is the decreases occurring in the plant height. In our study, the average plant height of 65 advanced bread wheat mutant lines in M6 generations ranged from 69.13-119.56 cm, and the average plant height of standard genotypes ranged from 92.86-117.74 cm (Table 2). The shortest plant height among the standard genotypes was determined for IBWSN-4 line, followed by Kate A1 with 100.52 cm. The longest plant height among standard genotypes was measured in Australian variety (Table 2). 23 mutant lines gave a shorter plant height than the standard genotype IBWSN-4, which has the shortest plant height. Among them, 13 lines were below the plant height value of 90.00 cm, which is recommended for our region to avoid lodging problems.

Table 2. Mean performance of checks and adjusted performance of mutant bread wheat lines.

Genotypes	PH (cm)	SL (cm)	NGS (no)	GWS (g)	HI (%)	TGW (g)	GY (kgda^{-1})
C1	117.74 abc	10.78 a-l	42.98 e-x	1.91 k-u	35.39 o-t	36.9 w-z	607.40 p-B
C2	100.52 f-u	10.46 b-t	47.76 b-p	2.12 g-t	40.60 a-t	42.78 n-x	855.60 a-h
C3	92.86 n-A	10.51 a-s	48.56 a-o	2.30 c-r	40.50 a-t	44.94 ı-v	836.20 a-j
C4	105.80 c-k	10.35 b-u	43.46 e-x	2.11 g-t	38.00 g-t	47.04 c-s	683.60 k-y
MT1	93.08 m-A	9.64 ı-y	41.14 g-y	2.02 j-u	39.53 e-t	44.98 h-v	715.20 g-t
MT2	95.58 h-z	9.78 g-x	45.94 d-u	2.03 j-u	40.15 b-t	41.18 p-y	536.20 w-B
MT3	106.48 c-ı	8.08 AB	36.14 p-y	1.63 stu	35.61 n-t	45.88 e-u	664.20 k-y
MT4	107.48 b-h	7.61 B	38.54 k-y	1.97 j-u	44.23 a-l	45.68 e-v	622.20 o-B
MT5	110.18 a-f	8.18 zAB	31.94 wxy	1.92 k-u	36.94 ı-t	53.48 a-d	620.20 o-B
MT6	108.08 b-g	9.78 g-x	34.94 q-y	2.41 b-l	42.96 a-p	48.88 b-o	695.20 j-x
MT7	102.98 e-r	9.26 q-A	46.44 d-t	2.41 b-m	43.47 a-o	50.88 a-k	717.20 g-t
MT8	93.31 l-z	9.15 t-A	31.44 xy	1.43 u	38.26 f-t	51.68 a-j	585.20 q-B
MT9	103.18 e-p	9.69 h-y	37.94 n-y	2.01 j-u	40.62 a-t	48.38 c-p	586.20 q-B
MT10	109.88 a-g	10.11 c-v	45.04 d-v	2.17 g-t	42.02 a-r	45.38 g-v	626.20 m-B
MT11	105.08 d-l	9.73 g-y	42.34 e-y	2.33 c-p	47.53 a-e	46.38 d-u	770.20 c-o
MT12	109.88 a-g	9.32 n-z	34.74 q-y	1.74 o-u	38.67 f-t	47.48 c-r	757.20 c-p
MT13	113.78 a-e	10.00 d-x	44.54 d-v	1.98 j-u	38.68 f-t	44.28 l-v	739.20 f-r
MT14	90.53 r-A	11.78 a	53.09 a-h	3.13 a	42.37 a-q	46.33 d-u	911.20 a-e
MT15	88.33 t-A	10.58 a-p	48.39 a-p	2.27 d-r	42.05 a-q	44.73 k-v	854.20 a-ı
MT16	89.53 s-A	10.35 b-u	41.49 f-y	2.03 j-u	36.94 ı-t	42.63 n-x	794.20 a-m
MT17	69.13 B	10.96 a-h	44.19 d-w	2.05 ı-u	43.86 a-m	36.43 xyz	746.20 e-r
MT18	90.63 r-A	10.70 a-l	37.89 o-y	1.88 k-u	38.94 f-t	48.53 c-p	648.20 l-A
MT19	94.13 k-z	9.45 m-z	44.59 d-v	2.36 c-o	43.92 a-m	50.03 a-m	941.20 ab
MT20	90.83 r-A	9.80 f-x	45.09 d-v	2.15 g-t	43.57 a-n	50.53 a-l	908.20 a-e
MT21	89.53 s-A	10.04d-v	37.99 m-y	2.09 g-t	44.93 a-ı	52.43 a-ı	682.20 k-y
MT22	86.73 v-A	10.27 b-u	49.89 a-o	2.31 c-q	40.94 a-s	46.93 c-s	686.20 k-y
MT23	97.33 g-y	10.31 b-u	43.29 e-x	2.19 g-t	35.79 m-t	48.73 b-p	648.20 l-A
MT24	98.13 f-v	8.74 w-B	46.19 d-t	2.24 e-s	39.75 d-t	48.73 b-p	542.20 v-B
MT25	108.53 a-g	10.90 a-k	33.99 t-y	2.07 h-t	33.89 rst	47.13 c-s	471.20B
MT26	103.03 e-r	9.81 f-x	34.49 r-y	1.95 k-u	36.50 l-t	47.03 c-s	641.20 m-A
MT27	88.70 t-A	11.07 a-g	53.44 a-g	2.85 a-e	45.81 a-g	44.71 k-v	863.20 a-h
MT28	91.90 o-A	10.62 a-o	54.04 a-f	3.01 ab	45.21 a-h	49.41 a-n	779.20 b-o
MT29	87.41 u-A	10.22 b-u	50.54 a-m	2.70 a-h	46.26 a-f	50.51 a-l	794.20 a-n
MT30	90.70 r-A	10.33 b-u	56.44 a-d	2.87 a-d	48.16 abc	49.41 a-n	714.20 h-u
MT31	97.71 f-y	11.38 abc	54.74 a-e	3.02 ab	44.49 a-l	49.91 a-m	727.20 g-s
MT32	94.91 h-z	9.06 u-A	51.30 a-j	2.81 a-f	45.67 a-g	47.31 c-r	798.20 a-l
MT33	94.61 ı-z	10.57 a-p	51.84 a-j	2.92 abc	48.37 a	45.71 e-v	923.20 abc
MT34	80.40 AB	10.50 a-s	44.54 d-v	2.13 g-t	35.17 p-t	46.01 e-u	707.20 j-v
MT35	86.20 v-A	11.45 ab	51.44 a-j	2.72 a-g	44.54 a-l	43.21 m-x	912.20 a-d
MT36	87.51 u-A	11.20 a-e	50.44 a-o	2.67 a-ı	41.00 a-s	49.41 a-n	644.20 m-A
MT37	91.30 o-A	10.84 a-l	44.74 d-v	2.71 a-g	48.28 abc	47.91 c-q	712.20 ı-u
MT38	88.50 t-A	10.53 a-s	41.14 g-y	2.27 d-s	40.10 c-t	49.31 a-n	710.20 ı-v
MT39	92.30 o-A	8.80 v-B	33.04 v-y	1.86 k-u	42.16 a-q	52.01 a-j	525.20 y-B
MT40	106.93 b-j	10.86 a-l	41.29 g-y	2.12 g-t	36.53 k-t	50.13 a-l	881.95 a-g
MT41	99.23 f-v	10.83 a-l	43.59 e-x	1.96 k-u	37.81 g-t	55.53 ab	615.95 o-B
MT42	97.63 f-y	10.81 a-l	52.19 a-ı	2.58 a-j	46.18 a-f	44.83 j-v	883.95 a-f
MT43	92.53 o-A	9.26 q-A	33.39 u-y	1.84 l-u	40.83 a-s	55.93 a	758.95 c-o
MT44	94.33 k-z	10.54 a-r	59.09 abc	2.44 b-k	45.90 a-f	43.43 m-w	824.95 a-k
MT45	107.23 b-ı	9.86 e-x	47.29 b-p	1.84 l-u	36.58 k-t	45.33 h-v	528.95 x-B
MT46	94.73 ı-z	9.62 j-y	44.49 d-w	2.23 f-t	45.85 a-f	52.13 a-j	612.95 o-B
MT47	97.33 g-y	9.96 d-x	50.89 a-k	2.15 g-t	47.82 a-d	46.03 e-u	595.95 p-B
MT48	102.03 e-s	11.24 a-d	49.19 a-o	2.15 g-t	45.90 a-f	45.43 f-v	579.95 r-B
MT49	92.73 o-A	8.43 y-B	30.59 y	1.70 r-u	39.16 f-t	53.33 a-d	611.95 o-B
MT50	89.03 t-A	9.16 s-A	51.19 a-k	1.98 j-u	46.12 a-f	40.33 q-z	495.95 zAB
MT51	104.63 e-n	9.28 p-A	50.09 a-o	2.27 d-r	44.41 a-l	47.03 c-s	616.95 o-B
MT52	83.03 yzA	10.68 a-m	39.39 j-y	2.10 g-t	42.17 a-q	50.33 a-l	672.95 k-y
MT53	101.86 e-s	8.24 zAB	50.04 a-o	2.36 c-o	39.47 e-t	47.13 c-s	951.45 a
MT54	117.66 a-d	10.19 b-u	49.24 a-o	2.09 g-t	38.61 f-t	39.63 u-z	656.45 k-z
MT55	118.96 ab	9.60 k-y	40.84 h-y	1.59 tu	35.45 n-t	37.03 w-z	702.45 j-w
MT56	103.86 e-o	9.94 d-x	44.34 d-w	1.79 m-u	37.29 h-t	39.93 s-z	714.45 h-t
MT57	100.96 f-t	9.22 r-A	46.84 c-r	1.96 k-u	39.42 e-t	42.03 o-y	749.45 d-q
MT58	103.16 e-r	8.69 x-B	47.54 b-p	2.05 j-u	41.60 a-r	43.93 l-v	758.45 c-p
MT59	117.66 a-d	10.50 a-s	54.24 a-e	2.00 j-u	38.81 f-t	34.13 z	627.45 m-B
MT60	101.76 e-s	9.74 g-y	39.74 ı-y	1.70 r-u	39.65 d-t	36.73 w-z	556.45 s-B
MT61	110.05 a-f	9.69 h-y	59.84 a	1.99 j-u	35.02 p-t	34.23z	552.45 t-B
MT62	97.76 f-y	10.02 d-w	44.44 d-w	1.85 k-u	41.97 a-r	38.93 v-z	545.45 u-B
MT63	105.06 e-m	9.30 o-A	44.34 d-w	1.72 p-u	34.38 q-t	44.53 k-v	487.45 AB
MT64	119.56 a	10.86 a-k	38.24 l-y	1.70 r-u	32.57 t	39.73 t-z	586.45 q-B
MT65	91.25 p-A	9.58 l-y	34.14 s-y	1.88 k-u	40.19 a-t	52.03 a-j	585.45 q-B

As a result, it is understood that these 13 advanced bread wheat mutant lines should be evaluated in the next generations in terms of plant height. Our results are supported by findings of Shubhra *et al.* (2013), explaining that mutant lines with shorter plant length can be obtained as a result of gamma irradiation.

Spike length (cm): One of the most important yield components affecting wheat yield is the spike length and it is accepted as one of the most important selection criteria in wheat breeding as well. Previous studies have shown that mutagen applications cause significant changes in spike length in wheat (Mohammad *et al*., 2004, Farag and El-Khawaga, 2013) In the study, the spike length of 65 advanced bread wheat mutant lines ranged from 7.61 to 11.78 cm, and the spike length of standard genotypes ranged from 10.46 to 10.78 cm (Table 2). Among the mutant lines examined in the experiment, 13 lines (MT14, 35, 31, 48, 36, 27, 17, 25, 64, 40, 37, 41 and 42) formed longer ears than all standard genotypes. These results are similar to those of Githinji and Birithia (2015), who also reported highly significant differences for spike length and mutant bread wheat lines produced the longest spike as compared to the parent wheat variety.

Number of grains per spike (no): In the case of wheat improvement, the number of grains per spike is one of the selection criteria considered as the most important main yield component in the wheat breeding studies. It has been determined that 65 advanced bread wheat mutant lines tested in the M_6 genome exhibit a wide variation in the number of seeds per spike from 30.59 to 59.88, while it is as low as 42.98 to 47.76 no (Table 2). Among the tested lines, 21 lines (MT61, 44, 40, 31, 59, 28, 27, 14, 42, 33, 35, 32, 50, 47, 29, 36, 51, 53, 22, 54 and 48) was found to have a higher number of grains per spike than the standard IBWSN-4 genotype, giving the highest mean. These lines showed an increase in number of grains per spike as compared to the parent varieties. These results collaborate with those of Ahmad *et al.* (2011) who found a highly significant difference ($p \leq 0.01$) in genotypes showing diverse types of wheat. Rachovska and Dimova (2000) stated that while, the number of grains were the most sensitive traits to mutagenic action, and were suggested as criteria for investigating the action of gamma rays.

Grain weight per spike (g): One of the most important yielding factors affecting wheat yield is the grain weight per spike and it is accepted as one of the most important selection criteria in wheat breeding as well. In terms of this character, means of the grain weight per spike for the 65 advanced bread wheat mutant lines in the M_6 generation ranged from 1.43 to 3.13 g, while the standard genotypes ranged from 1.95 to 2.30 g indicate variation was wider in lines than that of standard genotypes (Table 2). Among them, 19 mutant lines (MT14, 31, 28, 33, 30, 27, 32, 35, 37, 29, 36, 42, 44, 6, 7, 53, 19, 11 and 22) were a higher grain weight per spike than the IBWSN-4 standard genotype, which gave highest averages. These results suggest that these 31 mutant lines should be considered as priority in terms of grain weight per spike and agree with results of Sobieh and Ragab (2000) who have reported that increased doses of mutations have resulted in increases for grain weight per spike.

Harvest index (%): The harvest index, which is the ratio of economic yield to biological yield, is an important selection criterion. It is desirable that the harvest index be as high as possible (even closer to 50%) in wheat breeding. The harvest index values of sixty-five advanced bread wheat mutant lines ranged between 32.57% and 48.37%, and between 35.39% and 40.60% in standard genotypes. 35 mutant lines (MT33, 37, 30, 47, 11, 29, 42, 50, 44, 48, 46, 27, 32, 28, 21, 35, 31, 51, 4, 19, 17, 20, 7, 6, 14, 52, 39, 15, 10, 62, 58, 36, 22, 43 and 9) were the highest harvest index value compared to standard varieties. Fourteen mutant lines (MT33, 37, 30, 47, 11, 29, 42, 50, 44, 48, 46, 27, 32 and 28) performed well with harvest index averages of over 45%. The findings of Rahimi *et al.* (2011), which explains that lines with higher harvest index averages than the rootstocks can be developed with gamma irradiation, proves our results.

Thousand grain weight (g): Thousand grain weight (g) or seed index which is indicator of grain size and flour yield in wheat is important main yield contributing trait. In our study, the thousand grain weight means of the 65 advanced bread wheat mutant lines ranged from 34.13 to 55.93 g, while the standard genotypes' means ranged from 36.9 to 47.04 g. Among the advanced bread wheat mutant lines, 30 lines (MT43, 41, 5, 49, 21, 46, 65, 39, 8, 7, 20, 29, 52, 40, 19, 31, 28, 30, 36, 38, 6, 23, 24, 18, 9, 37, 12, 35, 25 and 53) were a higher 1000-grain weight than the standard genotype. Fifteen mutant lines (MT43, 41, 5, 49, 21, 46, 65, 39, 8, 7, 20, 29, 52, 40 and 19) draw attention with a averages of thousand grain weight above 50 g (Table 2). Our results show that a wide variation can be achieved with the application of mutagen in terms of 1000 grain weights similar to results of Singh and Balyan (2009) who illustrated mutant lines with larger grains than the parents can be obtained as a result of gamma irradiation.

Grain yield (kg da^{-1}): The main purpose of plant improvement is to increase grain yield obtained from unit area. Grain yield being polygenic and is highly influenced by cultivar, environment and cultivar x environment interactions. Taking into consideration the other features examined, it is seen that mutagen application causes a lower variation in grain yield. The average performance of grain yield (kgda^{-1}) of all genotypes is depicted in (Table 2). In the study, grain yield of advanced bread wheat mutant lines ranged from 471.20-941.45 kg da^{-1}, while grain yield of standard genotypes ranged from 607.40-855.60 kg da^{-1} (Table 2). Results regarding to the trait revealed that the higher grain yield was observed in 10 mutant lines (MT53, 19, 33, 35, 14, 20, 42, 40, 27 and 15) as compared to the other genotypes, our results are in confirmation with those of Ayub *et al.* (1989) who stated that the effect of different dose of gamma rays irradiation on grain yield of wheat varieties responded differently in different varieties. As a result, it can be said that it would be appropriate to take these 10 mutant lines into the pre-registration yield trials in terms of grain yield.

Conclusion

The mutant MT53, MT19, MT33, MT35, MT14, MT20, MT42, MT40, MT27 and MT15 out yielded among all lines and stood first as compared to other mutants and check varieties in yield associated traits studied. Among them, MT35, MT14 and MT27 over performed for all yield components except thousand grain weight. As a result, MT35, MT14, MT27, MT19, MT20 and MT15 should be included in further breeding programs for exploitation of its genetic potential in new cross combinations. When the pedigrees of these promising mutant lines were examined (Table 1), the majority of the mutants originated from populations irradiated with gamma rays of 100 and 200 Gy suggesting that these gamma doses may be more beneficial for yield improvement in wheat mutation breeding.

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EFFECT OF ROOT PARAMETERS ON SURVIVAL OF *IN VITRO* GROWN STRAWBERRY

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Abstract

This study carried out in Çukurova University of Turkey was started with six runner strawberry varieties and genotypes: 'Sevgi', 'Rubygem' 'Kaşka' cultivars and '291', '60' and '36' hybrid genotypes. These materials were waited in 15% sodium hypochlorite solution for 10 minutes in order to surface sterilization and then were rinsed with sterile water in tissue culture laboratory. After sterilization, shoot tips of plants were extracted by sterile forceps and scalpels under a stereobinocular microscope in laminar flow and placed on hormone free Murashige and Skoog (MS) nutrient medium for initiation development during four weeks. Then, the explants were transferred to the MS medium containing 1 mg L^{-1} 6-Benzylaminopurine (BAP) for shoot proliferation. After four weeks, the effects of genotypes on root number, root length and explant weight were investigated. The number of root varied from 4.11 ('Sevgi') to 2.33 ('36'). Root length was changed between 1.61 ('36') and 0.64 cm ('Sevgi'). Our results showed that root length and number were negatively correlated with each other due to nutrition competition. At the same time, there was the difference in rooting capacity even in the same genotype. Therefore, at the end of study, the explants were divided to tree classes (well rooted, medium rooted and non-rooted) to determine the effect of root on the percentage of survival *in vitro* plants during acclimatization stage. The genotype, amount of root and their interaction were significantly effective on the acclimatized plants successfully. As expected, the percentage of survival plants was influenced from the number of roots. The highest percentage for rooting obtained was 75.8%. Also, *in vitro* plants obtained from 'Rubygem' variety did not need many roots to survive. However, any *in vitro* plants could not live under non rooted condition in this cultivar. This study presents preliminary results of a project aimed testing different types of plant growth regulators and their different doses on important breeding varieties and genotypes using shoot tip culture. These pre-results will be useful for us in terms of knowing the tissue culture capacities of our genotypes and varieties.

Key words: Strawberry, *in vitro*, Shoot tip, Rooting

Introduction

Strawberry (*Fragaria x ananassa*) is one of the most preferred fruits due to their rich vitamin content and their unique flavor. According to Turkish Statistical Institute (TUİK) data, in the Mediterranean region of Turkey, strawberry cultivation covers 61 023 hectares area in 2016 (TUİK, 2017), demanding 335-366 million seedlings annualy in this region. The choice of proper and high quality variety, is the first step of successful production in agriculture. . Seedling diameter is a very important quality parameter in strawberry cultivation and therefore, A+ seedlings having ≥15 mm diameter should be considered (Paydaş Kargı and Sarıdaş, 2012). In addition, selection of varieties resistant to soil-borne diseases and growing these varieties in disease-free areas are important for efficient production. In recent years, meristem culture studies have been carried out in order to produce high number of disease and virus free plantletsRunners are normally used for mass propagation of strawberry. Soil-borne disease in areas where material for seedling production are used can spread easily with

planting materials when vegetative methods are used and causes loss of yield. Meristem culture is known as one of tissue culture methods used efficiently in obtaining disease and viruses free seedlings. Meristem culture also ensures that the important genetic materials obtained are preserved. Success of tissue culture is determined by genotype, explant type, season, nutrient medium, plant growth regulators.

In this study, the effect of the amount of roots *in vitro* propagated plantlets on viability of plants at the acclimatization step was investigated. Number of roots, root length and explant weight were considered comparatively among tested genotypes and cultivars. In addition to, survival percentage of plants was also determined.

Materials and methods

Three strawberry variety candidates obtained by crossing, namely: '291' (Rubygem x Kaşka), '60' (Fortuna x Sevgi) and '36' (Fortuna x Sevgi) and their parents (except Fortuna) were used. These three candidates were selected by considering of aroma and firm-flesh features among F1 hybrid individuals. Surface sterilization was performed by washing materials in 15% sodium hypochlorite solution including 1-2 drops Tween-20 for 10 minutes followed by rinsing with sterile water 4-5 times. Following sterilization, shoot tips were extracted using sterile forceps and scalpels under a stereobinocular microscope in laminar flow and cultured in glass tubes containing growth regulator free Murashige and Skoog (MS) (Murashige and Skoog, 1962) medium for initiation development. The cultures were incubated at the 25±2°C under 3000 lx in 16 h photoperiod. After establishment they were transferred in to MS medium supplemented with 1.0 mg L^{-1} BAP After four weeks, root number, root length (cm) and explant weight (g) were investigated in each genotype and cultivar.

The experiment was conducted as two factors randomized complete block design as split plot with three replications. The obtained data were analyzed with the statistical program JMP version 5.0.1 (SAS Institute Inc., Cary, NC). ANOVA was carried out to determine the effects of the different genotype and rooting conditions (non rooting, medium rooting and well rooting) on root number, root length, explant weight and survival percentage. A least significant difference (LSD) test was carried out to examine the differences among groups. Comparisons that yielded $P \leq 0.05$ were considered to be statistically significant.

Results and discussion

Data obtained from this study are summerized in Table 1-4. In this study, the *in vitro* plants were evaluated in three groups; non rooted, medium rooted and well rooted for each genotype and variety. And the genotypes and varieties were compared among each other.

There was a significant differences between root lengths of different rooting levels and genotypes-varieties. The differences between interactions of these two factors were not significant. In this respect, the highest values were obtained from the genotypes '36' (1.61 cm), '60' (1.57 cm) and '36' (1.51 cm) from the same statistical group. The root length of the 'Sevgi' variety revealed the lowest value with 0.64 cm. The root lengths of medium and well-rooted plants were found to be close to each other and the difference of 0.30 cm between them was statistically insignificant (Table 1).

Table 1. Root length of different genotypes under various root properties (cm)

Rooting	Genotype						Mean
	291	**Rubygem**	**Kaşka**	**36**	**60**	**Sevgi**	
Non rooting	0	0	0	0	0	0	**0 B**
Medium rooting	1.90	1.50	1.75	2.00	1.70	1.10	**1.66 A**
Well rooting	2.63	1.43	1.02	2.83	3.00	0.83	**1.96 A**
Mean of genotypes	1.51 ABC	0.98 BCD	0.92 CD	1.61 A	1.57 AB	0.64 D	
LSD_{gen}*= 0.62			**LSD_{r}***= 0.44**		**LSD_{genxr}= N. S.**		

(1): Differences between the means were showed with different letters
(2): N.S.: Not Significant, ***: p<0.001; **: p<0.01; * : p<0.05

Root number of genotypes and varieties were determined between 4.11 ('Sevgi') and 2.33 ('36'). The effects of rooting levels on root numbers were found statistically significant. As expected, the root numbers of well-rooted plants were determined to be 6.39 (Table 2).

Table 2. Root number of different genotypes under various root properties

Rooting	Genotype						Mean
	291	**Rubygem**	**Kaşka**	**36**	**60**	**Sevgi**	
Non rooting	0	0	0	0	0	0	0 C
Medium rooting	1.00	2.33	2.67	2.00	2.00	4.00	2.33 B
Well rooting	7.67	7.00	5.00	5.00	5.33	8.33	6.39 A
Mean of genotypes	2.89	3.11	2.56	2.33	2.44	4.11	
LSD_{gen}= N. S			**LSD_{r}***= 1.26**		**LSD_{genxr}= N. S..**		

(1): Differences between the means were showed with different letters
(2): N.S.: Not Significant, ***: p<0.001; **: p<0.01; * : p<0.05

Varieties and genotypes, rooting levels and differences between interactions of these two factors were found statistically insignificant on the explant weight. The heaviest explants were observed as 1.67 g in the 'Kaşka' variety, as 1.44 g in the well-rooted plants and as 2.15 g in the well-rooted plants of the 'Rubygem' variety (Table 3).

Table 3. Explant weight of different genotypes under various root properties (g)

Rooting	Genotype						Mean
	291	**Rubygem**	**Kaşka**	**36**	**60**	**Sevgi**	
Non rooting	1.15	0.97	1.76	1.41	1.39	1.41	**1.35**
Medium rooting	1.35	1.10	1.67	0.35	1.43	1.77	**1.28**
Well rooting	1.40	2.15	1.57	1.23	1.45	0.82	**1.44**
Mean of genotypes	1.29	1.41	1.67	0.99	1.42	1.34	
LSD_{gen}= N. S.			**LSD_{r}= N. S.**		**LSD_{genxr}= N. S.**		

(1): Differences between the means were showed with different letters
(2): N.S.: Not Significant, ***: p<0.001; **: p<0.01; * : p<0.05

The factors investigated and their interactions showed statistically significant effect on the viability levels of the plants. The highest viable plants were obtained from the best rooted plants at 75.8%, and non-rooted plants gave the lowest value at 7.08%. 'Rubygem' variety and the genotype '36' having 66.7% survival plants were important in terms of the highest viability rate, they were followed by the genotype '60' (55.0%) and 'Kaşka' variety (47.5%). 'Rubygem' variety and the genotype '36' having medium and well-rooted plants and 'Kaşka' variety having well rooted plants were remarkable with 100% viability (Table 4).

Table 4. Survival rate of different genotypes under various root properties (%)

Rooting	**Genotype**						**Mean**
	291	**Rubygem**	**Kaşka**	**36**	**60**	**Sevgi**	
Non rooting	0 g (0)	0 g (0)	12.5 f (20.6)	0 g (0)	30.0 de (33.2)	0 g (0)	**7.08 C (8.97)**
Medium rooting	0 g (0)	100 a (90.0)	30.0 de (33.2)	100 a (90.0)	60.0 c (50.9)	20.0 ef (26.1)	**51.7 B (48.4)**
Well rooting	40.0 d (39.2)	100 a (90.0)	100 a (90.0)	100 a (90.0)	75.0 b (60.8.0)	40 d (38.9)	**75.8 A (68.1)**
Mean of genotypes	13.3 D (13.07)	66.7 A (60.0)	47.5 B (47.9)	66.7 A (60.0)	55.0 B (48.3)	20.0 C (21.6)	
LSD_{gen}*= 5.07**			**LSD_{r}***= 3.59**		**LSD_{genxr}***= 8.78**		

(1): Differences between the means were showed with different letters
(2): N. S.: Not Significant, ***: $p<0.001$; **: $p<0.01$; *: $p<0.05$
(3): Angle transform data were bracketed

From the parameters tested it was observed that there is an effect of the rooting levels and the genotypes-varieties on root length and survival rates and the effect of the rooting levels on the number of roots. In addition to, no effect was observed on explants weight of parameters selected. It is also interesting that the surviving plants are also affected by the interaction of two selected parameters. Thus, it has been demonstrated that non-rooted (the genotype '60' and 'Kaşka' variety) and medium-rooted (except genotype '291') plants of some varieties and genotypes can survive.

Many studies have been carried out on strawberry meristem and shoot tips culture until today. These studies are based on different plant growth regulators and their varying doses. In a study carried out by Fatemeh et al. (2010), effect of the plant growth regulators at different concentrations on the rate of micropropagation, rooting and survival *in vitro* plants of the 'Camarosa' strawberry variety was examined using shoot tip technique. As a result, while 2.0 µM Thidiazuron (TDZ) and 4.0 µM BAP combination was found better than others, the highest number of roots, the number of rooted plants and the longest roots were determined in 1.0 µM Naphthaleneacetic acid (NAA), 2.0 µM Indole-3-butyric acid (IBA) and growth regulator-free MS medium, respectively. Yonghua et al. (2005) reported that when IBA concentration was increased to 0.4 mg L^{-1} from 0.2 mg L^{-1} and 1.5 mg L^{-1} TDZ was added to nutrient medium, the percentage of shoot regeneration and the number of shoots per plant increased significantly. Ko et al. (2009) recorded that 1.0 mg L^{-1} BA and 0.1 mg L^{-1} NAA added to MS nutrient medium was better than 0.5 mg L^{-1} BA and 0.02 mg L^{-1} NAA in terms of micropropagation and the multiplication capacity of plant materials was increased by this medium. Adak et al. (2009) examined the effects of different types of hormones and their combinations on the propagation of Camarosa strawberry variety by tissue culture. According to the results obtained from the study, it was reported that TDZ and its application together with IAA significantly affected micropropagation. In addition to, 1.5 mg L^{-1} TDZ and 1 mg L^{-1} Indole-3-acetic acid (IAA) was determined to be the best combination for micropropagation and average 28.46 shoots were obtained using this combination. It was also found that

increased IBA concentration reduced rooting and the highest root growth rate was obtained from medium containing activated charcoal. In a study carried out by Lee et al. (2010), different nutrient mediums (MS, Gamborg B5 and White), their different strength (1/3, 1/2, 1, 2 and 3) and different sucrose doses (1%, 3%, 5% and 8%) were tested in the microprogation success of 'Goha' strawberry variety. Among all of them, 1/2 MS and 1% sucrose were found to be better than the other ones. Increased sucrose content negatively affected shoot length. Adak (2011) recorded reaction of strawberry varieties to different Gibberellic acid (GA_3) doses during the meristem culture process. In terms of micropropagation, 0.4, 0.8, 1.0 mg L^{-1} GA_3, 0.1 mg L^{-1} GA_3 and 0.4 mg L^{-1} GA_3 doses gave the best results for Camarosa, Chandler and Oso Grande varieties, respectively. In the same study, media containing BAP more than 1.0 mg L^{-1} significantly reduced the number of micro shoots in strawberry varieties tested. The best hormone combination was found to be 1.0 mg L^{-1} BAP , 1.5 mg L^{-1} TDZ, 1.0 mg L^{-1} IAA for micropropagation of strawberry by Adak (2016). For rooting, using activated charcoal (AC) instead of auxin was recommended in the same study. For all varieties used in the research, the highest number and the longest roots were obtained from media with 5 g L^{-1} AC. It was found that IBA + AC combination and NAA + AC combination responded similarly and while higher NAA concentration increased callus formation, it decreased rooting capacity.

As seen in these studies, different dosses BAP, IAA, IBA, TDZ, NAA and GA_3 and their combination have been tested in the meristem and shoot tip culture studies of strawberry. Root parameters such as root length, root number, the number of rooted plants, shoot parameters such as shoot regeneration and the number of shoots per plant and the percentage of survival *in vitro* plants were considered.

Conclusion

The breeding studies were supported by biotechnological methods such as tissue culture to the process will accelerate these studies. As our results showed that root length and number were negatively correlated with each other due to nutrition competition. At the same time, there was the difference in rooting capacity even in the same genotype. Therefore, at the end of study, the explants were divided to tree classes (well rooted, medium rooted and non-rooted) to determine the effect of root on the percentage of survival in vitro plants during acclimatization stage. The genotype, amount of root and their interaction were significantly effective on the acclimatized plants successfully. As expected, the percentage of survival plants was influenced from the number of roots. The highest percentage for rooting obtained was 75.8%. Also, in vitro plants obtained from 'Rubygem' variety did not need many roots to survive. However, any in vitro plants could not live under non rooted condition in this cultivar. Shoot tip culture performances of some important breeding genotypes and varieties have been presented with this study. In future studies, it is aimed to obtain more useful information by using with different plant growth regulator combinations and doses.

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DETERMINATION OF THE NITROGEN DOSES EFFECTS ON GRAIN YIELD AND YIELD COMPONENTS OF SOME OAT GENOTYPES

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Abstract

In order to healthy ruminant feeding, high quality forage must be eaten and at least half of feed should consist of forage. Though oat is commonly used as food and feed in the world. In Turkey oats are mainly used for feeding as forage and kernel. Its kernel has high Content of carbohydrate, protein, oil, minerals and vitamins. Protein level of oat kernel can be reached up to 16% and its biological value is similar to the other cereals. Oat kernel has one of the highest oil content among cool season cereals with 6.5%. These properties increased oat kernel nutritive value, palatability and voluntary intake of oat by ruminants. The climatic characteristics of Samsun are very suitable for oat requirements. The aim of this study was to determine the effects of 5 different nitrogen doses, on grain yield and yield components of 2 oat varieties and 1 oat line. The experiment was established according to split plots design with 3 replicates on 11 November 2016. In the study, grain yield, 1000 seed weight, height of plant, number of panicle per square meter and harvest index of oat genotypes were determined. Average grain yields were 2.53, 2.86, 3.37, 3.62 and 3.93 t ha^{-1}, respectively, depending on N doses (0, 50, 100, 150 and 200 kg ha^{-1}). Panicle numbers were also increased with N fertilization, the highest value was obtained from 200 kg ha^{-1} N rate with 467 panicle number m^{-2}. As the N doses increased, grain yield was also increased even though 1000 seed weight decreased.

Keywords: *Oat, Nitrogen dose, Cultivar, Grain yield, Yield components*

Introduction

Oat (*Avena sativa L.*) is an important cereal crop and is commonly grown for food and feed. As compared to the other cereals, oat is considered to be more suitable for growing under marginal lands, including cool-wet regions and unfertile-arid lands (Buerstmayr et al., 2007, Mut et al., 2011). Though oat commonly was used as food and feed in the world, main usage area of oat is feeding as forage and kernel in Turkey. In order to healthy ruminant feeding, they must eat high quality forage and at least half of feed should consist of forage. Its kernel has high content of carbohydrate, protein, oil, minerals and vitamins. Protein level of oat kernel can be reached up to 16% and its biological value is similar to the other cereals. Oat kernel has one of the highest oil content among cool season cereals with 6.5%. These properties increased oat kernel nutritive value, palatability and voluntary intake of oat by ruminants. The climatic characteristics of Samsun are very suitable for oat requirements. The proof of this fact, there is a huge variation of local oat population or ecotypes in Samsun (Kün, 1983). Oat would be very important crop in this region because of its really good adaptation, higher yield, nutritious and palatable for ruminants (Aydin et al., 2010). Oat can be grown as a catch crop from October to July for grain production. Yield potential and response of nitrogen fertilizer of oat is higher than the other cool season cereals (Hartman, 2000; Frazer and Mc Cartney., 2004). Higher N use efficiency can qualify the recommendations and provide technologies that are more adjusted, economically viable and of lower environmental impact, which are decisive conditions in the search for a more

sustainable agriculture (Parry et al., 2011; Prando et al., 2013). Nitrogen fertilization is still very complex and it is worth to research closely. Its effect is very different due to environmental factors (soil conditions, and climate especially rainfall) and genotype. The aim of this study is to determine the effects of 5 different doses of nitrogen, on grain yield and yield components of 2 oat varieties and 1 oat cultivarline.

Materials and methods

This study was conducted to determine the effects of 5 N doses (0, 50, 100, 150 and 200 kg ha^{-1}) on hay yield and mineral contents of 2 oat cultivars (Kahraman and Faikbey) and 1 oat line in Agricultural Research Center of Ondokuz Mayis University. Monthly temperature and rainfall data from November 2016 to May 2017 of the experiment site were 12.5, 5.9, 6.2, 7.5, 9.4, 10.2, 15.3 ^{o}C and 42.4, 81.5, 68.8, 40.1, 65.1, 85.8, 70.9 mm, respectively. The soil of the experiment site was poor in terms of organic matter (1.36 %) and phosphorus (mg/100 g of soil) and medium for potassium (mg/100 g of soil). In experiment, oat planted with 20 cm row spacing and each plot was consist of 6 rows. Consider soil sample analysis, 80 kg ha^{-1} P_2O_5 was applied the whole experiment area. Half of the N doses were applied with seed cultivation, the other part was given when the plants started active growing in spring which BBCH growth stage. There was no irrigation. The experiment was established according to split plots design with 3 replicates in 11 November 2016. Plots were harvested when the oat seeds matured. Plots were harvested at The following traits were performed: grain yield per hectare at 13% moisture; yield components, height of plant, number of panicle per square meter, mass of one thousand grains and harvest index. Resultant data were subjected to statistical analyses according to split plot design in SPSS 17.0. Differences in means were tested with Duncan's multiple range test.

Results and discussion

Significant differences were determined amongst the genotypes and N doses in the point of grain yields. While the highest grain yield was obtained from Faikbey cultivar (3630 kg ha^{-1}) as an average of N doses, Line 38 genotype took place in the same statistical group. Regard the mean of genotypes, the highest grain yields was determined to N200, N150 and N100 doses (3930, 3620 and 3370 kg ha^{-1}, respectively). The lowest grain yield was 2530 kg ha^{-1}, obtained from N0 dose (Table 1). Previous works were also reported similar results indicating that grain yield values increased due to increased nitrogen rates (Rocquigny et al., 2004; Muurinen et al., 2007). The responses of genotypes to N doses were different. While grain yields of Faikbey cultivar and Line 38 linearly increased parallel to N dose increase, grain yield of the Kahraman cultivar increased until N10 dose and then decreased and then increased again (Table 1 and Figure 1).

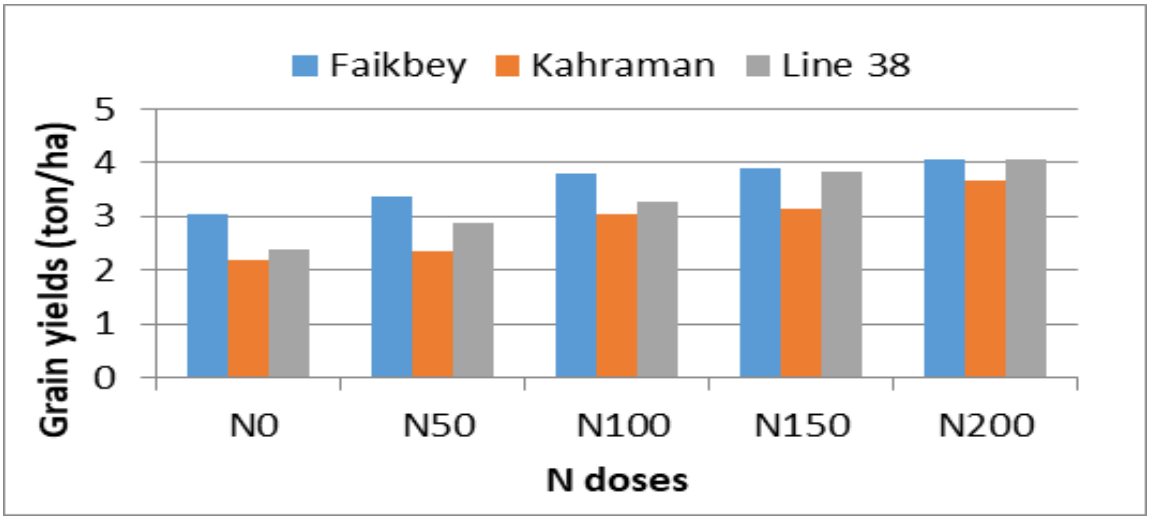

Figure 1. Grain yields of oat genotypes according to N doses

In terms of panicle number there were no differences amongst the genotypes, but differences amongst the N doses were significant. Panicle number m^{-2} were also increased with N fertilization, the highest value was obtained from 200 kg ha^{-1} N rate with 467 panicle number m^{-2} and N150 and N100 doses were in the same statistical group (Table 1). Similar results were also reported in previous works, indicating increased panicle number m^{-2} values with N fertilization (Peltonen-Sainio, 1997; Browne et al., 2006; Maral et al., 2013). Plant height of cultivars/line varied between 130.11 cm and 110.13 cm (Table 1). The average plant height was 111.79 cm for the control plots. It was increased to 115.70, 119.90, 125.80 and 127.50 cm at 50, 100, 150 and 200 kg ha^{-1} N rates, respectively (Table 1). Previous researchers reported that plant height is increased by increasing N fertilization, which supports our results (Chalmers et al., 1998; Shah et al., 2002; Rocquigny et al., 2004; Mohr et al., 2007; Maral et al., 2013). 1000-grain weight (1000-GW) of cultivars varied between 37.36 and 48.80 g. Line 38 had the lowest 1000-GW with 37.36 g (Table 1). Previous oat researchers also reported significant differences among cultivars in terms of 1000-GW (Gul et al., 1999; Shah et al, 2002; Inan et al., 2005). N doses caused to decrease of 1000-GW. It was determined as 43.7 g for control plots. It was decreased to 43.68, 42.29, 41.92 and 41.43 g in 50,100, 150 and 200 kg ha^{-1} N doses. As N doses increases, panicle number m^{-2}, kernel number and yields were also increased, in consequence 1000-GW was decreased.

In terms of harvest indexes there were no differences amongst the genotypes and N doses. The harvest indexes were 15.5%, 15.58% and 16.16% for Faikbey, Kahraman and Line 38, respectively (Table 1). The results were consistent with the findings of Shah et al., (2002); Inan et al., (2005) and Maral et al., (2013). Even though there was not any differences, harvest indexes were slightly increased in n150 and N200 doses.

Table 1. The values about grain yield and some agricultural characteristics obtained from some oat genotypes that was applied different N doses

Cultıvar-line / N Doses	**Plant height (cm)**					
	N0	**N5**	**N10**	**N15**	**N20**	**Average**
Faikbey	118.33	127.93	130.8	135.2	138.27	130.11a
Kahraman	104.43	105.33	109.07	115.53	116.27	110.13c
Hat 38	112.6	113.83	119.83	126.67	127.97	120.18b
Average	111.79b	115.70b	119.90ab	125.80a	127.50a	
Panicle number (m^2)						
Faikbey	323.33	356.67	413.33	436.67	463.33	398.67
Kahraman	335.33	359.33	424.67	442.33	483	408.93
Hat 38	310.00	320.00	410.00	426.67	454.67	384.27
Average	322.89c	345.33bc	416.00ab	435.22ab	467.00a	
1000-Grain weight (g)						
Faikbey	49.91	49.83	48.58	47.94	47.73	48.80a
Kahraman	42.38	42.59	41.41	41.09	40.78	41.65b
Hat 38	38.81	38.61	36.88	36.72	35.79	37.36c
Average	43.7	43.68	42.29	41.92	41.43	
Grain yield (ton ha^{-1})						
Faikbey	3.05	3.36	3.8	3.89	4.05	3.63a
Kahraman	2.17	2.35	3.04	3.15	3.68	2.87b
Hat 38	2.38	2.86	3.28	3.84	4.06	3.28ab
Average	2.53c	2.86bc	3.37ab	3.62a	3.93a	
Harvest index (%)						
Faikbey	15.09	15.93	16.35	17.19	17.95	16.5
Kahraman	14.93	15.21	15.58	16.04	16.15	15.58
Hat 38	15.33	15.73	15.89	16.83	17.01	16.16
Average	15.12	15.63	15.94	16.69	17.04	

*There is no difference amongst the number indicated same letter in the same column

Conclusion

In the study faikbey cultivar were appeared dominant in all parameters except panicle number were kahraman cultivar become superior. Doses of Nitrogen fertilizer were showed great effect at all cultivars on grain yield, plant height and panicle number m^{-2} N15 and/or N20 doses can be recommended in similar ecological conditions regarding the genotype. But, to clear the recommendation this type study should carry out at least 2 year.

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THE EFFECTS OF LIME DOSES ON SOME MORPHOLOGICAL AND FRUIT CHARACTERISTICS OF SOME STRAWBERRY (*FRAGARIA X ANANASSA* DUCH.) CULTIVARS

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Abstract

Calcareous soil causes iron (Fe) deficiency in many cultivated plants. Strawberries are among those most affected by lime stress among plants. In high-lime soils, sensitive plants such as strawberries, give less yield by not taking certain nutrients, especially iron. In the recent study, some morphological and fruit characteristics of four (Amiga, Festival, San Andreas and YFL) strawberry cultivars were examined in 5 lime doses (0%, 5%, 7.5%, 10%, and 12.5%). The plants were grown in a mix of peat and perlite (2: 1) containing lime at rates reported in 9-liter pots. After planting, fruits were harvested for four months. After this time the plants were removed and the root and stem characteristics were examined. According to the data obtained from a recent study, although there were differences between the varieties of strawberries, little amount of lime was needed for fruitful and high-quality fruit production. However, in high doses, lime led to reduced strawberry yield and quality, and even plant mortality. While 5% lime dose had a positive effect on root collar diameter (33.40 mm 'Amiga', 32.78 mm 'YFL2), crown per plant (7.42 per plant 'Amiga', 6.00 per plant 'YFL') and number of leaves (41.17 per plant 'YFL', 38.50 per plant 'Amiga'), 7.5% lime had a positive effect on root volume (54.67 ml 'Amiga', 41.33 ml 'YFL'), fruit yield per plant (96.99 g 'Amiga', 82.31 g 'Festival'), fruit size (35.68 mm 'San Andreas', 33.20 g 'Amiga') and fruit width (27.71 mm 'San Andreas', 26.21 mm 'Festival'). However, the number of chlorophyll decreased with increase in lime doses. As a result, more specific work is needed to determine the types of strawberries that can be grown in calcareous soils.

Keywords: *Lime, strawberry, high pH and chlorophyll.*

Introduction

Alkaline soils (pH > 7) are the dominant soil type in arid and semi-arid climates. These soils cover more than 30% of the earth's surface (Chen and Barak, 1982). The pH value of these soils varies between 7.5-8.5 depending on calcium carbonate content (Marchner, 2002).

Iron deficiency chlorosis (FeDC) was recorded as the first known nutrient deficiency (Vose, 1982). The FeDC is a common condition in calcareous soils in many crops such as strawberries (*Fragaria x ananassa*). Lime-induced chlorosis is the most common nutritional disorder of plants growing in soil which includes more than 20% $CaCO_3$ (Schinas and Rowell, 1977). As a result of this, significant loss of yields is experienced (Wallace and Lunt, 1960; Zaiter et al., 1991). Although strawberries yield loss, grown in high-lime soils cannot yet be clearly determined, this rate can reach up to 20% in soybeans (Froehlich and Fehr, 1981; Kinkaid, 1986).

Although the iron content in mineral soils is about 2%, and most plants can remove only 1-2 kg/ha of iron from the soil annually. The main underlying cause of iron chlorosis in soils containing high lime is not the poor iron content in the soil. The main reason is that the iron in the soil is converted to relatively insoluble complex molecules, such as $Fe(OH)_2^+$, $Fe(OH)_3$, and $Fe(OH)_4^-$, by the calcium causing the high pH values (Marchner, 2002). In addition, zinc (Melton et al., 1973; Moraghan and Mascagni, 1991), manganese (Jauregui and Reisenauer,

1982), boron (Kluge and Beer, 1979; Keren et al., 1981), and phosphorus (Welp et al., 1983) bonds similarly as relatively insoluble complex molecules in soils containing high lime.
The roots of the plants grown in calcareous soil are adversely affected by the lime-induced chlorosis seen on the leaves and weaker growth (Bertoni, 1995; Perret and Koblet, 1981; Perret and Koblet, 1984). However, since the iron is taken up by capillary roots, the anomalies that may occur in roots may cause the chlorosis (Bavaresco et al., 1991; Perret and Koblet, 1997; Colugnati et al., 1998). Iron uptake and reduction were closely related to the root diameter in *Vitis vinifera* (Bavaresco et al., 1991), and the shoots of the grapevine grown in calcareous soil are shorter than those grown in relatively non-calcareous soils (Bavaresco et al., 1993).
While the high lime content in the soil may be harmful to plants (at different rates depending on species and cultivars), the lack of lime also damages the plants by causing the washing of the nutrients and causing acidity. The Ca^{+2} ion, which stored by binding plant nutrients such as nitrogen (N) and potassium (K), is present in calcareous soils. In addition, the absence of this ion causes the formation of strongly bonded compounds between phosphorus (P)-iron (Fe) and phosphorus (P)-aluminium (Al) (İnal, 1991).
Lime contributes to the relaxation of compact soils (Aydeniz, 1985). In addition, lime promotes the growth of microorganisms and roots by contributing to the aeration of the soil and the water cycle (İnal, 1991). The root length, plant height, and dry matter are positively affected in tomato up to a certain level with the increase of the lime ratio (20%), and the effect becomes negative if the dose increase continues (Aydeniz and Brohi, 1990). In addition, Schaumberger (1990) reported that the increase in pH up to a certain level in corn cultivars 'Mutin' and 'Anjou' affects iron content positively in the roots and leaves. Similarly, Cutcliffe and Blatt (1984) reported that the addition of lime to low pH (4.9) soils up to a certain level, increased the yield and the mean fruit weight in the strawberry cultivar 'Redcoat'.
In this study, the effects of different lime doses on some plant and fruit characteristics of 4 strawberry cultivars were investigated.

Material and Method

This study was carried out in the Greenhouse of Department of Horticulture, Faculty of Agriculture at Selçuk University in 2016. In this study, effects of 5 lime doses (0%, 5%, 7.5%, 10% and 12.5%) on some plant (root collar diameter, root volume, crown number per plant, leaf number per plant) and fruit characteristics (fruit yield per plant, fruit length, fruit width and mean fruit weight) of 4 strawberries (*Fragaria x ananassa*) cultivars (*cv*.) (Amiga, Festival, San Andreas and YFL) were investigated.
Seedlings was planted in 9-liter pots containing 3:1 peat, perlite, and lime (0%, 5%, 7.5%, 10% and 12.5%) in March. The study was set up with 3 replications and 5 plants per replicate, according to randomized complete blocks design. Fertilization, irrigation, pest and weed control has been regularly treated according to strawberry producing protocols.
Root collar diameter (with Absolute Digimatic model CD-15CPX produced by Mitutoyo Corporation in Japan), root volume (immerse into water), crown number per plant (number of branch crown) and leaf number per plant were investigated in 5 plants in each replication at the middle of November 2016. At the same time, fruit yield per plant, fruit width (fruit diameter), fruit length (with Absolute Digimatic model CD-15CPX produced by Mitutoyo Corporation in Japan), fruit width (fruit diameter) and mean fruit weight (with CAS MW-II 600 precision scale) were investigated from July to October 2016. Because the cultivars used in the experiment were short day cultivars, they were covered with black plastic in order to get fruit in July and August.

The experimental data were collected and subjected to analysis of variance (ANOVA) using SPSS 20.0 software (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). The means were separated by multiple Duncan's test at $p \leq 0.05$.

Results and Discussions

1.Morphological Characteristics

The crown diameter (CD) was measured in November 2016 by digital caliper when plants were removed. As can be seen in Table 1, the differences between the results obtained from the study (both between treatments and cultivars) were statistically significant ($p \leq 0.05$). The highest CD values of 4 strawberry cultivars (Amiga: 33.40 mm, Festival: 31.26 mm, San Andreas: 23.96 mm, and YFL: 32.78 mm) were obtained from 5% lime treated plants. Second highest values were 0% treatment in Amiga (30.45 mm), Festival (23.91 mm), and San Andreas (21.49 mm), while 7.5% lime treatment in YFL (28.99 mm). The lowest values were obtained on 12.5% lime treatment as expected (at 10% in San Andreas "15.14 mm" and YFL "22.64 mm") in Amiga (17.55 mm), and Festival (15.21 mm). As can be understood from the CD values, the best results were obtained from the 5% lime treatment. These results suggest that the strawberry cultivars need some lime to grow strongly.

Root volume (RV) was measured by placing the roots in a measuring cylinder containing water after the plants have been removed in November 2016. After the roots are placed in the measuring cylinder, the increase in water level was measured in ml. As can be seen in Table 1, the results obtained are statistically significant ($p \leq 0.05$). The highest RV values were obtained from 7.5% lime treatment for 4 strawberry cultivars (Amiga: 54.67 ml, Festival: 33.56 ml, San Andreas: 25.58 ml, and YFL: 41.33 ml). The most effective treatment, compared with CD, was 7.5% lime treatment, while the second highest values of 5% lime treatment in Amiga (38.67 ml) and YFL (39.17 ml). The second highest values were obtained from 10% lime treatment in Festival (30.56 ml) and San Andreas (18.33 ml). The lowest values were obtained from 12.5% lime treatment in Amiga (21.33 ml), Festival (20.00 ml), and San Andreas (14.00 ml), except YFL (0%: 22.00 ml). Compared with CD, the most effective treatment on RV was 7.5% lime treatment. In addition, while the worst results in YFL (22.00 ml) were obtained from 0% lime treatment, the difference between 0% and 12.5% lime treatment was very low in Festival (respectively, 21.33 ml, and 20.00 ml) and in San Andreas (respectively, 15.42 ml, and 14.00 ml).

The crown number per plant (CNPP) was counted after the plants were removed. The results were statistically significant as shown in Table 1 ($p \leq 0.05$). The highest CNPP values were obtained from 5% lime treatment in all strawberry cultivars (Amiga: 7.42, Festival: 4.11, San Andreas: 4.25, and YFL: 6.00 pieces per plant). Second high values were obtained from 0% lime treatment in Amiga (6.44 pieces per plant), Festival (3.44 pieces per plant) and San Andreas (3.83 pieces per plant), while second high value (5.83 pieces per plant) on YFL was obtained from 7.5% lime treatment. The lowest values were obtained from 12.5% lime treatment in Amiga (2.00 pieces per plant) and San Andreas (2.17 pieces per plant, while it was obtained from 7.5% lime treatment in Festival (2.22 pieces per plant) and from 10% lime treatment in YFL (4.44 pieces per plant). If the results are compared with CD and RV, it seems to be incompatible with CD while compatible with RV. The affinity with the CD can be considered to be based on the relationship between both characters.

Table 1. Effect of lime doses on crown diameter (CD), root volume (RV), crown number per plant (CNPP), and leaf number per plant (LNPP) in Amiga, Festival, San Andreas and YFL (mean ± std. dev.).

Doses	Cultivars	CD (mm)	RV (ml)	CNPP	LNPP
%0.00	Amiga	30.45 ± 0.56^{b}	31.33 ± 0.63^{e}	6.44 ± 0.16^{b}	32.00 ± 0.66^{c}
	Festival	23.91 ± 0.78^{f}	21.33 ± 0.34^{hi}	3.44 ± 0.20^{hij}	15.55 ± 0.69^{jk}
	San Andreas	21.49 ± 0.57^{h}	15.42 ± 0.38^{k}	3.83 ± 0.29^{gh}	16.92 ± 1.01^{hi}
	YFL	27.28 ± 0.77^{d}	22.00 ± 0.50^{h}	5.33 ± 0.14^{d}	26.17 ± 0.52^{e}
%5.00	Amiga	33.40 ± 0.82^{a}	38.67 ± 0.38^{c}	7.42 ± 0.14^{a}	38.50 ± 0.75^{b}
	Festival	31.26 ± 0.49^{b}	29.33 ± 0.34^{f}	4.11 ± 0.38^{fg}	26.00 ± 0.33^{e}
	San Andreas	23.96 ± 0.59^{f}	18.00 ± 0.50^{j}	4.25 ± 0.25^{efg}	20.58 ± 0.80^{g}
	YFL	32.78 ± 0.68^{a}	39.17 ± 0.76^{c}	6.00 ± 0.50^{bc}	41.17 ± 1.26^{a}
%7.50	Amiga	25.12 ± 0.40^{e}	54.67 ± 1.01^{a}	3.17 ± 0.29^{j}	23.83 ± 0.76^{f}
	Festival	22.67 ± 0.67^{g}	33.56 ± 1.02^{d}	2.22 ± 0.19^{kl}	14.33 ± 0.34^{kl}
	San Andreas	20.27 ± 0.66^{i}	25.58 ± 0.63^{g}	2.50 ± 0.25^{k}	14.92 ± 0.14^{jkl}
	YFL	28.99 ± 0.60^{c}	41.33 ± 0.76^{b}	5.83 ± 0.29^{c}	39.17 ± 0.76^{b}
%10.00	Amiga	21.35 ± 0.91^{h}	30.33 ± 0.88^{ef}	3.33 ± 0.00^{ij}	17.78 ± 0.69^{h}
	Festival	22.13 ± 0.67^{gh}	30.56 ± 0.84^{ef}	3.78 ± 0.19^{ghi}	15.78 ± 0.19^{ij}
	San Andreas	15.14 ± 0.12^{k}	18.33 ± 0.38^{j}	3.33 ± 0.14^{ij}	14.25 ± 0.25^{l}
	YFL	22.64 ± 0.48^{g}	33.44 ± 1.17^{d}	4.44 ± 0.20^{ef}	22.78 ± 0.69^{f}
%12.50	Amiga	17.55 ± 0.47^{j}	21.33 ± 1.53^{hi}	2.00 ± 0.00^{l}	15.00 ± 1.00^{jkl}
	Festival	15.21 ± 0.77^{k}	20.00 ± 1.00^{i}	2.50 ± 0.50^{k}	12.83 ± 0.76^{m}
	San Andreas	15.27 ± 0.27^{k}	14.00 ± 0.50^{l}	2.17 ± 0.29^{kl}	8.17 ± 0.76^{n}
	YFL	23.10 ± 0.19^{fg}	30.50 ± 1.00^{ef}	4.67 ± 0.29^{e}	28.00 ± 0.50^{d}
p (0.05)		0.000	0.000	0.000	0.000

The leaf number per plant (LNPP) was counted after the plants were removed. As shown in Table 1, differences between treatments and cultivars are statistically significant ($p \leq 0.05$). The highest LNPP values were obtained from 5% lime treatment for all cultivars (Amiga: 38.50, Festival: 26.00, San Andreas: 20.58, and YFL: 41.17 pieces per plant). Second high values in Amiga (32.00 pieces per plant), and San Andreas (16.92 pieces per plant) were obtained from %0 lime treatment. However, the second-high values in Festival (15.78 pieces per plant) was obtained from 10% lime treatment, and in YFL (39.17 pieces per plant) it was obtained from 7.5% lime treatment. The lowest LNPP values were obtained from 12.5% lime treatment in Amiga (15.00 pieces per plant), Festival (12.83 pieces per plant) and San Andreas (8.17 pieces per plant), and 10% lime treatment in YFL (22.78 pieces per plant). The next lowest value in YFL cultivar (26.17 pieces per plant) was obtained from 0% lime treatment. When the obtained LNPP results were examined, it was similar to CD and CNPP, not RV. The reason for this maybe a relationship between CD and CNPP with LNPP. Relations are as follows. The increase in LNPP was due to the increase in CNPP number, and the CD was increased as a result of the increase in LNPP and CNPP numbers.

As can be seen in the previous studies, there is a need for a certain amount of lime for the growth and development of almost all plants in a healthy way, albeit vary according to species and cultivars (Schaumberger, 1990; Cutcliffe and Blatt, 1984; Aydeniz and Brohi, 1990; Inal, 1991). The results obtained from this study support previous studies. Depending on the strawberry cultivar used in this study, lime treatments have positive effects on morphological characteristics up to certain levels. While the most effective treatment was 5% lime treatment in Amiga, Festival and San Andreas on CD, CNPP and LNPP, 5% lime treatment on CD, and 7.5% lime treatment on CNPP and LNPP were effective on YFL. On the RV, 7.5% lime treatment was the most effective treatment in all cultivars. According to Schaumberger (1990), the increase in pH up to a certain level, which varies according to cultivar, has increased the content of iron in corn root and leaves. According to Aydeniz and Brohi (1990), increased lime rates increased root length, plant height and dry matter content in tomato. However, in both studies, pH level and increasing amount of lime caused negative effects. In

this study too, lime treatments over 7.5% led to negative effects on morphological characteristics. The values of CD, CNPP, and LNPP on Amiga, Festival, and San Andreas were lower in 7.5% and higher lime treatments more than 0% lime treatment. Also, RV values were lower in 10% lime treatment in Amiga and in 12.5% lime treatment in Festival and San Andreas than 0% in lime treatment While in YFL, CD CNPP, and LNPP values were lower in 10% and higher lime treatment than 0% lime treatment, RV values were higher in all treatments than control.

Fruit Characteristics

Fruits were harvested during July, August, September, and October 2016. Fruits harvested in the four-month period were calculated as the mean fruit yield per plant. As seen in Table 2, the productivity differences between treatments and cultivars are statistically significant ($p \leq 0.05$). The highest fruit yield per plant (FYPP) values were obtained from 7.5% lime treatment in Amiga (484.92 g) and Festival (411.53 g), 5% lime treatment in San Andreas (522.42 g) and 0% lime treatment in YFL (285.37 g). However, the difference between the yields obtained from 7.5% (238.49 g) and 5% (218.28 g) lime treatments and the yield obtained from 0% lime treatment in. YFL, although statistically significant, it was not so much. On the other hand, the second-high FYPP value was obtained from 0% lime treatment in Amiga (392.32 g) and San Andreas (468.90 g), while 5% for Festival (391.19 g) and 7.5% for YFL (238.49 g). The lowest FYPP values were obtained from 12.5% lime treatment for all cultivars (Amiga: 37.40 g, Festival: 79.07 g, San Andreas: 116.21 g, and YFL: 70.34 g). When tables (1 and 2) are examined, it shows that the treatment that gives the best results changes depending on the cultivar. However, it was seen that RV (Table 1) and FYPP (Table 2) values in Amiga, Festival, and YFL have largely overlapped.

After ripening, the fruits were harvested and the average fruit length (FL) was calculated. As can be seen in Table 2, the differences between the results are statistically significant ($p \leq 0.05$). The longest fruits were obtained from 12.5% (Amiga: 34.68 mm, YFL: 32.31 mm), and 7.5% (Festival: 30.56 mm, San Andreas: 35.68 mm) lime treatments. However, the shortest fruits were obtained from 0% (Amiga: 30.97 mm, Festival: 28.52 mm), 12.5% (San Andreas: 31.20 mm) and 10% (YFL: 28.78 mm) lime treatments. When the results were evaluated together with FYPP and mean fruit weight (MFW), it was seen that the differences are mostly related to the fruit load of the plants. However, the longest fruit in San Andreas (35.58 mm) was obtained from the 7.5% lime treatment which had the second-highest yield and the heaviest fruits. On the other hand, although the fruit load was low, relatively short fruit was obtained from Festival (29.08 mm) and San Andreas (31.20 mm) from 12.5% lime treatment. Taking these results into consideration, fruit load and lime treatments act together on FL.

After ripening, the diameter of the harvested fruits was measured and the average fruit width (FW) was calculated. As shown in Table 2, the differences between the results are statistically significant. The best results were obtained from different treatments depending on the cultivars. The largest fruits were obtained from 12.5% in Amiga (26.97 mm), 7.5% and 10% in Festival (26.21 mm for both treatments), 7.5% in San Andreas (27.71 mm) and 5% in YFL (25.83 mm). The second-highest fruit diameters were 7.5% in Amiga (25.34 mm), 5% in Festival (25.62 mm) and San Andreas (26.71 mm) and 12.5% in YFL (24.70 mm). The lowest values were different depending on the cultivar. The lowest values were obtained from 0% in Amiga (23.26 mm) and Festival (23.94 mm, 12.5% in San Andreas (25.31 mm) and 7.5% in YFL (22.96 mm). When the results were examined, it was seen that there are significant differences between the treatments and the cultivars. These differences appear to be due to the differences in fruit loads and the durability of the cultivars against lime.

Table 2. Effect of lime doses on fruit yield per plant (FYPP), fruit length (FS), fruit width (FW), and mean fruit weight (MFW) in Amiga, Festival, San Andreas and YFL.

Doses	Cultivars	FYPP (g)	FL (mm)	FW (mm)	MFW (g)
%0.00	Amiga	392.32 ± 1.12^{g}	30.97 ± 0.06^{h}	23.26 ± 0.19^{l}	7.38 ± 0.04^{n}
	Festival	379.82 ± 1.82^{h}	28.52 ± 0.07^{n}	23.94 ± 0.13^{k}	7.45 ± 0.02^{n}
	San Andreas	468.90 ± 3.19^{c}	32.81 ± 0.17^{d}	25.95 ± 0.05^{e}	10.30 ± 0.06^{d}
	YFL	285.37 ± 2.50^{f}	29.28 ± 0.07^{k}	23.94 ± 0.05^{k}	7.92 ± 0.07^{m}
%5.00	Amiga	359.53 ± 2.71^{i}	31.85 ± 0.08^{f}	24.17 ± 0.05^{j}	8.05 ± 0.05^{l}
	Festival	391.19 ± 1.15^{g}	30.45 ± 0.14^{ij}	25.62 ± 0.05^{f}	8.64 ± 0.03^{k}
	San Andreas	522.42 ± 2.72^{a}	34.58 ± 0.06^{b}	26.71 ± 0.03^{c}	10.57 ± 0.03^{c}
	YFL	218.28 ± 7.90^{l}	30.87 ± 0.11^{h}	25.83 ± 0.06^{e}	9.77 ± 0.06^{f}
%7.50	Amiga	484.92 ± 5.62^{b}	33.20 ± 0.09^{c}	25.34 ± 0.04^{g}	8.99 ± 0.14^{i}
	Festival	411.53 ± 0.58^{d}	30.56 ± 0.02^{i}	26.21 ± 0.36^{d}	9.14 ± 0.05^{h}
	San Andreas	464.82 ± 3.38^{c}	35.68 ± 0.12^{a}	27.71 ± 0.09^{a}	11.97 ± 0.08^{a}
	YFL	238.49 ± 0.34^{k}	30.87 ± 0.04^{h}	22.96 ± 0.12^{m}	7.45 ± 0.02^{n}
%10.00	Amiga	201.69 ± 0.71^{m}	32.02 ± 0.06^{f}	24.22 ± 0.06^{j}	8.68 ± 0.03^{k}
	Festival	283.64 ± 2.31^{j}	30.30 ± 0.21^{j}	26.21 ± 0.10^{d}	9.36 ± 0.03^{g}
	San Andreas	406.23 ± 1.25^{f}	31.86 ± 0.15^{f}	26.64 ± 0.03^{c}	11.37 ± 0.02^{b}
	YFL	134.08 ± 0.57^{n}	28.78 ± 0.26^{m}	23.88 ± 0.06^{k}	8.06 ± 0.03^{l}
%12.50	Amiga	37.40 ± 0.17^{r}	34.68 ± 0.04^{b}	26.97 ± 0.19^{b}	9.94 ± 0.05^{e}
	Festival	79.07 ± 0.38^{p}	29.08 ± 0.12^{l}	24.93 ± 0.15^{h}	8.88 ± 0.05^{j}
	San Andreas	116.21 ± 0.08^{o}	31.20 ± 0.11^{g}	25.31 ± 0.09^{g}	10.56 ± 0.01^{c}
	YFL	70.34 ± 0.46^{q}	32.31 ± 0.10^{e}	24.70 ± 0.07^{i}	9.41 ± 0.04^{g}
p (0.05)		0.000	0.000	0.000	0.000

As fruits ripened, the harvested fruits were weighed and the mean fruit weight (MFW) was calculated. As can be seen in Table 2, the differences between treatments and cultivars were statistically significant ($p \leq 0.05$). The best results were obtained from different treatments depending on the cultivar. The MFW values of the cultivars were affected differently by the fruit load and lime treatments, as in FL and FW. The highest MFW values were obtained from 12.5% in Amiga (9.94 g), 10% in Festival (9.36 g), 7.5% in San Andreas (11.97 g) and 5% lime treatment in YFL (9.77 g). The second-highest values were obtained from 7.5% in Amiga (8.99 g) and Festival (9.14 g), 10% in San Andreas (11.37 g) and 12.5% lime treatment in YFL (9.41 g). However, the lowest values were obtained from 0% lime treatment in all cultivars (Amiga: 7.38 g, Festival: 7.45 g, and San Andreas: 10.30 g) except YFL (7.5%: 7.45 g). In addition, the next lowest MFW in San Andreas (7.92 g) was obtained from 0% lime treatment.

The lime content in the soil and the too low or too high soil pH adversely affected the vast majority of the cultivated species. Especially, the pH demand of the cultivated plant species is around 7 (Gençkan, 1992; Manga et al., 1995; Açıkgöz, 2001; Dungalic et al., 2012; Widyati-Slamet et al., 2012; Schaumberger, 1990; Cutcliffe and Blatt, 1984; Aydeniz and Brohi, 1990; İnal, 1991). Lime is a compound that must be present in agricultural soils in order to provide both pH balance and to meet the Ca needs of plants (Aydeniz, 1985; Aydeniz and Brohi, 1990; İnal, 1991). The results obtained from this study support the above-mentioned studies. In this study, lime content of the growing medium up to a certain level (5%-7.5%) increased fruit yield and quality. Depending on the cultivars, fruit yield and quality (except. YFL) of 5% and 7.5% lime treated strawberry plants were higher than 0% lime treated plants. In this study, the highest yields in Amiga and Festival were obtained from plants subjected to 7.5% lime, while those obtained from 5% lime treated plants in San Andreas. In addition, the height, diameter, and weights of the fruits obtained from the plants with high lime treatment (10% and 12.5%) were higher than those obtained from plants with low lime treatment. This was may be due to the well-fed existing fruits because of the low yield from plants that have been treated with high (especially 12.5%) lime. On the other hand, the highest yield in YFL was obtained from 0% lime treatment. However, the yield obtained from 7.5% lime treatment

was close to that obtained from 0% lime treatment. According to Cutcliffe and Blatt (1984), increasing the pH level increases the fruit yield and fruit weight in strawberry (Redcoat), while the excessive increase in pH decreases yield and fruit weight. The results obtained from this study similarly increased the yield first but decreased with the increased dose. However, MFW, FL, and FW were high in the high lime treatments in Amiga and Festival which had low fruit load. In San Andreas, the highest values were found in 7.5% lime treatment. This seems to be due to the relatively high yield at high lime treatments. On the other hand, in the YFL, the best values were obtained from different treatments, whereas 5% and 7.5% lime treatment were more effective in terms of fruit characteristics. Besides, the sizes of the fruits obtained from 5% and 7.5% lime treatments in all cultivars (except in FW in YFL) were higher than the fruits obtained from 0% lime treated plants.

Conclusions

In this study, San Andreas was the most resistant cultivar to high lime doses among the four cultivars studied. The yield of San Andreas in 10% lime treatment was considerably higher than other cultivars. Moreover, San Andreas has been the most productive cultivar in all treatments except 7.5%. On the other hand, the highest yield of Amiga and Festival was obtained by 7.5% lime treatment. However, the yields of cultivars except San Andreas and Festival have decreased considerably at 10% lime treatment. In 12.5% lime treatment, the yields of all cultivars decreased dramatically.

According to the results, the following conclusions can be drawn. First, it may be beneficial for the soil to contain some lime so that the pH balance can be maintained and the calcium needs of the plants can be met. However, the amount required varies by species and cultivars. Second, the tolerance of strawberry cultivars to different lime levels varies. In this study, the number of cultivars used and the doses of lime were limited. Determining the maximum lime and pH levels that strawberry cultivars can tolerate more extensive studies will contribute to multicultural diversity in agriculture.

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REMOTE SENSING TECHNIQUES FOR RESEARCH AND CLASSIFICATION OF THE PHOSPHORUS AMOUNT REQUIRED FOR SUGAR BEET PLANT

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Abstract

Remote sensing and its terrestrial components provide practical and effective solutions in agricultural applications. Remote sensing in such areas as plant stress, irrigation, fertilization and investigation of chlorophyll content does not only provide a quick solution, but also provides precision agriculture with high accuracy. One of these application areas is to investigate the proper amount of fertilizers for plant health and development. Phosphorus was used as fertilizer in this study. Phosphorus is an element that people, plants and animals must take, however, phosphorus deficiency is a common occurrence. In addition, excessive amounts of phosphorus could be harmful to both plant health and to the ecosystem. Therefore, it is significant to apply the correct amount of phosphorus fertilizer in agriculture. In this study, an agricultural land in Sivas province was divided into three parts and phosphorus fertilizer was introduced at three different doses (0 kg P ha^{-1}, 150 kg P ha^{-1} and 300 kg P ha^{-1}) were, then sugar beet was planted to these areas. After the plants developed, spectroradiometer measurements were made on their leaves to obtain the spectral signatures of the plants in each phosphorus group. Spectroradiometer measurements were performed simultaneously with Sentinel 2 satellite transition. A spectral classification was carried out by resampling the reference spectra from the terrestrial measurements to the Sentinel 2 satellite band intervals. As a result, the growth of plants was monitored and sugar beet, which was treated with different amounts of phosphorus fertilizer, could be detected by using a satellite image.

Keywords: *Remote sensing, Spectral classification, Sugar beet, Phosphorus.*

Introduction

The use of remote sensing technology and its terrestrial components is increasing day by day. The accuracy of remote sensing technology is increasing accordingly along with the development of the technology. Remote sensing provides quick, easy and fast access to information. The areas of use of remote sensing vary in such fields as geology, mining, forestry, hydrology, mapping, construction, military industry, and so on. One of the most frequently used areas of remote sensing is agriculture (Tilling *et al.*, 2007*;* Wei *et al.*, 2008*;* Meggio *et al.*, 2010*;* Ramoelo *et al.*, 2015*;* Caturegli *et al.*, 2015*;* Argyrokastritis *et al.*, 2015). Managing and implementing agricultural policies sensitively is utmost importance for the economy of a country. Managing agriculture agreeable is one of the keys to sustainable development as well. Remote sensing, on the other hand, makes it possible to apply agriculture well due to its technological advantages.

Sugar beet is an important agricultural product grown in Turkey. Turkey ranks 5th in world production of sugar beet in 2008, according to statistics (Topak *et al.*, 2010). Sugar beet has a wide range of uses. It covers a large part of the sugar production in the country and is also used as animal feed. In addition, it is used in the production of alcohol and spirit.

Fertilizer is an extremely important factor for the development of plants. Some elements are limited in soil depending on the structure of it. Therefore, they must be given to the soil as fertilizer. Phosphorus is an element, one of this that plants must absolutely take. However,

excessive amounts of phosphorus can be harmful to both plant and soil. Therefore, applying the right amount of phosphorus fertilizer is extremely important for both plant and ecosystem. In this study, phosphorus fertilization was applied to sugar beet plant in different doses. Then, the development of the sugar beets were monitored according to the different amounts of fertilizer. As a result, sugar beets applied different amounts of fertilizer were detected by spectral classification through satellite image and terrestrial remote sensing measurement data.

Material and Methods

A zone of sugar beet was planted in a field belonging to Ulaş area (figure 1) of Sivas city as a study area, within the scope of the study. It was determined that the level of soil clay, limey and salinity used in the study is low. Valentina was used as a type of sugar beet.

Figure 1. Study area

European Space Agency's Sentinel 2 sensor was utilized as a satellite image (Clevers and Gitelson, 2013). Radiometric and atmospheric corrections were made to remove atmospheric and radiometric defects on the image.

Three different phosphorus fertilizers were applied to the study area: 0 kg P ha^{-1}, 150 kg P ha^{-1} and 300 kg P ha^{-1}. Then, spectroradiometer measurements were made on the leaves when the sugar beets grown in order to obtain end members required for spectral classification. Spectroradiometer measurements reflectance values were then resampled to Sentinel 2 band intervals in order to use them to spectral classification.

Results and Discussion

As a result of spectroradiometer measurements, it was determined in which region of the electromagnetic spectrum of the sugar beet that the phosphorus fertilization was applied in different doses. A steady change is observed between 2000 and 2500 nanometres depending on the amount of phosphorus, but no linearity exists in other regions. As a result of the

spectral classification, different amounts of phosphorus fertilization treated areas could be detected on the satellite image (figure 2). The Sentinel 2 satellite, on the other hand, shows the productivity and usability of agricultural applications in remote sensing. In order to increase the accuracy of the study, it has been shown that it is necessary to integrate the terrestrial remote sensing measurement data with the satellite image.

Because the spatial resolution of the satellite image used in classification is limited, classification result does not overlap with the resultant phosphorus dose in some regions where phosphorus is applied in different amounts. Using a higher resolution satellite image will improve classification accuracy.

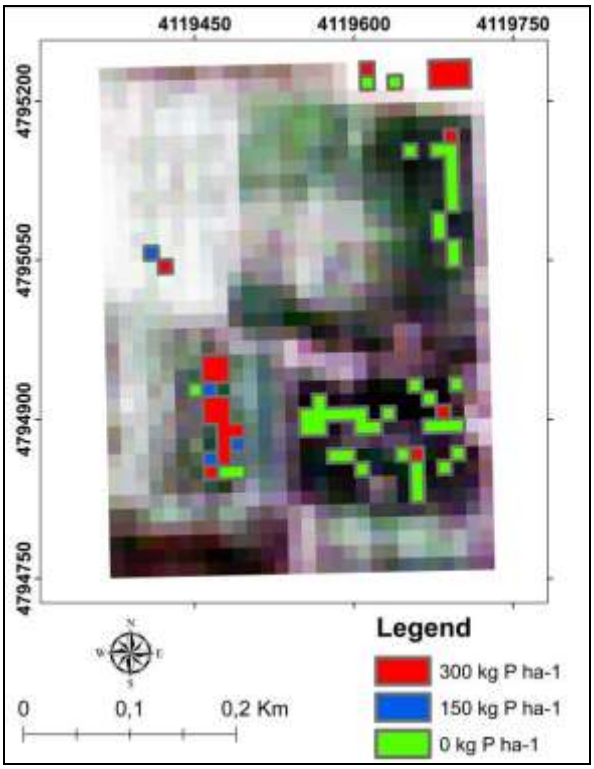

Figure 2. Result of spectral classification on Sentinel 2 image

Conclusions

As a result of the study, it was understood that how fast, practical and accurate results of remote sensing in agricultural applications. It has been seen that the study done is a basis for future work. Fertilizer quantities applied in very large areas can also be detected on the satellite image by applying the technique to different zones. Thus, sustainable development can be achieved while the manageability of sensitive agricultural policies is increased.

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SOME PROPERTIES DETERMINED IN CONFECTIONARY SUNFLOWER PLANT IN PROGRESSIVE SELFING-GENERATIONS

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Abstract

Sunflower (Helianthus annuus L.) is cultivated in many countries of the world due to its high adaptability and is being used for various purposes. It is primarily produced for sunflower, confectionery, ornamental plant, fodder, and other purposes for agriculture in order to obtain oil. As a confectionery production and consumption have an economy that can not be underestimated. In this respect, there is a need to develop varieties of confectionery sunflower. Approximately for 10 years, 36 lines have been selected by single plant selection and have the ability to be combined with each other. In the study, promising lines in the third year of the self-fertilization phase were used. In each line, 10 plants were isolated before flowering and prevented from taking polen from the outside. Results of the study were shared in 2017. Characteristics discussed: self-fertilization rate, plant height, disc diameter, disc shape, full achene, empty achene, achene length and achene width. Significant changes were observed in the plant height and table size in the lines due to depression of the self-fertilization. In addition, deformations were detected in the shape of disc. An increase in the rate of full achene in the subsequent selfing generations has been observed as a positive development.

Keywords: *Sunflower, breeding, confectionery, selection.*

Introduction

Today, sunflower is used to meet the need for a large amount of cooking oil. In some countries, varieties of oil seed sunflowers as well as snack varieties are produced (Lofgren, 1978). Seeds of these varieties are rich in nutrients and are mixed with salt, butter, and honey to make confectionery, added as a condiment on vegetables, fish, meat and salads, also consumed in the form of cookies in roasted or unroasted (Millete, 1974).

Sunflower production in Turkey, Black Sea and Thrace-Marmara, Aegean, Central Anatolia and Çukurova are made. It can grow in dry or wet conditions in almost every region of Turkey. With the adaptation of the sunflower being good, our sowing areas have remained at 500-600 thousand hectares. Turkey confectionary sunflower production is 180 thousand tons and yield is 1550 kg per hectare (Tüik, 2015).

Sunflower should use as appetizer in Turkey, as well as is quite common in the world's different countries, is the most consumed cookies by people in many countries. Sunflower has long been used by humans as a snack and has been used in more than a hundred food types around the world, such as bread, pastry, ice cream, chocolate, cookies etc. sunflower seeds are used internally (Lofgren, 1997). Although the sunflower is described separately as oil and confectionary, the statistics of the confectionary are not given much in the country agricultural organizations (OECD, FAO, ISA etc.). Confectionary sunflower cultivation in the province made economic sense in Turkey; Kahramanmaraş, Elazığ, Ankara, Aksaray, Balıkesir, Bursa, Uşak, Burdur, Yozgat, Kırşehir, Amasya, Çorum, Erzurum, Kayseri, Iğdır, Isparta, Eskişehir and Tekirdağ and Edirne (Tan, 2011). The seeds used are mostly known as open-dusted village populations (Tan, 2011), but in recent years certified varieties have been used. Certified varieties used as seeds have been rehabilitated abroad (Avesa Çrz 2012,

Marker, Confeta CL and Shelly), as well as varieties of domestic confectionery sunflowers that have been improved in our country (Çiğdem 1, Palancı 1, 09 TR Ç 004, Çetinbey ve İnegöl alası).

Generally, according to the physical appearance in the confectionary sunflower grown in the Central and Eastern Anatolia provinces; Alaca, Cyprus, İnegöl and so on. The use of village populations, the majority of which is derived from the unit area due to the cultivation of the vast majority in arid conditions, is much lower than the type of oil used in hybrid varieties (Kaya, 2004). According to most researchers; confectionery sunflower type; low oil content should contain high protein. In addition, low shell ratio and wide grain size are also desirable characteristics of snack types (Kaya at al., 2008; Hladni et al., 2011).

In this study, it is aimed to purify the varieties of confectionery sunflower with the characteristics demanded by the consumer and high adaptation ability. In this way, it will be possible to find out the types that will respond to the needs of the country and to register them or to develop advanced stages. In accordance with this purpose; The breeding trial is ongoing. The results obtained in 2017 are shared between lines that are self-healing. .

Material and Methods

The study was conducted in Central and Eastern Anatolia to purify genotypes collected in 2002 and reduced to 13 by selection over a period of 10 years. In the purification study, the plants of 13 confectionary sunflower genotypes (12, 18, 27, 28, 50, 77, 84, 85, 87, 90, 93, 100, 110) were isolated to prevent pollen from the outside and the plant was self-fertilized. Thus, in addition to the purification of the line, the rate of self-fertilization of lines, which is an important feature for sunflower breeding, has also been determined. 13 lines obtained by single plant selection were sown in May 2017 as a single line. The necessary maintenance procedures (hoeing, irrigation and fertilization) were carried out in accordance with the procedure. Prior to flowering (tabla formation), the plant was forced to fertilize itself by passing 5 herbal isolation bags randomly selected in each order. After the seed formation was completed, the insulation bags were removed. The tables belonging to each line were harvested and necessary measurement and counting processes were performed. The results of 13 lines which have been successful in self-fertilization were evaluated.

In genotypes that achieve self-success;

- self-fertilization rate (%),
- plant height (cm),
- full achene (unit),
- empty achene (unit)
- disc diameter (cm),
- disc shape,
- achene length (mm) and
- achene width (mm) have been examined.

Results and Discussion

The results obtained and the evaluation of the statistics are given in Table 1. When examining 13 lines taken at the stage of self-healing, with the highest rate of self-fertilization belonging to the 77th line with 93.5%. In descending order, 87 lines (92.9%), 84 lines (91.0%) and 93 line (90.2%) were observed. Snow et al (1998) reported; the rate of self-fertilization in sunflower ranges between 15-95%, on average, self-fertilization rate is 68%.

therefore, the lines with a self-fertilization rate below 68% will be disabled. The lines of 12 and 90 which have more than 68% value in themselves have been found promising. When examined in terms of disc diameter; It is determined that the 18th line forms larger tables than

the other lines. The main factor in this study is the selection of lines with high self-fertilization rates. However, the large diameter of the table is important for productivity. On the other hand, the achene length is the reason why consumers prefer it. When all these issues are dealt with, 77 is the front line. However, lines 84 and 87 appear to be good parental candidates for further breeding processes.

Table 1. Evaluating the results and statistics obtained in the study

Line Number	Replications	Full achene (unit)	Empty achene (unit)	Self-fertilization rate (%)	Disc shape	Plant height (cm)	Disc diameter (cm)	Achene length (mm)	Achene width (mm)
12	7	25.7±19.0	8.4±7.1	75.3	Smooth conical	166.4±12.5	19.4±8.1	**16.2±3.4**	7.3±1.8
18	3	41±24.1	1588.6±512.6	2.5	Smooth conical	151.6±7.2	**21.3**±9.2	**16.0±2.5**	5.3±1.7
27	7	73.4±42.3	820.4±389.3	8.2	Smooth conical	163.7±14.1	15.3±5.2	14.9±2.4	6.0±1.1
28	7	**287.2±122.9**	570.1±215.4	33.4	Straight reverse conical	175.4±25.4	15.6±5.1	13.4±3.2	4.5±1.2
50	4	142.5±41.4	451±325.7	24.0	Smooth conical	143.2±7.6	13.1±5.1	13.1±3.4	4.4±1.4
77	3	**252.3 ±120.6**	17.3±9.6	**93.5**	Straight reverse conical	181.2±25.8	18.6±5.0	**16.9±3.3**	4.9±2.1
84	8	157±36.3	143±27.7	**91.0**	Smooth conical	153.4±41.4	13.2±3.8	12.1±4.2	5.0±1.9
85	4	75.2 ±60.4	604±421.5	11.07	Straight reverse conical	205±15.4	14.3±4.4	15.9±2.5	4.7±1.4
87	5	**226±92.3**	17.2±14.6	**92.9**	Smooth	154.8±22.6	8.0±3.2	10.5±3.1	3.9±1.2
90	3	**212.3±75.7**	89±24.3	70.4	Straight reverse conical	180.6±17.6	13.5±3.4	13.8±5.2	4.7±1.7
93	4	**207.2±114.9**	22.2±8.4	**90.2**	straight reverse conical	176.5±6.3	13.8±3.5	15.2±2.5	5.3±1.2
100	3	25.6±20.3	933.6±325.4	2.6	straight reverse conical	178.3±15.4	17±5.4	13.9±2.7	5.3±1.6
110	4	12.2±7.0	46.5±41.6	20.7	smooth conical	118.2±6.9	8.5±4.3	10.0±2.6	4.1±2.2

Conclusion

When examined in terms of disc diameter; lines 12, 18, 77 and 100, in terms of achene length; lines 12, 18, 77 and 85, in terms of the number of full achene; lines 28, 77, 87, 90 and 93 are prominent. On the other hand, in terms of self fertilization rate, which is the most important criterion of this study; the lines 12, 77, 84, 87, 90 and 93 were above the specified level (68%). It has been decided that lines 12, 77, 84, 87, 90 and 93 with overall performance ability should be included in the hybridization program.

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THE EFFECTS OF 2,4-D AND BAP ON *IN VITRO* SOMATIC EMBRYOGENESIS IN QUINOA (*CHENOPODIUM QUINOA* WILLD.)

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Abstract

Chenopodium quinoa Willd. (Quinoa) is a pseudocereal, an annual broad-leaved plant, 1–2 m height with deep penetrating roots and can be cultivated from sea level up to an altitude of 3800 m. The plant shows tolerance to frost, salinity and drought, and has the ability to grow on marginal soil conditions. Also, Quinoa is highly nutritious due to its outstanding protein quality and wide range of minerals and vitamins. The grain protein is rich in amino acids like lysine and methionine that are deficient in many other cereals. Somatic embryogenesis is the main plant tissue culture method used in *in vitro* mass production, synthetic seed production, and gene transformation. The effect of different concentrations of BAP (6-benzylaminopurine)-2,4-D (2,4-dichlorophenoxyaceticacid) on *in vitro* somatic embryogenesis from shoot apex and stem explants of quinoa was studied. The most commonly used auxin type to promote callus formation and somatic embryogenesis was 2,4-D in previous studies. Highest percentage of seed germination (100%) was obtained in full strength MS medium on 5% NaOCl application for 5-10 minutes. Best embryonic callus induction (100%) was observed on MS medium supplemented with 0.5 mg/l 2,4-D from shoot apex explants. The callus which was produced in this treatment was compact and of high quality for somatic embryogenesis. This work can be used to develop the virus-free and saponin level reduced cultivars on quinoa.

Keywords: *Chenopodium quinoa, Quinoa, somatic embryogenesis, 2,4-D, BAP*.

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a dicotyledon, annual, herbaceous plant from the goosefoot (*Chenopodiaceae*) family and adapt well to cold and dry weather conditions. Although quinoa can grow in saline, alkaline, and dry soils, quinoa plants grown in saline soils and dry climate conditions have a high yield, albeit their seeds are rich in saponin. The origin center of quinoa is the Andean Mountains region in South America (Weber, 1978).

Recent advances in *in vitro* culture methods give rise to the possibility of more effective rehabilitation programs. High-yielding, disease-resistant, saponin-free, and lysine-rich quinoa cultivars can be developed using *in vitro* methods. Homozygous plants are also of advantage in obtaining saponin-rich lines, which are used for pharmaceutical purposes. Haploids with homozygous genes are either produced by anthers or stimulated by direct embryogenesis and haploid callus formation (Nitsch, 1977). The use of saponins in quinoa as compounds with pharmacological properties in agriculture, medicine, and industry should not be overlooked.

The number of the studies focusing on the *in vitro* methods applied to the *Chenopodiaceae* family is limited. Somatic embryogenesis is the main plant tissue culture method used in clonal propagation, synthetic seed production, and gene transformation.

This study investigated the effects of the alone and combined applications of 2,4-D and BAP to the explants derived from shoot apices and stems on somatic embryogenesis.

Material and Methods

Limtar Red quinoa cultivar was used as the plant material for research purposes. Seeds, harvested in 2016, were procured from the Central Directorate of Seed Registration and Certification, Ankara, Turkey. The study was carried out in 2016-2017 in the Plant Tissue Culture Laboratory of the Agricultural Biotechnology Department of the Ahi Evran University Agriculture Faculty, Ahi Evran University/Turkey.

In the trials, different ratios and combinations of 2,4-D and BAP was added to MS (Murashige and Skoog, 1962), 3% saccharose, and 0.5% plant agar were used. pH to 5.7±0.1 using either 1N NaOH or 1N HCl, the medium was sterilized 20 minutes by keeping at 121^{o}C under 105 kPa pressure. All cultures were kept under a photoperiod of 16 h and in a climate chamber at 24±1 $^{\circ}$C. In the somatic embryogenesis trials for quinoa, 2,4-D and BAP were used. Stock solutions at 1 mg/l concentration were prepared for all growth regulators used in the study, and different doses of a growth regulator alone and its combinations with other growth regulators were added into the MS medium. For the stock solution of 2,4- D, 1-2 drops of ethyl alcohol (EtOH), for the stock solution of BAP, 1-2 drops of 1 N NaOH were used as dissolvers; then, the solutions were completed using pure water, and were vortexed to obtain homogenous mixtures. The stock solutions were transferred to falcon tubes and kept in a refrigerator at 4°C, and re-prepared when necessary.

The effect of 2,4-D and BAP on somatic embryogenesis from the explants derived from shoot apices and stems

To determine the effect of different 2,4-D concentrations on *in vitro* somatic embryogenesis 0.50, 1.00, 1.50, and 2.00 mg/l concentrations of 2,4-D were used and the growth regulator free medium was used as the control. Shoot apices and stem parts from *in vitro* germinated seedlings were used as explants. Cultures were observed after 14 days for embryonic callus formation. The most commonly used auxin type to promote callus formation and somatic embryogenesis was 2,4-D in several of the previous studies (Burbulis et al., 2007). In a study on somatic embryo culture for quinoa, hypocotyl explants were sown on0.45 mg/l 2,4-D-added MS mediumand callus formation was observed after 2 weeks; somatic embryo development was observed when calluses were also sown on a 2,4-D-free MS medium (Eisa et al., 2004).

In another set of experiments to determine the combined effects of 2,4-D and BAP on *in vitro* somatic embryogenesis of the quinoa seeds, 0.5 mg/l 2,4-D and 0.50, 1.00, 1.50, and 2.00 mg/l concentrations of BAP were used with the growth regulator-free medium as the control. Cultures with shoot apex and stem explants were observed after 14 days.

All trials were carried out following the Randomized Blocks Experimental Design. Each treatment was repeated three times with magenta vessels (GA-7) containing 5 explants in each. The data were analyzed using the "SPSS for Windows 24" computer software and the differences among means were compared using the Duncan test. Prior the statistical analysis, values in percentages were converted to arcsine values (Snedecor and Cochran 1967).

Results and Discussion

Stem and shoot apex explants were placed in 0.50, 1.00, 1.50, and 2.00 mg/l 2,4-D-containing MS mediums to promote embryonic callus formation, and the statistical analysis of the callus formation ratios was performed and the results were compared to each other. The difference between the applications was significant at the level of 0.05. Results are summerized in Table 1, hundred percent (100%) callus formation was observed in the shoot apex explant in the presence of 0.50 and 1.00 mg/l 2,4-D, while 100% callus formation was observed in the stem explant with 0.50, 1.00, and 2.00 mg/l 2,4-D applications. Calli produced are embryonic and the callus formation in the shoot apices decreased with the increasing doses of 2,4-D.

Table 1. Ratio (%) of callus formation from shoot apex and stem explants in the Limtar Red quinoa cultivar

2,4-D (mg/l)	(%) Callus formation **	
	Shoot apex	Stem
0.50	100.00a	100.00a
1.00	100.00a	100.00a
1.50	93.33b	86.67b
2.00	80.00c	100.00a

** There was a significant difference at the level of 0.05 between the means with different letters in the same column.

Figure 1. The initial stage of somatic embryogenesis in the shoot apex explants of quinoa after 2 weeks

Figure 1 shows the visible condition of the explants kept in different doses of 2,4-D for 2 weeks. After 4 weeks, the calluses were transferred to MSO mediums to continue embryonic callus formation. All embryonic stages were observed in the calluses in these mediums.
Statistical analysis revealed that there is a significant difference in the callus formation ratios in the Limtar Red quinoa cultivars at the level of 0.05. With 40%, highest callus formation in the shoot apex explant was observed in the presence of 0.50 mg/l 2,4-D and 2.00 mg/l BAP, while highest callus formation in the stem explant was observed in the presence of 0.50 mg/l 2,4-D and 1.00 mg/l BAP (Table 2.). The increasing amounts of BAP decreased the callus formation in stem explants. Figure 2. shows the callus formation from the stem explants of the Limtar Red quinoa cultivar in the 0.50 mg/l 2,4-D and 1.00 mg/l BAP-containing nutrient medium. Hesami and Daneshvar, (2016) investigated the callus formation from the hypocotyl explant derived from quinoa in different MS medium concentrations and observed the highest callus formation as 93.33% in the 0.5 mg/l 2,4-D and 0.05 mg/l BAP-added MS medium.

Table 2. Callus formation ratio in the shoot apex and stem explants of the Limtar Red quinoa cultivar in 0.50 mg/l 2,4-D and different doses of BAP (%)

Treatment		(%) Callus formation*	
2,4-D (mg/l)	BAP (mg/l)	Shoot apex	Stem
0.50	0.50	13.33a	6.67b
0.50	1.00	6.67b	40.00a
0.50	1.50	26.70a	6.67b
0.50	2.00	40.00a	6.67b

* There was a significant difference at the level of 0.05 between the means with different letters in the same column.

Figure 2. Callus formation from the stem explants of the Limtar Red quinoa cultivar in the presence of 0.50 mg/l 2,4-D and 1.00 mg/l BAP in MS medium

The co-examination of the data and Table 1 showed that although the use of 0.5 mg/l 2,4-D alone promoted 100% callus formation both in the shoot apex explant and in the stem explant, the combined use of 2,4-D with BAP resulted in a decrease in the callus formation ratios in both explant sources.

Conclusion

The most commonly used auxin to promote embryogenesis is 2,4- dichlorophenoxyacetic acid, which is usually added into 2,4-D nutrient medium at ratiosranging from 0.5 mg/l to 2.0 mg/l. If somatic cells are cultured in 2,4-D-containing medium in the first culture stage, and then, transferred to a new auxin-free medium, they develop the ability to produce embryos. With 100%, the highest callus formations in the shoot apex and stem explants were observed in the control group, and in0.50 mg/l and 1.00 mg/l doses of 2,4-D. After 45 days in the 0.5 mg/l 2,4-D and 1.00 mg/l BAP-added culture, callus formation and red coloring were observed in stem explants, which led to the conclusion that BAP addition at high concentrations caused color changes, and coloring in red tones was attributed to saponin.

Scientific knowledge on quinoa species is still insufficient, albeit widespread popularity of quinoa among consumers. Obtaining clones with low saponin content is an important

rehabilitation goal, and developing cultivars that will not regain their saponin production capacities should be emphasized. This can be obtained through the improvement of genetic properties. The results of this study will serve as an important foundation for this goal.

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EFFECTS OF ROW SPACING AND SEEDING RATES ON SEED YIELD AND QUALITY OF BIRDSFOOT TREFOIL (*Lotus corniculatus* L.) İN CENTRAL BLACK SEA REGION OF TURKEY

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Abstract

Birdsfoot trefoil a series of abiotic stress adaptation is an important component of grassland ecosystems and natural life, apart from producing feed in restricted areas in the direction of environment. One of the solutions for generalize the birdsfoot trefoil breeding is to produce quality seeds in sufficient quantity. This research was conducted to determine the effects of various row spacing (20,40,60 and 80cm) and seeding rate (5,10,15 and 20 kgha-1) on birdsfoot trefoil (cultivar candidate) seed yield and quality components. The experiment was conducted at the Tokat Agricultural Research Institute, using a randomized complete block split-plot design with three replications in 2016-2017 growing season. Research result; while the row spacing and seed amount applications had significant influences on the seed yield and the weight of thousand seeds for the first harvest and on the germination rate for the second, they didn't have a statistically relevant effect on other parameters. The interaction between row spacing and seed rate had no correlation except for the seed yield performance of the first harvest. Seed yield was to increase with narrowest spacing and increased seed rate. Highest yield for both harvests (226.1 and 85.4 kgha-1 respectively) were achieved with 40 cm row spacing and 10 and 15 kgha-1[1] seed amounts. Highest weight of 1000 seeds performance was achieved with 40 cm row spacing with 5-10 kgha-1 seed rate, while the highest germination rate was found to occur in 40-60 cm row spacing with 10 kgha^{-1} seed rate.

Keywords: *Birdsfoot trefoil, Sowing frequency, Thousand seed weight, Seed germination,*

Introduction

Birdsfoot trefoil *(Lotus corniculatus* L.), is a short to medium-lived, perennial special legume plant. Birdsfoot trefoil is with a high protein content (15-28% DM) and is a valuable forage that can be grazed or cut for hay or silage, and does not cause bloat in ruminants. The condensed tannins in birdsfoot trefoil elicit reductions in CH4 emissions and urinary N in dairy cattle (Williams et al., 2011). These tannins Unlike other legumes prevent bloat (by reducing methane and ammonia emissions), together with anthelminthic activity in ruminants (Hannaway et al., 2004; Waghorn, 2008). It is also helpful for erosion control, revegetation and as a wildlife habitat (Bush, 2002; FAO, 2014). Its flowers produce abundant and concentrated nectar and it is considered a valuable honey plant (Canpolin, 2012). Birdsfoot trefoil is an N-fixing legume, takes significant place in sustainable agricultural and organic production (Tomić, 2007). It is tolerant of wet acidic soil (pH = 4.5) and has some drought tolerance. Birdsfoot trefoil can withstand some soil salinity. It thrives in places where alfalfa and other forage legumes cannot grow because of soil acidity and moisture (FAO, 2014; Hannaway et al., 2004).

Potential seed yield of the plant is estimated at 1200 kg ha-1, while the average yields on the global level are below 200 kg ha-1 (Turkington et Franco, 1980; Gullien, 2007).

Regarding the seed cultivation performance of the birdsfoot trefoil, (Winch et al.1985) report that 1000 seed weight for the plant is between 0,78-1,26 grams, while seed germination rate

varies between 43-80%. Stevovich et al. (2013) have reported that the Rocco type of the foil has increased seed yield compared to the K-37 and Zora types (408 kg ha^{-1} against 85 kg ha^{-1} and 54 kg ha^{-1}, respectively), while the 1000 seed weight for the plant varies between 1.22-1.24 grams. Pankiw et al. (1977) have achieved the highest seed yield for three consecutive years (537 kg/ha) using 15 cm row spacing and 4.5-9 kg/ha seed rates (for 443-456 kg/ha yield, respectively). Gatarić et al. (1990), have achieved the highest seed yield with 60 cm row spacing and 12 kg/ha seed rate (1^{st} part 311 kg/ha, 2^{nd} part 192 kg/ha). In his studies, (Petrović, 2011) has found that the lowest seed yield (97.1 kg/ha) was obtained with 12.5 cm row spacing, while the highest (155.9 kg/ha) was achieved with 25 and 50 cm rows (155.7 kg/ha) using the highest seed rate norm available (8kg/ha). Ayres et al. (2008), on the other hand, report 1000 seed weight for the plant between 0.72-1.17 grams.

The difficulty of obtaining consistently high yields of seeds has limited the use of the bird's foot trefoil significantly: the beans are easily crumbled and the seeds often spill over and are lost (Fairey, 1994). Significant losses occur during the seed harvesting of bird's foot trefoil, causing a varied seed yield of up to 85% (Winch et al., 1985). To test the plant's seed yield, McGrew et al. (1986) have conducted a research in three separate locations and have detected that genotype and environment had significant interactions between each other. For that reason, the researchers have concluded that the seed yield tests have to be conducted in the location where the plant is commercially cultivated. Steiner et al. (1995) have reported that the seed yield of perennial leguminous plants is determined primarily by the plant's genetic basis, which was followed by environment conditions, initial harvest time, presence of insects & pollinators, and the interaction between genotype and environment.

The most important condition for more widespread birdsfoot trefoil cultivation is to be able to obtain proper amounts of high-quality seeds(Petrovic 2011). It is also known that plant spacing has significant effects on seed yield and quality. Very high or very low plant spacing may cause adverse effects like diseases and increased pressure from other plants. Many cultivators are seeking production guidelines that will help them create production programs to maximize their yields and to achieve agricultural and commercial success (Ekiz et al.2009). Regarding the effects of the plant population density on the birdsfoot trefoil seed production and quality is little information exists on for our country. Furthermore, since the plant is perennial, determining the optimum density is a complicated process.

This study aims to determine the optimum row spacing and seeding rate in order to obtain maximized high-quality seed yield and net gains, and to gain insight on the effects of plant population on the seed yield and quality components of birdsfoot trefoil. The results obtained from this study can be used as scientific and practical guidelines to increase birdsfoot trefoil cultivation and to improve high-quality roughage production.

Material and Methods

The research took place under the agro-ecologic conditions of Central Black Sea Region of Turkey within the province of Tokat, in the Central Black Sea Transition Zone Agricultural Research Institute fields, during the 2016-2017 cultivation periods. The coordinates for the research field are 40° 18' latitude and 36° 34' longitude and has an altitude of 585 meters.

The soil analysis of the field has revealed the following properties: the soil had medium-heavy (clayey-loamy) nature, salinity was almost non-existent (0.028%), pH was slightly alkaline (pH 7.6), and it was poor in plant-consumable phosphor (P: 13.5 kg/ha) and organic materials (N: 9.5 kg/ha). On the other hand, it had medium amounts of lime ($CaCO_3$:77,0 kg/ha), and ample amounts of potassium (K_2O: 812,9 kg/ha).

The regional climate of the Tokat district carries the marks of a transition zone between the steppe climate of the Central Anatolia region and the Black Sea region. There was no significant average temperature variation for the years of 2016 and 2017 in which the study

was conducted, but the year of 2017 had significantly less rainfall during the growth period compared to previous years. The minimum and maximum temperatures were also considerably lower and higher compared to previous years as well, respectively. Table 1 below contains some of the climate data for the study area, spanning the years the study took place.

Table 1. Temperature and rainfall values of the experimental years in and long-term average (1929- 2017) in TOKAT*.

Month	Highest monthly temperature m (°C)			Lowest monthly temperature mean (°C)			Raining monthly averages (mm)		
	2016	2017	Long term	2016	2017	Long term	2016	2017	Long term
January	16.0	14.0	6.1	-18.8	-13.5	-1.7	79.2	53.6	40.9
February	20.6	21.4	8.2	-8.0	-12.2	-0.6	55.2	3.3	33.3
March	25.8	23.6	13.0	-5.6	-4.2	2.4	47.0	27.5	40.5
April	30.9	29.5	19.0	-1.9	-2.7	6.6	18.1	32.6	54.1
May	29.7	32.2	23.5	3.6	0.3	10.0	99.7	66.6	59,3
June	36.4	34.3	26.8	5.3	5.4	13.0	34.1	102.4	38.9
July	37.0	41.8	29.0	8.9	7.5	15.4	22.3	0.0	11.0
August	36.4	39.4	29.7	13.1	12.8	15.6	2.0	0.7	5.6
September	32.8	36.9	26.5	2.4	4.8	12.1	6.7	53.6	17.9
October	30.3	29.3	20.7	-1.7	0.2	8.1	2.1	4.0	39.3
November	26.1	20.4	13.7	-10.7	-6.1	3.3	4.1	31.3	44.0
December	11.8	17,9	7.8	-10.3	- 5,8	0.2	21.0	33.5	47.1
Mean/Total	**27.8**	**28.3**	**18.7**	**-1.9**	**0.6**	**6,4**	**391.5**	**409.1**	**431.9**

*Data source: The General Directorate of State Hidraulic Works. (1929- 2017)

In this study, cultivar candidate improved for birdsfoot trefoil was used as plant material. Genotype is of erect or semi-erect growth type. It has a forage yield of 32.0/51.0 tons/ha under various conditions, while it has a seed yield that varies between 287.0 and 347.0 kg/ha. The crude protein content is around 18.5 to 19.7%, while the Acid detergent fibre (ADF) content is 31.1% and Neutral detergent fibre (NDF) 39.9 % (Karadağ et al.,2016; Çınar et al.2016).

The experiment was conducted at using a randomized complete block split-plot design with three replications. Before the sowing, 200 kg ha- 1 N18P46K0 fertilizer was applied to the soil. Weed control was done hand hoe and mechanically on two occasions. Plants were grown without irrigation. İn research main plot was four rows spacing (20,40, 60 and 80 cm) and sub plots were different seeding rate (5, 10, 15 and 20 kg seed ha-1) were tested.

The seed planting was carried out with a row seeding machine at a depth of 1.0-1.5 cm, and on March 12, 2016.

Seed yield and quality components were determined from the first cut in the year of the second growing season. The following components of quality were determined on the laboratory: seed yield, seed germination rate, and of thousand seed weight. Seed yield was determined during the stage where 70 to 80% of the pods were turning brown. Half of every sub-parcel was harvested twice in 5 to 7 cm height using a parcel harvester (first harvest in 21 July, second in 6 September, 2017). All the harvested plant materials were gathered and laid to rest in an air-dry environment for 4 weeks. The parcels were then blending using a parcel thresher machine. Every seed sample was sifted using 1.0 to 2.0 mm sifters and cleaned using a portable fan set. The obtained seed weights were recorded in kg/ha format.

The seed germination rates (%) were calculated using the internationally accepted methodology (ISTA 1993), where 50 seeds were germinated on petri dishes over germination papers in four iterations in 25-28 ^{0}C for 4-7 days and were counted. 1000 seed weight was determined counting 100 seeds 4 times over (ISTA 1993).
The research data were analyzed by the analysis of variance of the two-factorial trial using JMP software. The significance of differences in mean values was tested by LSD test.

Results and discussion

Seed yield

As can be seen in Table 2, the seed yield increases as the row spacing is reduced and seeding rate is increased, and the difference is statistically relevant. The highest yield was achieved with 40 cm row spacing, while the lowest was achieved with 60 cm. For the different seeding rate applications, the highest yield was achieved with 10.0 kg/ha for both harvests, and in 15.0 kg/ha which is within the same statistical group.

Table 2. Comparing yield component averages and birdsfoot trefoil seed yield and row spacing and seed rate treatments of during experimental year.

Row spacing A	Seed yield (kg ha-1)		Thousand seed weight		Seed germination rate	
	I. cut	II.cut	I. cut	II.cut	I. cut	II.cut
20 cm	200.2b	79.2	0.96	0.70	62.6	71.4c
40 cm	226.1a	85.4	1.08	0.71	68.3	76.0b
60 cm	143.8d	59.7	1.00	0.68	62.6	79.8a
80 cm	168.6c	70.6	0.96	0.70	65.1	72.8c
F-test	**	NS	NS	NS	NS	**
Lsd value	2.19	2.26	0.10	0.01	6.89	3.1
Seeding ratesB						
0,5 kg/da	161.2b	68.5	1.07a	0.69	61.2	74.8
1,0 kg/da	202.9a	78.4	1.00b	0.71	66.0	76.7
1,5 kg/da	201.3a	74.7	0.94c	0.70	65.3	76.6
2,0 kg/da	173.4b	73.3	1.00bc	0.69	66.3	71.8
F test	**	NS	**	NS	NS	NS
Lsd value	1.86	0.97	0.05	0.01	5.15	3.93
CV(%):	**11.9****	**15.6**	**6.6****	**3.1**	**9.4**	**6.2***
AXB	3.72**	ns	ns	ns	ns	ns

Values followed by different letters within columns are significantly different ($P < 0.05$) according to the LSD test *F test signifi cant at $P \leq 0.05$; **F test significant at $P \leq 0.01$; NS = not significant

The lowest seed yield was achieved with 5.0 kg/ha seeding rate. The seed yield values with regards to row spacing – seeding rate interaction varied between 132.4-281.7 kg/ha, while the highest seed yield was achieved in the 40 cm row spacing with 10.0 kg/ha seeding rate, where the yield amount was 281.7 kg/ha.
The results of our study are in compliance with the results of many previous studies (Turkington and Franco, 1980; Gullien, 2007; Fairey and Smith, 1999; Vučkovic et al., 1997).

Thousand-Seed Weight

Thousand-seed weight is an important seed quality component indicating seed size and seed fill. The results of our study show that the row spacing has no statistically relevant influence over the seed weight, while seeding rate was found to be the primary factor for the first harvest and had no effect for the second. The highest average 1000 seed weight for both

harvests (1.08 and 0.71 g, respectively) were achieved with 40 cm row spacing, and with 5.0 kg/ha seeding rate for first harvest, and with 10.0 kg/ha seeding rate for the second (Table 2). While our results are in line with the findings of Ayres et al. (2008), Vinch et al. (1985), and Gatarić et al. (1990), they are lower compared to the findings of Stevovich et al. (2013)

Seed Germination Rate

The row spacing was effective on the germination rate for the 2nd harvest in a statistically relevant manner, while the seeding rate and row spacing and seed rate interaction were not effective for other harvests. Average seed germination rate was lower for the first harvest and was found to be between 62.6-68.3%, while it increased to 71.4-79.8% for the second (Table 2). This change can be attributed to changes in temperature and other environmental conditions and may also be influenced by the seed quality and the time of harvest.

Conclusions

In this study, the data for the second year of cultivation (2017) were evaluated, and it was found that the seed yield increased with the reduced row spacing, and increased seeding rate. This study shows that 20-40 cm row spacing and 10.0 -15.0 kg/ha seeding rate is enough to obtain proper seed yield for the bird's foot trefoil in the Central Black Sea Transition Region agro-ecologic conditions, and conditions that are similar to it. Furthermore, the results of this test also indicate that close row spacing (20-40 cm) has positive effects on weed control.

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INFLUENCE OF GROWTH REGULATORS ON PROPAGATION OF *Zamioculcas zamiifolia* ENGL. – AN INDOOR ORNAMENTAL PLANT

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Abstract

Zamioculcas zamiifolia Engl. (ZZ), native of Eastern Africa, is a stem-less herbaceous monocotyledon belonging to Family Araceae. It produces attractive dark green and glossy foliage with succulent rhizomes at the base. This foliage ornamental is gaining popularity as an indoor plant showing best performance even under reduced light and restricted water. ZZ plants are propagated vegetatively using leaflets. Large scale multiplication is very expensive due to its slow growth and low multiplication rate. The use of growth regulators in the propagation of ZZ plants has been attempted to address the issue. Mature leaflets collected from healthy stock plants were treated with rooting hormones *viz*: IAA, IBA and NAA at 1000, 2000, 3000, 4000 and 5000 ppm. IAA at 2000 ppm was found to initiate rhizomes in 30 days and 30% of the treated leaflets produced plantlets in five months. IBA at 2000 and 3000 ppm also resulted in initiation of rhizome in 30 days. However, the number of plantlets formed was less (12.5%). Early tuberization and shoot initiation occurred in three weeks and subsequently plantlet formation was observed in twelve weeks at 3000 ppm NAA and maximum shoot length of 11.42 cm at 5000 ppm. Among the growth regulators tested, NAA 3000 ppm gave promising results in terms of time taken for initiation of tubers, tuberization and formation of new shoots and its employability in nursery production. The findings of the research are useful for largescale and faster multiplication of ZZ plant in nursery production to meet the demand.

Keywords: *ZZ, propagation, IAA, NAA, IBA.*

Introduction

Zamioculcas zamiifolia Engl. (ZZ), is stem-less herbaceous monocotyledonous plant belonging to family Araceae (Feng,2006; Wong, 2009). The plant is sub-erect, evergreen perennial, native to Eastern Africa. It produces succulent rhizomes at the base of attractive dark green and glossy foliage. (Lopez, *et al.*, 2009). The plant consists of a large tuber and a short, thick underground stem from which compound leaves arise. Above the ground, each compound leaf consists of 4 to 8 pairs of oblong–elliptic, glabrous, coriaceous, slightly succulent leaflets borne on an elongate rachis with a succulent petiole (Mayo *et al.*, 1997). The petiolule of the leaflet is very short and attached to the central rachis.

The plant is commonly known as "Zanzibar Gem" "Fat Boy" "ZZ Plant", "Eternity Plant" or "Golden tree". This indoor foliage plant is 'unique' in its exquisite appearance. It has no apparent limiting insect or disease problems under interior conditions and is extremely tolerant to low light and drought (Chen *et al*., 2002). The ability of the plant to withstand water stress and low light has elevated its horticultural importance at international level. (Chen and Henny, 2003). Since its introduction in the Dutch nurseries during the year 1996 (Horwood, 2007), it has emerged as an important ornamental plant for 'interiorscaping' (Papafotiou and Martini, 2009). *Z. zamiifolia*, is gaining popularity as an indoor plant in India too, for its elite characteristics and performance under reduced light and restricted water.

ZZ plants are propagated vegetatively by leaf cuttings using leaflets (Chen and Henny, 2003). As ZZ plant is slow growing even under favourable production conditions, their productivity is low when leaflets used as propagation material. The bottleneck in its large-scale production is its slow rate of growth and lower rate of multiplication making it very expensive. Methods to enhance rate of multiplication can augment its production and decrease the cost of production. Hence, the present study aimed at use of growth regulators for early tuberization of leaves for faster multiplication.

Material and Methods

Stock plants of *Z. zamiifolia* were maintained in the low cost polyhouse of 'D' Block, GKVK, University of Agricultural Sciences, Bengaluru -560065. Individual leaflets from third and fourth pair of matured leaflets from the base of the petiole were collected from healthy stock plants (Fig 1, & 1a). Cut end of the leaflets were dipped in rooting hormones viz; IAA, IBA and NAA at 1000 ppm, 2000ppm, 3000ppm, 4000ppm, and 5000ppm for 30 seconds and planted in the rooting medium Vermicompost and coirpith in combination in equal proportions (1:1 by volume) used as rooting medium. Totally, there were sixteen treatments, in each treatment, five leaflets were planted in a pot constituting to represent five replications. The pots were regularly watered once in a week till they attained field capacity during the experimental period of 90 days. The observations were recorded on size of rhizomes, number of roots, root length, and number of sprouts at 30th, 60^{th,} and 90th day of plating. The experiment was laid out in Completely Randomized Design and data was analysed using appropriate statistical tools.

Stock plant

Habit

Leaflet - propagule

Results and Discussion

The initiation and formation of rhizome follows a definite pathway in ZZ during vegetative propagation (Fig. 2). Rhizome initiation was noticed at the cut distal end of the leaflet. The white or creamy white inner tissue emerges out which gradually develops into rhizome. As the rhizome develops, roots emerge from the dorsal surface of the newly formed rhizome. Cutter (1962) also reports that the detached leaflets form a tuberous swelling, from where roots and one or more buds emerge, at the base of the leaflet stalk (petiolule).

Leaflet

30 days

60days

90days

Shoot initiation at 90th day

Fig. 2. Tuber formation, root and shoot initiation from growth regulator treated leaflets of *Z. zamiifolia*

Similar regeneration occurs at the proximal end of any cut across midrib and in the absence of the mid rib, at the base of the main lateral veins. It is always associated with vascular tissue. These rhizomes are formed by the downward-movement of food material and its accumulation in the distal end. Engler (1881), reported that growth hormones may be involved in rhizome formation due to biologically active hormones present in the leaflet of *Z. zamiifolia*, with the complete formation of the rhizomes, adventitious shoot buds appear on the dorsal surface of the rhizome.

Table 1. Influence of growth regulators on development of rhizome, root and shoot formation in *Zamioculcas zamiifolia* Engl. (at 90th day)

Treatments			Rhizome diameter (cm)			Number of roots			Root length (cm)			Shoot length (cm)
Growth regulator concentration (ppm)			30th day	60th day	90th day	30th day	60th day	90th day	30th day	60th day	90th day	90th day
T1	IAA	1000	0.34	1.26	2.20	2.60	5.60	8.20	1.40	3.30	5.52	0.00
T2	IAA	2000	0.54	1.04	2.68	2.80	5.80	9.00	2.10	3.24	10.08	0.00
T3	IAA	3000	0.50	1.02	2.62	3.80	4.80	6.20	2.02	2.78	8.44	0.00
T4	IAA	4000	0.56	1.14	2.22	4.00	4.80	5.40	1.76	2.32	3.16	0.00
T5	IAA	5000	0.50	1.16	1.96	3.60	7.20	11.20	1.20	4.10	6.30	0.00
T6	IBA	1000	0.52	0.96	1.36	2.60	4.60	8.20	1.24	5.36	8.86	0.00
T7	IBA	2000	0.62	1.16	1.16	2.80	4.80	8.80	0.94	6.00	14.36	0.00
T8	IBA	3000	0.56	1.26	2.12	3.80	6.00	6.60	0.90	5.22	10.88	0.00
T9	IBA	4000	0.54	0.86	2.26	4.00	5.00	6.00	0.86	5.42	10.54	0.00
T10	IBA	5000	0.54	1.22	2.38	4.00	7.80	11.20	0.76	6.86	13.24	0.00
T11	NAA	1000	1.26	2.14	2.78	2.60	4.80	8.80	1.00	3.80	10.60	8.32
T12	NAA	2000	1.04	1.52	1.82	2.80	3.80	11.20	1.74	4.24	10.42	8.26
T13	NAA	3000	1.02	1.8	1.88	3.80	7.80	8.80	1.72	5.16	8.70	7.50
T14	NAA	4000	1.14	1.86	2.24	4.00	4.80	9.60	1.38	5.12	8.10	7.96
T15	NAA	5000	1.16	1.14	1.78	3.60	7.20	10.80	1.06	5.46	10.60	11.42
T16	Control		0.50	0.7	1.06	0.00	3.20	4.20	0.00	2.72	5.10	3.14
	F test		S	S	S	S	S	S	S	S	S	S
	SEm±		0.093	0.171	0.213	0.429	0.642	0.943	0.184	0.614	1.084	0.582
	CD		0.263	0.483	0.601	1.211	1.815	2.663	0.521	1.735	3.063	1.644

Plant growth regulators have been used to induce roots from stem cuttings in several ornamental plants. However, use of growth regulators for induction of rhizomes or rooting of leaves are limited. In the present study, among the three auxins tried, significantly higher rhizome diameter was recorded at 30th day in leaflets treated with NAA 1000ppm and diameter increased significantly as the days advanced at 60th and 90th day.

Number of roots observed differed significantly at 30th , 60th and 90th day and more roots were produced when treated with IAA 4000 ppm, IBA 4000 & 5000 ppm and NAA 4000 ppm at 30th day. However, at 60th and 90th days significantly more roots had formed when treated with IBA 5000 ppm and NAA 3000 ppm. Similarly, significant difference was also noticed in the length of roots at 30th, 60th and 90th days which was more in IAA 2000 ppm treated leaves at 30th day and IBA 5000 treated leaves at 60th, IBA 3000 at 90th days. Formation of plantlets was noticed at 90th day, only in NAA treated leaflets (Table 1).

Early tuberization and shoot initiation at 30th day and subsequent plantlet formation at 90th day with maximum number of plantlets (100% of leaflets developing into plantlets) was noticed in leaflets treated with 3000 ppm NAA. Further, NAA 5000 ppm resulted in maximum shoot length (11.42 cm). Among the growth regulators tested, NAA 3000 ppm has given better results in terms of time taken for initiation of tubers, tuberization and formation of new shoots.

Seneviratne *et al*. (2013) in their study reported basal leaflets *Z. zamiifolia* without rachis as best cutting for rapid propagation while Lopez *et al*. (2009) reported rapid propagation of *Z. zamiifolia* can be done by apical leaflet cuttings under relatively low light intensity. Single leaflets were also used in the present study after treating them with growth regulators. There are no earlier reports on use of growth regulators to enhance the production of plantlets in *Z. zamiifolia.* The present results proved that use of growth regulators contributed for the development of plants. This has a practical application in largescale production of plants.

Conclusion

The growth regulators employed for the induction of development of rhizome, root and shoot have influenced the plantlet formation at a faster rate. NAA 3000 ppm is very effective in inducing the developmental stages in accelerated manner and formation of plantlets at a shorter period.

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VARIATION OF RYE GENOTYPES FOR SOME MORPHOLOGIC TRAITS VIA BIPLOT METHODOLOGY

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Abstract

Limited information is available on trait relations of rye landraces in world and the objectives of this study were to understand the relationships among traits, and to document trait profile of some rye landraces. Effective interpretation of the data is important at all stages of plant improvement and the genotype by trait (GT) biplot was used for two-way wheat dataset as genotypes with multiple traits. For this propose, 18 rye genotypes with specific characteristics were evaluated in randomized block design with four replications. The GT biplot explained 61% of the total variation (the first two principal components explained 40 and 21%, respectively). The polygon view of GT presented for 11 different traits of rye landraces showed six vertex landraces as genotypes G1, G3, G6, G8, G11 and G13. The landrace G8 had the highest values for most of the measured traits. The genotypes G8 and G7 following to genotypes G3, G18 and G19 could be considered for the developing of desirable progenies in the selection strategy of rye improvement programs. The landraces used in this study were found to be useful sources for genetic variability for future breeding that targets to improve grain yield and other agronomic traits of rye.

Key words: genotype-by-trait, principal components, trait associations

Introduction

The insufficient knowledge of the interrelationship among various characters and the practice of unilateral selection for agronomic characters frequently results in retrograde or less than optimal results in plant breeding program. Correlations between different traits is an aspect which should be kept in mind for better planning of selection programs because they show relationships among independent characters and the degree of linear relation between these characters (Seibel and Weiper, 2001). Seed yield is a quantitative trait, which is largely influenced by the environment and the response to direct selection for seed yield may be unpredictable and breeders are interested to use good criteria for seed yield. Therefore, there is the need to examine the relationships among various traits, especially between seed yield and other agronomic traits. Genetic studies in winter cereals like wheat and triticale have established that traits like the number of plant per area, the number of seeds per spike and thousand seed weight are the major traits of yield potential and genetic gains through selection based on these components have been realized in practice (Zečević et al. 2004; Dogan and Senyigit, 2016). Bhutta (2006) has concluded that the number of seeds per spike, the number of plant per area and spike length correlate with seed yield per plant and according to Koutis et al. (2012) seed yield correlates with plant height, the number of plant per area and thousand seed weight.

According to Del-Blanco et al. (2001) some yield components such as the number of plant per area, the number of seeds per spike and thousand seed weight have significant contribution to seed yield. It has been reported that the number of plant per plant, plant height, thousand seed weight and spike length have significant correlation with seed yield (9). Effective interpretation of the data on breeding programs is important at all stages of plant improvement, particularly when it is only possible to select yield components. Several

methods have been used in exploration for an understanding of the data structure which may differ in overall appropriateness; different methods usually lead to the similar conclusions for a given dataset. Yan et al. (2000) have developed a genotype main effect (G) plus genotype by environment (GE) biplot methodology for the graphical analysis of multi-environment trial data. A biplot is a plot that simultaneously displays the effects of genotypes and the environment and the GGE refers to G main effect plus GE interaction effect. The GGE biplot can also be used for all types of two-way dataset such as genotypes with multiple traits. Yan and Rajcan (2002) have used a genotype by trait (GT) biplot, which is an application of the GGE biplot to study the genotype by trait data.

The present study was performed to study the interrelationship of rye yield components and their contribution to seed yield using GT biplot technique. The information so derived could be employed in reaching further breeding strategies and selection of methods to develop new high yielding rye genotypes.

Materials and methods

The experimental field design (Maragheh, Iran) was a randomized complete block design (RCBD) arrangement with four replications. The experiment included 18 rye genotypes, which represent a range of phenotypic variation in maturity, adaptation zone, yield potential and date of release. The small samples of experimental seed materials were obtained from Genebank Department of Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany. These samples were propagated in growing season 2014 and their name and origin are given. Tillage of all plots was performed prior to sowing date and fertility was constrained by low organic matter and phosphorus contents.

The fertilizer application was performed before sowing, 50 kg $ha^{-1}$of N, 25 kg ha^{-1} of P and 15 kg ha^{-1} of K were spread on the surface and tilled into the soil and the weeds were controlled chemically by MCPA (2-methyl-4-chlorophenoxyacetic acid). Plant density was 250–300 plants m^{-2}. Plant height (PH), spike length (SL), awn length (AL), seed number per spike (SNS), first internode weight (FIW), spike weight (SW), flag leaf length (FLL), and number of spike per area (NSA) were measured based on ten guarded plants, which were randomly selected from each plot. Also, thousand seed weight (TSW) and seed yield (SY) of each plot were measured and harvest index (HI) were computed based on ratio of SY to biological yield. The genotype × trait (GT) biplot was used to show the two-way pattern of rye genotypes' traits in a biplot. Because the traits were measured in different units, the biplots were generated using the standardized values of the trait means using GGE biplot software (Yan, 2001).

Results and discussion

The GT biplot explained 61% (40 and 21%, for the first two principal components, respectively) of the total variation of the standardized data and this relatively moderate percentage reflects the complexity of the relationships among the measured traits. The polygon view of the GT biplot helps identify genotypes with the highest values for one or more traits and provides the best way for visualizing the interaction patterns between genotypes and traits and to effectively interpret a biplot. For this purpose, the genotypes that are connected with straight lines so that a polygon is formed with all other traits contained within the polygon. The Fig. 1, as polygon view, presents data of 18 rye genotypes in eleven traits and the following information can be understood: the vertex genotypes in this investigation are G1, G3, G6, G8, G11 and G13. These genotypes are the best or the poorest genotypes in some or all of the traits since they had the longest distance from the origin of biplot. Therefore, genotype G8 had the highest values for seed number per spike (SNS), first internode weight (FIW), plant height (PH), spike weight (SW) and thousand seed weight

(TSW) traits while genotype G3 had the highest values for spike length (SL) and awn length (AL). Genotype G11 had the highest values for number of spike per area (NSA), seed yield (SY) and harvest index (HI) while genotype G3 had the highest values for flag leaf length (FLL). The vertex genotype and the other genotypes which fell in related sector had good amounts of the above mentioned traits. The other vertex cultivars (G1 and G13) and related sectors were not suitable performance for the measured traits (Fig. 1). Different vertex genotypes and their related genotypes which are located in different sections of biplot are good candidates for examination heterosis (Yan et al. 2007) for hybrid production in rye using these genotypes or pure lines. Also, genotype G11 could be used for improving seed yield in breeding program.

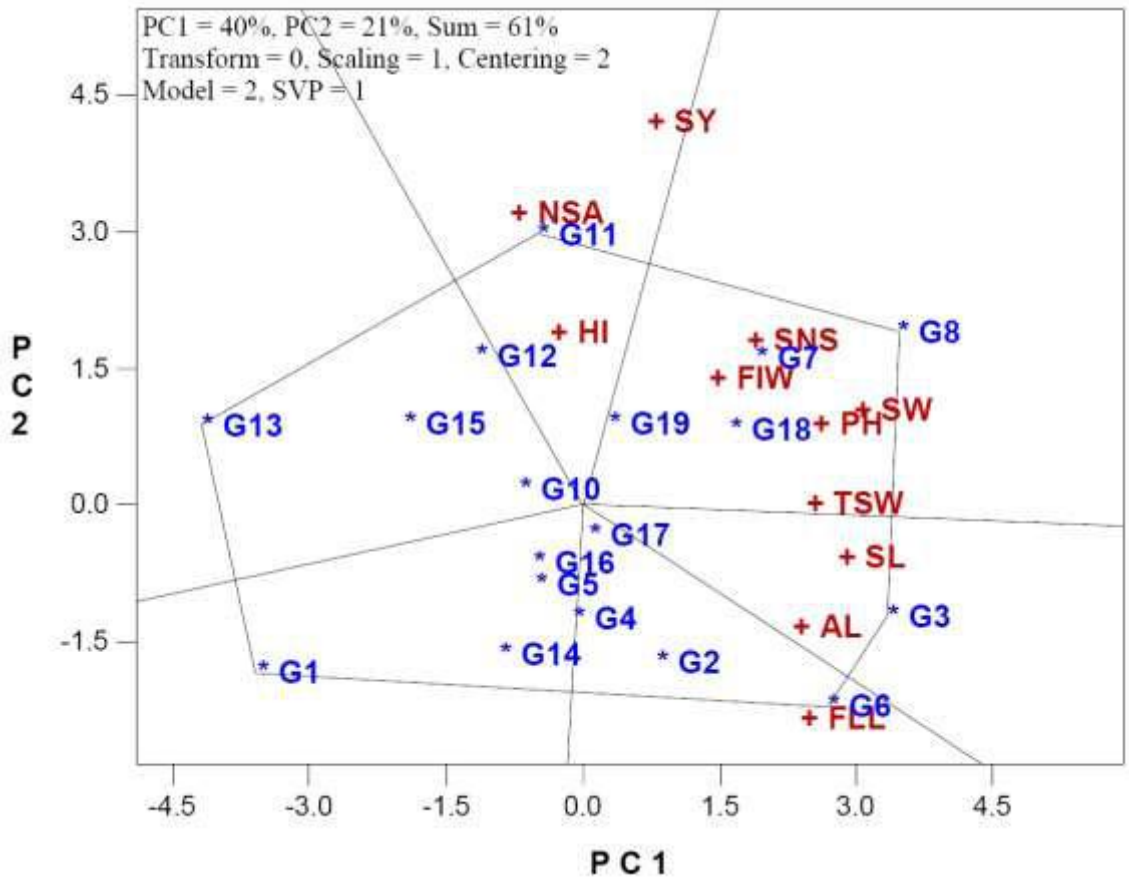

Fig. 1. Polygon view genotype by trait biplot, showing which genotype had the highest values for which traits for 18 different rye genotypes

Provided that the GT biplot model described relatively a sufficient amount of the total variation, the correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors (Yan and Kang, 2003). The most prominent relations by vector-view biplot at the first year (Fig. 2) are: a strong positive association between SW and PH; between TSW and SL; between FIW and SNS; between AL and FLL; and between HI and NSA; as indicated by the small obtuse angles between their vectors. There was a near zero correlation between FLL with FIW and SNS, between SY with TSW and SL, and between PH and SW with NAS and HI (Fig. 2) as indicated by the near perpendicular vectors. There was a negative correlation between FLL with NAS and HI as indicated by the near an angle of approximately 180 degrees. Some above discrepancies of the biplot predictions and original data were expected because the biplot accounted for the total variation. Potential sources of genetic variation are important to allow rye breeders to deal with changing

environmental condition and large collections of genetic stocks do not exist for rye and this point is best illustrated by the fact that in the past decade a few high yielding cultivars have developed. While large collections of genetic stocks do not exist, there are some local landraces available to breeders but variation exists for most traits have not been investigated. The data obtained in this study could be useful for rye breeders in efforts to increase yield. The correlation coefficients between the seed yield and yield components showed good variation, and the results suggest that the seed number per spike, first internode weight, number of spike per area and harvest index are the primary selection criteria for higher seed yield in rye.

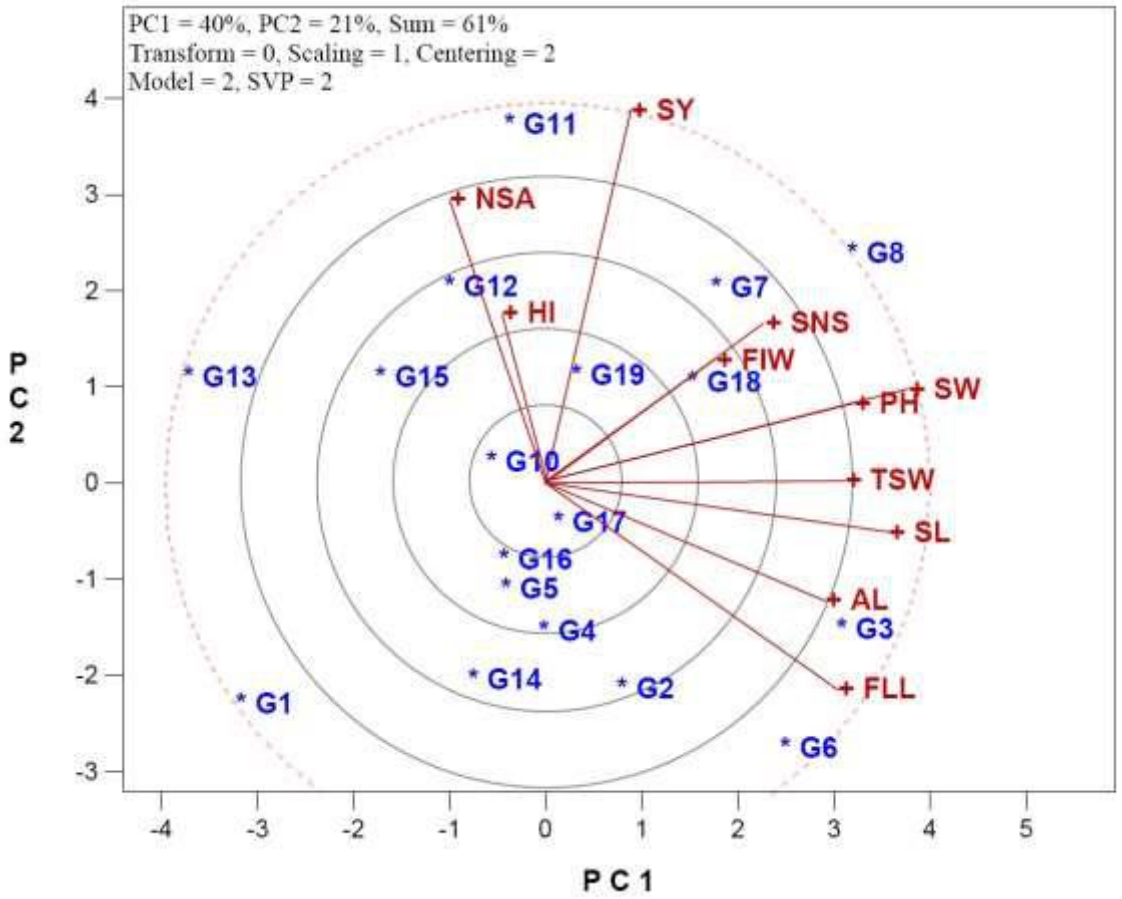

Fig. 2. Vector view genotype by trait biplot, showing the interrelationship among all measured traits for 18 different rye genotypes

The GT biplot method is an excellent tool for visual genotype by trait data analysis because it is a powerful tool and can be used to graphically address research questions (Yan, 2014). Compared with conventional methods, the GT biplot approach has some advantages: (i) graphical presentation of the data, which greatly enhances our ability to understand the patterns of the data, (ii) more interpretative and facilitates pair-wise genotype or trait comparisons and effectively reveals the interrelationships among the rye traits, (iii) it facilitates identification of possible which-won-where patterns or which-lost-where patterns, and (iv) it can be used in independent culling based on multiple traits and in comparing selection strategies. (Yan and Kang, 2003; Yan, 2014). In conclusion, based on the GT biplot, seed number per spike (SNS), first internode weight (FIW), number of spike per area (NSA), and harvest index (HI) were identified as traits suitable for selection for yield improvement in rye. Thus, selecting for these traits is expected to lead to improved yield and this suggests that selection index that incorporates these traits will not only result in the development of high

yielding cultivars but with other desirable agronomic traits. From my observations, it appears possible to improve exotic rye landraces by selecting for genotypes with higher values of the above mentioned traits. Also, almost half of studied genotypes including G3, G7, G6, G11, G17, G18 and G19 are good candidates for improving most of the measured traits due to existence of good genetic variability in this materials.

Conclusions

The biplot is a useful methods for visual genotype by trait data analysis and compared with conventional methods, the biplot has some advantages. The seed number per spike (SNS), first internode weight (FIW), number of spike per area (NSA), and harvest index (HI) were identified as traits suitable for selection for yield improvement in rye. Also, genotypes G3, G7, G6, G11, G17, G18 and G19 are good candidates for developing new cultivars.

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EVALUATION OF EARLY PLUM CULTIVARS IN THE REGION OF BELGRADE (SERBIA)

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Abstract

Phenological traits, yield, and fruit characteristics of nine plum cultivars of early maturation time were studied in the region of Belgrade (Serbia) in the five-year period (2013-2017). The control cultivar for comparison was 'Čačanska Rana'. The average time of flowering of tested cultivars was in the first half of April, and the average duration of flowering varied from 7.4 to 10.4 days. The average time of maturation ranged from June, 22 ('Boranka') to July, 14 ('Minerva'). The average yield per tree was lowest in the control cultivar, 'Čačanska Rana' (8.4 kg) and highest in the cultivar 'Dalikatnaya' (25.5 kg). Compared to control, significantly higher yield was achieved in four cultivars: 'Dalikatnaya', 'Opal', 'Minerva', and 'Katinka'. The lowest vigor was recorded in the cultivar 'Katinka', and the highest in the cultivar 'Opal'. The average fruit weight ranged from 22.9 g in the cultivar 'Katinka' to 55.9 g in the cultivar 'Čačanska Rana'. Compared to control, fruit weight was significantly lower in all cultivars except 'California Blue'. Soluble solids content was lowest in the cultivar 'Boranka' and highest in cultivars 'Opal', 'Herman' and 'Minerva'. The best rated cultivars for fruit appearance were 'Čačanska Rana' and 'California Blue', while cultivars 'Opal' and 'Katinka' had the best scores for taste.

Key words: *Prunus domestica, flowering, maturation, yield, fruit characteristics*

Introduction

Plum (*Prunus domestica* L.) is the most important fruit species in Serbia. The average annual production of 403.060 t in the period of 2014-2016 ranks Serbia on the third place in the world, after China and Romania (FAOSTAT, 2018). The assortment of plum cultivars is dominated by those intended for processing. The most of produced plum fruits is processed into brandy (more than 70%), while much smaller amounts are dried, frozen, and processed into other products. Fresh consumption of plums is quite small. The highest profit in plum production is gained by growing table cultivars, especially that of early maturing time.The most important cultivars for fresh consumption are 'Čačanska Lepotica' and 'Čačanska Rana'. In recent years, there is a tendency to increase export of fresh fruits, mostly to Russia (Milatović, 2013). A lot of work has been done in the world on creation of new European plum cultivars with improved characteristics, such as better adaptation to different environmental conditions, increased disease resistance, self-fertility, higher yield and better fruit quality. In the last 20 years more than 170 new plum cultivars were released in Europe (Butac et al., 2013). The introduction of new foreign cultivars and their study in Serbian climatic and soil conditions allow better choice of cultivars, and may improve the production of plums. The aim of this study was to evaluate phenological traits, yield and fruit characteristics of European plum cultivars of early maturation time. The best performing cultivars will be recommended for growing in the region of Belgrade, as well as in other regions with similar environmental conditions.

Material and Methods

The study was conducted in the plum collection orchard at the Experimental Station "Radmilovac" of the Faculty of Agriculture in Belgrade during the period of five years (2013–2017). The orchard was planted in 2009. The rootstock is Myrobalan (*Prunus cerasifera* Ehrh.) seedling, training system is central leader, and planting distance is 4.5 m × 3 m. The study included eight plum cultivars: 'Boranka' (from Serbia), 'Dalikatnaya' (Belarus), 'Herman' (Sweden), 'California Blue' (USA), 'Katinka' (Germany), 'Minerva' (Romania), 'Opal' (Sweden) and 'Ruth Gerstetter' (Germany). Control cultivar for comparison was 'Čačanska Rana'. All cultivars are represented by five trees.

Flowering was recorded according to recommendations of the International Working Group for pollination: start of flowering – 10% open flowers, full bloom – 80% open flowers, end of flowering – 90% of the petal fall (Wertheim, 1996). Trunk cross-sectional area (TCSA) was calculated on the basis of trunk circumference measured at the height of 30 cm above the ground level. Cumulative yield efficiency was calculated by dividing the cumulative yield over five years by TCSA in the last year (2017). Fruit characteristics were measured on a sample of 25 fruits per cultivar. Fruit shape index was calculated using the formula: length × length / width × thickness. Soluble solids were determined by refractometer and total acids (expressed as malic acid) by titration with 0.1 N NaOH. Sensory characteristics of the fruit (appearance and taste) were evaluated by a five-member jury, scoring the cultivars using the scale from 1 to 5 points.

The obtained data were statistically analyzed using analysis of variance. The significance of differences between mean values was determined using Duncan's multiple range test at 0.05 level of probability.

Results and Discussion

Average time of flowering of tested cultivars was in the first half of April (Table 1). The earliest start of flowering was recorded in the cultivar 'Dalikatnaya' (March, 30), and the latest in cultivars 'Herman' and 'California Blue' (April, 4). The average difference between cultivars with earliest and latest flowering was six days.

Table 1. Phenological characteristics of plum cultivars (average, 2013–2017).

Cultivar	Flowering dates			Duration of flowering (days)	Abundance of flowering (0-5 scale)	Harvest date
	Start	Full	End			
Čačanska Rana (control)	1 April	4 April	11 April	9.8	3.9	3 July
Boranka	2 April	5 April	11 April	8.8	4.2	22 June
Dalikatnaya	30 March	2 April	9 April	10.4	4.2	13 July
Herman	4 April	6 April	11 April	7.4	4.0	30 June
California Blue	4 April	6 April	11 April	7.6	3.9	5 July
Katinka	2 April	5 April	10 April	8.0	3.7	5 July
Minerva	2 April	5 April	11 April	8.2	3.6	14 July
Opal	2 April	5 April	11 April	9.0	2.7	10 July
Ruth Gerstetter	2 April	5 April	11 April	8.4	4.5	25 June

Among years, the earliest flowering was in 2014, when the average date of the flowering onset for all cultivars was March, 24. The latest flowering was in 2013 when the average date of the flowering onset was April, 17. The difference between years with earliest and latest flowering was 24 days and it was much bigger than the difference between cultivars.

The average duration of flowering ranged from 7.4 days ('Herman') to 10.4 days ('Dalikatnaya'). Among years, the average duration of flowering for all cultivars ranged from

6.8 days in 2015 to 10.6 days in 2016. The most abundant flowering was recorded in cultivar 'Ruth Gerstetter' (score 4.5 on the 0-5 scale). The lowest flowering intensity (score 2.7) was recorded in the cultivar 'Opal'.

The range of fruit maturity was from June 22 ('Boranka') to July 14 ('Minerva'). For most cultivars the earliest fruit maturation was in 2016, and the latest in 2013 or 2014. Difference between years with earliest and latest fruit maturation varied from 4 to 8 days.

Both flowering and fruit maturation of plum cultivars in the region of Belgrade were earlier comparing to Czech Republic (Blažek et al., 2004), Central Bulgaria (Dragoyski et al., 2010), Northern Montenegro (Božović and Jaćimović, 2012) and Poland (Markuszewski and Kopytowski, 2013). These differences can be explained by different environmental conditions between the study regions.

The average yield per tree was lowest in the control cultivar 'Čačanska Rana' (8.4 kg) and highest in the cultivar 'Dalikatnaya' (25.5 kg) (Table 2). Compared to control, significantly higher yield was achieved in four cultivars: 'Dalikatnaya', 'Opal', 'Minerva', and 'Katinka'.

Table 2. Yield, trunk cross-sectional area (TCSA), and cumulative yield efficiency (CYE) of plum cultivars.

Cultivar	Yield (kg per tree)						TCSA	CYE
	2013	2014	2015	2016	2017	Average	(cm^2)	(kg/cm^2)
Čačanska Rana (control)	2.8	12.1	9.8	7.4	9.9	8.4 d	156.5 ab	0.27
Boranka	3.9	7.7	11.9	4.4	19.6	9.5 cd	137.1 bc	0.35
Dalikatnaya	8.7	51.4	14.4	47.4	5.6	25.5 a	163.9 ab	0.78
Herman	4.8	21.3	21.9	11.2	1.6	12.2 bcd	121.1 bc	0.50
California Blue	5.3	14.7	19.3	13.2	21.7	14.8 bcd	119.6 bc	0.62
Katinka	5.9	34.7	4.5	28.1	7.3	16.1 bc	101.5 c	0.79
Minerva	9.7	31.3	9.0	29.8	5.4	17.0 b	131.7 bc	0.65
Opal	8.9	45.5	7.5	26.9	6.2	19.0 ab	186.5 a	0.51
Ruth Gerstetter	2.7	17.2	15.1	3.5	5.3	8.8 d	166.6 ab	0.26

Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at $P \leq 0.05$.

Among studied cultivars, the lowest vigor was found in the cultivar 'Katinka', and the highest in the cultivar 'Opal'. Our results of low vigor and high yield of cultivar 'Katinka' confirm previous findings of Blažek and Pišteková (2009).

Cumulative yield efficiency ranged from 0.26 to 0.79 kg/cm^2. Cultivars 'Katinka' and 'Dalikatnaya' stands out for high cumulative yield efficiency. On the other side, cultivars 'Ruth Gerstetter', 'Čačanska Rana' and 'Boranka' are characterized by low yield efficiency.

Fruit weight ranged from 22.9 g in the cultivar 'Katinka' to 55.9 g in the cultivar 'Čačanska Rana' (Table 3). Compared to control, fruit weight was significantly lower in all cultivars except 'California Blue'.

Stone weight ranged from 1.12 g ('Herman') to 2.80 g ('Čačanska Rana'), and its share in the fruit weight ranged from 3.0% ('California Blue') to 5.2% ('Katinka'). Significant differences were found between cultivars for fruit dimensions. Fruit length was highest in the control cultivar ('Čačanska Rana'), whereas width and thickness were highest in the cultivar 'California Blue'. Based on the fruit dimensions the shape index was calculated, whose values ranged from 1.03 in 'California Blue' (round shape) to 1.83 in 'Čačanska Rana' (elliptic shape). Stalk length was shortest in 'Herman' (0.8 cm), and longest in the control cultivar, 'Čačanska Rana' (2.1 cm).

Results of fruit characteristics are in accordance with the previous findings for some cultivars (Hodun et al., 1998; Blažek et al., 2004; Ogašanović et al, 2007; Milatović et al., 2011; Milošević and Milošević, 2011; Glišić et al., 2015).

Table 3. Fruit characteristics of plum cultivars (average, 2013–2017).

Cultivar	Fruit weight (g)	Stone weight (g)	Stone share (%)	Fruit dimensions (cm)			Shape index	Stalk length (cm)
				Length	Width	Thickness		
Čačanska Rana (control)	55.9 a	2.80 a	5.0	5.2 a	4.2 a	3.6 bc	1.83	2.1 a
Boranka	34.8 c	1.38 cd	4.0	3.9 cd	3.7 bc	3.7 bc	1.08	0.9 fg
Dalikatnaya	45.3 b	2.28 b	5.0	4.2 bc	4.0 ab	4.0 ab	1.07	1.8 b
Herman	30.0 cd	1.12 d	3.7	3.6 d	3.3 de	3.3 cd	1.15	0.8 g
California Blue	52.4 ab	1.60 c	3.0	4.3 b	4.3 a	4.3 a	1.03	1.5 ed
Katinka	22.9 d	1.18 d	5.2	3.8 cd	3.1 e	3.1 d	1.53	1.3 de
Minerva	33.9 c	1.29 cd	3.8	4.2 bc	3.5 cde	3.6 bc	1.42	1.1 ef
Opal	31.7 c	1.33 cd	4.2	3.8 cd	3.6 cd	3.5 cd	1.15	1.6 bc
Ruth Gerstetter	29.1 cd	1.14 d	3.9	3.7 d	3.5 cde	3.4 cd	1.15	1.0 efg

Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at P≤0.05.

Cultivars 'Opal', 'Herman' and 'Minerva' are characterized by high soluble solids content (15.6-16.0%). On the other hand, lowest soluble solids content (12.8%), significantly lower than in previous cultivars, was found in the earliest maturing cultivar - 'Boranka' (Table 4). Cultivar 'Dalikatnaya' is characterized by high total acids content (1.94%), that was significantly higher than in all other cultivars.

Table 4. Indicators of fruit quality of plum cultivars (average, 2013–2017).

Cultivar	Soluble solids (%)	Total acids (%)	Soluble solids /Total acids	Sensory evaluation (1-5 scale)	
				Appearance	Taste
Čačanska Rana (control)	13.8 ab	1.13 b	12.2	4.6 a	3.7 ab
Boranka	12.8 b	1.06 bc	12.1	3.2 c	3.1 b
Dalikatnaya	15.2 ab	1.94 a	7.8	3.7 bc	3.6 ab
Herman	15.8 a	0.88 bc	17.9	3.7 bc	3.6 ab
California Blue	14.7 ab	1.14 b	12.9	4.2 ab	3.4 b
Katinka	14.8 ab	0.84 c	17.6	3.6 c	4.1 a
Minerva	15.6 a	0.95 bc	16.4	3.5 c	3.3 b
Opal	16.0 a	1.09 bc	14.7	3.3 c	4.2 a
Ruth Gerstetter	14.6 ab	1.02 bc	14.3	3.8 bc	3.6 b

Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at *P≤0.05*.

The ratio between contents of soluble solids and total acids (SSC/TA), rather than the very content of soluble solids, represents a reliable indicator of a cultivar's suitability for acceptance by consumers (Crisosto et al., 2004). Cultivars 'Herman' and 'Katinka' are characterized by high SSC/TA ratio (17.6-17.9).

The data on the chemical composition of fruits are in good agreement with most of the previous findings (Bohačenko et al., 2010; Milatović et al., 2011; Glišić et al., 2015).

The best rated cultivars for fruit appearance were 'Čačanska Rana' and 'California Blue', while cultivars 'Opal' and 'Katinka' were best scored for taste. Our results of good taste for cultivar 'Katinka' are in accordance with those of Hartmann (1998) and for cultivar 'Opal' with those of Kemp and Wustenberghs (1998).

Conclusion

Control cultivar, 'Čačanska Rana' is characterized by the best fruit appearance, especially by large fruit size. All other cultivars had significantly smaller fruit size, except 'California Blue'. However, productivity of 'Čačanska Rana' cultivar is low. Significantly higher yield was found in four cultivars: 'Dalikatnaya', 'Opal', 'Minerva', and 'Katinka'. Besides, cultivars 'Opal' and 'Katinka' had the best scores for taste.

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MORPHOLOGICAL DIFFERENCES AMONG STRAINS OF OYSTER MUSHROOM GROWN ON DIFFERENT SUBSTRATES

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Abstract

Distribution of oyster mushroom production worldwide is based on its cultivation on available and low-priced substrates prepared using residues of cellulose materials from agricultural production. It is also based on its nutritive value and content of medically active matters that favourably affect the human organism, as well as the specific taste, which offers wide possibilities in cooking. Forms of oyster mushroom differ by colour, size and shape of the cap, length and width of stems, taste, as well as the physiological requirements during cultivation on different substrates in various climate conditions. Three isolated and determined strains of oyster mushroom (*Pleurotus ostreatus*) were studied - NS 77, NS 355 and NS 244. They were grown on residues of agricultural production most frequent on the territory of Vojvodina, such as wheat and soybean stems, and stalks of maize and sunflower, as individual substrates and in combinations with wheat straw. Variability was recorded among strains in most important morphological traits. The study monitored the ratios of cap weight (CW) and stem weight (SW), cap length (CL), cap width (CWi), stem length (SL) and stem width (SWi), as well as the number of mushrooms per bunch (NM). The monitored morphological traits have been expressed differently most of the time, depending on the strain, while in some cases the substrate was the determining factor. The number of fruiting bodies in strain NS 244 therefore varied in range 4.2-5.5, depending on the substrate. Strain NS 77 ranged from 7.4 to 11.2, while strain NS 355 ranged from 8.6 to 10.7. However, the highest values of cap weight of all three strains were recorded on maize stalk substrate.

Key words: *oyster mushroom, morphological traits, different substrates.*

Introduction

Oyster mushroom is a cosmopolitan plant which grows on all continents. It has long been adapted to different growing environments (Chang and Miles, 2004), and it can be cultivated on any surface which contains residues of cellulose materials from agricultural production - cellulose, hemicellulose, and nitrogen (Diana et al. 2012). It appears in different forms, varied in colour, size, shape and taste. Material included in the substrates prepared for oyster mushroom cultivation depends on the growing area (Bugarski *et al*., 2012). Agricultural waste area, as it is physically more accessible and therefore more cost-effective, is usually the substrate chosen for mushroom cultivation. For the purpose of increased yields and quality, mushroom cultivation substrates have lately been enriched with different supplements. In Spain, Pardo-Giménez et al. (2015) enriched wheat straw with 5, 10 and 15% degreased pistachios. In China, Yang et al. (2013) added cotton seed hulls and bran to wheat straw and rice straw. Mushrooms grown on substrates with a proportional content of cotton seed hulls developed larger caps, with greater cap length and shorter stems. As the quantity of cotton seed hulls in the substrates decreased 40-30-20, cap diameter decreased proportionally, ranging from 9.4 cm, 8.8 cm, to 7.8 cm. Cap weight also decreased from 22.93 g, 19.19 g, to 17.77 g, while stem length increased from 2.6 cm, 1.9 cm, to 3.2 cm.

In Taiwan, Hoa et al. (2015) showed significant differences between the morphological parameters of different species of P. *ostreatus* and P. *cystidiosus* grown on various substrates

(combination of sawdust, sugarcane and corncob). The highest cap length in both mushrooms was obtained if they were grown on corncob 100%, and lowest on sawdust 100%. Besides the effect of the substrate, type of mushroom also has a significant role; the number of obtained fruiting bodies in P. *cystidiosus* therefore ranged from 2.32 on sawdust 100% to 1.85 on substrate formula 50% sawdust +50% sugarcane bagasse, while it ranged from 10.32 on sawdust 100%, to 7.93 on corncob 100% in P. *ostreatus*. Stem length and stem width of oyster mushroom P. *ostreatus* (35.28 -39.21 mm) were lower on all substrates compared to P. *cystidiosus* (46.06 -57.84 mm).

In India, Mondal et al. (2013) enriched rice straw with different quantities of banana leaves, and the number of obtained fruiting bodies varied from 8.5 on banana leaves to 37.25 on sawdust. Stem length of oyster mushrooms grown on substrate formula banana leaves + rice straw 1:1 (2.47 cm) was significantly reduced compared to those grown on sawdust (3.80 cm), while cap diameter was significantly smaller on substrates banana leaves + rice straw 1:3 (4.13 cm), banana leaves + rice straw 3:1 (3.70 cm), and banana leaves + rice straw 1:3 (3.11 cm) compared to sawdust (7.79 cm). Contrary to the above, cap thickness was lower on sawdust in 2. (0.49 cm), and larger on banana leaves + rice straw mixture 3:1 (0.66 cm) and rice straw (0.64 cm).

Kitamoto et al (1995) explained the emergence of more fruiting bodies on some substrates by the higher content of carbohydrates (glucose, fructose and trehalose) in the substrate, while Shah et al. (2004) explained variations in cap weight by the relatively better availability of nitrogen, carbon and minerals from the substrates.

In Vojvodina, wheat straw is mainly used as the substrate for oyster mushroom growing. Wheat straw can be baled automatically after combining and thus made accessible for removal from the growing plots and transport. Since it is used in livestock breeding, it can be obtained throughout the year, so planning its purchase in advance is not necessary. Although soybean straw, corn stalks and sunflower stalks have to be collected and stored, previous studies have shown that they are better substrates than pure wheat straw. We have therefore focused our further studies on examining their effect on mushroom quality.

Material and Methods

Determination of morphological traits of oyster mushroom (*Pleurotus ostreatus*), produced in different substrates during three-year long studies, was conducted under the auspices of Institute of Field and Vegetable Crops Novi Sad, using the strains of *Pleurotus ostreatus* NS 77, *Pleurotus ostreatus* NS 355 and *Pleurotus ostreatus* NS 244 maintained in the collection of the Vegetable Crops Department (Bugarski, *et al*., 2012).

The substrates were prepared individually or with wheat straw (the substrate most often used in oyster mushroom cultivation in Serbia), in the following combinations: 1. wheat straw (S1), 2. wheat straw 50% + soybean straw 50% (S2), 3. wheat straw 50% + corn stalks 50% (S3), 4. wheat straw 50% + sunflower stalks 50% (S4), 5. soybean straw (S5), 6. corn stalks (S6), and 7. sunflower stalks (S7).

In order to obtain morphological traits of strains from each individual sample, three first bunches were taken and total fruiting bodies were counted and weighed individually. Cap weight and stem weight was measured, and cap-stalk ratio was determined. Cap diameters were measured using nonius (vernier) lines from stem root to tip and cross the widest part of the stem, at an angle of 90° to the previous diameter. Stem diameter was measured in the central part, while stem length was measured from the root, along the cap to the point of separation from the substrate.

For the analysis of morphological traits of P. *ostreatus*, principal components (PCA) were visualized so as to reduce the multi-dimensional nature of experimental data. Principal components are orthogonal and synthetic variables obtained as a linear combination of the

optimally-weighted observed variables, whose significance is defined by the regression coefficient (or weight) for observed variable as used in creating a principal component. The total number of components equals the total number of original variables. Since most of the obtained principal components are weighted with non-systematic components or noise to graphically display the results of PCA method, the first two artificial components are used to compute the scores on the principal component. Interpretation of a graphical display of data follows the geometry of a biplot (Kroonenberg, 1995).
Analysis and visualization of data was carried out in the R environment for statistical computing (R Core Team, 2015).

Results and Discussion

In strain NS 77, cap weight (CW) was uniform across all substrates, ranging from 10.08 to 11.24 g (Table 1.), while strain NS 355 on substrate Corn (S6) weighed 17.96 g, which was significantly higher than cap weight (CW) 14.90 g found on substrate wheat+soybean (S2). In strain NS 244, cap weight (CW) 26.17 g on substrate Corn (S6) was significantly higher compared to other substrates. Cap weight (CW) variations across substrates were the highest in strain NS 244 21.32%, compared to strain NS 355 17.03%, and strain NS 77 weighing only 10.32%. Cap weight (CW) was lowest in strain NS 77, and highest in strain NS 244, i.e. double the weight of strain NS 77.
Cap weight (CW) in all three NS strains was significnatly greater than cap weight obtained by Mondal et al. (2010) for oyster mushrooms grown on six different substrates, ranging from 1.77 g on sawdust to 6.41 g on rice straw, and equal to cap weight obtained by Yang et al., (2013) in their analysis of oyster mushroom growth on nine substrates (16.66-25.13 g).

Table 1. Cap weight (CW) and stem weight (SW) in NS strains of P. *ostreatus*

Substrate	Cap weight (CW) (g)			Stem weight (SW) (g)		
	NS 77	NS 355	NS 244	NS 77	NS 355	NS 244
Wheat (S1)	10.82a	15.38ab	21.44ab	3.53ab	4.80ab	5.43ab
Wheat+ soybean (S2)	11.24a	14.90a	21.65a	4.47ab	4.47a	6.24a
Wheat +corn (S3)	11.02a	16.18ab	20.75a	3.33b	5.35ab	6.44a
Wheat +sunflower (S4)	10.29a	16.61ab	20.83ab	4.19ab	4.71ab	3.67c
Soybean (S5)	10.76a	15.74ab	25.16bc	4.52a	4.37a	5.86a
Corn (S6)	11.13a	17.96b	26.17c	3.79ab	5.80b	5.79a
Sunflower (S7)	10.08a	16.58ab	20.59a	3.85ab	5.46ab	4.00cd

Note: a, b, c, d, - Means followed by the same letters within the columns are not significant (Tukey's multiple comparison test)

Stem weight (SW) in strain NS 77 on the substrate soybean (S5) was significantly greater than stem weight on the substrate wheat+corn (S3) (Table 1). Stem weight (SW) of strain NS 355 on substrate Corn (S6) was significantly greater than stem weight on the substrate soybean (S5) and wheat+soybean (S2), while significantly lower in strain NS 244 on wheat+sunflower (S4) and sunflower (S7), compared to other substrates. Stem weight variations according to substrates were the highest in strain NS 244 43.01%, compared to only 24.65% in strain NS 355, and 26.33% in strain NS 77. Stem weight (SW) was the lowest in strain NS 77, while the highest in strain NS 244.
Cap length (CL) (Table 2), in strain NS 77 was significantly lower on wheat+sunflower mixture (S4) compared to soybean (S5) and wheat (S1). In strain NS 355, it was significanly lower on wheat (S1) compared to wheat+corn (S3), whereas in strain NS 244 it was significantly higher on wheat+soybean (S2) compared to soybean (S5) and corn (S6). The

observed cap length (CL) variations, depending on the substrate, were the highest in strain NS 244 7.28%, compared to 7.12% in strain NS 355, and 6.27% in strain NS 77.
Depending on the substrate, cap width (CWi) (Table 2.) in strains NS 77 and NS 244 was uniform, but significantly lower in strain NS 355 on substrate soybean (S5) compared wheat+corn (S3). Cap width (CWi) variations were insignificant across substrates; the highest CWi was observed in strains NS 355, NS 244, and NS 77: 7.79%, 5.45%, and 4.07%, respectively.
Cap length (CL) and cap width (CWi) determined the shape of the cap, which was uniform across all the substrates, but varied depending on the strain; the cap of strain NS 355, especially strain NS 77 was almost round shaped, but an elongated cap was observed in strain NS 244 .

Table 2. Cap length (CL) and cap width (CWi) in NS strains of P. *ostreatus*

	Cap length (CL) (mm)			Cap width (CWi) (mm)		
Substrate	NS 77	NS 355	NS 244	NS 77	NS 355	NS 244
Wheat (S1)	65.83a	77.03a	93.01ab	59.85a	70.05ab	70.15a
Wheat+ soybean (S2)	64.99ab	78.01ab	99.05b	58.95a	69.56ab	73.35a
Wheat +corn (S3)	62.55ab	83.79b	97.33ab	60.41a	74.03b	74.29a
Wheat +sunflower (S4)	61.70b	78.75ab	97.26ab	59.05a	71.27ab	74.02a
Soybean (S5)	66.17a	79.46ab	91.93a	59.46a	68.26a	70.57a
Corn (S6)	65.25ab	79.98ab	91.84a	59.80a	71.25ab	70.82a
Sunflower (S7)	63.65ab	78.53ab	93.06ab	57.95a	69.69ab	71.05a

Note: a, b, - Means followed by the same letters within the columns are not significant (Tukey's multiple comparison test)

Mondal et al. (2010) obtained the results of cap diameters, ranging from 41.3 mm on banana leaves+rice straw (1:1) to 77.9 mm on sawdust, while Tupatkar & Jadhao (2006) obtained cap diameters of 28.38 cm^2 (equal to diameter of 60 mm) on wheat+soybean, 27.80 cm^2 on soybean straw, 27.05 cm^2 on corn stalk and leaves, and 24.50 cm2 on wheat straw, which is at the threshold of obtained NS values. Cap diameters of 63 mm on wheat straw 80%+wheat bran 20% and 104 mm on cotton seed hulls 80%+ wheat bran 20%, obtained by Yang et al. (2013), were the closest to CL and CWi values of NS strains.

Table 3. Stem length (SL) and stem width (SWi) in NS strains of P. *ostreatus*

Substrate	Stem length (SL) (mm)			Stem width (SWi) (mm)		
	NS 77	NS 355	NS 244	NS 77	NS 355	NS 244
Wheat (S1)	52.18a	38.72ab	30.76ab	11.34abc	12.23a	18.00a
Wheat+ soybean (S2)	54.32a	38.99ab	30.32ab	12.25ab	12.07a	21.55b
Wheat +corn (S3)	49.98a	41.88ab	34.82b	9.82c	12.73a	20.35ab
Wheat +sunflower (S4)	54.73a	37.98b	46.01c	13.95a	12.62a	46.99c
Soybean (S5)	54.53a	40.38ab	31.56ab	12.22ab	13.38a	20.54ab
Corn (S6)	56.38a	44.71a	33.52ab	10.59ac	12.68a	19.06ab
Sunflower (S7)	52.97a	40.28ab	27.91a	11.70abc	14.17a	18.40ab

Note: a, b, c, - Means followed by the same letters within the columns are not significant (Tukey's multiple comparison test)

Stem length (SL) (Table 3) in strain NS 77 was uniform on all substrates. It was significantly lower in strain NS 355 on wheat+sunflower (S4) compared to corn (S6), and significantly lower in strain NS 244 on sunflower (S7) compared to wheat+sunflower (S4), although higher in wheat+sunflower (S4) compared to other substrates. Stem length (SL) in strains NS 355

and NS 244 equaled the range of SL from 35.20 mm on substrate 50% sawdust+50% corn straw to 39.21mm on substrate 80% sawdust+20% sugarcane, as obtained by Hoa et al. (2015), while it was significantly higher in strain NS 77. Mondal et al. (2010) reported lower SL values than the values found in NS strains, ranging from 24.7 mm on banana leaves+rice straw mixture (1:1) to 38.0 mm on sawdust. Tupatkar & Jadhao (2006) obtained the SL values of 35.6 mm on wheat+soybean, 32.9 mm on soybean straw, 25.4 mm on corn stalk and leaves, and 22.6 mm on wheat straw. Jonathan et al. (2013) reported SL values within the range of 53-71 mm on sawdust, the closest to the SL value of strain NS 355.

Stem width (SWi) (Table 3) was uniform on all substrates for strain NS 355 (12.07-14.17 mm). Strain NS 77 had significantly lower SWi on wheat+corn mixture (S3) compared to wheat+sunflower (S4), wheat+soybean (S2) and soybean (S5), while significantly higher values were observed on wheat+sunflower mixture (S4) compared to wheat+corn (S3). The obtained SWi values were close to SWi of 8.52 mm on sawdust and 11.06 mm on corn straw, as reported by Hoa et al. (2015). The value of SWi in strain NS 244 was significantly lower on wheat (S1) compared to wheat+soybean (S2) and wheat+sunflower (S4), but significantly higher on wheat+sunflower (S4) compared to other substrates.

The highest SL with the variable SWi was obtained in strain NS 77, where SL was 5.3 times higher than SWi (56.38 x 10.59 mm) on substrate corn (S6), and 3.9 times higher than SWi (54.73 x 13.95 mm) on wheat+sunflower (S4) (Table 3). The lowest SL and highest SWi were observed in strain NS 244, often giving away the impression that the cap is attached to the substrate. On wheat+sunflower mixture (S4), SL was lower than SW (46.01 mm x 46.99 mm), while the stem was uniform in strain NS 355 where SL/SWi ratio ranged from 2.84 (40.28 mm x 14.17 mm) on sunflower (S7) to 3.61 (44.71 mm x 12.68 mm) on corn (S6).

Table 4. The number of fruiting bodies (NM) in NS strains of P. *ostreatus*

Substrate	NS 77	NS 355	NS 244
Wheat (S1)	7.44a	8.96ab	4.93a
Wheat+soybean (S2)	9.44ab	10.67a	4.22a
Wheat+corn (S3)	8.19ab	8.63b	4.70a
Wheat+sunflower (S4)	11.22b	10.56a	5.52a
Soybean (S5)	10.74ab	10.59a	5.04a
Corn (S6)	10.48ab	9.93ab	5.22a
Sunflower (S7)	10.52ab	9.04ab	5.52a

Note: a, b, - Means followed by the same letters within the columns are not significant (Tukey's multiple comparison test)

The number of fruiting bodies was uniform in strain NS 244 (4.22-5.52) (Table 4). In strain NS 77, it was significantly lower on substrate wheat (S1) compared to wheat+sunflower (S4), and significantly lower on wheat+corn mixture (S3) in strain NS 355 compared to wheat+sunflower (S4), wheat+soybean (S2), and soybean (S5). Kitamoto et al. (1995) explained the variation of fruiting bodies on certain substrates by the higher content of glucose, fructose and trehalose in the substrates.

The biplot revealed 72.9% of sample variability in the first dimension, and 14.8% in the second dimension (Fig. 1.). The first dimension separated the substrates which established morphological traits of strain NS 244, from the substrates defining morphological traits of strain NS 77, whereas substrates establishing morphological traits in strain NS 355, as the strain with the lowest variability, were displayed along the first axis located in the coordinate origin of the biplot. Strain NS 244 connected to stem width (SWi) was isolated in the second dimension, while other strains along the axis were located in the coordinate origin of the biplot as the substrates and strains with the lowest variation coefficient.

Cap weight (CW), stem weight (SW), cap length (CL) and cap width (CWi), as well as stem width (SWi) were positively correlated variables, with high values observed in strain NS 244. High values for the mushroom number (NM) were observed in strains NS 77 and NS 355. Positively correlated group of traits - cap weight (CW), stem weight (SW), cap length (CL), cap width (CWi), and stem width (SWi) - were negatively correlated with stem length (SL) and mushroom number (NM).

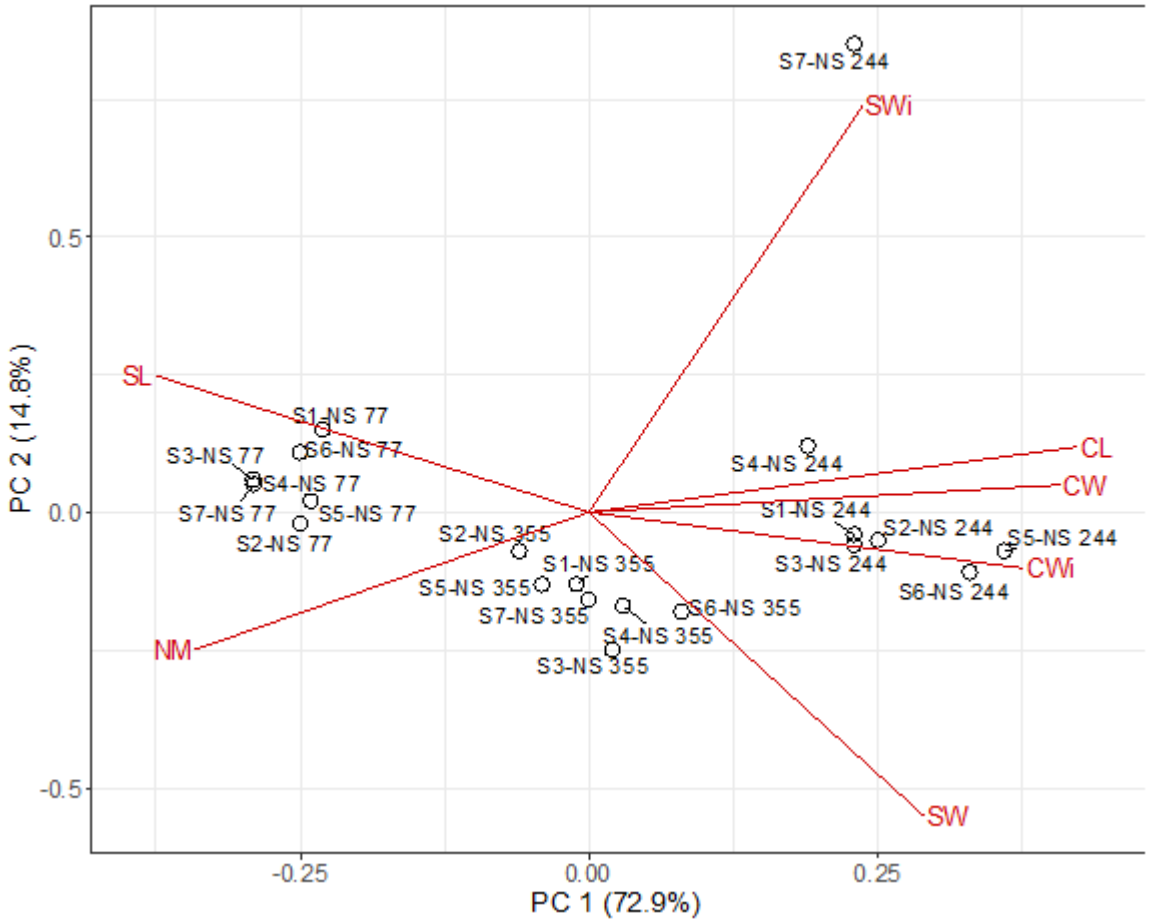

Fig. 1. Biplot display of morphological traits of NS strains of P. *ostreatus*

Conclusions

Comparison of morphological traits of the caps (cap weight CW, cap length CL and cap width CWi) revealed high variability between strains P. *ostreatus* (cap weight CW was doubled in strain NS 244 compared to strain NS 77); across substrates they were strain-specific and not significant.

The obtained results of stem weight (SW) and stem length (SL) revealed high variability between strains (NS 77> NS 355 > NS 244) as well as the results of stem width (SWi) (NS 244> NS 355 > NS 77), depending on the substrate. Soybean substrate (S5) had the highest effect on stem length (SL) in all three strains (NS 77, NS 355, and NS 244); a significant effect of substrate wheat+soybean (S2) was observed in strains NS 77 and NS 244; wheat+corn mixture (S3) significantly affected strains NS 355 and NS 244.

The number of fruiting bodies was uniform in strain NS 244, depending on the substrate, while strains NS 77 and NS 355 were significantly higher in wheat+sunflower (S4).

The biplot indicated a positive correlation between the morphological traits of stems - stem width (SWi) and stem weight (SW), a positive correlation between cap morphological traits - cap length (CL), cap width (CWi) and cap weight (CW), but a negative correlation with stem

length (SL) and the number of fruiting bodies in a bunch (NM). Depending on the composition of the tested substrates, high values of trait correlations - stem length (SL) and cap width (CWi) - were found on corn (S6); the highest values of cap width (CWi) and stem width (SWi) were observed on wheat+corn mixture (S3) as a group of positive variables, while the number of fruiting bodies (NM) had high values on sunflower stem substrates (S4 and S7).

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PREDICTION OF SOIL MOISTURE IN DOUBLE CROPPING USING THE FAO AQUACROP MODEL

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Abstract

Double cropping in the growing conditions in Serbia can be successful only with irrigation. For the establishment of an adequate irrigation schedule and water dosage it is necessary to monitor soil moisture in the rhizosphere layer. Soil moisture assessment instruments are not yet in mass use by irrigation system users. Prediction of soil moisture can also be performed by crop models. The aim of this paper is to test the reliability of soil moisture simulation in double cropping obtained by use of FAO Aquacrop model. The testing was carried out on the basis of the results of experimental research carried out on the chernozem type of the soil on a loessial terrace in Zemun Polje (Serbia). The soil moisture content in the rhizosphere horizon was measured in two fields. One field was double cropped with soybeans following wheat, and the other with green beans following wheat. The fields were irrigated by the sprinkling method. The soil moisture measurements in the experimental field were performed by a gravimetric method. Soil samples were taken at different depths, in three repetitions, at 10cm intervals up to a depth of 60cm, and then at every 20cm up to a depth of 1m. The reliability of simulations obtained using the Aquacrop model was rated by use of statistical parameters: root mean square error (RMSE), normalized root-mean-square error (nRMSE), the Willmott index of agreement (d) and the correlation coefficient (R). Simulation of soil moisture at the rhizosphere horizon shows good concurrence with measured values (RMSE<2.0, nRMSE<8.31, d>0.82, R>0.82). The one year results are showing that the Aquacrop model is able to simulate chernozem moisture of root zone of succession plants (in irrigation condition) accurately. This finding should be confirmed by the results of the research during several more vegetation seasons.

Key words: *Aquacrop model, soil moisture, soybeans, green beans, double cropping*

Introduction

The double cropping or succession planting system of plant production is the basis for growing the plants in sustainable production. In the climate zone in which Serbia is situated, a double cropping system is not possible without the use of irrigation. Since the succession planting is usually done at the end of June or early in July, it is almost certain that the plants will be exposed to severe drought at the very beginning of their vegetative life, since agro-meteorological analyzes indicates that in our region July and August are the periods with the largest climate deficit of water (Matović et al., 2013) and with very high air temperatures (Ruml et al., 2017). With the application of irrigation a water regimen of the soil, which provides conditions for the normal functioning of the physiological processes of succession planting crops from sowing to ripening, can be established. To assess the impact of water deficit and irrigation scheduling crop-models with different approaches are used in order to simulate the production process.

In this paper, the FAO crop yield model AquaCrop (Steduto et al., 2012; Raes et al., 2012) which simulate crop biomass and yield in response to water and other abiotic factors was used. Previously conducted studies in which model reliability was tested in simulation of

yields of different crops (Araya et al., 2010; Stricevic et al., 2011; Hussein et al., 2011; Heng et al., 2009; Hsiao et al., 2009; Zinyengere et al., 2011) all have reported positive results.
In addition to the yield simulation and irrigation regimen, the FAO AquaCrop model simulates moisture in the soil, based on input data on soil characteristics, climate and plant cover. In contrast to the studies in which the AquaCrop model was used to simulate yield and biomass, a significantly smaller number of studies focused on simulation of moisture in the soil (Farahani et al., 2009; Katerji et al., 2013; Paredes et al., 2015; Mkhabela and Bullock, 2012).
The aim of this paper is to evaluate the justification of the use of the FAO AquaCrop model for moisture simulation in the rhizosphere layer of soil, based on data from a one-year experiment conducted on two irrigation fields (succession green beans and succession soybeans).

Material and Methods

The experimental field was located in Zemun Polje, on Chernozem soil on a loesial terrace. The land is characterized by favourable water-physical properties and is rich in humus. The texture composition of the surface horizon up to 50 cm of depth is clay loam, and from 50 cm to 200 cm there is the loam. Field water capacity was determined by the method of monolith, and the wilting point by Richards method. Values of field water capacity (FC) and wilting point (WP) are shown in Figure 1.

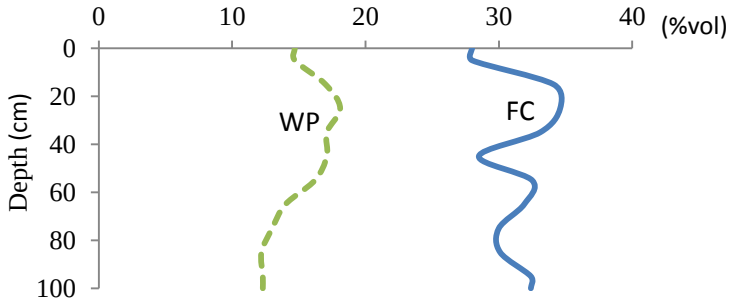

Figure 1. Field capacity (FC) and wilting point (WP) values on chernozem at experimental field in Zemun Polje.

The irrigation fields where the succession soybeans and succession green beans were grown are 0.76 ha. The test measurements were made on chosen basic plots. Those basic plots were square shaped, measuring 12 m by 1 2m. In the corners of the square, sprinklers of 10 m range have been installed. A uniform distribution of water deposits over the whole basic plot was achieved, which provided the same soil moisture conditions for all plants.
The soil moisture was controlled by a thermogravimetric method. Within a time span of about ten days, soil samples were taken at every 10 cm to a depth of up to 60 cm, and then every 20 cm to a depth of 1 m. The soil moisture control was carried out in three repetitions. The paper presents the average values of three measurements.
The sowing of succession crops was made on June 29th, a few days after wheat harvest. The harvest of green beans was on September 19th and the harvest of soybeans on October 30th. During the entire vegetation season records of the phases of growth and development of the plants were made (Table 1).

Table 1. Lasting of the phases of growth and development of the succession plants from the first day after sowing

Growth Stages	Soybeans		Green beans	
	Length (days)	Date	Lenght (days)	Date
From day 1 after sowing:		30-Jun		30-Jun
to emergence	6	06-Jul	6	06-Jul
to maximum canopy cover	54	23-Aug	34	03-Aug
to start of canopy senescence	98	06-Oct	69	07-Sep
to maturity	123	30-Oct	82	19-Sep

Soil humidity was simulated using the FAO AquaCrop crop model (http://www.fao.org/aquacrop). The model is user-friendly and requires a relatively small number of input parameters for climate, soil, agro-technical measures and plant characteristics. Climatic data from the Zemun Polje meteorological station provided the data necessary for the calculation of evapotranspiration by the Penman-Montheith method (minimal and maximal air temperature, relative humidity, insolation, wind speed). Data relates to 1987 when research was carried out. The amount of precipitation was measured on a daily basis, using a pluviometer located on the experimental field itself. Phenological phases of the development and development of the observed plant species were recorded on site (Tab.1). The stratigraphic and aqueous physical properties of the soil were determined in the described way (Figure 1). The missing data were taken from the crop model itself.

Soil moisture is simulated for the depth of the effective zone of the root system. In case of succession soybean the humidity is simulated for depth 0-80 cm, and for succession green beans 0-50 cm. These depths have been selected because they represent the depth of soil moistening by irrigation, which is the basis for calculating the amount of water needed for irrigation.

For the assessment of the reliability of soil humidity simulations, statistical parameters were used: root mean square error RMSE, normalized root-mean-square error nRMSE, Willmott index agreement d (Willmott et al., 1985) and correlation coefficient R.

Results and Discussion

According to the amount of precipitation, the vegetation season, in which the research was carried out, was approximate to the perennial average (Matović, 2000). The schedule of precipitation during the vegetation period is shown on Figure 2.

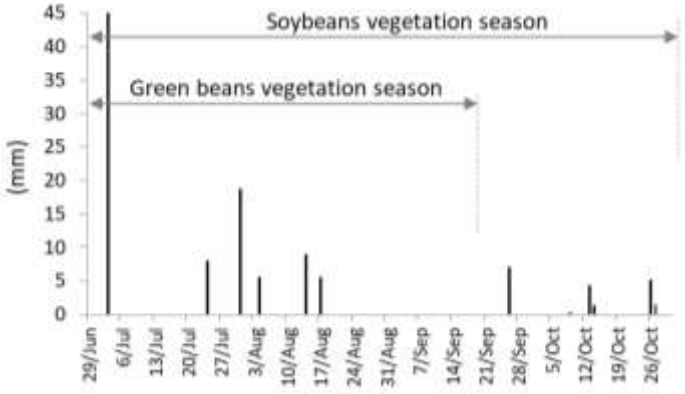

Figure 2. Precipitation schedule in the experimental field in Zemun Polje, starting from the date of sowing, until the harvest of succession soybeans and green beans

During its growing season, succession soybeans received 117 mm of rainfall and succession green beans 92 mm. Soybean was watered in 10 occasions, with a total quantity of 220 mm of

water, while green beans was watered, with a total of 197 mm of water also in 10 watering. That means that, excluding initial and final soil moisture, the soybeans had at its disposal a total of 337 mm of water, while green beans had 289 mm of water. Groundwater is deep (about 10 m depth), so it is considered that there is no ascendent touch of water from the underground.

The average values of chernozem humidity measured under the succession soybean (depth 0-0.8 m) and succession green beans (depth 0-0.5 m) are shown by dots in Figure 3. Simulation of soil moisture for the mentioned depths is represented by the curve line composed of daily values of soil moisture obtained by the water balance calculation (Raes et al., 2018).

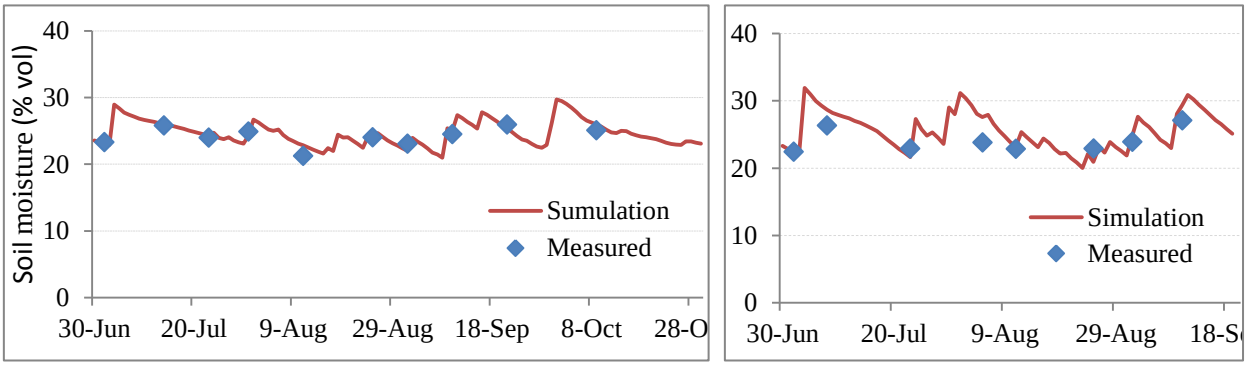

Figure 3. Left: Measured soil moisture and simulation of soil moisture under succession soybeans, at a depth of 0-0.8 m. Right: Measured soil moisture and simulation of the humidity of the soil under the succession green beans, at a depth of 0-0.5 m, in the experimental field in Zemun Polje

Measured and simulated soil moisture under succession soybeans were in a similar range. The measured values were from 21.2 - 25.9% vol., and simulated from 22.0 - 26.0% vol. In the case of soil under green beans, the simulation showed a higher range of values (20.9-29.4% vol) compared to the measured values (22.4-27.1% vol). Mkhabela and Bullock (2012), have concluded that the model tended to slightly underestimate the soil water at high water content in wet conditions and overestimate it at low soil water contents during dry years using the FAO AquaCrop model for simulation of total soil water content (0-120 cm layer) in the field under wheat. Similar results came from Paredes et al. (2015), comparing the results from a four-year experiment of the soybean growing in North China Plain with simulated soil moisture results obtained by the AquaCrop model. Our research can not confirm this conclusion, since the measurements always show the moderate humidity of the soil (Figure 3). At no time there was no high moisture content, nor the large water deficit registered.

The estimation of the concurrence of the measured and simulated soil moisture values was made using the statistical parameters: the Willmott index of agreement (d), absolute and normalized root mean square error (RMSE, nRMSE) and the correlation coefficient (R) (Table 2).

Table 2. - Evaluation of the agreement of simulations and observed (measured) soil moisture data under succession soybean and green beans in the experimental field in Zemun Polje (n - number of observed data)

	Willmott d	RMSE	nRMSE	R	n
Soybeans (0-0.8m)	0.90363	0.793465	3.2817	0.8231	10
Green beans (0-0.5m)	0.821808	1.995971	8.3079	0.8897	8

Based on the Willmott index of agreement (d), the conformity of the measured and simulated values of soil moisture obtained by the Aquacrop model is better in soybean (d=0.90363) than in the green bean (d=0.821808). The calculated values of RMSE and nRMSE, were 0.79% and 3.28% for soybean; 2% and 8.31% for green bean, respectively. In both cases simulation is considered as excellent (nRMSE is less than 10%). The correlation coefficient shows a high degree of agreement of the results and it is 0.82 for the soil under the succession soybean and 0.89 for the soil under the succession green beans.

The evaluation of the AquaCrop model at different location, indicated that the model was able to simulate soil moisture of root zone accurately (Andarziana et al., 2011; Xiangxiang et al., 2013; Mkhabela and Bullock, 2012).

Conclusion

Testing of the FAO AquaCrop model, on two irrigation fields sowed by succession soybean and succession green beans, showed good agreement between measured i.e. observed and simulated soil moisture values. It can be concluded that the FAO AquaCrop model can be successfully used for predicting the moisture of chernozem on the loessial terrace of the Zemun Polje, for the purposes of determining the irrigation regimen of succession crops. However, since the research was carried out during the one vegetation season, with a relatively small number of observed data, this finding should be confirmed by the results of the research during several more vegetation seasons.

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IMPROVED MAIZE CROPPING TECHNOLOGY TO REDUCE THE IMPACT OF CLIMATE CHANGES

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Abstract

Maize (*Zea mays* L.) is the highest ranking crop in terms of area and production in Serbia. During the whole growing period maize requires high temperatures and sufficient amounts of precipitation. High yields could be achieved on loose and porous soil with good capacity for water and those containing easily-accessible nutrients. The agro-ecological conditions for maize production differ over various regions of Serbia and meteorological parameters can seriously influence maize cultivation. Natural hazards, such as the occurrence of frosts, heavy rains in spring, floods, storms, hail, droughts, cause stress and to a significant degree can reduce or destroy maize yields. The application of appropriate cropping practices mitigates adverse effects of climate and soil and provides conditions for the maximum utilisation of the genetic yielding potential of maize hybrids. A system of measures that encompasses proper crop rotation, primary soil tillage, fertilisation, sowing date and density, irrigation, as well as some ecological measures such as intercropping or cover crops, are basic prerequisites for successful production of maize in Serbia. Many producers do not use these measures at the appropriate level even though results shows that fertilisation can improve maize yields from 7.87 t/ha to 10.00 t/ha under rainfed and from 9.42 t/ha to 12.32 t/ha under irrigated conditions. Irrigation can also increase maize yields by more than 30%. In the study, good agricultural practices as components of an improved technologies that could help to reduce the impacts of natural hazards and obtain high and stable yields, are identified and validated.

Key words: *Maize, Cropping practices, Yield, Natural disasters, Drought*

Introduction

Of a total of 5 million ha of agricultural land in Serbia, 3.3 million hectares are arable land. Maize is traditionally grown on 1.2 or even 1.3 million ha, but in 2018, maize was sown on about 0.9 million ha (http://www.zitasrbije.rs). Maize hybrids with the genetic potential of 10-15 t ha^{-1} have been grown for over 40 years in Serbia, but yielded 5.5 to 6.5 t/ha in average (Videnovic et al., 2013b). The main reasons for such a difference are drought; soils that are not suitable in the same degree for intensive production; the insufficient application of mineral fertilisers, outdated machinery and small and fragmented arable farms (Kovačević et al., 2012a; Videnović et al., 2013b). Drought often occurs, particularly in Eastern Serbia and only 1/3 of the total maize/soybean production is carried out on favourable soils; the irrigation is applied in only 2% of the maize production areas. Despite this, the Republic of Serbia is a significant producer of cereals within the European frames and the largest regional producer of maize with an average production of about 6 million tons (Statistical Office of the Republic of Serbia, http://webrzs.stat.gov.rs/WebSite/public/ReportView.aspx).

The effects of climate change, which affect the Western Balkans include increased temperature, reduced total precipitation, increased number of summer days, extended period between the last spring and first autumn frost, increased sum of active air temperature above 10 °C, etc. (Čustović et al., 2012; Kovačević et al., 2013). Drought is a multidimensional stress affecting photosynthesis and maize growth. No other environmental factor limits global crop production more severely than water deficit and it is the most important abiotic stress

that seriously decreases the final grain yield of maize (Videnović et al., 2013a). In addition, heavy rains, floods, storms, hails, as well as, extremely low and high temperatures, can cause stress and may, to a significant degree, reduce or destroy the yield of maize (Kovačević et al., 2012b). According to the 2015 World Risk Index, the exposure of the population in the Western Balkan countries to such hazards is the highest within the European region.

Maximum yield achievement is still the main strategy in agriculture and could be reached by innovative research orientated towards better understanding of soil-plant relations within the agro-ecosystem, maximum utilisation of the genetic potential of new genotypes and optimisation of cropping technology (inputs-outputs) in regard to climate changes (Byerlee et al., 2014). Crop growing practices can give advantages to the growing plant to maximise its genetic potential in years with regular and extreme conditions (Dragičević et al., 2015). It is very important that maize plant is well-supplied with moisture until the end of flowering, during the cob formation, and grain filling (Kovačević et al., 2013). In the fight against drought, regular and specific cultural practices could be used along with irrigation as the most direct practice by which water can be added in desired quantities independently of precipitation and with far-reaching effects (Kovačević et al., 2012a). The economic effect of climate changes on agriculture requires different practices for rain-fed (Schlenker et al. 2002) and for irrigated regions. In dry land farming areas, climate changes are equivalent to an exogenous shift in the fixed input associated with new supplies. In irrigated regions, the local climate is not directly connected to water supply. A risk reduction present in forecasting is a necessary part of any cropping practice (Iglesias et al., 2011).

Cropping technology determines the effectiveness of certain cultivation measures on morphological and productive traits of the crop, as well as effects of some components of the agro-environmental complex such as weeds and insects. Variations in cropping practices enable environmental factors to be overcome. A cropping practices that can help in overcoming the negative influence of climate changes on maize production and increase grain yield includes crop rotation, soil tillage, fertilisation, irrigation, sowing time and density, appropriate choice of hybrids, intercropping, cover crops, etc. (http://www.fao.org/3/I8848RS/i8848rs.pdf). In the three-crop rotation system with soybean as a legume crop, maize grain yield was higher by 11.4% than in the three-crop rotation system with winter wheat (Videnović et al., 2013a); conventional soil tillage that includes autumn ploughing at a depth of 25 cm or more resulted in the highest ten-year average maize yield of 10.61 t/ha (Videnović et al., 2011); fertilisation is one of the most important aspects in increasing yield and its stability (Berzsenyi et al., 2000; Varvel, 2000); sowing should be done earlier in case of drought and with a proper density for each hybrid (Kresović et al., 2011); irrigation needs to be conducted with adjusted norms in respect to the soil moisture and monthly water needs of maize (Kresović et al., 2013). Moreover, some ecological measures, such as intercropping or cover crops contribute to higher yield achievement (Dolijanović and Simić, 2016; Janosevic et al., 2017). Previous investigations also showed that soil moisture was slightly higher during anthesis under no-till in rain-fed cropping in relation to conventional and reduced tillage (Dragičević et al., 2012). All of the listed practices are basic prerequisites for successful production of maize in Serbia, but many producers do not apply these measures at the appropriate level even though results show that they are effective. Research results of good agricultural practices and improved technologies that could help producers to reduce the impacts of natural hazards and obtain high and stable yields are presented in the manuscript.

Material and Methods

Effects of various cropping practices on maize production have been observed within a long-term research program of experiments that have been carried out in the fields of the Maize

Research Institute, Zemun Polje. Maize was cultivated in different crop rotations, soil tillage systems, under recommended and altered densities, with application of cover crops and within intercropping systems, as well as with and without irrigation. Standard grain quality hybrids were grown with modified sowing densities and the application of organic, mineral and microbiological fertilisers, and various forms of mineral nitrogen.
The soil type in the experimental field was slightly calcareous chernozem with 53% sand, 30% silt, 17% clay; with good fertility and 3.3% of organic matter content and moderate drainage. The pH was 6.9 and soil structure was a silty clay loam. The meteorological conditions varied during and between the seasons as evident through results.
Maize grain yield was the main parameter that was evaluated. This parameter was estimated in the two inner rows and then calculated to 14% moisture.

Results and discussion

The cropping systems were basically defined by **crop rotation** which means proper arrangement of crops in time and space to better utilise soil potentials and climate (Kovačević, 2010). The crop rotation improved soil physical and chemical properties, contributed to biodiversity by growing different crops and genotypes, and influenced the composition and structure of field weed communities (Dolijanović and Simić, 2016). Rotation sequences with row and dense crops, legumes and cereals, also included rotation of cropping technologies and herbicides with different modes of action (Simić and Dolijanović, 2016). Efficient crop rotation encompasses crops with deep and shallow root systems, crops that uptake large quantities of nutrients and crops that enrich the soil, crops that extract and consume large amounts of water from the soil vs. crops that accumulate and maintain the water in the soil.

Table 1. Efficacy of crop rotation in maize cultivation (grain yield), Zemun Polje, 1998-2015

Cropping systems	Fertiliser level				Average
	F1	F2	F3	F4	
Continuous maize	3.89	6.39	6.69	6.90	5.97
Maize - Soybean	6.95	7.99	7.80	7.66	7.60
Maize - Winter wheat	6.33	7.67	7.81	7.66	7.37
Maize - W. wheat - Soybean	8.01	8.78	8.77	8.65	8.55
Average (t ha^{-1})	6.29	7.71	7.77	7.72	7.37

F1 - 0 kg ha^{-1} NPK; F2 - 180 kg ha^{-1} NPK; F3 - 270 kg ha^{-1} NPK; F4 - 360 kg ha^{-1} NPK

In Serbia, maize is grown on approximately 70% of areas after small grains, usually winter wheat (Videnović et al., 2013a). These crops are good preceding crops, as they are dense and inhibit the development of weeds and there is also enough time after harvest for summer and autumn tillage. Continuous cropping or maize monoculture is applied on approximately 20% of arable areas in Serbia. It brings higher infestations with perennial, especially grass weeds potentially resistant to herbicides, and increased damage caused by western corn rootworm. For these reasons, this system is not spreading and in some regions its application even declines. Maize is grown on about 10% of areas in which soybean was a preceding crop which is a very important advancement in the improvement of maize growing practices in Serbia; it is well-known that soybean is one of the best preceding crops for maize, Table 1. Results from investigations showed that presence of plants from the family *Fabaceae* significantly contributes to the efficiency of crop rotation through the reduction of mineral nitrogen fertilisers by 50%, maize yields increase, accompanied by soil preservation and improvement (Videnović et al., 2013a; Jovanović et al., 2004). A cropping system such as

double-crop rotation of maize and winter wheat and three-crop rotation of maize, soybean and winter wheat influences maize plant height and grain yield after only one rotation in comparison with maize continuous cropping (Spasojević, 2014; Simić et al. 2017). In years with unfavourable conditions for maize production, the beneficial effects of the crop rotation are even more pronounced.

Soil tillage supports efficient water use and prevents erosion, increases crop competitiveness, reduces the concentration of CO_2 and soil compaction and gives better texture etc. In most production regions in Serbia, a variety of measures such as proper tillage and fertilisation, autumn ploughing, summer ploughing etc., are employed to collect and store moisture in the soil. Maize plants can use the stored water during periods of drought. It is well known that accumulated water increases maize yield.

Recently, systems of reduced tillage, particularly direct sowing, have entered into widespread use (Fulton, 2010). In dry years and on chernozem as a rich and quality soil, no-till could be an effective measure for soil moisture preservation in relation to conventional and reduced tillage (Dragičević et al., 2012). The effectiveness of reduced tillage systems should be tested for other types of soils and agro-ecological conditions in Serbia. However, these systems require the implementation of measures for intensive weed control (herbicides).

Table 2. Effects of soil tillage on maize grain yield (t ha^{-1}), Zemun Polje, 2000-2013

Tillage system	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Average
No till	3.0	6.0	8.5	6.0	11.5	8.9	8.0	6.4	3.9	11.6	8.1	6.7	2.8	5.5	6.9
Reduced tillage	5.2	8.0	8.8	7.5	12.8	12.4	10.8	7.6	6.3	11.1	8.9	8.0	3.5	7.6	8.5
Conv. tillage	8.8	9.0	10.3	8.8	14.3	13.9	12.1	8.3	9.6	12.5	10.3	10.4	3.3	8.7	10.0
Average	5.7	7.7	9.2	7.4	12.9	11.7	10.3	7.4	6.6	11.7	9.1	8.4	3.2	7.3	8.5

Under the agro-ecological conditions of Serbia, the highest maize yields and the most efficient weed suppression, especially perennial, are achieved by using a conventional soil tillage which is also most suitable in regard to changeable climatic conditions. In average for fourteen years, conventional tillage contributed to higher yield of maize for 1,5 t/ha and 3,1 t/ha in comparison to reduced and no-till under agro-ecological conditions of Zemun Polje, Table 2. The conventional tillage includes ploughing in autumn (20-25 cm) - soil remains in the form of open furrows to freeze during winter; a single pass land preparation is applied in spring - shallow tillage 10-12 cm. In such a way a good soil structure is provided, moisture is maintained, the soil is not trampled, and weed seedlings are destroyed, which is all of particular importance for efficient herbicide actions.

Fertilisation provides the necessary elements for growth and development of maize. Fertilisation with manure improves soil properties, particularly water - air capacity, which is especially evident when plants are exposed to stress in conditions of natural disasters (Simić et al., 2016a). The application of mineral fertilisers to maize can be managed by time, place and amount of fertilisers (Liu and Wiatrak (2011). Fertilisation changes the relationship in crop competition to weeds, not only for nutrients, but also for other resources, so it is essential that the optimal quantity of fertilisers is provided to the plant at the right time and at the place from which it will best be used (Simić et al., 2016b). The most intensive and the highest rate of nitrogen intake occurs at the beginning of the growing season, and in the period of flowering, respectively. After the start of the milk grain stage, the absorption of nitrogen

slows down. Phosphorus is taken by plants in significantly smaller amounts than potassium and nitrogen, in the period of tillering until the beginning of flowering. Potassium is most quickly absorbed from the soil during germination and the formation of tillers. On heavy and substantially sandy soils, maize responds very well to manure fertilisation. Application of mineral fertilisers in maize cultivation can contribute to obtaining higher yields, under rain-fed and especially irrigated soils, Table 3. In Central Serbia, soils are porous with a good water availability, but recently the climate has altered towards an increased frequency of drought years. The use of N fertilisers with inhibitors may reduce the risk of environmental N loss associated with application of conventional N fertiliser sources under variable-climatic conditions (Simić et al., 2016c). The highest yield of a late-maturing hybrid in the experimental plot with long-term continuous cropping (over 35 years) was achieved when manure was applied, harvest residues ploughed down completely and N fertiliser applied in spring at the beginning of the growing season (Simić et al., 2013).

Table 3. The influence of fertiliser level on maize grain yield (t ha^{-1}) in different soil tillage systems, Zemun Polje, 2000-2017

	No till	Reduced tillage	Convent. tillage	Average
		Rainfed		
F1 - 0 kg NPK	5.84	7.39	10.39	7.87
F2 - 150 N, 105 P, 75 K	7.76	9.43	11.50	9.56
F3 - 330 , 211 P, 150 K	8.85	9.93	11.22	10.00
Average	7.48	8.92	11.04	9.14
		Irrigation		
F1 - 0 kg NPK	7.24	9.15	11.88	9.42
F2 - 150 N, 105 P, 75 K	10.15	11.27	13.40	11.61
F3 - 330 , 211 P, 150 K	11.02	12.19	13.74	12.32
Average	9.47	10.87	13.00	11.12

Irrigation helps to reduce the impact of soil and air drought on maize. This concerns the application of water in controlled quantities and certain intervals to the soil or plants to support the optimal growth and development of crops. The achievement of high yields under conditions in Serbia is limited by two factors: - the uneven distribution and deficit in precipitation. In Serbia, six of ten years are dry (extreme to moderate and weak drought), two years have moderate conditions and two are optimum for maize production. In the case of maize, the critical period begins 15–20 days prior to tasselling and lasts until the beginning of the milk stage. When the years are average in terms of weather conditions, maize production under irrigation conditions will result in higher yields by 15-30% or even by 50% in seed maize production (Simic et al., 2016d). Irrigation provides the optimum water supply, activates soil microorganisms and nutrient reserves, and contributes to a better utilisation of incorporated fertilisers.

Sowing and crop density - Maize sowing involves several operations (selection of hybrids, preparation of seed for sowing, sowing time, sowing depth, number of plants per ha, i.e. sowing density, and plant arrangement). High yields of today's maize hybrids can be obtained only if the necessary number of plants is sown per area unit: 70-80,000, 60-70,000 and 60,000 plants ha^{-1} for hybrids of FAO 300-400, FAO 500-600 and FAO 700, respectively.

The sowing time and density are important because they affect crop biomass, lodging, efficiency of nutrient use, harvest time, etc. The optimum sowing time of maize should be harmonized with weather forecasts for a given year, with particular reference to the possible occurrence of drought, floods, etc. Late sowing does not give satisfactory yields, especially in

no-irrigation conditions, and in the case of FAO 600-700 hybrids. In such conditions, it often happens that later FAO groups do not complete their vegetation.

Producers in the regions with frequent occurrence of drought such as Banat, East Serbia and South Serbia, usually grow hybrids with shorter vegetation, FAO 300-400 or FAO 500, with 115-125 days of vegetation period. In dry years, it is more efficient to conduct early sowing at the beginning of April with maximum density of 55-65,000 plants/ha and adapt cropping technology to dry conditions.

A B

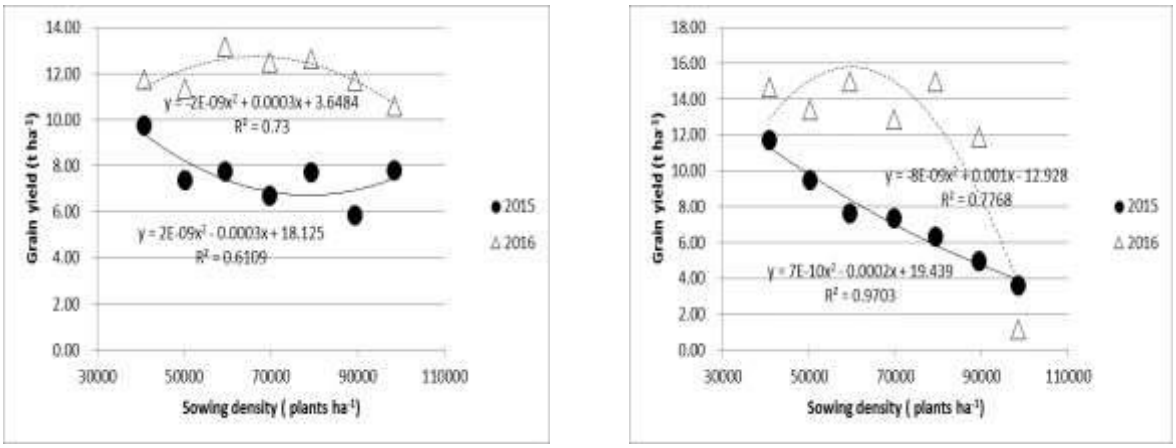

Figure 1. Average yields (t/ha) of maize hybrids of different FAO groups – ZP 366 (A) and ZP 606 (B) in different sowing densities under irrigation conditions (2015-2016 in Zemun Polje)

Increasing the number of plants per hectare up to a certain extent increased the grain yield of maize, but once this limit was achieved the yield decreased. This is, for both hybrids, particularly observable in 2016, a very favourable year for maize production. In this year, yield slowly decreased only when maize was sown in very high densities (more than 70,000 plants per ha), Figure 1. On the other hand, in the dry 2015 year, increased plant density lead to the reduction of grain yield of maize hybrids. The intraspecific competition occurs when the number of maize plants per ha increases and the full yield potentials are manifested only in good years, such as 2016. The impact of meteorological conditions in some years could be very important and similar results were presented for other years, which were not optimal regarding precipitation (Kresovic et al., 2011).

Maize hybrids with a rapid initial growth rate are more competitive than the other hybrids and, a careful selection of competitive hybrids can dramatically reduce grain yield losses and weed biomass, as well as seed production (Simić et al., 2009; Travlos et al., 2011).

Cover crops and intercropping - The cultivation of cover crops is a special way of overlaying/covering the soil. Thus cover crops contribute to the sustainable management of soil structure and soil functioning for water infiltration and storage in crop production (Bodner et al., 2014). Cover crops reduce the runoff/leaching of nutrients from the soil and increase the content of organic matter and they should be cultivated to preserve the fertility of the soil. In a study recently conducted in Zemun Polje, sweet maize yield was highest after a fodder kale and field pea cultivation, but smallest after winter oat and common vetch as cover crops. Different autmn-winter cover crops and microbiological fertiliser showed more efficiency in the dry year on nitrogen utilisation in the soil and sweet maize yield (Janosevic et al., 2017).

Inter-cropping provides greater stability and diversity of production and better use of nutrients, light, heat, air, and vegetation space. Maize can successfully be intercropped with legumes (soybeans, beans and lupines). The choice of genotype in intercropping depends on

the aim of the cultivation. For stock production, usually specific maize hybrids are grown that would be most productive in intercropping with soybean. However, in addition to numerous advantages, this cropping system encounters numerous limitations (e.g. mechanical sowing and harvesting, weed control), which prevent its greater application in practice. Despite this, many results suggest that intercropping is a potential strategy to increase agro-ecosystem preservation by enhancing crop production, improving soil quality and mitigating greenhouse gas emissions (Dolijanović and Simić, 2015).

Conclusion

Based on the analysis of many results it could be concluded that maize production in Serbia significantly depends on meteorological conditions. Genetic potential of maize hybrids was not used enough. Good agricultural practices within maize cropping technology proved in the experimental work must be implemented in order to increase maize yield. In regard to present climate changes, precipitation deficiency is the main limiting factor in maize production in Serbia, so the irrigation is one of the most important measures which could contribute to significant maize production improving. All recommended practices contribute to the crop withstanding negative effects of climate changes and maintaining yield and grain quality with the least possible adverse consequences. Recommendations for their application are particularly emphasised when certain natural hazards are expected or it is known that they will occur.

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DETERMINATION OF SOME QUALITY PARAMETERS IN SILAGE CORN AFTER DIFFERENT HUNGARIAN VETCH + CEREAL MİXTURES

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Abstract

This study was conducted to determine some quality traits of silage corn (*Zea mays* L.) after Hungarian vetch (*Vicia pannonica* Crantz) intercropping with barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) and triticale (*Triticosecale* Wittmack) sown at different mixture rates (100:0 70:30, 60:40, 50:50 and 40:60%) and cut at the flowering and milk dough stages in the ecological condition of Yozgat during 2014 and 2015 growing seasons. The experiment was arranged in split plot-design with four replications, main plots were sowing time and sub plots were previous crop (mixture rates). Crude protein ratio and crude ash ratio, ADF, NDF, K, P, Ca and Mg contents were determined. Crude protein and ash ratio were ranged between 5.90 – 7.75% and 6.18 – 7.38% of respectively. The lowest ADF and NDF ratios were determined sole barley (34.19 – 61.38%), while the highest were determined 50HV:50T% (36.88 – 65.99%). K, P, Ca ve Mg contents of silage corn were ranged 1.96 – 2.17%, 0.271 – 0.289%, 0.23 – 0.30% and 0.18 – 0.22% of respectively. According to two years results, it has been determined that it is decreases quality when delayed silage maize sowing time and also, some corn plots that were planted after the Hungarian vetch + cereal mixtures of previous crop are not difference to stubble plot in terms of hay quality.

Key words: *Pre-plant, sowing time, quality, silage corn*

Introduction

In the crop rotation, first sown plant is previous plant (pre-plant) and second sown plant is following plant. The effect of the pre-plant to following plants is called the pre-plant effect. Each following plant demands precious sowing time and growing condition from the pre-plant. The effect of the pre-plant to following plants is called the pre-plant effect. The pre-plant effect is affected by climate, soil and environmental factors and the long or short duration of the vegetation. The length of the vegetation period is important for legumes. Because, legumes fix nitrogen and organic matter to soil and also improve the structure. Legumes affect the yield and quality of following plants. Kavut and Geren (2005) reported that legumes supply nitrogen for corn and increase of their yield. Therefore, legumes are very good pre-plants for corn.

Silage corn has a great importance for feeding ruminants, such as cattle. Corn is use intensively in silage production and it is constitute approximately 80% of silage production in Turkey (Dumlu and Tan, 2009). Silage corn can be planted as main and double crop, planting time show the differences year by year according to the climatic conditions and pre-plant harvest time, therefore especially silage quality, plant development and plant nutrient contents show differences (Oten, 2017).

This study was aimed to determine some quality traits of silage corn (*Zea mays* L.) after Hungarian vetch + cereal intercropping sown in the ecological condition of Yozgat during 2014 and 2015 growing seasons.

Material and Methods

This study was conducted to determine some quality traits of silage corn after Hungarian vetch (HV) intercropping with barley (B), wheat (W) and triticale (T) sown at different mixture rates (100:0 70:30, 60:40, 50:50 and 40:60%) and cut at the flowering (first sowing time) and milk dough stages (second sowing time) in the ecological condition of Yozgat during 2014 and 2015 years.

Soil properties of the experimental area of both year were clay –loam type with pH of 8.20-8.12 and 7.93-7.99% CaCO3, 86.2-82.1 kg ha^{-1} phosphorus, and 1.98-1.78% organic matter at the depth of 30 cm (Table 1).

Table 1. Physical and Chemical Properties of Soil in Research Area*

Properties	2014	2015
Structure	Clay-loam	Clay-loam
CaCO3 (%)	7.93	7.99
Total salt (%)	0.018	0.020
P_2O_5 (kg ha^{-1})	86.2	82.1
K_2O (kg ha^{-1})	484.7	454.7
Ph	8.20	8.12
Organic matter (%)	1.98	1.78

* Turkish chamber of agriculture cooperation.

Table 2 shows the some meteorological parameters of experimental area in 2014 and 2015, including monthly average temperature, monthly total precipitation and relative humidity. Total precipitation of the long-term 147.7 mm and growing season was 260.1mm for 2014 and 264.1 mm for 2015 (Table 2).

Table 2. Some meteorological parameters of experimental areas in 2014 and 2015**.

Months	Average temperature (^{O}C)			Total precipitation (mm)			Relative humudity (mm)		
	LTM*	2014	2015	LTM*	2014	2015	LTM*	2014	2015
May	13.0	13.3	14.1	65.0	121.3	131.6	64.2	60.4	59.9
June	16.8	16.6	16.0	43.5	79.8	95.3	60.5	56.0	71.5
July	19.7	21.5	19.8	12.3	3.7	7.1	56.8	43.2	54.7
August	19.6	22.4	21.3	8.90	27.1	5.4	55.7	43.5	56.7
September	15.5	14.5	20.1	18.0	28.2	24.7	58.1	58.1	49.4
Mean/Total	**16.92**	**17.66**	**18.26**	**147.7**	**260.1**	**264.1**	**59.06**	**52.24**	**58.44**

*LTM: Long term mean; ** Tukish State Meterogical Service

Firstly, Hungarian vetch and cereals (Barley, Wheat and Triticale) were sown as binary mixtures with different seed rates (100:0%, 70:30%, 60:40%, 50:50% 40:60%) and harvested at the flowering and milk dough stages. Then silage corn (*Zea mays* L. "Cadız") was sown. The experiments were arranged in split plot design with four replications, main plots were sowing times and sub plots were previous crop (mixtures). The sowing was done manually after harvesting of hungarian vetch: cereal mixtures. Plot size was 5 x 1.5 m = 7.5 m^2 and distance between rows was 50 cm. Also Hungarian vetch: cereals mixtures were comparison stubble as a control. Plant density was 120.000 seed for per hectare. The full dose nitrogen (50 kg N ha^{-1}), phosphate (80 kg P_2O_5 ha^{-1}) were applied at sowing time. Additional nitrogen fertilizer (50 kg N ha^{-1}) was applied at the stage when the plants were 40-50 cm. In the study, silage maize was irrigated five times and weeds were controlled by hoeing. Silage corn was harvested in dough stage.

Plant samples were dried 65 °C until constant weight to determine their hay yield. Crude protein, ADF, NDF, K, Ca, P and Mg contents of hay were determined by using Near Reflectance Spectroscopy (NIRS, 'Foss 6500') with software package program 'IC-0904FE'. Data were statistically analysed by repeated measure analysis in MSTAT-C statistical programme and means were separated by Duncan's Multiple Range test.

Results and Discussion

According to combined years, in terms of crude protein ratio there was significant difference ($p \leq 0.01$) between sowing time and previous crop, while it was not significant sowing time*previous crop interaction and years (Table 3). Crude protein ratio was ranged 5.90% (previous crop 50HV:50W%) – 7.75% (Stubble). Parlak and Sevimay (2007) reported that crude protein ratio of silage corn, grown after barley and wheat, was 7.23%.

The lowest crude ash ratio was 6.18% (previous crop 40HV:60T%), while the highest was 7.38% (previous crop 70HV:30W%) (Table 3). Some silage corn samples after previous crop had more crude ash ratio than hay stubble plots (Table 4). This can be due to the previous crop that can supply nutrients to silage corn.

Table 3. Crude protein and ash ratio of silage corn

Previous crop	**Crude protein ratio (%)**			**Crude ash ratio (%)**		
(Mixtures)	**FST**	**SST**	**Average**	**FST**	**SST**	**Average**
100HV	7.58	5.90	6.74 b-d	6.78 a-h	6.69 a-h	6.74
100B	7.66	6.69	7.18 a-c	6.81 a-g	5.82 h-ı	6.32
70HV:30B	7.59	7.33	7.46 ab	5.52 ı	7.05 a-e	6.29
60HV:40B	8.07	6.09	7.08 bc	6.07 f-ı	7.02 a-f	6.55
50HV:50B	7.20	6.04	6.62 b-d	6.27 d-ı	6.29 d-ı	6.28
40HV:60B	6.73	6.05	6.39 cd	6.54 b-h	6.24 d-ı	6.39
100W	6.47	5.69	6.08 d	6.49 c-h	7.12 a-d	6.81
70HV:30W	7.32	5.75	6.54 cd	7.27 a-c	7.49 ab	7.38
60HV:40W	6.66	5.52	6.09 d	7.64 a	6.71 a-h	7.18
50HV:50W	6.50	5.29	5.90 d	6.18 d-ı	6.73 a-h	6.46
40HV:60W	7.43	5.91	6.67 b-d	6.99 a-f	5.95 g-ı	6.47
100T	6.90	5.94	6.42 cd	6.79 a-h	6.28 d-ı	6.54
70HV:30T	7.41	5.63	6.52 cd	6.75 a-h	6.61 b-h	6.68
60HV:40T	6.50	6.48	6.49 cd	6.34 c-ı	6.64 b-h	6.49
50HV:50T	6.56	6.08	6.32 cd	7.48 ab	6.05 f-ı	6.77
40HV:60T	7.36	6.01	6.69 b-d	6.22 d-ı	6.13 e-ı	6.18
STUBBLE	8.03	7.46	7.75 a	6.46 c-ı	6.43 c-ı	6.45
Average	**7.18 A****	**6.11 B****		**6.62**	**6.54**	
Years	**2014**	**6.88**		**6.79 A****		
	2015	**6.42**		**6.38 B****		

(*) 0.05, (**) 0.01; FST: First sowing time; SST: Second sowing time; HV: Hungarian vetch; B: Barley, W: Wheat; T: Triticale

The highest ADF content was determined 36.88% (previous crop 50HV:50T%), while the lowest was 34.19% (previous crop 100B%). NDF content was ranged 61.38% (previous crop 100B%) – 66.18% (previous crop 60HV:40W%) (Table 4). Previous studies, ADF and NDF content were ranged 24.22% – 43.29% and 48.11% – 73.85% of respectively (Balmuk, 2012; Carpıcı, 2016).

Stem ratio, cellulose and hemicellulose accumulation in cell wall are increase with the progress of sowing time. This can be occur due to the short vegetation period and high temperature (Buxton ve Casler; 1993; Tan and Mentese, 2003). Therefore, crude protein ratio,

crude ash ratio, ADF and NDF content of the plants for the second sowing time were higher than the first sowing time (Table 3 and 4).

Table 4. ADF (Acid detergent fibre) and NDF (Neutral detergent fibre) content of silage corn

Previous crop	**ADF content (%)**			**NDF content (%)**		
(Mixtures)	**FST**	**SST**	**Average**	**FST**	**SST**	**Average**
100HV	33.04	36.94	34.99	61.57	66.92	64.25
100B	33.43	34.94	34.19	59.42	63.34	61.38
70HV:30B	32.66	35.82	34.24	60.36	65.40	62.88
60HV:40B	32.46	37.66	35.06	60.79	66.68	63.74
50HV:50B	33.60	36.51	35.06	61.80	65.44	63.62
40HV:60B	33.43	36.65	35.04	61.38	66.75	64.07
100W	34.10	38.13	36.12	62.18	67.00	64.59
70HV:30W	34.71	37.62	36.17	61.81	67.46	64.64
60HV:40W	35.22	36.93	36.08	64.83	67.52	66.18
50HV:50W	35.17	37.15	36.16	63.47	66.50	64.99
40HV:60W	33.64	36.56	35.10	60.06	65.59	62.83
100T	33.28	37.07	35.18	62.94	67.28	65.11
70HV:30T	33.61	37.13	35.37	61.12	67.38	64.25
60HV:40T	34.41	37.19	35.80	63.21	68.25	65.73
50HV:50T	36.53	37.22	36.88	65.43	66.55	65.99
40HV:60T	34.00	35.64	34.82	62.94	64.83	63.89
STUBBLE	33.95	35.41	34.68	63.16	64.42	63.79
Average**	**33.95 B**	**36.74 A**		**62.25 B**	**66.31 A**	
Years**	**2014**	**37.57 A**		**67.87 A**		
	2015	**33.13 B**		**60.59 B**		

(*) 0.05, (**) 0.01; FST: First sowing time; SST: Second sowing time; HV: Hungarian vetch; B: Barley, W: Wheat; T: Triticale

The potassium (K) content was ranged 1.96% (previous crop 50HV:50W%) – 2.17% (previous crop 100T%). On average, K content of silage corn that observed most of the sites is at satisfactory level for dairy cows (NRC, 2005) (Table 5). Different researches reported that silage corn K content was ranged 1.33% – 2.97% (Erdem, 2011; Ozata at al., 2012).
According to combined years, the highest phosphorus (P) content was determined as 0.289% (previous crop 100B%) while the lowest was 0.271% (previous crop 60HV:40W%). NRC 0.2% phosphorus level is meet the demand of cattle (NRC, 2005). On average, the content of P in silage corn in all treatments is above the specified limits (Table 6). Erdem (2011) and Ozata et al. (2012) reported that silage corn P content was ranged 0.120% – 0.260%.
Silage corn Ca content was ranged between 0.23% (previous crop 50HV:50B% and 100T%) – 0.30% (previous crop 70HV:30W%) (Table 6). Average Ca content of the first sowing time was higher than the second sowing time. Ozata et al. (2012) reported that silage corn Ca content was ranged from 0.17% to 0.37%.

Table 5. Potassium (K) and Phosphorus (P) contents of silage corn

Previous crop	**K content (%)**			**P content (%)**		
(Mixtures)	**FST**	**SST**	**Average**	**FST**	**SST**	**Average**
100HV	2.12	1.91	2.02	0.298	0.259	0.279
100B	2.04	1.92	1.98	0.283	0.295	0.289
70HV:30B	2.06	2.06	2.06	0.284	0.285	0.285
60HV:40B	2.17	2.06	2.12	0.288	0.274	0.281
50HV:50B	2.05	2.05	2.05	0.275	0.291	0.283
40HV:60B	2.04	2.00	2.02	0.268	0.288	0.278
100W	2.19	1.98	2.09	0.285	0.270	0.278
70HV:30W	2.19	1.93	2.06	0.290	0.264	0.277
60HV:40W	2.06	1.90	1.98	0.268	0.274	0.271
50HV:50W	2.04	1.87	1.96	0.269	0.278	0.274
40HV:60W	2.15	1.97	2.06	0.301	0.274	0.288
100T	2.23	2.11	2.17	0.277	0.298	0.288
70HV:30T	2.08	1.90	1.99	0.293	0.275	0.284
60HV:40T	2.16	2.12	2.14	0.287	0.259	0.273
50HV:50T	2.07	1.93	2.00	0.274	0.271	0.273
40HV:60T	2.02	2.14	2.08	0.287	0.278	0.283
STUBBLE	2.08	2.10	2.09	0.264	0.281	0.273
Average	**2.10 A****	**1.99 B****		**0.282**	**0.277**	
Years	**2014**	**2.00 B****		**0.275**		
	2015	**2.09 A****		**0.285**		

(*) 0.05, (**) 0.01; FST: First sowing time; SST: Second sowing time; HV: Hungarian vetch; B: Barley, W: Wheat; T: Triticale

Table 6. Calcium (Ca) and Magnessium content (Mg) of silage corn

Previous crop	**Ca content (%)**			**Mg content (%)**		
(Mixtures)	**FST**	**SST**	**Average**	**FST**	**SST**	**Average**
100HV	0.31	0.25	0.28	0.20	0.21	0.21
100B	0.28	0.29	0.29	0.22	0.21	0.22
70HV:30B	0.30	0.28	0.29	0.19	0.23	0.21
60HV:40B	0.23	0.24	0.24	0.16	0.24	0.20
50HV:50B	0.23	0.22	0.23	0.16	0.20	0.18
40HV:60B	0.24	0.28	0.26	0.18	0.19	0.19
100W	0.25	0.32	0.29	0.18	0.20	0.19
70HV:30W	0.27	0.32	0.30	0.19	0.21	0.20
60HV:40W	0.28	0.21	0.25	0.21	0.20	0.21
50HV:50W	0.25	0.30	0.28	0.19	0.22	0.21
40HV:60W	0.29	0.23	0.26	0.18	0.20	0.19
100T	0.24	0.21	0.23	0.19	0.19	0.19
70HV:30T	0.27	0.27	0.27	0.19	0.20	0.20
60HV:40T	0.27	0.21	0.24	0.18	0.19	0.19
50HV:50T	0.29	0.26	0.28	0.20	0.21	0.21
40HV:60T	0.27	0.23	0.25	0.21	0.17	0.19
STUBBLE	0.32	0.23	0.28	0.20	0.19	0.20
Average**	**0.27 A**	**0.25 B**		**0.19**	**0.20**	
Years**	**1. yıl**	**0.24 A**		**0.18 B**		
	2. yıl	**0.28 B**		**0.21 A**		

(*) 0.05, (**) 0.01; FST: First sowing time; SST: Second sowing time; HV: Hungarian vetch; B: Barley, W: Wheat; T: Triticale

The lowest Mg content was 0.18% (previous crop 50HV:50B%),while the highest was 0.22% (previous crop 100B%) (Table 6). The Mg content in all analysed silage corn samples are above the critical limit of 0.1% DM (NRC, 2005).

Conclusion

According to two years results, it has been determined that quality of silage corn decreases when sowing time delayed in Yozgat ecological conditions. Also, some corn plots that were planted after the Hungarian vetch + cereal mixtures of previous crop are not different to stubble plot in terms of hay quality.

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THE TOTAL PHENOLS CONTENT OF AUTOCHTHONOUS CULTIVARS OF APPLE IN MAJEVICA AREA (BOSNIA AND HERZEGOVINA)

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Abstract

The autochthonous apple cultivars are the result of a long selection process by human population and edafish, climatic and geomorphological conditions. Many autochthonous cultivars and populations of many species of fruit trees are present in Bosnia and Herzegovina. The paper presents the one-year results of the study influence of cultivars on the total phenols content in six autochthonous cultivarst of apple (Petrovaca, Bjelicnik, Zelenika, Bobovec, Ljepocvjetka and Sampanjka) in Majevica area. The results of the research showed that the highest phenolic content was in the cultivar Ljepocvjetka (542.10 mg GAE/100 g), then in cultivar Bobovec (419.52 mg GAE/100 g) and Sampanjka (345.28 mg GAE/100 g), while the lowest content of phenols was in the cultivar Bjelcnik (247.45 mg GAE/100 g). Based on the results of research, there are significant variations in the total phenols content and there is no regularity in the content change.

Keywords: *apple, autochthonous cultivar, total phenols content, Majevica area.*

Introduction

The autochthonous cultivars of apple are the result of a long process of selection by human and natural conditions. There are many autochthonous cultivars and populations of many species of fruit trees in Bosnia and Herzegovina, thanks to the diversity of its edafish, climatic and geomorphological conditions. The most of the orchards of old cultivars neglected, but it is necessary to restore and protect, because it gradually create a market for their fruits.

Old cultivars represent a rich source of genetic material, but some cultivars also have very positive properties (eg. high and regular yield), which is very important from the aspect of growing (Mitre et al., 2009), and can have a significant role in selection in the future.

Most of traditional cultivars have a balanced composition of nutrients and bioactive components (Feliciano et al., 2010) and some of cultivars, usually, contain larger amounts of certain compounds, such as total phenols and flavonoids (Veberič et al., 2005) which gives them characteristic properties of color, aroma and taste. The fruit of apple contains several phenolic compounds, and also possesses a high total antioxidant capacity (Lotito & Frei, 2004; Biedrzycka & Amarowicz, 2008). The content of total polyphenols and antioxidant activity depends on cultivar, agroecological contitionals, maturity of fruit, exposure to sunlight, storage and ways of processing (Van der Sluis et al., 2001; Alonso-Salces et al., 2004). The apple is in second place by antioxidant activity and second place (immediately after cranberry) at the total concentration of phenolic content in United States of America (Boyer & Liu, 2004). The aim of the paper was to determine the phenolic content in six autochthonous cultivars of apple from the Majevica area and based on their positive results, their remarkable nutritional potential, based on the high content of natural antioxidants (Ding et al., 2006; Tulipani et al., 2008). There is a large number of studies which relate to the analysis of nutritional and other components in fruits of apple, but it was of particular importance to establish the deviations, ie the correlation of the obtained results with the literature data, because it will use autochthonous cultivars from the Majevica area for which such data are not yet fully available.

Materials and Methods

The research of phenolic content of autochthonous cultivars of apple (Petrovaca, Bjelicnik, Zelenika, Bobovec, Lijepocvjetka and Sampanjka) from the Majevica area in Bosnia and Herzegovina was carried out during 2017. The fruit of cultivars (meat + epidermis) was used to determine the influence of cultivar on total phenol content. The fruits for research were taken in the period of their full maturity from old apple trees placed at several locations.

Total phenolic content assay was conducted using the Folin-Ciocalteu reaction (Singleton et al., 1999), with gallic acid as the standard. The method is based on the measurement of the reducing capacity of polyphenolic compounds whose dissociation results in proton and phenoxide anion. The phenoxy anion reduces the Folin Ciocalteu's reagent to-blue colored ions $(Fenol\text{-}MoW_{12}O_{40})^{4-}$. The reaction mixture was prepared by mixing the fruit extract (0.1 cm^3), 7,9 cm^3 distilled water, 0,5 cm^3 Folin Ciocalteu's reagent and 1,5 cm^3 20% Na_2CO_3.

As a blank test a mixture was used in which 0.1 cm^3 of distilled water was added instead of 0.1 cm^3 of the sample. After incubation at room temperature for two hours, the optical density of the solution at 760 nm was measured. Total phenolics was expressed as mg of gallic acid equivalents (GAE) per kilogram of fresh weight of edible part of fruit (Amerine & Ough, 1980). The results were processed statistically using the analysis of variance. The significance of differences between mean values was evaluated using Duncan's multiple range test for significance level of 0.05. Data analysis was performed using the statistical software package IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

According to Jakobek & Barron (2016) ecological factors such as temperature, light, humidity, but also and the applied method of extraction significantly influence on the total phenols content in the fruit. The cultivar has an influence on the content of phenols in fruit (Anttonen & Karjalainen (2008). According to these authors, the fruits of cultivars, which grown under the same conditions, are significantly different in total phenolic content.

Tabart et al. (2006) state that the content of phenolic in fruits of apples depending on the genotype, the conditions during maturing, the moment of harvest, but also treatment with fruits after harvest. The phenolic content have a significant influence in the resistance of the cultivar to Venturia inaequalis (Cooke) Wint. According to Usenik et al. (2004), the total phenols content in plant tissues affects the level of susceptibility/tolerance of genotype of apple to fungal infections and pathogens. Treutter & Feucht (1990) state that the content of total flavanols in the epidermis of the fruit resistant cultivars of apple was three times higher than the cultivars of apple susceptible to this pathogen.

The fruit of six autochthonous cultivars (meat+epidermis) was used to determine the influence of cultivar on total phenol content and the results of these studies were presented in Table 1.

Table 1. The total phenols content in fruit of cultivars of apple from Majevica ara

Cultivar of apple	**Total phenols content (TP mg GAE/100 g FW)**
Petrovaca	276,41 ± 1,15 **a**
Bjelicnik	247,45 ± 1,31 **a**
Zelenika	306,80 ± 1,17 **b**
Bobovec	419,52 ± 1,52 **c**
Ljepocvjetka	542,10 ± 1,12 **d**
Sampanjka	345,28 ± 1,11 **ab**

**1mg of gallic acid equivalents (GAE)/100 g FW; **Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at P≤0.05.*

The results of research show that the highest phenolic content was in cultivar Ljepovjetka (542.10 mg GAE/100 g) than in cultivar Bobovec (419.52 mg GAE/100 g) and cultivar Sampanjka (345.28 mg GAE/100 g). The lowest phenolic content was in cultivar Bjelicnik (247.45 mg GAE/100 g). Using Duncan's multiple range test interval is clearly observed differences in total phenol content in the tested cultivars of apple ($P < 0.05$).

Similar results have also come up and Valvanidis et al. (2009); Li et al. (2013) and other. Oszmianski & Wojdylo (2008) and Ayub Ali et al. (2010) show lower values of the total phenol content. Our results are similar to data reported by the Wolfe et al. (2003), as and authors Escarpa & Gonzaleza (2001). Delian et al. (2011) have determined the lower content of total phenol content in the apidermis of apple, while the higher values of total phenol content was in researches Drogoudi et al. (2008) and Vieira et al. (2009).

Based on the results of this research, as well as the literature data, it can be concluded that there are significant variations in the phenolic content and that there is no regularity in the change content of phenolic. Such variations in the phenolic content in fruit of different cultivars of apples are only confirmed results and conclusions in this research and it is that is cultivar and geographic origin have an important influence on its ultimate phenolic content.

Conclusion

Based on the study of phenolic content of the autochthonous cultivars of apple from the Majevica area, we can conclude the following:

- Autochthonous cultivars of apple during a long period og time have been adapted to the agro-ecological conditions of Republika Srpska and Bosnia and Herzegovina and have economic importance.
- Autochthonous cultivars are a very rich source of genetic variability
- The highest phenolic content was in cultivar Ljepovjetka (542.10 mg GAE/100 g) than in cultivar Bobovec (419.52 mg GAE/100 g) and cultivar Sampanjka (345.28 mg GAE/100 g). The lowest phenolic content was in cultivar Bjelicnik (247.45 mg GAE/100 g).
- Based on the results of these researches, there are significant variations and that there is no regularity in the change content of phenolic.
- Such variations in the phenolic content of different cultivars of apple are only confirmed results and conclusions of our research, and that the cultivar and geographic origin have an important impact on his final phenolic content.

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A STUDY ON THE PHENOLOGICAL STAGES OF THE VARIETY CABERNET SAUVIGNON UNDER THE EFFECT OF CLIMATE CHANGE GROWN IN THE REGION OF WEST BEKAA-LEBANON

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Abstract

Lebanon is one of the oldest sites for wine production in the world. The sector of viniculture is witnessing a general development in the last decades, where more than 65 wineries are found. Worldwide, the reality of climate change being a problem is affecting agricultural aspects by modifying phenological growth of crops. Therefore, in the current study, the performance of the variety Cabernet sauvignon as affected by climate modification was observed during the last decade in the west Bekaa. In fact, the effect of increasing temperature and decreasing precipitation between 2009 and 2017 was tested on dates of budburst, flowering, fruit set and fruit maturity. All dates were calculated considering the first of January as an initial date ($date_0$). As a result, in 2017, budburst, flowering and fruit set occurred earlier by 15 days, 12 days and 28 days respectively compared to 2009. Accordingly, the number of days between beginning budburst and fruit set was later in 2009 (52 days) by 3 days than the one in 2017 (49 days). Consequently, the phenomenon of earliness in all phenological stages was detected on the entire growth cycle presented by the harvest date. This latter was earlier by 23 days in 2017 (262 days after $date_0$) compared to 2009 (285 days after $date_0$). The phenological stages of the variety Cabernet Sauvignon were highly affected by the climate variation occurring in the last years.

Keywords: *Lebanon, Cabernet Sauvignon, viniculture, phenology, climate.*

Introduction

Intergovernmental Panel on Climate Change (IPCC), in its latest announcement affirmed that the frequency and intensity of drought in the Mediterranean region will possibly increase into the early and late 21st century. The average of precipitation in the eastern Mediterranean region for the period 1986-2005 to 2081-2100 will supposedly decrease between 20% and 30%, also an increase in temperature of 2°C to 3°C (IPCC, 2013).Wet and warmer conditions result in an elongated hot and dry climate. Temperature and precipitation extremes will also increase. The drought periods, will become 9 days longer by 2040 and 18 days longer by 2090 (MoE, 2011).

The connection between temperature and wine production in wine grape growth with conductive climates is supported through historical evidence (Le Roy Ladurie, 1971; Pfister, 1988). In fact, the dramatic decline in temperatures during the "Little Ice Age" (14–19th centuries) led to northern vineyards to die out and to short growth seasons that made grape growth and harvesting extremely difficult in southern Europe. Moreover, geographic and varietal changes on viticulture due to climate change are most likely to be highly variable. Early analysis suggests that European growth seasons will lengthen and that there would be an increase in precipitation in the North and a decrease in the South (Lough et al., 1983). *Vitis vinifera* grapevines are phenologically distinct with defined development stages: budburst, flowering, fruit set, and grape maturity. The timing of each stage varies with grapevine variety, climate, and geographic location. In cool climate regions with short growing seasons,

early ripening varieties are needed, while in hot climates late ripening varieties have sufficient time to mature. Developmental stage timing is related to the ability of the vine to yield fruit, where early and fully expressed phenological events usually lead to larger yields. Furthermore, phenological timing has been found to relate to the quality of the vintage with early harvests resulting in high quality vintages (Jones and Davis, 2000).

Viticulture is an excellent example in showing the importance of studying the effect of climate change on agriculture. Grape growth history has associated fine wines with distinct viticulture regions (Johnson, 1985; Penning-Rowsell, 1989; Unwin, 1991) mainly found in Mediterranean climate regions around the world. Lebanon, being part of the Mediterranean region is characterized by a climate with four different seasons. This encloses a rainy period followed by dryness from November to March. Annual precipitation ranges between 600 mm to 800 mm, 1000 mm to 1400 mm, 200 mm to 600 mm and 600 mm to 1000 mm in the coastal plain, Mount Lebanon, Central and Northern Bekaa and Southern regions respectively (Ministry of Environment/Ecodit, 2010).

Since the Phoenician era, the existence of Lebanese wine has been an ideal example of the country's viticulture. This is a representation of the story-making activity of wine production. The wines and vines of the Lebanese territory have portrayed an indispensible role throughout the Mediterranean. In 2016, this number reached 60 (Roselier, 2016) when a total of 8 million bottles were produced per year.

The most substantial factor in manufacturing great wines is grapes quality, especially when it is associated with a know-how expertise in the professional vinification course. This ensures optimization of the organoleptic expression of the wines and its terroir characteristics. Lebanese terroirs are characterized by plains and hillsides, typical calcareous-clay and stony soils, wetness during winter and dryness in the summer (Mohasseb and Sassine, 2010), 300 days of sunshine, which accommodates flawless maturity and ultimate biosynthesis (Link 1 ; Le Commerce du Levant, 2009). Freshness of wines regardless of the sun and temperature elevation is perpetuated through thermal amplitudes between summer nights and days. This causes the fluctuation of sugars to berries (Navarre and Langlade, 2010).Increase in anthocyanin synthesis through the enhanced reflection of the sunlight is owed to the white color induced by limestone soil copiousness. Different grape varieties could be found in Lebanese vineyards. Each grape variety has its own characteristics, flavor and aromas that could be expressed in the finished wines. The grape varieties planted in Lebanon are mostly noble varieties (LaMar, 2001) as Cinsault, Cabernet Sauvignon, Syrah, Tempranillo, Pinot Noir, Obeidi, Clairette, Chardonnay, Sauvignon Blanc, and Muscat.

Throughout the course of nine consecutive years, our aim was to observe the differences in duration of the phenological stages in West Bekaa as affected by changes in temperature and precipitation between the year 2009 and 2017.

Material and methods

Experimental field

Observations were done in the fields of KSARA winery situated in West Bekaa at an altitude of 1020 meters above sea level, latitude of 33.6406, and longitude of 35.7169 on a surface of 8300 m^2 planted by the variety Cabernet Sauvignon at a 2.5 m × 1.25 m distance between plants and grafted R110 rootstock. Pruning was done using the double Guyot technique leaving twenty buds per vine. Fertilization was done by applying cow manure and organic wastes (plant leaves) in a ratio 50:50 and a dose of 30 Kg/plant.

Data collection

The methods sectioned the season's growth into multiple time periods ranging from each phenological event to the other (budburst, flowering, fruit set, and fruit maturity), where in each period climatic influences were added up and analyzed.

Weekly data collection was performed in order to record the date of each phonological stage on vines that was expressed as number of days after first of January. Phenological stages assessed were dates of budburst, flowering, fruit set and harvest, total production and quality. Records of temperature, relative humidity and precipitations were provided by the Lebanese Agricultural Research Institute (LARI) and soil analysis was performed at the same institute.

Data analysis

A correlation was done among the tested parameters in order to determine positive and negative correlation, using SPSS23 program in addition all graphs were performed applying excel 2007.

Results and discussion

Throughout the previous years, days to full budburst (Figure 1) were as follows: 118 days (in 2009), 109 days (in 2010), 111 days (in 2011), 115 days (2012), 111 days (2014), 115 days (2015), 111 days (2016), and 103 days (2017). This shows that budburst occurred earlier in later years (2016 and 2017) compared to 2009 with the exception of the year 2013 where it was the latest among all years (127 days). The number of days for flowering (Figure 2) declined in 2016 and 2017 (139 and 138 respectively) compared to earlier years mainly 2009 (150 days), 2012 (140 days), 2013 (142 days) and 2014 (143 days) where flowering took place later. With respect to the variety Cabernet Sauvignon, 166 days were needed to obtain the first fruit of the set in 2009. This number was higher than all following years, which required 144, 156, 156, 155, 153, 157, 160, 138 days from 2010 till 2017 respectively. A gradual decrease in number of days to fruit set (Figure 3) was detected over the past 9 years. Similarly, maturity dates (harvest dates) in 2009 (285 days) and 2011 (287 days) surpassed that of the remaining years where it was respectively of 254, 268, 263, 265, 264, 260 and 262 days in 2010, 2012, 2013, 2014, 2015, 2016 and 2017 (Figure 4).

Figure 1: days to full budburst from 2009 till 2017

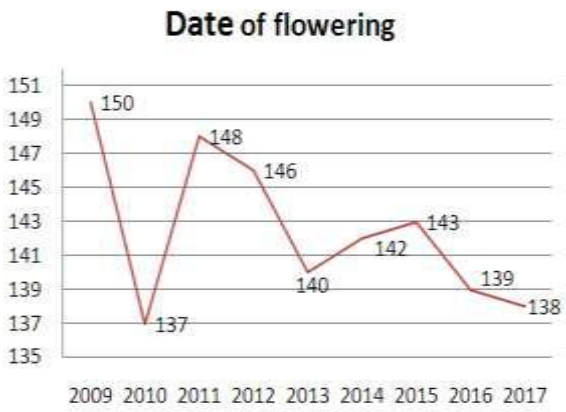

Figure 2: Date of flowering from 2009 till 2017

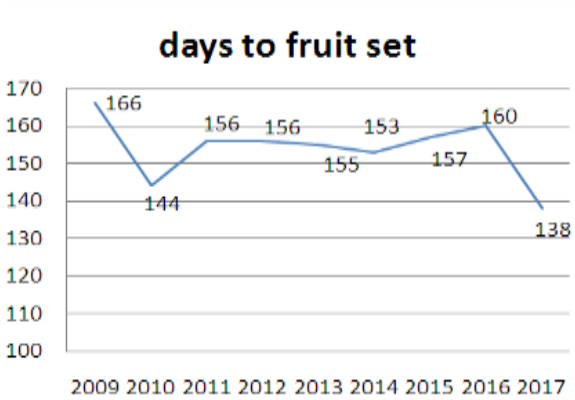

Figure 3: days to fruit set from 2009 till 2017

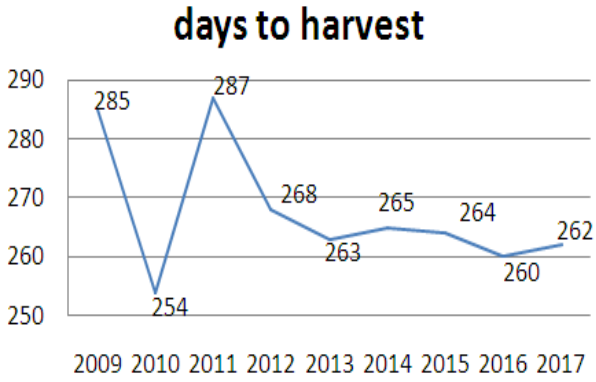

Figure 4: days to harvest from 2009 till 2017

Budburst and flowering shared a decreasing number of days in between them from 2009 till 2017. 36 to 56 days from 2009 till 2016 were recorded, followed by 49 days in 2017. Moreover, there was a mild difference in the number of days between budburst and fruit set in the 9 consecutive years, in which there was a cutback in this number during the first two years (52 days declined to 41 days in 2009 and 2010 respectively). It later escalated to 52 days in 2011, 57 days in 2013 and 59 days in 2014. On the other hand, the number of days between budburst and fruit set in 2015 and 2017 was identical (49 days). However, the highest number of days to fruit set took place in 2016: 77 days. As a result, in 2017, the phenological stages budburst, flowering and fruit set occurred earlier by 15 days, 12 days and 28 days respectively compared to 2009. Accordingly, the number of days between budburst and fruit set was later in 2009 (52 days) by 3 days than the one in 2017 (49 days). Consequently, the phenomenon of earliness in all phenological stages was detected on the entire growth cycle presented by the harvest date. This latter was earlier by 23 days in 2017 (262 days after $date_0$) compared to 2009 (285 days after $date_0$). The phenological stages of the variety Cabernet Sauvignon were highly affected by the climate variation occurring in the last years.

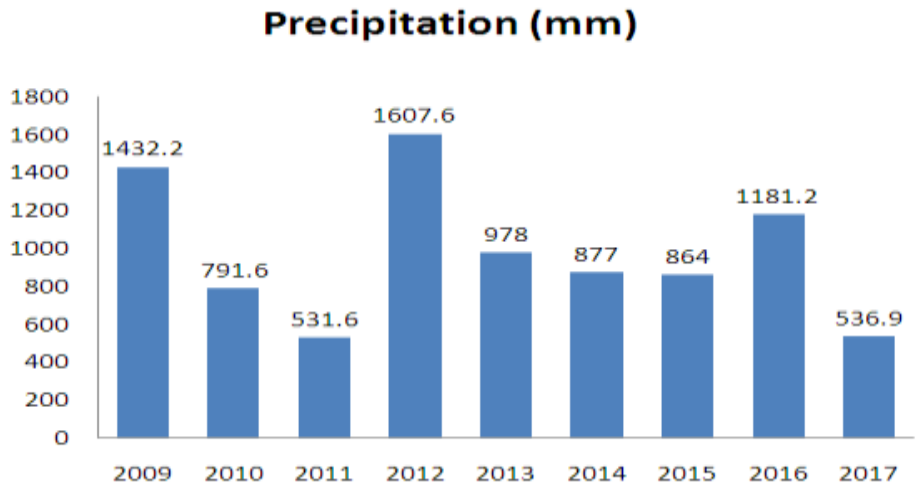

Figure 5: Precipitation levels during 2009 till 2017

Precipitation in Qanafar region varied every year as shown in this graph (Figure 5). It practically began to decline as of 2009 (1432.2 mm), in which precipitation was lower in following years, with the exception of 2012. The latter had 1607.6 mm of rain, while the other years measured 791.6 mm in 2010, 531.6 mm in 2011, 978 mm in 2013, 877 mm in 2014, 854 mm in 2015, 1181.2 mm in 2016, 536.9 mm in 2017.

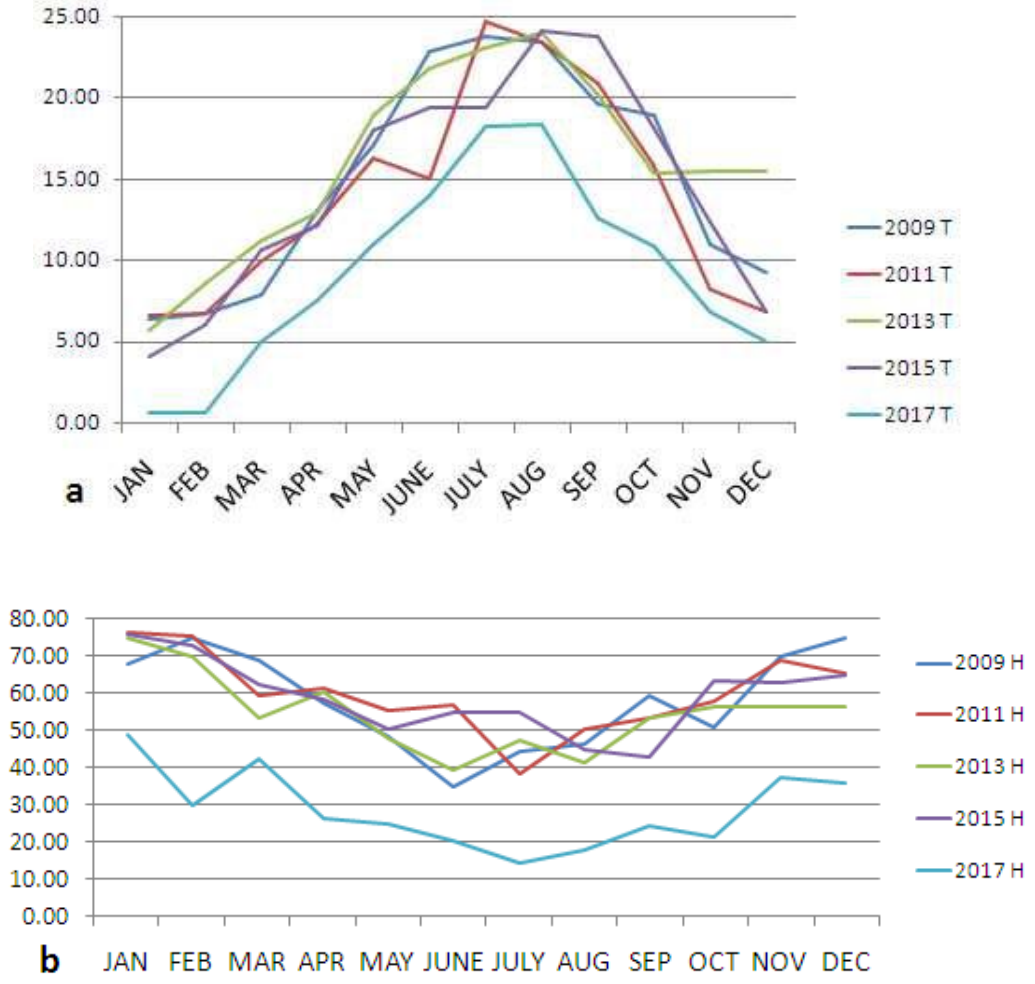

Figure 6: Monthly variation of and temperature (a) humidity (a) during the 9 consecutive years

In 2009, average monthly temperature (Figure 6a) and humidity (Figure 6b) differed between the four seasons. During winter, temperature was 6.4^{o} C and the humidity was 67.5% in January, and 9.24^{o}C, 74.7% in December. Spring recordings however showed 7.8 ^{o}C and 68.9 % in March, 13^{o}C and 57.3% in April. July and August had a temperature and humidity of 23.7^{o}C, 43.8%, and 23.04^{o}C, 46.1% respectively during the summer of 2009. Furthermore, 19.6^{o}C and 59.3% and 18.9^{o}C and 50.6% were the average temperature and humidity measured in autumn. In winter of 2011, temperature and humidity during January and February were 6.6^{o} C, 76%, and 6.75 ^{o}C, 74.9%, and 6.8^{o}C and 64.9% in December. 9.9 ^{o}C and 59.3% in March, 12.28^{o}C and 61.3% in April, represent the average temperature and humidity during spring. Moreover, summer of 2011 showed that in July, and August, temperatures and humidity were 24.6^{o}C and 38.19%, 23.4^{o}C and 49.9% respectively. In addition to that, an average of 20.8^{o}C and 53.2% in September and an average of 15.8^{o}C and 57.7% in October, were recorded in autumn 2011. Temperature and Humidity during winter of 2013 as shown in the graph, states that in January and December, we obtained an average 5.69^{o} C 74.6%, and 15.43^{o}C 55.98% respectively. Moving to spring of 2013, temperature was 11.2 ^{o}C and humidity was 53.06% in March. In summer, July, and August temperatures were 24.03^{o}C, while humidity was 46.9%, 41.2% respectively. Furthermore, autumn of 2013 showed an average of 15.35^{o}C and 56.1% in October.

Temperature and Humidity during winter of 2015 were 4.1^{o} C, 75.8%, and 6.04 ^{o}C, 75.8% in January and February. March and April showed an average temperature and humidity of 10.5 ^{o}C, 62.1%, and 12.1^{o}C, 58.1%. Meanwhile, summer of 2009 revealed that in July, and August, temperatures and humidity were 19.3^{o}C, 54.6%, and 24.06^{o}C, 44.7% respectively. Finally, autumn of 2015 showed an average of 18.1^{o}C, 63.3% in October. Temperature and Humidity during winter of 2017 were 0.5^{o} C, 48.7% in January, and 4.9^{o}C, 35.8% in December. In spring, the average temperature and humidity consisted 4.9^{o}C, 42.4% in March, and 7.5^{o}C, 26.2% in April. July, and August temperature and humidity were 18.1^{o}C, 14.1%and 18.3^{o}C, 17.3% respectively. Last but not least, autumn of 2017 showed an average temperature and humidity of 12.6^{o}C, 23.9% in September and 10.8^{o}C, 21.1% in October.

The overall average of the years 2009, 2011, 2013, 2015, 2017 included a temperature of 14.9^{o}C, 13.8^{o}C, 16.05^{o}C, 14.5^{o}C, 9.19^{o}C, and a humidity of 57.9%, 59.6%., 54.43%., 58.8%, and 28.5% respectively. As a final conclusion, temperature during winter (November, December, January and February,) decreased from 2009 till 2017. However, temperature during summer (June, July, August, and September) increased from 2009 till 2015, and then decreased from 2016 and 2017. With respect to budburst, earliness in this phenological stage was affected by the climatic conditions. In 2017 the temperature was very low in winter compared to other years, in addition to the sudden raise in temperature which led to shortening of the phenological stage budburst. This was also a product of the low precipitation during that year. On the other hand, full budburst, flowering, and fruit set occurred in the longest number of days in 2009. This was the result of the gradual change in temperature and thus budburst and flowering took the optimal time needed to occur in this year.

Due the intensely cold, short-lived winter in 2017 and then an unanticipated upsurge in temperature, premature budburst, flowering, and fruit set transpired. These conditions might have created favorable situations to break vine dormancy earlier than normally expected dates. Early season growth was slightly influenced by temperature extremes (Jones and Davis, 2000), minimal or maximal. The short winter is causing an earlier spring, so we're witnessing an increase in temperature during the final month of winter in February till mid march. Not to mention that this increase in temperature is happening during the final stage of winter so these phenomena created a shock for the vines normal functioning due to the risk of a sudden decrease in temperature in the final stage of winter. Climate of West Bekaa has been witnessing a reduction in precipitation levels and a decrease in temperature during the month of September, so autumn season occurred earlier, in September which is the final month of the summer. New climatic characteristics turned out in the past 15 years, unusual changes are happening during the 4 seasons, like rains and some heavy winds during the final month of summer and dryness during the whole season of winter. Considering the correlation between fruit maturity, budburst, fruit set and flowering, the earliness which took place in the last three stages mentioned also had an impact on harvest. This promoted a similar precedence in harvest occurrence in the 2017 season. However, this caused a disadvantage in the maturity of the fruit, in which the suitable time needed to achieve full maturity was minimized. Contrarily in 2009 harvest went through the normal time needed for maturity, not to mention the prior phenological stages which had the same outcome. This portrays 2009 as taking the highest number of days to achieve flowering.

Lebanon witnessed a mild winter in the period between 2009 till 2017. This was indicated by the low precipitation and insufficient snow on mountains tops. In addition to that, there was a prolongation in spring season which generated the formation of frost on vines, and thus resulted in a prominent damage and decrease in grape quantity. Furthermore, extreme temperature elevation during summer throughout the mentioned years provoked earlier maturity

Table 1: Correlation between all investigated indicators

	BB	FB	DF	DFS	DH	NDBF	NDBFS	
BB	1	0.51	0.77	0.46	0.56	-0.9	-0.64	BB
FB	0.51	1	0.46	0.63	0.29	-0.42	0.01	FB
DF	0.77	0.46	1	0.69	0.85	-0.41	-0.2	DF
DFS	0.46	0.63	0.69	1	0.65	-0.19	0.38	DFS
DH	0.56	0.29	0.85	0.65	1	-0.22	-0.02	DH
NDBF	-0.9	-0.42	-0.41	-0.19	-0.22	1	0.78	NDBF
NDBFS	-0.64	0.01	-0.2	0.38	-0.02	0.78	1	NDBFS

BB: beginning of budburst, FB: full budburst, DF: date of flowering, DH: date of harvest, NDBF: number of days between beginning of budburst and flowering, NDBFS: number of days between budburst and fruit set

The Table 1 shows existing correlation among various indicators (Table 1). Initially, beginning of budburst and full budburst were strongly and positively correlated with flowering, fruit set and fruit maturity dates. However, beginning of budburst was negatively correlated with the number of days between beginning of budburst and flowering and between beginning of budburst and fruit set. This was also the case in flowering which showed the same results.

With respect to the fruit set, there was a strong and positive correlation between the latter, and beginning of budburst, full budburst, flowering, fruit maturity and the number of days between beginning of budburst and fruit set. On the other hand, fruit set had a negative correlation with the number of days between budburst and flowering.

Fruit maturity was strongly and positively correlated with all the phenological stages excluding the number of days between beginning of budburst and flowering, and the number of days between beginning of budburst and fruit set.

Earliness in the four phenological stages led to an increase in the time interval of the number of days between beginning of budburst and flowering, and between beginning of budburst and fruit set.

Conclusion

Conclusively, this change in climate prompted earliness in the phenological stages which include, budburst, flowering, fruit set and fruit maturity. Additionally, premature harvest occurred in the beginning of August instead of the end of August as accustomed.

In order to adapt to these climatic variations, vines ought to be treated in the onset of each season firstly. Secondly, irrigation should be applied when there is a deprivation in precipitation. Thirdly, cultivators should utilize frost resistant varieties and avoid hydric stress during hot summers.

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THE SUNFLOWER PRODUCTIVITY IN FUNCTION BY THE NUTRITION LEVEL ON CHERNOZEM CAMBIC IN LONG-TERM EXPERIENCES

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Abstract

The evaluation results on the sunflower productivity cultivated on the chernozem cambic in according to the fertilization level and agro-meteorological conditions in the years 2011-2017 are presented. Sunflower seed production obtained from the unfertilized (witness) variant ranged from 0.85 t/ha to 1.93 t/ha. Administration of fertilizers on the natural background on average for 5 years led to the increase of the sunflower yields from 1.48 t/ha to 2.47 t/ha, the production increase being 19-67%. At phosphorus levels the crop yield increased from 10% containing 1.5 mg/100g of soil to 48% - 3.0-3.5 mg/100g of mobile phosphorus soil versus the $N_{45}K_{30}$ background. In the $P_{3.5}K_{30}$ variant, the increase in harvest versus control variant was 38%. In nitrogen-based versions of PK at doses of 30-90 kg/ha the increase in sunflower production was 51-67% compared to the control variant and 13-29% relative to PK. The optimal soil phosphorus level for chernozem cambic in the sunflower cultivation was 3.0-3.5 mg/100g of soil (Machigin method) and optimal nitrogen were 45-60 kg/ha.

Keywords: *Chernozem Cambic, Fertilization, Nutrition level, Productivity, Sunflower*

Introduction

Productivity of agricultural crops largely depends on the moisture and the level of soil fertility. Research in the Republic of Moldova has shown that the average multiannual rainfall ensures obtaining of 2.7 t/ha of sunflower seed. From the account of the soil natural fertility the 1.4 tons of sunflower seeds can be obtained (Andries, 2011; Почвы Молдавии, 1986).
The untapped value of sunflower seed production under soil humidity conditions is 1.3 t/ha. This value can be covered by increasing soil fertility by fertilizer management and improving recommendations on their rational use. The agricultural soils in Moldova are relatively rich in humus, the weighted average being 3.1%. During the process of organic matter mineralization in the soils, about 74 kg/ha of the nitrogen is produced, which is not enough to produce profitable sunflower productions. According to the phosphorus content the soils of Moldova are poor. According to the results of the last cycle of the soil agrochemical cartography, about 60% of the surveyed area has an assurance degree under the optimal content of mobile phosphorus in the soils. Up to 90% of the soils are relatively optimally ensured with accessible form of potassium for plants. The main available potassium reserve is the exchangeable form, which is largely restored based on the disintegration of potassium minerals in soils of Moldova (Andries, 2007; Burlacu, 2000; Recomandări, 2012). From the soil nutrition regimes in the first minimum are nitrogen and phosphorus. In order to improve the fertilization system of the chernozem cambic (leached), the productivity and the quality of the sunflower were evaluated according to the level of fertilization and the agro-meteorological conditions of the 2011-2017 years.

Materials and methods

Field investigations were conducted within the Long-term Experimental Station of the "Nicolae Dimo" Institute of Soil Science, Agrochemistry and Soil Protection from Ivancea commune, Orhei district, founded in 1964 on the clayey-loamy cambic chernozem.

The humus content in the arable layer was 3.4%; the value of pH_{H2O} - 6.8; the sum of Ca and Mg - 37.4 me/100g of soil. In 2000 year, the experimental station was registered in the EUROSOMNET (European Soil Organic Matter Network). In the crop rotation are grown: winter wheat, corn for grain, sunflower, winter barley, rape and legumes (alfalfa, peas, beans, soybeans). The sunflower preceding culture were winter wheat and winter barley. The experiments were performed in 4 repetition. The surface of the experimental plot - 200 m^2. Investigations were carried out on the following levels of mineral nutrition: mobile phosphorus in the soil - 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0 and 4.5 mg/100g of soil; exchangeable potassium in soil - 29-32 mg/100g of soil (Macighin method). The levels of mobile phosphorus in the soil were maintained by offsetting the phosphorus export from preceding culture by applying phosphorus fertilizer to the base soil tillage. Potassium fertilizers from 2010 to present do not apply in the experiments. Nitrogen doses were applied annually in doses - 0, 30, 45, 60, 75 and 90 kg/ha.

Results and discussions

The amount of rainfall, as well as their distribution during plant growing, has conditioned the sunflower's productivity. During the agricultural investigation years, agro-meteorological conditions were different. From five years of research at "Ivancea" station - two years have been relatively dry (2012 and 2015), with a humidity deficiency of 17-21% over the multiannual average, fewer droughts were 2014 and 2016. Close to normal was 2011 with 563 mm, making up 102%, over norms or so-called "wet years" were 2013 and 2017, respectively 115% and 108%. The average of 7-year atmospheric precipitations was 23 mm less than the multiannual average, constituting 529 mm (Table 1).

Table 1. Atmospheric precipitation at the "Ivancea" Station in 2011-2017 period

Year	Months														Agricultural Year*	
	IX-III		IV		V		VI		VII		VIII		IV-VIII			
	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%
2011	245	95	49	117	26	49	195	247	31	51	17	28	318	108	563	102
2012	153	60	38	90	114	215	48	61	59	97	22	37	281	95	434	79
2013	293	114	20	47	64	121	84	106	126	206	46	77	340	115	633	115
2014	261	102	25	60	112	211	36	46	55	90	20	33	248	84	509	92
2015	325	127	39	93	10	19	33	42	37	61	15	25	134	45	459	83
2016	252	98	31	74	57	107	133	168	3	5	36	60	260	88	512	89
2017	251	97	99	236	46	87	60	76	91	149	49	82	345	117	596	108
Average - 7 years	254	99	43	102	61	115	84	106	58	95	29	48	275	93	529	96
Multi-annual	257	100	42	100	53	100	79	100	61	100	60	100	295	100	552	100

*Note. *The period of the agricultural years is considered 01.09.2010 - 31.08.2017*

Precipitation during the cold season of the year (September-March) has created favorable humidity conditions in the early spring, which have influenced normal growth and development of sunflower plants. The amount of precipitation during the cold season at the station was almost normal, accounting for 97-114% of the multiannual average, except for 2012 with only 60% and 2015 - by 127% over the norm.

The atmospheric precipitation for the active grow period of crop field (April to August) in research years of research decreased by 7% on over the multiannual average, and by 2015 they were 55% less, constituting 134 mm. The drought effect was most pronounced in July

and August, where the monthly rainfall in the years 2015-2016 decreased by 75-95% over the multiannual average and the air temperatures exceeded the norm by 2.0-3.9^{0}C (Table 1).
The application of mineral fertilizers on the chernozem cambic has positively influenced the growth and development of sunflower culture. Sunflower production increased on average from 1.48 t/ha in the control variant to 2.47 t/ha on fertilized variants (Table 2).

Table 2. Sunflower yields on the chernozem cambic in according to fertilization level, t/ha

Variant		Year of cultivation					Average, t/ha	Increase, %
Nitrogen, kg/ha	P_2O_5 mg/100 g	2011	2012	2013	2015	2017		
Control	1,0	1,90	1,42	1,93	0,85	1,31	1,48	-
45	1,0	2,21	1,57	2,21	1,43	1,45	1,77	19
45	1,5	2,30	1,65	2,36	1,49	1,77	1,91	29
45	2,0	2,36	1,71	2,79	1,56	2,20	2,12	43
45	2,5	2,40	1,90	3,14	1,69	2,55	2,34	58
45	3,0	2,50	2,15	3,21	1,71	2,60	2,42	63
45	3,5	2,54	2,27	3,19	1,78	2,59	2,47	67
45	4,0	2,57	2,13	3,18	1,83	2,51	2,44	65
45	4,5	2,51	2,23	3,21	1,85	2,53	2,47	67
0	3,5	2,32	1,78	2,64	1,64	1,89	2,05	38
30	3,5	2,40	1,90	3,00	1,70	2,22	2,24	51
45	3,5	2,49	2,25	3,21	1,72	2,50	2,43	64
60	3,5	2,55	2,22	3,26	1,81	2,49	2,47	67
75	3,5	2,50	2,17	3,27	1,82	2,57	2,47	67
90	3,5	2,34	2,20	3,22	1,84	2,49	2,42	63

The increase in sunflower seed yield for fertilized variants increased from 19% to 67% compared to the natural background. On the phosphorus fertilization levels, the sunflower production increased from 10% on the background of 1.5 mg of mobile phosphorus to 48% - 3.5 mg/100g of soil toward the $N_{45}K_{29\text{-}32}$ fund. On the variant with $P_{3,5}K_{29\text{-}32}$ background, the increase in crop yield versus control variant was 38%. On nitrogen variants in doses of 30-90 kg/ha on the $P_{3,0}K_{29\text{-}32}$ background**,** the sunflower seed production rate was 0.76-0.99 t/ha or 51-67% against the control variant and 13-29% - versus PK variant (Table 2).
In drought years the fertilizers have significantly contributed to the formation of sunflower yield. Although the global harvest has fallen in these years, the sunflower productivity compared to the unfertilized variant in 2012 year increased by 11-60%, and in 2015 year it was doubled (from 0.85 t/ha to 1.70-1.85 t/ha). The role of phosphorus fertilizers has been decisive in the formation of sunflower production. The optimal level of mobile phosphorus in the arable layer of the cambic chernozem was 3.5 mg/100g of soil, and in the wet years the optimum phosphorus level decreased to 3.0 mg/100g of soil.
Oil content in sunflower seeds in research years ranged from 38.2% to 51.1%, the five-year average was 45.1-46.8%. In the control variant the quantity of oil was 0.3-5.4% higher compared to the fertilized variants, the average being 1.7% (Table 3).
In fertilized variants with higher yields, the seed oil content did not increase. The so-called "dilution effect" was obtained in the production of sunflower seed.
The oil content obtained per unit area is an integral indicator of crop productivity evaluation. This indicator gives the possibility to determine the agronomic efficacy or yield of fertilizers in order to obtain seed production. The administration of mineral fertilizers practically doubled the amount of oil obtained at 1 ha from the natural background (Table 4).

Table 3. The oil content of the sunflower seed cultivated on the cambic chernozem,%

Variant	Year of cultivation					Average, %
	2011	2012	2013	2015	2017	
Control	48,0	50,5	43,6	48,6	45,5	46,8
$N_{45}P_{1,0}$	46,5	50,8	39,8	47,3	46,7	46,2
$N_{45}P_{1,5}$	46,0	50,2	42,7	49,6	45,5	46,8
$N_{45}P_{2,5}$	46,8	49,3	39,2	48,1	45,8	45,8
$N_{45}P_{3,5}$	47,6	48,3	40,4	48,6	46,2	46,2
$N_{45}P_{4,5}$	46,5	50,0	41,7	47,9	47,2	46,7
$P_{3,5}$	45,5	48,3	38,2	46,2	47,2	45,1
$N_{45}P_{3,5}$	48,7	46,9	42,8	47,8	45,7	46,4
$N_{60}P_{3,5}$	46,1	49,4	39,1	45,2	46,9	45,3
$N_{75}P_{3,5}$	46,0	50,8	40,1	46,3	45,2	45,7
$N_{90}P_{3,5}$	45,9	51,1	41,1	46,4	45,5	46,0

On average, during research years on the levels of phosphorus fertilization, the amount of oil increased from 695 kg/ha to 1140 kg/ha. The role of nitrogen fertilizers was significant. The application of nitrogen fertilizers at doses of 30-45 kg/ha on the background of PK led to an increase in the amount of oil from 914 kg to 1121 kg/ha. Increasing nitrogen doses from 45 kg to 90 kg/ha did not increase the content of sunflower oil per hectare (Table 4).

Table 4. The content of sunflower oil obtained in dependence of the fertilization level, kg/ha

Variant	Cultivation year					Average, kg/ha	Increase, %
	2011	2012	2013	2015	2017		
Control	912	717	841	413	596	695,8	-
$N_{45}P_{1,0}$	1027	797	879	676	677	811,2	16,6
$N_{45}P_{1,5}$	1058	828	1008	739	805	887,6	27,5
$N_{45}P_{2,5}$	1123	937	1231	813	805	981,8	41,1
$N_{45}P_{3,5}$	1209	1096	1289	865	1196	1131	62,5
$N_{45}P_{4,5}$	1167	1115	1338	886	1194	1140	63,9
$P_{3,5}$	1055	860	1008	757	892	914,4	31,4
$N_{45}P_{3,5}$	1212	1055	1374	822	1142	1121	61,1
$N_{60}P_{3,5}$	1175	1097	1275	818	1168	1106,6	59,0
$N_{75}P_{3,5}$	1150	1102	1311	842	1161	1113,2	60,0
$N_{90}P_{3,5}$	1074	1124	1323	854	1133	1101,6	58,3

The increase of sunflower oil production from the application of mineral fertilizers increased from 16.6% to 63.9% compared to the control variant (Table 4). On the phosphorus levels ($N_{45}P_{1,0}K_{29\text{-}32}$ background), the yields increased from 10.9% to 47.3%. When applying nitrogen fertilizers at doses of 30-90 kg/ha on the $P_{3,5}K_{29\text{-}32}$, the yield of oil was decreasing compared to the administered doses and constituted 29.7-26.9%. The maximum yield of sunflower oil production was obtained on the $N_{45}P_{3,5}K_{29\text{-}32}$.

Conclusion

The application of mineral fertilizers on the natural background of cambic chernozem led to an increase in the production of sunflower seeds by 19-67%. Phosphorus fertilization levels (1.5-4.5 mg/100g of mobile phosphorus) led to 10-48% increase in sunflower yield, and nitrogen fertilizers at 30-90 kg/ha increased the seed harvest with 13-29%. The maximum yield of sunflower oil production was obtained on the $N_{45}P_{3,5}K_{29\text{-}32}$ variant. The optimal level of phosphorus in the soil for chernozem cambic in the cultivation of sunflower is 3.0-3.5 mg/100g of soil (Machigin method) and the optimal nitrogen doses are 45-60 kg/ha.

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EFFECT OF ALTITUDE ON THE POMOLOGICAL AND PHYSICO-CHEMICAL TRAITS OF DELLAHIA PRICKLY PEAR FRUITS IN NORTHERN MOROCCO

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Abstract

Opuntia sp., commonly known as cactus pear, originates from Mexico and illustrates the impressive genetic diversity of Cactaceae with 400 species and a great number of varieties for Opuntia ficus indica. Moroccan cactus presents a very high genetic variability and several cultivars exist. They are distinguished by the flowering period (early, late), the flower color (yellow, orange and pink), fruit and pulp colors (green, yellow, orange, red and purple), fruit shape (oval, round or oblong), organoleptic characteristics and antioxidant content of the fruit. Cactus crop plays an important role on the ecological and economical scales, but unfortunately, in Morocco, its production is faced with a high lost due to the lack of valorization prospects. Dellahia prickly pear variety, widespread in northern Morocco, is characterized by the green pulp color of the fruit. It is among the lowest valorized cactus varieties due to the low oil content of its seeds. Therefore, its fruits are mainly used for fresh consumption. The aim of this study is to discuss the effect of the altitude on the pomological (fruit dimensions, seediness and weight) and phytochemical (juiciness, sugariness…) characteristics of the fruits of this variety in northern Morocco in order to reconsider the different possibilities to transform the excess of the production.

Key words: *Opuntia ficus-indica, Dellahia, altitude, pomology, phytochemistry.*

Introduction

Cactus pear (*Opuntia* sp.), native from Mexico, illustrates the impressive genetic diversity of Cactaceae with 400 species and a great number of varieties for *Opuntia* (Odoux et al., 1996). The prickly pear cactus (*Opuntia ficus indica*) is a plant highly distributed in the Mediterranean area, Central and South America, and South Africa (Barbera and Inglese, 1993; Muñoz de Chávez et al., 1995).The pericarp and the edible pulp of its fruit may have soft green, greenish white, canary yellow, lemon yellow, red, cherry red or purple hues (Muñoz de Chávez et al., 1995). In Morocco, this crop is witnessing a growing interest and an unprecedently spread thanks to the Green Morocco Plan. In fact, the cactus pear crop presents a lot of ecological and economical advantages. Unfortunately in Morocco, its production is faced with a high loss due to the lack of valorization prospects. Cactus pear also knows a renewed interest in several countries due to its ecological, environmental and socio-economic role: the fight against erosion and desertification, and fruit production fodder (Bouzoubaâ et al., 2014). Its ecological advantages can be attributed to its crassulacean acid metabolism, which allows CO_2 uptake at night, thus minimizing water loss during photosynthesis (Kluge and Ting, 1978 ; Winter and Smith, 1996). Low water requirements and a high water use efficiency ratio (Nobel, 1995) make cactus pear suitable for cultivation in arid and semi-arid marginal regions (Singh and Felker, 1998). In the light of changes in the global climate, cactus pear is suggested as a promising crop by the Food and Agriculture Organisation (Barbera, 1995 ; Nobel and Israel, 1994). The prickly pear is also a source of products and by-products to diverse industrial uses, medicinal, pharmaceutical and cosmetics

(Barbera, 1995). Moroccan cactus presents a very high genetic variability and several cultivars exist. They are distinguished by the flowering period (early, late), the flower color (yellow, orange and pink), fruit and pulp colors (green, yellow, orange, red and purple), fruit shape (oval, round or oblong), organoleptic characteristics and antioxidant content of the fruit. The Dellahia prickly pear variety, widespread in northern Morocco, is characterized by the green pulp color of the fruit. It is among the lowest valorized cactus varieties due to the low oil content of its seeds. Therefore, its fruits are mainly used for fresh consumption. The aim of this study is to discuss the effect of the altitude on the pomological (fruit dimensions, seediness and weight) and physicochemical (juiciness, sugariness…) traits of the fruits of this variety in northern Morocco in order to reconsider the different possibilities to transform the excess of the production.

Materials and Methods

Vegetative Material

The fruits have been collected in August 2016 at four sites with different altitudes in the North of Morocco as resumed in Tab. 1.

Tab. 1. Sites of study with their geographic coordinates and climatic characteristics

	Latitude	Longitude	Altitude (m)	Annual Rainfall (mm)	Mean Temperature (°C)
Mestassa (Mes)	35.10460	-4.41633	119	400 – 500	10 – 30
Wahran (Wah)	35.03564	-4.46452	482	500 – 600	10 – 30
Boujibar (Bou)	35.01616	-4.46961	573	600 – 700	10 – 30
Tizakhte (Tiz)	35.00597	-4.47130	712	700 – 800	10 – 30

Pomological traits measurement

From the samples kept cold at 6°C, 30 fruits per batch were randomly selected in order to determine the various physicochemical parameters. The lenght and diameter of the fruits have been measured with a digital caliper (Stainless Hardened). The weight of the whole fruits has been measured with a 0.0000g precision balance (S-203). Once weighed, the fruits have been carefully peeled manually and the peels as well as the pulp with seeds have been weighed separately. Then, the thickness of the peels was measured with a Stainless Hardened digital caliper. Then, 10 fruits per sample have been used for juice extraction. The fruits were washed, manually peeled and the pulp with seeds of each fruit was malaxed in a porcelain mortar separately then filtered. The juice obtained was quantified and kept in stoppered flasks at -4°C in a refrigerator for further analyses. The experiment was carried in triplicate and the juice yield was expressed in percentage of sample weigh. The seeds recovered from each fruit were then washed, spread on a paper and dried at room temperature for 3h. They were then manually separated from the dried pulp, weighed and counted. 10 seeds per fruit were also selected randomly and weighed separately in order to determine the mean weight of seeds. The sugar content of the juice was determined using a refractometer (DIGIT 032). The results are expressed as °Brix. The free acidity expressed as a percentage of citric acid was determined by titration; 10 mL of the juice of prickly pear was placed in a 100 ml beaker equipped with a magnetic stirrer, 20 mL of distilled water was added and the homogenized mixture is titrated with NaOH (0.1 N) to pH = 8.1 (Medina et al., 2007). For the determination of dry matter, fruits were washed and peeled. Then, 5 g of the pulp with seeds obtained was precisely weighed and placed in clean glass Petri dishes and pre-weighed. Then they were

placed in an oven at 103±1°C. After 24h, periodical weighing was carried at intervals of 2h until a constant weight. (A.O.A.C, 1990). The experiment was carried in triplicate and results have been expressed in percentage of fresh weight. As for the ash determination, it has been carried by incineration (A.O.A.C, 1990). In fact 2g of sample was placed in a porcelain crucible and incinerated in a Lenthon Thermal LTD oven at 550°C for 2 h. The porcelain crucibles were weighed again after the complete combustion in order to determine the weight of ash. Each experiment was carried in triplicate and results have been expressed in percentage of fresh weight.

Physico-chemical traits determination

Standards of sugars and organic acids commonly found in prickly pear juice were used with concentration ranges typical of the matrice indicated in Tab. 2. Standards solutions have been prepared according to their mean concentration in prickly pear juice. For each standard, three concentrations were prepared. Sugars standards solutions were prepared separately from those of organic acids.

Tab. 2. Concentrations ranges of sugars and organic acids standards solutions (g/L)

Standard Solutions	Glc.	Frct.	Sucr.	Ox. Ac.	Citr. Ac.	Mal. Ac.	Asc. Ac.	Tar. Ac.	Fum. Ac.
	Sugars	(g/L)		*Organic Acids*		(mg/L)			
STD 0	0	0	0	0	0	0	0	0	0
STD 1	2	2	2	5	16	37	6	15	0.74
STD 2	4	4	4	10	32	74	12	30	1.48

Tab. 3. Chromatography parameters and concentration ranges of sugars and organic acids standard solutions

Compounds	t_R(min)	Detection System	Area (µV-sec)	Quantity (g/L)	Symetry Factor	Quantitation factor
Glucose	11.921	RI	753343	2 – 4	---	1
Fructose	13.067	RI	731911	2 – 4	---	1
Sucrose	10.233	RI	1432198	2 – 4	--	1
Oxalic acid	9.342	UV	1522865	$5.\ 10^{-3}$ – $10.\ 10^{-3}$	1.137	---
Citric acid	10.925	UV	378404	$16.\ 10^{-3}$ – $32.\ 10^{-3}$	1.149	---
Mal. + Asc. acids	12.750	UV	694392	$43.\ 10^{-3}$ – $86.\ 10^{-3}$	1.097	---
Tartric acid	11.600	UV	559185	$15.\ 10^{-3}$ – $30.\ 10^{-3}$	1.218	---
Fumaric acid	17.525	UV	734578	$0.74.\ 10^{-3}$ – $1.48.\ 10^{-3}$	1.043	---

Samples preparation

The fruit pulp with seeds has been carefully crushed in a porcelain mortar by avoiding crushing the seeds. The mixture has been filtered to recover the juice. The pH of the juice extracted has been adjusted to 9 - 10 with 1 N NaOH. Then 2g of juice was taken in a test-tube and 25mL of aqueous ethanol (90% v/v) was added. The mixture was centrifugated at

8000rpm and the supernatant was recovered in a test-tube. 5mL of the supernatant was evaporated to dryness on the boiling water bath. The residue obtained was then disolved in 5mL of the mobile phase (ultrapure water), filtered through 0.45 μm membrane and finally injected in the HPLC apparatus.

Sugars determination

The HPLC system was equipped with an isocratic pump (PU 980), a variable wavelength detector UV 970 set at 210nm (for organic acids) connected in series with a refractive index detector RI 830 (for sugars) and an injection valve fitted with a 20 μL loop. Sugars have been separated on a REZEX RHM ion-exclusion column (300*7.80 mm) operating at a flow rate of 0.5mL/min by injecting 20μL of sample in the system through the valve. The refractive index detector was working at 40°C while the mobile phase was a water of chromatographic purity (ultrapure water). The chromatographic runs were performed at a column temperature of 30°C. Individual sugars were identified by comparison of their retention times with those of authentic standards under analysis conditions and quantified by external standard method. Each analyse lasted 15 min and the injections were carried out in triplicate.

Organic acids determination

The same HPLC system with the same REZEX RHM ion-exclusion column operating at a flow rate of 0.5mL/min has been used for organic acids determination. The separation was made by the variable wavelength detector UV 970 set at 210nm and working at 40°C. The mobile phase was a 0.0005 M H_2SO_4. The column temperature was initially 40°C for 5 min, then the column was heated at a rate of 5°C/min up to 60°C. 20μL of sample was injected in the system through the valve and individual organic acids were identified by comparison of their retention times with those of authentic standards under analysis conditions and quantified by external standard method. Each analyse lasted 30 min and the injections were carried out in triplicate.

Gross energy determination

The gross energy is the amount of heat produced from unit feed when it is completely burnt down to its ultimate oxidation products (CO_2 and H_2O). The feed is burnt in a closed container (Bomb calorimeter) and heat produced from it is measured (AOAC, 1995). The hydrothermal equivalent of the Parr 1341Plain Oxygen Bomb Calorimeter which represents the energy required to raise the temperature of the calorimeter one degree, usually expressed as calories per degree Celsius, was first determined by burning 1g of a standard sample (benzoic acid pellets) in the calorimeter. Then, 1g of each lyoplilised sample was compressed into pellet with a mechanical compressor and put in a cruicible to be carefully placed in the bomb calorimeter. The bomb was then closed, filled with oxygen and placed in the calorimeter bucket containing 2000mL of distilled water with 1.5°C below room temperature. The temperatures of the system were recorded with a 6775 Digital Thermometer at one-minute intervals for 5min. At the 6th minute the bomb was fired and temperatures were also recorded until a constant value. The gross energy was calculated by multiplying the rise of temperature by the equivalent hydrothermal of the system and dividing the product by the sample weigh.

$$\mathbf{He = (W \times A - 14) / (Tf - Tf)} \quad \text{and} \quad \mathbf{GE = (Tf - Ti) \times He / W}$$

With :
- He : Hydrotermal equivalent of the system (J/°C)
- A : Caloric capacity of benzoic acid (26442 J/g).
- 14 : Correction of acid formation (J)
- GE : Gross Energy (J/g)
- Tf : Final temperature (°C)
- Ti : Initial temperature (°C)
- W : Sample weight (g)

Final gross energy was expressed in Kcal/100g of dry matter.

Statistical analysis

Descriptive statistical analysis (based on calculating the mean and standard deviation for each studied parameter), the analysis of variance (ANOVA test at one way of variation : « sites ») and means comparison were all performed by the SPSS software 22. The Newman–Keuls test was used to determine significant differences between means with a 95% confidence interval.

Results and discussion

Pomological traits

Tab. 4. Pomological traits

Pomological traits	Mes	Wah	Bou	Tiz
Fruit weight (g)	96.70±4.91^{a}	78.43±1.41^{a}	94.15±2.44^{a}	84.38±3.96^{a}
Edible part weight (g)	62.04±6.55^{a}	52.75±2.46^{a}	64.74±1.40^{a}	56.82±4.47^{a}
Peel weight (g)	32.77±1.53^{a}	28.77±0.81^{c}	30.56±2.50^{b}	28.34±1.94^{c}
Peel Thickness (mm)	4.82±0.05^{a}	4.62±0.37^{a}	4.38±0.08^{b}	4.64±0.31^{a}
Fruit Lengh (mm)	70.69±3.34^{a}	70.33±2.20^{a}	68.58±0.61^{b}	70.54±1.39^{a}
Fruit Diameter (mm)	50.25±1.06^{a}	46.22±0.96^{a}	49.01±0.73^{a}	47.97±1.99^{a}
Ratio D/L	0.71±0.02^{a}	0.66±0.03^{a}	0.71±0.01^{a}	0.68±0.02^{a}
Fruit Firmity (%)	95.48±0.27^{c}	96.46±1.72^{b}	96.79±1.04^{a}	96.42±0.71^{b}
Total seeds number	297±41^{b}	255±45^{c}	323±30^{a}	249±20^{c}
Total seeds weight (g)	2.99±0.64^{b}	2.58±0.38^{c}	3.4±0.29^{a}	2.89±0.20^{b}
Mean weight by seed (µg)	10.03±0.89^{c}	10.14±0.63^{c}	10.71±1.46^{b}	11.66±1.62^{a}
Juiciness (%)	71.24±1.70^{b}	72.02±2.3^{a}	68.74±1.85^{c}	70.96±2.01^{b}
Seediness (%)	3.08±0.49^{d}	3.29±0.50^{c}	3.61±0.26^{a}	3.43±0.34^{b}
Sugars content (°Brix)	14.22±0.09^{a}	13.56±0.09^{a}	14.89±0.47^{a}	14.33±0.58^{a}
Free Acidity (%)	0.07±0.00^{a}	0.08±0.01^{a}	0.07±0.01^{a}	0.09±0.02^{a}
Ratio A/S	0.005±0.000^{c}	0.006±0.001^{b}	0.005±0.000^{c}	0.007±0.001^{a}
pH	6.29±0.10^{a}	6.16±0.10^{a}	6.25±0.09^{a}	5.93±0.22^{b}
Volatile matter (%)	82.95±1.06^{a}	84.13±1.13^{a}	81.19±0.86^{a}	83.83±1.03^{a}
Dry matter (%)	17.05±1.06^{a}	15.87±1.13^{a}	18.81±0.86^{a}	16.17±1.03^{a}

The values in the same line followed by the same letter are not significantly different with a 95% confidence interval. D/L = Diameter/Lengh ; A/S= Acidity/Sugars content

No significant difference was noticed between samples about the whole fruit and pulp weight (respectively from 78.43±1.41g to 96.70±4.91g and from 52.75±2.46g to 64.74±1.40g), fruit diameter (from 46.22±0.96mm to 50.25±1.06mm), their total sugars content (from 13.56±0.9°Brix to 14.89±0.47°Brix), their free acidity (0.07±0.01 to 0.09±0.02), their volatile and dry matters (varying respectively from 81.19±0.86% to 84.13±1.13 % and from 15.87±1.13% to 18.81±0.86%). As for the ratio D/L (from 0.66±0.03 to 0.71±0.01), no significant was nor detected between samples. All fruits samples are therefore relatively oval (ratio D/L<1). Compared to previous studies, fruits weight are considerably high than those reported by Bouzabaâ et *al.* (2014) (50.58 – 64.55g).

Otherwise, the sugars content are consistent with those reported by Bouzabaâ et *al.,* (2014) (14.91 – 15.87°Brix for *Amouslem* cultivar and 13.15 – 15.87°Brix for *Moussa* cultivar) and by Mashope (2007) (11 – 16°Brix) and also by Sepúlveda and Sáenz (1990) (11 – 17°Brix). However, the sugar content is significantly influenced by cultural practices (fertilization, irrigation) and environmental factors, generally the fruits of dry lands are sweeter than those of wetlands (Mondragón et *al.,* 2001) or irrigated lands (Inglese et *al.,* 1995). Furthermore, the sugar concentration can be improved particularly by the nitrogen fertilization (Potgieter and Mkhari, 2002). The citric acid values comprised between 0.07% and 0.09% are also

consistent with those reported in the literature (0.05 – 0.18%) (Sepúlveda, 1998; Sáenz, 2000). The pH values comprised between 5.93 and 6.29 are instead relatively high compared to those reported by Bouzabaâ et *al.*, (2014) (5.80 – 5.92).
The rate of volatile matters found comprised between 81.19 and 84.13% is high compared to results reported by Bouzabaâ et *al.* (2014) (80%). This very high water content in prickly pears is a factor reflecting the high perishability of this type of fruit and therefore hinders its ability to storage at room temperature. As for the dry matter content, values found for Mestassa, Boujibar, and Tizakhte sites (respectively 17.05, 18.81, and 16.17%) are higher than the theoretical one (16%) reported by Askar and El Samahy, (1981). On the other hand, the dry matter found for Wahran site (15.87%) is lower than the theoretical one.
However, significant difference was noticed between samples about fruit lengh, their peel weigh and thickness, fruit firmity, their total seeds number, their total seeds weight, the mean weight by seed, fruits juiciness and seediness and the ratio free acidity/sugar content (A/S).
The values found for fruits lengh picked at Mestassa, Wahran and Tizakhte sites (respectively 70.69mm, 70.33mm and 70.54mm) are significantly higher than the Boujibar site one (68.58mm). Compared to the literature, these values are lower than the arverage one reported by Bekir (2006) (74.43mm). The peel weight of the the sample from Mestassa site (lowest altititude, 119m) is higher (32.77g) than the Wahran, Boujibar, Tizakhte ones (respectively 28.77, 30.56 and 28.34g). The fruits from the highest altititude (Tizakhte, 1109m) have the lowest peel weight. The values found are still lower than those reported by Bekir (2006) (37.2 to 55.24g). On the other hand, fruits from Mestassa, Wahran and Tizakhte sites have similar peel thickness (respectivement 4.82, 4.62 and 4.64mm). Those values are significantly higher than Boujibar site one (4.38mm). The peel thickness is correlated to the fruit firmity. Indeed, fruits from Boujibar wich present the lowest peel thickness also present the highest firmity (96.79%) while fruits from Mestassa presenting the highest peel thickness present the lowest firmity (95.48%). The firmity values of fruits from Wahran and Tizakhte are not significantly different (96.46 and 96.42% respectively). The values for fruits juiciness are comprised between 68.74% and 72.02%. A significant difference was found between samples, Boujibar samples being the less juicy (68.74%) and Wahran ones being the most juicy (72.02%) while Mestassa and Tizakhte samples had a similar juice rate (respectively 71.24% and 70.96%). This similarity is probably du to the sea influence on Mestassa crops (low altitude 119m) and the dew influence on Tizakhte ones (high altitude 712m). The total seeds number of Boujibar samples (323 seeds, representing 3.61% of the fruit) is significantly higher while Wahran (482m of altititude) and Tizakhte (712m of altitude) samples being thes less seedy (respctively 255 and 249 seeds representing 3.29 and 3.43% of the fruits). Mestassa samples (119m of altitude) have a total seeds number relatively high but still remains the less seedy ones (297 seeds representing 3.08% of the fruits). The total seeds weigh are comprised between 2.58 and 3.4g while the mean weigh per seed varies from 10.03 to 11.66µg Tizakhte samples (712m of altitude) being the more weighty and Mestassa ones (119m of altitude) the less weighty. The total seeds number found for Boujibar samples is higher than those reported by Bekir (2006) (187 to 262 seeds per fruit). However, values found from the other sites are consistent with those reported by Bekir (2006).

Physico-chemical traits

Tab. 5. Physico-chemical traits

Chemical traits	M	W	B	T
Glucose (mg/g DM)	63.16±5.77^{b}	60.35±4.32^{c}	68.46±2.14^{a}	61,78±6.61^{c}
Fructose (mg/g DM)	53.58±5.71^{b}	51.55±3.80^{c}	58.10±3.01^{a}	51.87±6.35^{c}
Citric acid (mg/100g DM)	185.74±10.97^{a}	176.10±9.77^{b}	126.92±4.12^{c}	64.94±2.91^{d}
Fumaric acid (mg/100g DM)	5.93±1.24^{c}	9.39±1.82^{b}	3.69±0.53^{d}	9.80±1.52^{a}
Gross energy (Kcal/100g DM)	16.49±1.15^{b}	16.21±0.17^{b}	17.94±1.65^{a}	15.09±0.21^{c}

The values in the same line followed by the same letter are not significantly different with a 95% confidence interval. DM means Dry Matter

Reports are available on nutrient composition (Benjamin and Old, 1939; Sawaya et al., 1983a), amino and non volatile acid composition (Teles et al., 1984) and soluble sugar composition (Laskshminarayana et al., 1979; Askar and El-Samahy,1981; Sawaya et al., 1983b; Wessels, 1989) of prickly pear fruit. The predominant soluble sugars in the fruit are glucose and fructose with smaller amounts of sucrose (Sawaya et al., 1983b). Other sugars such as galactose and maltose have been reported in minute amounts (Espinosa et al., 1973). Accumulation of hexoses in the final weeks of fruit development has also been reported (Barbera et al., 1992). This accumulation was probably due to seepage of mucilage from peel to the pulp (Laskshminarayana et al., 1979).

In our studies we just found glucose and fructose as the only sugars in catus pear with various content according to the altitude. Boujibar samples (573m of altitude) present the highest glucose and fructose content (respectively 68.46 and 58.10 mg/g of dry matter) while Wahran and Tizakhte ones recording the lowest content (respectively 60.35 and 61.78mg/g of DM for glucose and 51.55 and 51.87mg/g of DM for fructose). Mestassa samples have relative high content of glucose and fructose (63.16 and 53.58mg/g of DM respectively). The glucose content from Boujibar samples is higher than those reported by Gurrieri et al., (2000) (60 to 64mg/g of dry matter) but the fructose content for this site and for the other sites are consistent with those found by the same author (54 to 60 mg/g of dry matter). The glucose content Mestasaa, Wahran and Tizakhte sites are rather consistent with those reported by Gurrieri et al., (2000). Kuti and Galloway (1994) also reported data about sugars content in prickly pear juice (5.89 and 4.30mg/g of juice respectively for glucose and fructose).

A significant differencewas noticed between samples about citric acid content. The samples of Mestassa (119m of altitude) presents the highest citric acid content (185.74mg/g of DM) while Tizakhte ones presenting the lowest content (64.94mg/g of DM). We clearly see the effect of the altitude on the citric acid content. It may also be due to the nature of the soil. As for fumaric acid content, Tizakhte samples one is significantly higher (9.80mg/g of DM) while Boujibar ones presenting the lowest content (3.69mg/g of DM). Wahran samples have also a high content of fumaric acid (9.39mg/g of DM) when Mestassa samples one is relatively low (5.93mg/g of DM). Regarding the gross energy, Boujibar are significantly more caloric (17.94Kcal/100g of DM) while Tizakhte samples are the les sones (15.09Kcal/100g of DM). Values found for Mestassa and Wahran samples are not significantly different (16.49 and 16.21Kcal/100g of DM). The high gross energy of Boujibar samples can be explained, inter alia, by the their high sugars content. But, the gross energy is also influenced by the whole organic matter content of a sample while in this study we have not determined protein, lipids, among others organic matters content.

Conclusion

Samples from Boujibar site (573m of altitude) present a lot of important characteristics on pomological ant physico-chemical scales. These fruits present, among others, the highest edible part weigh (64.74g), the lowest peel thickness (4.38mm) against 4.82mm for Mestassa fruits (119m of altitude), the highest seediness (3.62%) against 3.08% for Mestassa samples (119m) and the highest firmity (96.79%) against 95.46% for Mestassa ones. Boujibar samples also presnt the highest glucose and fructose content (respectively 68.46 and 58.10mg/g of DM) and the highest gross energy (1794Kcal/100g of DM). As for citric acid content, it decreases according to the altitude, Mestassa samples having the higest content (185.74mg/100g of DM) and Tizakhte samples having the lowest content (64.94mg/100g of DM). The effect of altitude is substantial about these parameters but Tizakhte samples present situated at the highest altitude present a contradiction. This difference is probably due to dew influence on these crops, making them grow at similar conditions as if they were at sea level. We also notice that samples Tizakhte present the lowest pH value (5.93) compared to Mestassa, Wahran and Boujibar (respectively 6.29, 6.16 and 6.25). As a conclusion, the effect of altitude on the pomological and physico-chemical traits of cactus pear is notable for some traits but not for others. Other factors as soils composition or sunlight may be probably influencing these parameters.

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VARIABILITY OF LENGTH OF SPIKE AND NUMBER OF SPIKELETS PER SPIKE IN WHEAT (*Triticum aestivum* L.)

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Abstract

Variability of length of spike and number of spikelets spike^{-1} have share in forming of grain yield of wheat. The aim of this study was estimation of variability of length of spike and number of spikelets spike^{-1} in 20 genetically divergent wheat cultivars grown in different environmental conditions. The experiment was set up as a randomised block design in three replications. Obtained results indicated differences in average values of length of spike and number of spikelets spike^{-1} among tested cultivars in both years of experiment. In average for all cultivars length of spike was higher in the second year than in first year of experiment. Also, average value of number of spikelets spike^{-1} was higher in second year at the analysed wheat cultivars. The wheat cultivar Dejana expressed the highest length of spike (12.50cm) in average in the second experimental year while the wheat cultivar Sumadinka had the least length of spike (8.91cm) in average in the first year. Based on the results was established, variability of wheat cultivars for the both analysed traits of spike, as well as, significant differences between the wheat cultivars according to length of spike and number of spikelets spike^{-1}, which are in dependence of genetic and environmental factors.

Keywords: *wheat, variability, spike length, spikelets, cultivars.*

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops as a source of food for the people worldwide. Increasing of wheat grain yield is the main task of breeders which require effort in improving characteristics of spike traits, grain, stem, leaf and root traits. The long and fertile spike potentially can contribute to improvement of grain yield of wheat (Zečević et al., 2008; Knezevic et al., 2014). Spike length together with number of spikelets and number of florets per spike represent great potential for yield improvement (Zečević, et al., 2004; Dimitrijević et al., 2011) through developing grain number spike^{-1} and as a source of assimilate in grain filling period as well as forming grain yield. Increasing of number of spikelets potentially related to increasing of number of grains (Álvaro et al., 2008). Spike length had positive relationship with number of spikelets spike^{-1} at both genotypic and phenotypic levels (Akram et al., 2008). Floral development is an important part of the pre-anthesis stage. Anther and ovary growth as well efficient pollination connected to grain number per spike, grain size and grain weight (Guo et al., 2015). Grain number per spike is related to floret survival (Gonzalez et al., 2011; Sreenivasulu and Schnurbusch, 2012). Spike structure has advantages in utilizing light in compare to other parts of plant and contribute to

increasing of yield. Also, spike together with awns contribute to longer stay green area duration. All these characteristics of spike contribute to accumulate in average 20-30% of dry matter depends of genetic and environmental factors as well as their interaction (Knezevic et al., 2015: Branković et al., 2015). The effect of genetic and environmental factor at the length of spike and development of number of spikelets per spike need further investigation. Increasing of genetic capacity of spike traits is a potential direction of increasing grain yield of wheat (Knezevic et al., 2012).

The aim of this paper was investigation of variability of length of spike and number of spikelets spike^{-1} in genetically divergent wheat cultivars grown in different environmental conditions.

Materials and methods

The twenty geneticaly divergent winter wheat cultivars were used for study of length of spike and number of spikelets spike^{-1} during two season of vegetative growth year (2015/16 and 2016/17). The experiment was performed in randomized block design in three replication on the field in Kraljevo, Serbia. The seeds of varieties were sown at the distance of 0.05m in rows of 1m length among which was the distance of 0.2m. For analysis of length of spike and number of spikelets spike^{-1} were used 60 plants in full maturity stage (20 plants per replication). For statistical analysis used MSTAT C version 5.0. The significant differences between the average values were estimated by F-test values. The analysis of variance was performed according to a random block system with one factor significant difference was tested by means of the value of LSD test.

Weather conditions

In the first year experiments 2015/206, the average temperature was 9.9 °C and the total amount of precipitation was 651.00 mm. In the second year of experiment 2016/17 average temperature was 13.0 °C and the total amount of precipitation was 523.0 mm. The average rainfall was 651.00 mm in the first year and significantly higher than in the second year (523.1 mm), and significantly higher than average values for ten years - 417.8 mm (table 1). For plants growth in the second year was more favorable regime of temperature and precipitation.

Table 1. Average monthly temperature and total monthly precipitation in Kraljevo

	Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Xm	Total
Temperature ^{0}C	2015/16	11.6	7.3	3.3	-0.1	8.8	7.8	14.1	15.5	21.3	**9.96**	89.64
Temperature ^{0}C	2016/17	10.6	6.8	0.0	-4.7	5.2	10.8	11.1	16.8	22.1	**8.74**	78.66
2000-2010		11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	**8.5**	76.5
Precipitatin (mm)	2015/16	56.8	64.0	9.0	86.2	52.7	157.9	39.9	135.9	48.6	72.3	**651.0**
(mm)	2016/17	84.1	77.6	9.4	22.0	35.0	57.0	82.0	100.0	56.0	41.1	**523.1**
2000-2010		61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3	46.4	**417.8**

During October-November, a greater amount of water residue was in the second year (161.7 mm) than in the first (120.8 mm), which represents a favorable period of germination of plants and better condition of plants for survive in the coming winter period, while during the preiod February-April amount of precipitation in the first year (250.5 mm) was higher than in the second (174.0 mm), although the distribution of rainfall was favorable in the second year of the experiment. Also, in the two months (October-November) the average temperature values were similar, wherein the sum of the temperature for the period February april 30.7 °C in the first year, more than the amount for the same period in the second year - 27.1 °C (table 1).

Results and discussion

The length of spike in the first year of experiment varied in ratio of 8.91-11.11 cm with average value 9.92 cm, while in second year varied from 10.03 to 12.50 cm with average value 11.09 cm. Number of spikelets spike^{-1} in the first year of experiment varied in interval of 20.85 - 24.38 with mean value 22.79, while in second year number of spikelets spike^{-1} varied between 21.23 and 25.0 with average value 23.53 (table 2). The obtained results showed significant differences in the average values of length of spike and number of spikelets spike^{-1} per year, that indicating diversity of studied cultivars. Similar results were reported in previous investigation of Serbian wheat genotypes (Zečević et al., 2008; Knezevic et al., 2012) as well as for Italian and Spanish wheat cultivars (Álvaro et al., 2008).

Table 2. Variability of length of spike and number of spikelets spike^{-1}

	length of spike (cm)			*Number of spikelets spike^{-1}*		
Cultivars	First year	Second year	Average	First year	Second year	Average
Evropa 90	11.09a	12.07b	11.58	22.83cde	23.00defg	22.92
Dejana	11.11a	12.50a	11.80	24.38a	24.87ab	24.62
Sila	9.95bcd	10.54ijk	10.24	22.40de	24.38abc	23.39
Omega	10.06 bcd	11.27cd	10.66	22.80cde	23.50cdefg	23.15
Lasta	10.07 bcd	11.23cd	10.65	22.00ef	23.33cdefg	22.66
Milica	10.05 bcd	10.83efghi	10.44	23.63abc	23.65cdef	23.64
Partizanka	10.43abc	11.53c	10.98	23.67abc	24.00abcde	23.83
Pobeda	9.50de	10.73ghij	10.12	23.67abc	24.00abcde	23.83
Dična	9.99bcd	11.14cdef	10.56	23.00bcde	24.00abcde	23.50
NSR-5	9.96bcd	10.03l	10.00	23.10bcd	22.90efg	23.00
Alfa	9.59cde	11.07defg	10.33	24.00ab	25.00a	24.50
Rodna	9.50de	11.30cd	10.40	22.67cde	23.33cdefg	23.00
Agrounija	9.56cde	10.76fghij	10.16	22.27de	22.83fg	22.55
Zadruga	10.50ab	10.30kl	10.40	20.85g	21.23h	21.04
KG -75	10.55ab	12.35ab	11.45	21.08fg	23.20defg	22.14
Šumadinka	8.91e	10.97defgh	9.94	22.48de	22.40g	22.44
Levčanka	9.56cde	10.65hijk	10.10	23.67abc	23.73bcdef	23.70
Oplenka	8.98e	10.36jkl	9.67	23.08bcd	23.92abcdef	23.50
Gruža	9.56cde	10.93defghi	10.24	22.00ef	23.35cdefg	22.67
KG-56	9.41de	11.21cde	10.31	22.39de	24.05abcd	23.22
Average	9.92	11.09	10.50	22.79	23.53	23.16

The significant differences among the investigated wheat cultivars were established for the length of spike (table 3). Also, the values of length spike of analysed genotypes were significant different between first and second experimental years (table 2). Generally, in average all studied wheat cultivar in both year and in average expressed higher values of length of spike. This indicates response of genotypes to environmental conditions.

Differences among cultivars according to value of spike length are affected more by genotype than by relationships to the geographic origin (Dotlačil et al., 2003). The length of spike controls by additive and nonadditive gene with prevalence of additive gene effects (Ljubičić et al., 2014). Also, the sensitivity of length of spike under environmental variation noticed (Zečević et al., 2008; Knezevic et al., 2014) and represent important components of wheat yield. The environmental factors as well temperature values, precipitation, nutrition have influence on increasing of capacity of spike (Petrović et al. 2008; Knežević et al., 2016).

Table 3. Components of phenotypic variance for length of spike (cm) of wheat – in 1st and 2nd year

Source of variance	First Year							Second Year					
	DF	SS	MS	F	LSD			DF	SS	MS	F	LSD	
					0.05	0.01						0.05	0.01
Repetitions (R)	2	0.500	0.250	0.9270^{ns}	-	-		2	0.111	0.056	0.9978^{ns}	-	-
Genotypes (G)	19	20.606	1.085	4.0247**	0.886	1.212		19	23.938	1.260	22.6335**	0.404	0.553
Error	38	10.240	0.269	-	-	-		38	2.115	0.056	-	-	-
Total	59	31.345	-	-	-	-		59	26.165	-	-	-	-

The significant differences among the tested wheat cultivars were established for the number of pikelet spike^{-1} in both year of experiment (table 4). The number of spikelet spike^{-1} at the analysed wheat cultivars varied and were significantly different between the cultivars between and between the years of experiment. Generally at the all tested cultivars the number of spikelet spike^{-1} in second year was the higher than in first year of experiment (table 2).

Table 4. Components of phenotypic variance for number of spikelets spike^{-1} in wheat

Source of variance	First Year							Second Year					
	DF	SS	MS	F	LSD			DF	SS	MS	F	LSD	
					0.05	0.01						0.05	0.01
Repetitions (R)	2	1.265	0.632	1.7152^{ns}	-	-		2	0.709	0.355	0.7949^{ns}	-	-
Genotypes (G)	19	47.646	2.508	6.8009**	1.038	1.419		19	41.244	2.171	4.8668**	1.141	1.560
Error	38	14.012	0.369	-	-	-		38	16.949	0.446	-	-	-
Total	59	62.922	-	-	-	-		59	58.902	-	-	-	-

The investigated trait highly depended to genetic and environmental factors (Zečević et al. 2004; Dodig et al., 2008). The spike length is yield components which highly positively correlated to number of spikelets spike^{-1} (Akram et al., 2008). The spike length has strong indirect influence on yieald through number of spikelets spike^{-1} and futher on number of grain and size and weight of grain (Zečević et al., 2004). Improvements in the number of grains per spikelets mean increasing the number of grains spike^{-1}.

Conclusions

In this investigation were determined differences among wheat genotypes according to values of length of spike and number of spikelets spike^{-1}. The highest values of length of spike (12.50cm) in Dejana cultivar expressed in the first experimental year while the least (8.91cm) in wheat Šumadinka had in first experimental year. Breeding programs need conduct in the different environments in the aim of improvement of spike traits by using germplasm resources. Increasing of wheat grain yield is achievable through improving of all morphological, physiological characteristics of spike as well other organs of wheat.

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THE CONTENT OF ORGANIC MATTER IN THE SOIL OF NIS MUNICIPALITY (SERBIA)

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Abstract

One of the main components of soil, which affects the yield of plants, is the content of organic matter. The aim of this paper was to examine the saturation of the soil with organic matter on the territory of the Nis (Serbia) in 2015. An analysis of 284 soil samples from 110 farms at the location of Nis municipality, with total area of 115,28 ha, showed that 0.4% of the samples belonged to the class of very poorly humified soil, containing organic matter up to 1%. It was also found that 50.4 % of the samples belonged to the class of poorly humified soil, with the organic matter content ranging from 1% to 3%. Furthermore, 47,9% of the analyzed soil samples belonged to the class of well-humified soil, with the humus content ranging from 3% to 5%. The lowest value was found in one of the samples from the village of Trupale in Crveni Krst, and it was 0.88%, while the samples with the highest humus value were found in the village of Hum (5.,47%). Chemical analysis was performed in 2015, in the Agricultural Advisory and Professional Service - Nis, where the humus content was determined by using the Tyurin method. Soil samples were taken from a depth of 0-30 cm in field crops and vegetable cultures, or 0-60cm in orchards. The analysis showed that the soil of this area wais not humified enough, wherefore it was necessary to pay attention to the content of humus, increased the level of organic matter with organic fertilizers and reduced losses which happened by removing and burning the crop residues.

Keywords: *Organic matter, Humus, Soil, Serbia.*

Introduction

The fraction of organic matter in the soil, which is a result of decomposition by living organisms, is called humus. Humus has a certain duration in the soil, after which it is degraded, in this way, old humus is replaced with new humus. It is desirable to maintain this balance. Soil organic matter originates from dead animal and plant residues. Microorganisms decompose organic matter, which leads to the synthesis of high molecular compounds – humus. Humus, in a broad sense, is composed of all dead organic matter in the soil, and, in a narrow sense, humus is composed of humic substances (Marković, 2016)

The amount of humus in the soil serves us to determine soil fertility. The content of organic matter in the agricultural upper layer is usually in the range of 1-6%. Humus influences the water, air and thermal regime of soil, through which it influences plant development conditions. The trend of humus decrease has been observed in nearly the entire territory of the Republic of Serbia, in the amount of 0.5-1%. This decrease of humus in the soil is very high, because humus is created slowly; as a rule, 1 cm of humus is created in 100 years (Poljosfera, 2018). In the past 20 years, the organic matter in the soil in Serbia has decreased from 3.5% to below three percent (Gulan, 2018)

A long-term, heavy use of mineral fertilizers, heavy machinery and agricultural residues burning have caused a drastic decrease in humus in the arable land at a larger territory of Serbia. The beginning of intensive land use and the use of greater quantities of mineral fertilizers, have led to the fact that these intensive methods began to destroy humus

microbiologically. Organic matter does not disappear immediately, but gradually. In countries where there is enough cattle, i.e. the soil is fertilized with manure, organic matter remains preserved (Stevanović, 2017). Burning of crop residues is considered to be an undesirable practice and is prohibited by the legislation of the Republic of Serbia (Zakon o poljoprivrednom zemljištu, 2006).

Soil fertility, i.e. humus loss, occurs because more soil nutrients are taken out of the soil than returned to it. In Serbia, the numbers of sheep, pigs and cattle are in decline, and, for this reason, soil fertilization with organic fertilizers is reduced. One of the ways to increase soil fertility is plowing crop residues (Jaćimović et al., 2017). Apart from plowing crop residues, a number of experts recommends that we "let the soil rest," which indicates a certain period when it will not be used extensively. Humus is lost faster from bare soil, so it is recommended that the soil is sown with siderate crops, which are plowed, during the "rest." Planting and plowing of siderates increase humus in the soil from 0.2 to 0.4%, depending on the plant species to be plowed (Spoljar i sar., 2011).

The holders of soil fertility are not macro- and microelements, but humus with soil microorganisms. (Serenson, 1972; SARE, 2012).

The quality of humus, in addition to humic acids, is also determined by the C:N ratio (Abdellatif et al., 2017). It is envisaged that humus contains a constant content of carbon (C), which is 58%, while the nitrogen (N) content in humus varies from 3-6%. For this reason, the C:N ratio fluctuates from 10-20. A favorable composition of humus in the soil is achieved when the C:N ratio is approaching 10; the ideal ratio is C:N = 10, favorable 10-20:1, while adverse is greater (higher) than 20:1 (Marković, 2016)

Humic matter can be divided into three groups of organic matter: humins, fulvic acids and humic acids, which are natural sources of carbon (Humic Solutions, 2016)

The influence of soil irrigation on the humus content is contradictory. Based on the results Mačkić (2016), concluded that the mean value of humus (1.11%) in the studied chernozem was lower in the surface horizons on irrigated plots, compared to non-irrigated plots (1.61%). It was assumed that the increased soil moisture stimulated microbial activity, causing faster mineralization of organic matter. Similar results were also presented by Gajić (1999); Zamfir et al., (2003); Kalinitchenko et al., (2011).

On the other hand, creating conditions for a large increase in the aboveground mass of plants and their roots, irrigation may stimulate the formation of organic matter. In soil of a lighter mechanical composition, meadows and pastures, irrigation resulted in an increase in the content of organic matter, as well as in nitrogen and phosphorous, but it did not influence the pH values of soil and the potassium content (Zhao et al., 2007).

Irrigation increased the content of organic matter in the soil primarily due to large quantities of crop residues (Denef et al., 2008; Roland et al., 2005)

Erosion also causes a reduction in organic matter in the soil, most often in agricultural soils (Bossio et al., 2010).

Needs of plants for humus are different and depend on the plant species, as well as the spreading depth of their root system. For example, in Serbia, the dominant plant species is the plum (Grčak, 2017).). Plum roots are branched and develop mostly on the surface, although roots can grow even 2 m in depth. The optimum humus content in the soil for the plum and other fruit cultures is 2.5-3%. The most needed fertilizer for the plum is nitrogen fertilizer (Gavrilović, 1959). If the soil is rich in humus, it is unnecessary to fertilize it (Blagojević and Božić, 2012).

Material and Methods

During the research, soil of farmers from the municipalities of Nis was analyzed. A total of 284 plots from 110 farms was studied, ranging in size from 0.09 to 5.89 ha. The total surface area analyzed was 115.28 ha.

Chemical analyses were performed in 2015, in the Agricultural Advisory and Professional Service - Nis, where the humus content was determined by using the Tyurin method. Soil samples were taken from a depth of 0-30 cm in field crops and vegetables, and 0-60 cm in orchards. An individual sample consisted of 20 to 25 individual samples (in lots up to 5 ha) from a depth of 0-30 cm in plots under field or vegetable crops or in plots where field or vegetable crops were planned to be grown, and from a depth of 0-60 cm, in plots under orchards or vineyards or in plots where orchards and vineyards were planned to be located.

Results and Discussion

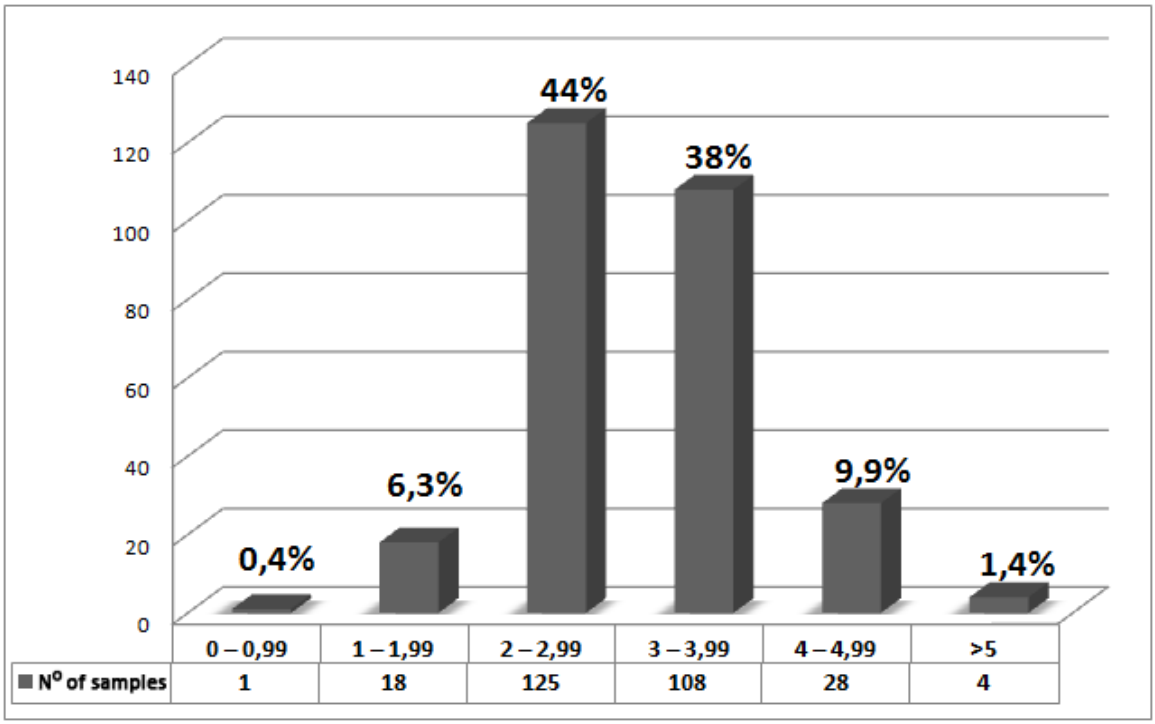
The analysis of soil inculded all 5 municipality of Niš. Most of the samples were taken from the farmlands of Crveni Krst municipality (75) and the smallest number of samples were from the local municipality of the Niska Banja (10).

As the control of soil fertility, chemical analyses of certain parameters were performed, among which was the humus content analysis. On the basis of the data obtained from a total of 248 plots on the territory of Nis, it was found that as much as 50.7% of the samples belonged to the class of land with poor or very poor humus content (below 3%). Only 1.4% of the plots belonged to the class of soil rich in humus, where the content of organic matter was higher than 5%.The lowest value was found in one of the samples from the village of Trupale in Crveni Krst, and it was 0.88%, while the samples with the highest humus value were found in the village of Hum (5.47%). The variability indicator was calculated through standard deviation using the computer, and the standard deviation was ± 0.175, which indicated that there were no major differences among the values of humus. Similar research led to similar results. The testing of humus in Central Serbia in 2015 found that a majority of samples belonged to the soil class with the percentage of soil organic matter from 3-5 (Ministarstvo poljoprivrde i zaštite životne sredine RS, 2017). The testing of the arable land in Kragujevac showed that as much as 59.5% of the samples belonged to the soil rich in humus, with its content above 3.29% (Milivojević *et al*., 2012).

Table 1. The proportion of samples that belong to certain classes of humus content, based on the analysis of 284 soil samples taken in the municipality of Niš in 2015

Soil	Number of plots	Percentage (%)	Cumulative percentage
very poorly humified (0-0.99%)	1	0.4	0.4
poorly humified (1-2.99 %)	143	50.4	50.7
humified (3-4,99 %)	136	47.9	98.6
well-humified (≥5 %)	4	1.4	100.0
Total	284	100.0	

Based on a detailed classification (Graph 1), the samples with the organic matter content ranging from 2% to 3% are dominant with 44% of all samples. Second most common samples with the organic matter contect is from ranging 3% to 4% with 38% of all samples.



Graph 1. The proportion of samples that belong to certain classes of humus content (%) in the municipality of Niš (number of samples 284).

The quantity of humus in the soil depends on the soil type, for example, in Vojvodina (Serbia), in chernozem, there is 3.5 - 5.5% of humus at a depth of 0 - 20 cm (Bajić, 2017), while in the continental part of Croatia, there is 2.99% of humus, Bensa et al. (2013). The mean content of humus in the top (surface, A) horizons of solodic soil indicates high humosity (4.43% of humus), which is a result of the presence of shallow dense grass vegetation, while the deeper horizons of solodic soil have a moderate content of humus (2.73% of humus). The humus content rapidly decreases with a depth increase (Nešić, 2004; Brady and Weil, 2008). Based on the reasrch of content of humus in Aleksinac Grčak D., and sar., (2017), which has the south-east border with the territory of Nis (Serbia), where the humus tests were performed for this study, the results are very similar. An analysis has found that 0.2% of the samples belong to the class of very poorly humified soil, containing organic matter up to 1%. It has also been found that 43.3% of the samples belong to the class of poorly humified soil, with the organic matter content ranging from 1% to 3%. Furthermore, 55.2% of the analyzed soil samples belong to the class of well-humified soil, with the humus content ranging from 3% to 5%.

Conclusions

The biggest problem in soil management in Serbia is permanent destruction of soil, its degradation and contamination. The quantity of humus has declined in a large surface area in Serbia in the last decades. The long and heavy use of mineral fertilizers, heavy equipment, burning of crop residues, irrigation in the absence of sufficient fertilizing with manure, larger quantities of nutrients being taken out of the soil than are returned to it, are the causes of the disappearance of the organic matter in soil. Based on the test results of the humus content in the soil on the territory of Nis, it was found that, on the basis of data obtained from a total of 248 analyzed plots on the territory of Nis, as much as 50.7% of the samples belonged to the class of soil whose humus content was poor or very poor (below 3%).

Due to improper use of the soil, large quantities of organic matter are removed from the soil. It is necessary to avoid crop residue burning, and reenter organic matter into the soil by plowing.

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INVESTIGATION OF CONTENT OF PRIMARY AND SECONDARY OXIDATION PRODUCTS IN SUNFLOWER OILS WITH A DIFFERENT CONTENT OF OLEIC ACID

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Abstract

Oxidative stability, *i.e.* shelf life of oil is related to the degree of the oxidative changes in the amount of the resulting primary and secondary oxidation products of unsaturated fatty acids. In order to improve oxidative stability, *i.e.* oil shelf life, sunflower hybrids with altered fatty acid composition have been created, *i.e.* significantly higher oleic acid (C18:1) content, even over 90% w/w, relative to the linoleic type with 25-30% w/w of oleic and 60-65% w/w of linoleic acid (C18:2). In order to examine the oxidative stability of the oil, three samples of sunflower seed oil NS hybrids (NS Oliva, NS Horizont and NS Romeo) of different fatty acid composition were analyzed. Samples were exposed to moderate temperatures (63±2°C) over a period of 8 days. Changes in the content of primary and secondary oxidation products based on changes in peroxide (PV) and anisidine (*p*-AnV) values, as well as changes in the content of conjugated dienes and trienes, were observed. The highest oxidative stability was observed in the oleic type oil sample. In this sample the determined values of the tested parameters of oxidative stability, after 8 days, were PV=4.85 mmol/kg and *p*-AnV=0.65, compared to the initial sample (PV=0.36 mmol/kg and *p*-AnV=0.57). The greatest oxidative changes occurred in the oil sample with the lowest content of oleic and the highest content of linoleic acid, as indicated by the PV=73.22 mmol/kg and *p*-AnV=3.60, after 8 days, in relation to PV=2.16 mmol/kg and *p*-AnV=0.50 in the initial linoleic type oil sample.

Keywords: *Sunflower oil, primary and secondary oxidation products, oleic acid, peroxide value, conjugated dienes.*

Introduction

Breeders all around the world try to create hybrids of sunflowers of different oil quality. The content of higher fatty acids, or their mutual relationship, is one of the basic parameters that determine the quality of the oil. Standard sunflower oil contains linoleic (62-72%), oleic (15-25%), palmitic (5-7%), stearic (4-6%) and several other higher fatty acid in traces (Purdy, 1985; Purdy, 1986; Dimić, 2005; Grompone, 2005; Miklič *et al.*, 2008; Cvejić *et al.*, 2014).
Because of the health and nutritional aspects of edible oil that is gaining increasing attention, it has become important to create the oil of the desired composition, which promotes its stability and improves nutritional value. "Standard" sunflower oil is considered to be "healthy" oil, because it contains high levels of essential fatty acid and vitamin E (*α*-tocopherol), the most important natural biological antioxidant, which functions in *in vivo* processes, while the proportion of saturated fatty acids is small (Holló *et al.*, 1998; Dimić, 2005). In Republic of Serbia, among the first in Europe, hybrids with high oleic acid content have been created, with NS Olivko the most famous, with the content of oil in the seed of 47 to 50%, and the content of oleic acid in oil exceeds 80%. This hybrid is also recognized in Italy under the name Soleado. In addition to NS Olivko, our oleic hybrids are Goleador, NS Delija and NS Oliva (Dimić, 2005, Miklič *et al.*, 2008, Cvejić *et al.*, 2014, Romanić, 2015).

In addition to increasing the nutritional value, the change in the composition of fatty acids of the oil also affects the oxidative stability of the oil. The content of oleic, linoleic and linolenic acid in oil affects its oxidative stability. Since standard sunflower oil can have up to 70% linoleic acid, it is highly susceptible to lipid oxidation. By modifying the composition of fatty acids in sunflower oil, the content of linoleic acid decreases with increasing oleic acid content, which can increase oxidative stability during storage and frying, which leads to improvement of the health status of consumers (Grompone, 2005).
Taking into account the above mentioned reasons, as well as the fact that the sunflower assortment permanently changes, the aim of this study is to analyze the oxidative stability of sunflower seeds oil of the latest NS hybrids of different fatty acid compositions based on changes in the content of primary and secondary products of oxidation at moderate temperatures. For the purpose of comparison, oxidative characteristics of oils with different oleic acid content were examined in the same way.

Material and Methods

Material: Samples of cold pressed sunflower oil of three different NS hybrids were used (NS Oliva, NS Horizont and NS Romeo). The seeds are cleaned, 6 months after harvesting and storage under normal storage conditions and without prior peeling, pressed with a worm press. The oil temperature at the output of the press was 45-50°C. Samples of cold pressed oils were taken after 72 hours of agitation at room temperature for the sedimentation, decantation and oil filtration through plain, wrinkled laboratory filter paper.
Methods: Samples of oil have been exposed in the laboratory conditions at moderate temperatures (63±2°C), in the presence of air, without the presence of light according to the methodology described by Pokorny *et al.* (1985). It was measured in 50 ml of each sample and subjected to test conditions for a period of 8 days. After the 4^{th} and 8^{th} day, the samples were taken and analysed and compared with the initial samples. The content of primary oxidation products is determined on the basis of the peroxide value (PV) (SRPS EN ISO 3960:2017) and specific absorbances at a wavelength of 232 nm, *i.e.* the content of conjugated dienes (SRPS EN ISO 3656 (2013/A1:2017). Based on anisidine value (SRPS EN ISO 6885:2017) and specific absorbances at a wavelength of 270 nm, *i.e.* the content of conjugated triers (SRPS EN ISO 3656 (2013/A1:2017), the content of secondary oxidation products has been determined. The composition of fatty acids in oils is determined by the application of gas chromatography - mass spectrometry (GC-MS) (SRPS EN ISO 12966-2:2015 SRPS EN ISO 12966-2:2017) using the HP 5890 gas chromatograph with HP 5971A mass detector ("Hewlett Packard", USA). All determinations were done in three replications, the results are tabulated as the mean value ± standard deviation, and only the mean value for the graphic representation was obtained. Statistical processing of the obtained results and graphical representations were made using Microsoft Excel 2010 (Microsoft, Washington, USA) and Statistica 13.0 (StatSoft, Tulsa, USA).

Results and Discussion

Table 1 shows the fatty acid composition of the tested samples. The highest content of oleic acid (C18:1) was found in the sample of NS Oliva and amounted to 90.04%. Oil with oleic acid content between 75.0-90.7% is defined as "high-oleic" (Codex, 1990), which classifies this sample in a group of "high-oleic" oils. The content of linoleic acid in this sample is very low, only 3.87%. The NS Horizont sample contains smaller but still significant oleic acid content of 65.63%, while the content of linoleic acid in this sample is 27.71%. According to the determined oleic acid content, the NS Horizont hybrid belongs to the category "mid-oleic" with the defined oleic acid content of 43.1-71.8% (Codex, 1999). The NS Romeo sample in its fatty acid composition is closest to the "standard" sunflower oil with 26.25% oleic acid and

66.41% linoleic acid. According to Codex (1999), oleic acid content of 14.0-39.4% and linoleic 48.3-74.0% was defined for "standard" sunflower oils, which NS Romeo sample included in this group of oils.

Table 1. Fatty acid composition (% w/w) of the tested samples

Fatty acids	**NS Oliva**	**NS Horizont**	**NS Romeo**
C14:0	nd	nd	0,03±0,00
C16:0	2,08±0,02	3,39±0,00	4,01±0,03
C16:1	nd	0,05±0,00	0,03±0,00
C18:0	2,25±0,02	1,92±0,00	2,27±0,00
C18:1c	**90,04±0,12**	**65,63±0,14**	**26,25±0,22**
C18:2c	**3,87±0,03**	**27,71±0,13**	**66,41±0,19**
C20:0	0,21±0,01	0,17±0,01	0,17±0,01
C20:1	0,14±0,02	0,13±0,00	0,11±0,00
C22:0	1,03±0,02	0,69±0,00	0,52±0,02
C24:0	0,37±0,00	0,30±0,01	0,21±0,02

nd - not detected

Figure 1 shows the results and the dependence of the peroxide value and the content of conjugated dienes.

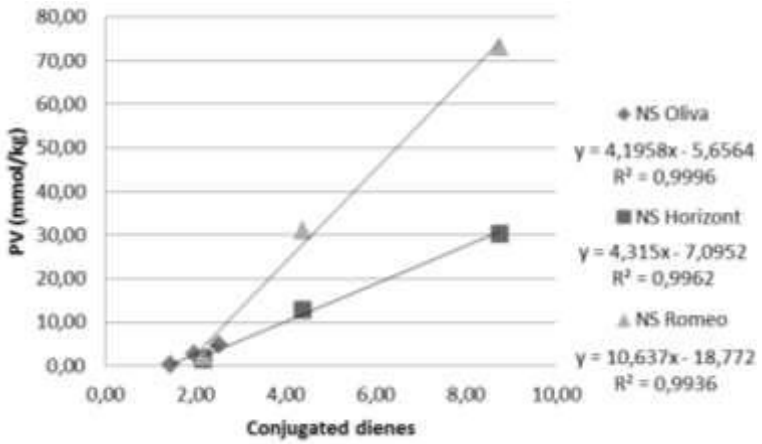

Figure 1. Results and dependence of the peroxide value and content of conjugated dienes

The highest PV in the initial samples was found in the sample NS Romeo (2.16 mmol/kg), while the lowest PV was determined in the NS Oliva sample (0.36 mmol/kg). The PV of sample NS Horizont was 1.59 mmol/kg. After 4 and 8 days, the lowest PV was found in the NS Oliva sample (2.66 and 4.85 mmol/kg) while the highest value was found in the NS Romeo sample (30.95 and 73.22 mmol/kg).

A similar situation was observed in the content of conjugated dienes. By increasing the exposure time of the test samples, the content of conjugated dienes increased. The lowest value of the conjugated dienes content in the initial samples was found in the NS Oliva sample (1.44), while the highest content of these compounds was found in the NS Romeo sample (2.69). In the NS Horizont sample, the content of conjugated dienes was 2.17. After 8 days of tempering, the trend remained the same. The largest change in content compared to the initial sample was recorded in the NS Horizont sample whose content increased 4.03 times, a slightly smaller increase was observed in the NS Romeo sample and amounted to 3.62, while the smallest increase was recorded in the NS Oliva sample, only 1.73 times.

The obtained dependence of the peroxide value and content of conjugated dienes is linear with correlation coefficient over 0,99, which indicates that the correlation between the tested parameters is very strong, Figure 1.

Figure 2 shows the results and the dependence of the anisidine value and content of conjugated trienes. The highest value of the anisidine value in the initial samples was 0.57, found in the NS Oliva sample, while the lowest value of the *p*-AnV was found in the NS Horizont sample (0.08). After 8 days, the highest value of the anisidine number was determined in the NS Romeo sample, which increased from baseline 0.5 to 3.6, while in the NS Oliva sample this increase was only a little, to 0.65.

The highest content of total trienes in the initial samples as well as after 8 days of tempering was found in the NS Romeo sample (0.23 and 0.42). The smallest content of these compounds in the initial samples was found in the NS Horizont sample (0.13). In this sample, after 8 days of tempering, the value of the content of conjugated trienes was found to be 0.31. The smallest value of the contents of these compounds after 8 days of tempering was found in the NS Oliva sample (0.28), in which the content of conjugated trienes in the initial sample was 0.21. In Figure 2 it can be seen that an insignificant linear dependence between the anisidine value and the conjugated triene content in NS Oliva and NS Horizont samples (the coefficient of correlation is less than 0.3) was found. In the NS Romeo sample, a strong correlation was found between the *p*-AnV and the content of conjugated trienes with a correlation coefficient of 0.72.

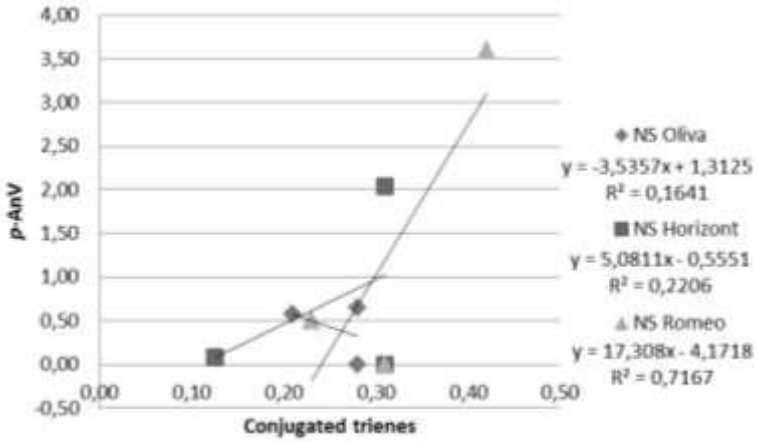

Figure 2. Results and dependence of anisidine value and content of conjugated trienes

Oxidation of oleic acid is far slower than linoleic (Romanić, 2015; Romanić and Kravić, 2017). Since, in the course of oxidation, the primary products first arise, then only secondary ones, such correlations are probably due to the high content of linoleic and low oleic acid content in the NS Romeo sample, ie the high content of oleic and low linoleic acid content in samples of NS Oliva and NS Horizont, which in any case opens the possibility of further testing. Similar results were also obtained by Romanić and Kravić (2017) who compared sunflower oil, cold-pressed high-oleic and linoleic type, and refined oil under similar conditions. The highest oxidative stability was observed in a sample of high-oleic oil. Merill *et al*. (2008) found that high-oleic sunflower oil has three times higher oxidative stability than sunflower oil of linoleic type.

Cluster method is based on PV, *p*-AnV, content on conjugated dienes and trienes in initial samples and after 4^{th} and 8^{th} day of testing. This method has determined that NS Horizont and NS Oliva samples constitute one subcluster, or from the aspect of oxidative stability, these two samples are similar in contrast to the sample NS Romeo which is separated from this group. The largest distance was recorded among NS Oliva and NS Romeo samples, 74.4 (expressed as Euclid's distance), somewhat lower distance was recorded among samples of NS Horizont and NS Romeo (46.6) while the lowest distance was 28.3 among the samples NS Oliva and NS Horizont, as can be clearly seen in Figure 3. The similarity obtained by the

clustering method among the samples of NS Oliva and NS Horizont is explained by their fatty acid composition as these samples belong to the oleic type, while the NS Romeo sample belongs to the linoleic type.

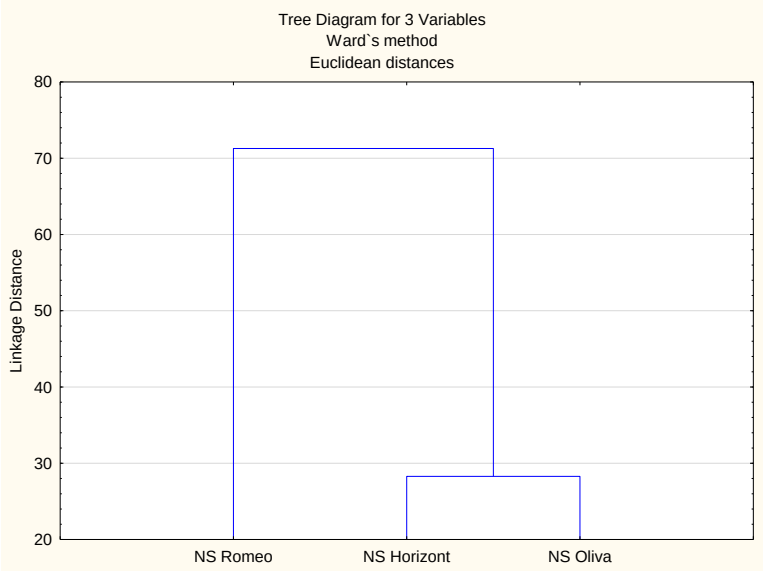

Figure 3. Cluster of oxidative stability of the tested samples

Conclusions

By comparing three NS hybrids with different oleic acid content, it was found that the best characteristics from the aspect of oxidative stability were demonstrated by NS Oliva, a sample with the highest oleic acid content (90.04%). In this sample the lowest peroxide value in the initial sample (0.36 mmol/kg) was determined, as well as for 8 days of tempering (4.85 mmol/kg). Also, the lowest value of the *p*-AnV was determined after 8 days of tempering and amounted to 0.65. The weakest characteristic was the NS Romeo sample with the lowest oleic acid content (only 26.25%). The PV in the initial sample was 2.16 mmol/kg, which represents the highest value in the initial samples, and after the 8 days of tempering in this sample, the highest PV was found at 73.22 mmol/kg. Also, in this sample the highest *p*-AnV was determined after 8 days of tempering and was 3.60.

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VEGETATIVE PROPAGATION OF CALLICARPA BODINIERI LEVL. BY HARDWOOD CUTTINGS

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Abstract

Callicarpa bodinieri is an ornamental, deciduous shrub that can be easily grown in well-drained soil in full sun to part shade. It is suitable for landscape use, for planting in group or mass, in bird gardens or in open woodland areas. For this research, two types of hardwood cuttings were used: t-cuttings with a part of two-year old wood and standard hardwood cuttings. The cuttings were treated with 0.8 % IBA (indole butyric acid) using a quick dip method. After rooting in mixture of peat and sand (2: 1), following parameters were recorded: number and length of primary roots, and presence of secondary roots. Considerably better results were achieved with t-cuttings, and there were no significant difference among those cutings treated with IBA and control. Rooting percentage was high, 95.3 % (IBA treatment) and 96 % (control), and there were no significant difference regarding other parameters, except root length. T-cuttings treated with IBA formed longer roots. Rooting rate of cuttings without two-year old wood was low - 21.3 % (IBA treatment) and 17.3 % (control). These results indicated that carbohydrate and endogenous hormone levels possibly significantly influenced rooting of hardwood cuttings of this species.

Keywords: *Bodinier beautyberry, vegetative propagation, cuttings, rooting medium, auxine.*

Introduction

Callicarpa bodinieri is an ornamental, deciduous shrub, native to Sichuan, Hubei and Shaanxi provinces in central and western China (Zhou *et al,* 2013). It grows up to 3 m, with upright slender branches covered with dark green leaves turning golden yellow and red in autumn. Bodinier beautyberry blooms in summer, producing small lilac flowers in the leaf axils, followed by small, decorative violet-purple berries in September and October. *C. bodinieri* can be easily grown in well-drained soil in full sun to part shade. It is suitable for landscape use, for planting in group or mass, in bird gardens or in open woodland areas (Brickell, 2008; Zhou *et al,* 2013). Bodinier beautyberry is a non-native species in Serbia, but due its low invasive potential (Hauser, 2017) it is suitable for use in green areas in the conditions of climate changes. *C. bodinieri* can be propagated by seed or cuttings (Brickell, 2008), but vegetative propagation is more convenienet in order to produce desired genotypes. There is no available records about detailed vegetative propagation of this species. There are recommendations that this species can be propagated by softwood cuttings or semi-hardwood cuttings (Tripp, 1995; Segall, 1997; Vermeulen, 1997; Brickell, 2008), but there is no data about expected rooting percentage of cuttings or suggested auxine pretreatments for this species. Also, there is no data about possibility of its propagation using hardwood cuttings. For this reason, we decided to investigate vegetative propagation of *C. bodinieri* using hardwood cuttings.

Material and Methods

The hardwood cuttings were collected early in the morning, at the begining of March. For this research, two types of hardwood cuttings were used: t-cuttings with a part of two-year old wood and standard hardwood cuttings; both types 4-7 cm long with 2 nodes. The cuttings

were disinfected with 0.6% solution of fungicide Benomyl (Benlate®). The bottom of cuttings was wounded and the cuttings were dipped in a 0.8% (8000 ppm) IBA (indole-3-butyric acid) solution for 3-5 seconds, using a quick dip method. After that, the cuttings were sticked in a mixture of peat and sand (2: 1) and kept in the Greenhouse of the Faculty of Forestry under intermittent mist (5 seconds mist with 15 minutes interval). Three replications of 50 cuttings per treatment were used.

After 5 weeks, the following parameters were determined: number and length of primary roots, and presence of secondary roots. The significance of differences between the means was determined by the analysis of variance (ANOVA, $p < 0.05$) and the least significant difference (LSD) test. Before the statistical analysis, the percentage data were converted using arcsine transformation.

Results and Discussion

After 5 weeks, rooting percentage was high, reaching more than 90% rooted cuttings (Table 1). Cutting type significantly influenced rooting, and t-cuttings with a part of two-year old wood rooted in a high percentage uncorrelated with the IBA pretreatment. Those cuttings also formed well developed root system, and more than 85% of rooted cuttings also developed secondary roots. As opposed to that, cuttings without a section of of two-year old wood rooted poorly, and IBA pretreatment also didn't influence rooting, because there was no significant difference among treated cuttings and a control (Table 1).

Table 1. State of cuttings after 5 weeks

Cutting type	treatment	Rooting (%)	No of primary roots	Length of primary roots (cm)	Secondary roots frequency* (%)
t-cuttings	0.8% IBA	95.3[a]	5.82[a]	1.77[a]	0.89[a]
t-cuttings	control	96.0[a]	5.37[a]	1.47 [b]	0.86[a]
standard cuttings	0.8% IBA	21.3[b]	0.66[b]	0.18[c]	0.07[b]
standard cuttings	control	17.3 [b]	0.53[b]	0.17[c]	0.09[b]

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

*Frequency represent the percentage of rooted cuttings which have formed secondary roots

Contrary to our findings, results obtained with some other *Callicarpa* species indicates that use of plant growth regulators (PGRs) is neccessary in their vegetative propagation (Fang, 2010; Zhang *et al.,* 2013; Chen *et al.,* 2010). Thus, Fang (2010) reported that the rooting rate, root length and root number of *C. macropylla* were greatly improved with the treatment of NAA (naphthalene acetic acid) and IBA, and similarly Chen *et al.* (2010) found that addition of NAA was neccessary for successful rooting of *C. dichotoma*. Additionaly, Zhang *et al.,* (2013) showed that rooting percentage could be significantly increased from 38.6% (control) to 94.6% by using a rooting agents during propagation of *C. nudiflora*. They also obtained better results with 2 year old cuttings, which is in accordance with our findings that 2 years old cuttings have a higher rooting percentage than younger ones, but Zhang *et al.,* (2013) also found that cutting diameter influenced rooting, and cuttings with 0.8 cm diameter had a higher rooting percentage than cuttings with 0.6 cm diameter. This can be expected probably due to higher carbohydrate reserves in cuttings with greater thickness relative to thinner cuttings (Biondi et al., 2008; Zhang et al., 2010), however there are also reports that rooting percentage of some species could decrease with increasing cutting diameter (Howard and Ridout, 1991). The cutting diameter could influence rooting differently, and for example,

during propagation of *Actinidia arguta*, thiner cuttings (<2 mm) had higher rooting percentage, but thicker cuttings (2-8 mm) developed longer roots in a higher number (Beyl *et al*., 1995). The cuttings used in our research were uniform, with 2 nodes, and some additional research could be conducted using a single node cuttings in order to obtain higher multiplication rate. However, rooting of single node cuttings can be poor, because cutting length can also significantly influence rooting and longer cuttings usually have a better rooting rate and root system is better developed (Gerrakakis and Özkaya, 2005; Gopale and Zunjarrao, 2011).

Besides PGRs, Bai *et al.* (2015) showed that rooting substrate can significantly influence the rooting of *Callicarpa bodinieri* Levl. var. *bodinieri* semi-hardwood cuttings, and they recommend use of perlite, riversand and the garden soil (1: 1: 1), but taking into account that rooting percentage obtained in our research was high, there is no need for further investigation of effect of other rooting media on rooting of *C. bodinieri.*

Conclusions

Obtained results indicated that *Callicarpa bodinieri* can be successfuly propagated using a hardwood t-cuttings with a part of two-year old wood, 4 - 7 cm long, with 2 nodes, wounded at the bottom. These cuttings should be rooted in a mixture of peat and sand (2: 1), under mist system, without addition of PGRs. Using this method, rooting percentage was high - 96%, and root system was well developed.

Acknowledgments

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THE QUALITY OF SOIL IN VOJVODINA AND ITS SUITABILITY FOR HAZELNUT PRODUCION

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Abstract

Over the course of the last decade, surfaces under hazelnut cultivation in Serbia have been under significant expansion thanks to the new market possibilities. Given the high investment required for establishment of hazelnut plantation, proper and thorough examination of agroecological conditions, above all climate and soil, is necessary for this type of cultivation. Research was, therefore, carried out in 2017 and 2018, including 120 agricultural plots in AP Vojvodina where 240 samples were collected from the depth of 0 – 30 cm and 30 – 60 cm. The tested parameters included the main agrochemical soil properties (pH, total $CaCO_3$, humus content, total nitrogen and content of readily-available phosphorus and potassium), content of active lime ($CaCO_3$) and mechanical content. The most important limiting factor of the research was high content of active $CaCO_3$, resulting in adverse conditions for hazelnut tree growing at 1/4 of the tested plots. A few plots had an acidic pH reaction, while 1/4 of the tested plots had very low humus content (below 2 %). Since most of the tested plots were used for field crop cultivation, nutrients were mainly concentrated within the surface soil horizon; deep tillage should therefore be encouraged, so that the nutrients were transferred into the active rhizosphere which was suitable for growing perrenial plants such as hazelnut trees. In addition, nine pedological profiles were open and the results showed that the tested soils belong to chernozem soil type, or its subtype or form. Having conducted the detailed research, we can conclude that the soils in AP Vojvodina are suitable for contemporary and intensive hazel growing, with the good prospect of obtaining high, top-quality hazelnut yields.

Keywords: *hazelnut, lime, basic agrochemical properties, soil.*

Introduction

Vojvodina is an important agricultural region in Southeast Europe and a gem of the Pannonian Plain for its soil properties. It provides a surplus of wheat, maize and industrial crop production (Živković *et al*., 1972). Thanks to the considerably improved market conditions in the recent years, an increasing number of perennial fruit and vine plantations have been established in many locations. A total of 2,239 ha of cultivated land was estimated in an agricultural survey conducted in 2012 (Keserović *et al*., 2014). Hazel cultivation has significantly expanded in recent years, amounting to 4,000 ha of hazel plantation surface according to unofficial estimates, and 1,200 ha more planned for 2018 according to contract with Agriser Ltd. Serbia and Ferrero as its parent company.

Hazel has long been grown in an extensive production system, mostly in private backyards without the application of suitable cultivation practices. However, new markets demand the security of product placement, and high standard of both the quantity and quality of product. To meet these requirements, production needs to be improved and adapted to specific agro-ecological conditions. High quality of soil is one of the most important preconditions for hazel production. Soil is a natural source of organic matter and primary base for organic matter production by cultivation of different plant species. It consists of mineral and organic substances, soil solution and soil air. Soil is a natural environment of plants, animals and

microrganisms, limited in its surface and destructable by nature. Soil formation is slow and gradual, but soil destruction can be very fast.

High-quality fruit production begins with the optimal use of soil with regard to local climate conditions (Keserović *et al.*, 2008). Human activities can have a significant influence on soil properties, primarily during plantation establishment. Although agro-ecological conditions in Vojvodina are suitable for intensive hazel production, certain existing limitations are the reason for soil quality testing of each plantation.

The aim of the paper is to put together a document on the global estimate of fertility and quality of soils under hazel cultivation in Vojvodina, as well as completely adopt the fertility control system and rational use of fertilizers by end users - producers and authorities, through education, promotion and dissemination of research results.

Material and Methods

Hazel production was chosen as the research subject due to the recent significant increase in its growing surfaces and ongoing tendencies for hazel cultivation, primarily at the territory of AP Vojvodina. The paper aims to examine research results obtained at 120 control plots, and 240 soil samples.

Picture 1. Soil sampling localities under hazel cultivation

Control plot is a plot with the uniform history, terrain, and cultivation practices. The samples were collected in 2017 and 2018 using an agrochemical probe at the depth of 0-30 and 30-60 cm, according to fertility control methodology, so that one submitted sample consisted of 20 individual sub-samples. Complete laboratory research was conducted at the Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops. The applied research methods were:

- determination of soil mechanical structure by the international pipette method (with Na-pyrophosphate)

- determination of potential soil acidity - pH in 1 M KCl – in soil suspension (10g:25cm^3) with potassium chloride, potentiometrically, with pH meter.
- determination of calcium-carbonate ($CaCO_3$) content – volumetric method, using the Scheibler calcimeter.
- determination of physiologically active calcium-carbonate ($CaCO_3$) by the Drouineau – Galet method (with ammonia oxalate)
- determination of humus (organic matter) content - by the method of Turin (with $K_2Cr_2O_7$)
- determination of ammonium lactate P_2O_5 – spectrophotometric determination of readily available phosphorus.
- determination of ammonium lactate K_2O –flamephotometric determination of readily available potassium.

Results and Discussion

Hazel has the widest growing areal of all woody fruit species, reaching over 1500 m.a.s.l. It grows on different types of soil, from the fertile humus soils to the poor alkaline skeletal soils. However, high-yielding and cost effective production requires cultivation in deep, fertile soils with an optimal water and air regime, such as different types of chernozem, alluvial soils and eutric cambisols. High – yielding cultivars have specific soil quality requirements (Šoškić, 2016).

Mechanical content (Granulometric analysis)

The solid phase of soil is a polydisperse system composed of different sized particles. Soil mechanical structure is therefore a quantitative content of variable soil particles, grouped into mechanical fractions by their limit fractal dimensions. The method of Atterberg and IUSS distinguishes clay (<0,002 mm), silt (0,002-0,02 mm), fine sand (0,02-0,2 mm), and rough sand (0,2-2 mm) (IUSS-International Union of Soil Science). The two fractions observed at the tested plots were loamy clay and clay loam, with the prevailing content of the first fraction.

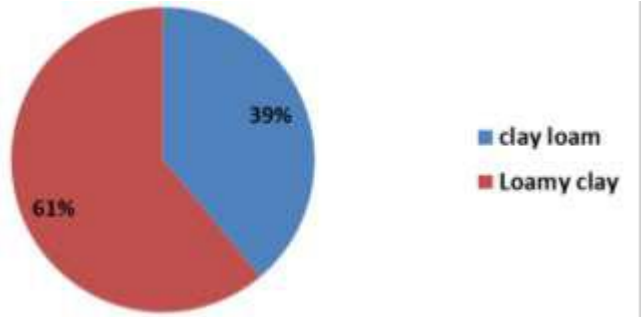

Figure 1. Granulometic content

Soil pH, total and active lime ($CaCO_3$)

The results of substitutional acidity tests and total $CaCO_3$ content confirm the fact that slightly alkaline soils are predominant in the territory of Vojvodina. The development of such soils occurs because of their geological substrate i.e. loess, which is rich in $CaCO_3$ and directly correlates to soil pH thus affecting its higher value. However, the content of physiologically active soil lime as determined by the method of Droineau – Galet (Hadžić *et al*., 2004) is not highly correlated with the total content but with its mean (r=0,62). This soil property should be given special attention as one of the most significant limiting factors of hazel production in Vojvodina. High $CaCO_3$ content in soil causes blockage of plant nutrients and, as a result, prevents their adoption by plants and leads to mild (in active lime values of 5-8 %) or strong chlorosis (in active lime values above 8 %) (Magazzini, 2018). Active lime content increases

with soil depth. The highest root mass of hazel and other perennial woody plants is in the soil layer of 30 to 60 cm. According to our research, one of the four tested plots has an active lime content above 8 %, which is unsuitable for hazel production.

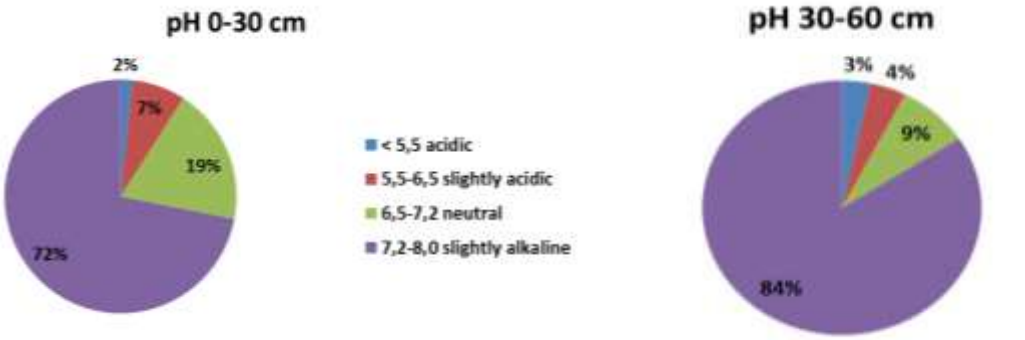

Figure 2. pH value in KCl (classification: Belić *et al., 2014)*

Figure 3. Total $CaCO_3$ (classification: Belić *et al., 2014)*

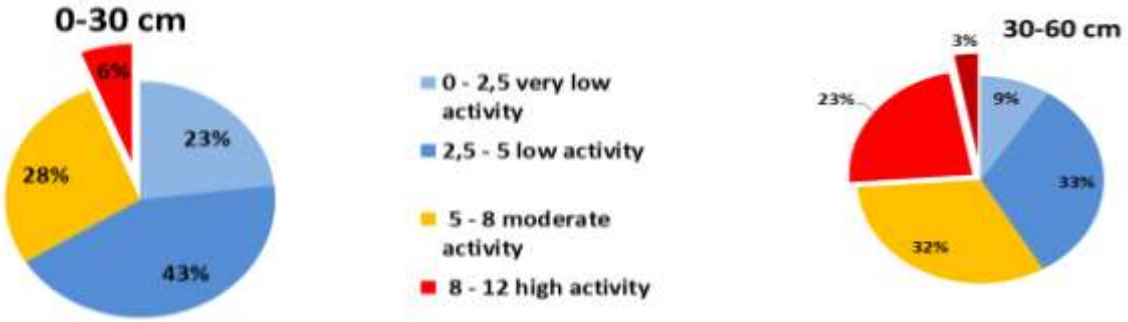

Figure 4. Active $CaCO_3$ (classification: Magazzini, 2018)

Content of organic matter

Humus is an important soil fertility indicator, the source of nutrients and factor of soil fertility preservation. Vojvodinian soils are still well-supplied with organic substances, regardless of the dramatic decrease observed in the last few decades due to minimalized use of organic fertilization and harvest residue burning.

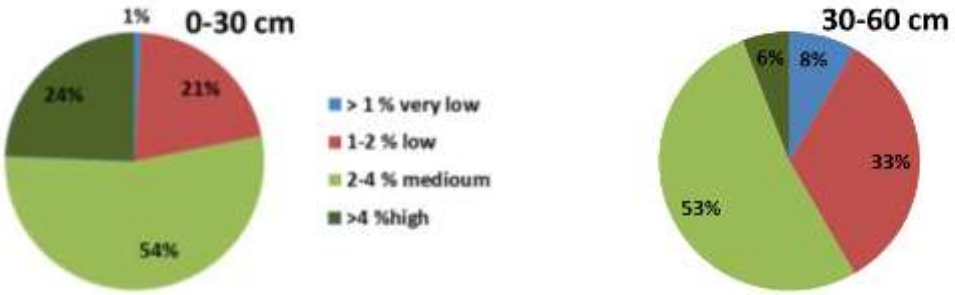

Figure 5. Content of organic matter (classification: Ninkov *et al.*,2014)

Content of readily available macronutrients – phosphorus and potassium

Phosphorus and potassium have a major role in plant growth and therefore belong to the group of primary nutrients (NPK). The tested soils are mostly well-supplied with the primary minerals, but there is a substantial difference between their content in the upper and lower soil layers. The upper soil layer generally prevails in terms of nutrient sufficiency, which can be explained by the previous use of the tested plots for field crop cultivation, focusing mainly on fertilization of the upper soil layer. Incorporation of nutrients into deeper soil layers is vital due to rooting depth of 30 to 60 cm found in perennial fruit plantations.

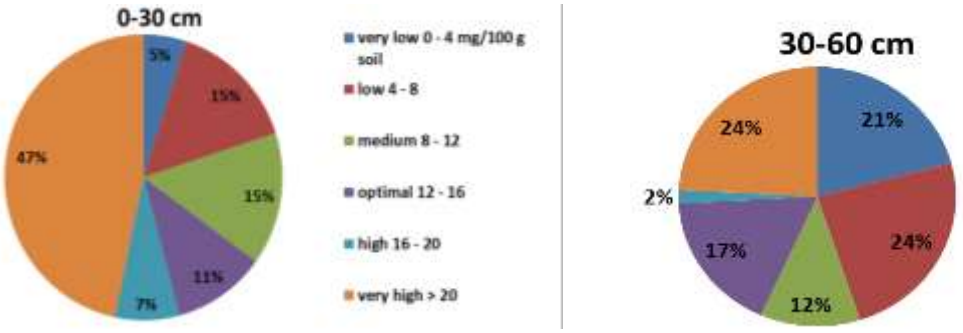

Figure 6. Readily available **phosphorus (P_2O_5)** (classification: Ninkov *et al.*,2014)

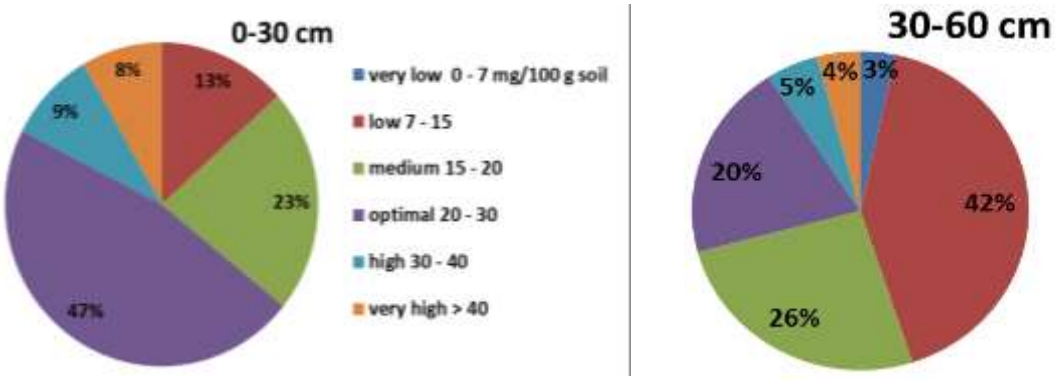

Figure 7. Readily available **potassium (K_2O)** (classification: Ninkov *et al.*,2014)

Conclusions

Only the well-prepared soils can provide optimal nutrition for economically viable yields of hazel, and ensure satisfactory resistance to attacks of pests and diseases. Mistakes made during the establishment of plantations are costly and very difficult to remove. All the factors directly or indirectly affecting this type of production must therefore be taken into consideration when hazel plantations are established. High content of physiologically active $CaCO_3$ and high levels of ground water, determined in additional field research by opening soil pedological profiles, are the major limiting factors for hazel production in Vojvodina.

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QUO VADIS, RES RUSTICA?

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Abstract

Humanity is facing the population growth, environmental erosion, and climate changes. Dramatic challenges require resolute solutions in agriculture. The agro-industrialization was the main goal of the 20th Century Green Revolution (GR). A new plant ideotype capable to respond to the new requirements was created. Though GR mitigated the hunger in the world, it opened some other problems. The prize was environmental erosion, climate changing, and over population. Using long term results of wheat breeding in the Institute of Field and Vegetable Crops in Novi Sad, Serbia, the authors comment plant breeding goals and selection criteria that were established to meeting growing demand for food, through the variation of some yield and quality marker in wheat, plant stature variation and creation a novel genetic variability for overcoming stressful growing conditions. Allelic variation of seed storage protein loci on 1B, wheat rye translocation, and 1D chromosomes, HMW glutenin alleles, mostly, as a consequence of selection pressure in wheat breeding showed that the main effort through selection criteria was to reconciling negative correlation between high yield and good quality. The variation of sink/source ratio and its effects studied, as well. On the basis of GR legacy, authors tried to anticipate future trends in agriculture. Whether a further genotype by environment interaction minimization by more controlled agro-production conditions in closed growth systems could be the next level? In order to feed more than 10 billion people are we forced to seek for radical solution in food production increment, rather than step by step improvements?

Keywords: *Agriculture, food, wheat, breeding, future.*

Introduction

The elephant in the room. The human population grew slowly for thousands of years, but in recent centuries, it has jumped dramatically. Between 1900 and 2000, the increase in world population was three times greater than during the entire previous history of humanity giving an increment from 1.5 to 6.1 billion in just 100 years (Roser, M., and Ortiz-Ospina, E., 2018). How to feeding 10 billion people sustainably in the late 21st century? In just 30 to 40 years, about 600 quadrillion calories every single day is going to be required. This is twice as much as today. The mass of 7-8 billion people will be squeezed in cities spending around 2800km^3 of fresh water a year. That is more than the whole of irrigation farming is using, today. Every second 750 tons of topsoil is lost even, today giving a warning that present food production is unsustainable in the long term. Moreover, we have lost 24% of our land, already, and we keep on losing 1% a year. The UK's Hadley Climate Centre projects that 40 per cent of the planet could be in regular drought by 2100. The hunger breeds war. If we failed to prevail what is coming, the consequences would affect every person on the planet. Fortunately, to some opinions, humanity has potentials and the technical ability to do limit the danger of extinction and the end of civilization, but things have to change. (Cribb, 2010, 2016). However, world demand for staple food, from 2010 to 2050, is projected to grow by 60%, contrasting about 10% growth of crop area in the same period and the trend of linear increment of wheat, rice and soybean yield that have started to gradually decline (Fischer *et al*., 2014). Furthermore, the ongoing processes are urbanization, environmental erosion including arable land degradation and climate change (Myers *et al*., 2017). Despite alarming voices, there is an

impression that neither agricultural scientific community, nor broader public do not address the issue adequately. That has to be changed. Mortal danger requires resolute and joint action, because if we had no ability to do so, there would be no one to light up the light in celebration of the next millennium, maybe the next century, even.

The road to hell is paved with good intentions. "The Earth provides enough to satisfy every man's need, but not enough for every man's greed" (*cit.* Mahatma Gandhi). The full intensification of agricultural production began in the middle of the previous century. The second half of the twentieth century was marked by the "green revolution", which significantly increased the yield of basic agricultural crops by a significant change in plant architecture. It was a project of great American foundations (Rockefeller, Ford) and governments of developing countries. The intensive agricultural production was based on new genotypes that were more distanced from their wild relatives, and highly dependent on human nursing. Though, the "Green agricultural revolution" has never solved "hunger problem in the world" as the proclaimed goal, the road of agricultural production was traced.

Evolution on steroids. The road of industrialization has started from instinctive selection and breeding that farmer did on the bases of field experience and personal goals. However, this was not fast enough. Controlled evolution required to be accelerated. The Green Revolution (GR) was a game changer, not only in primary food production as well as in plant breeder's philosophy, but also in relations of humans to nature, economic and political relations both locally and globally. It is widely spread opinion that genetics and plant breeding were the foundation of GR, particularly because American scientist and plant breeder Norman Borlaug was the symbol and a Nobel Prize laureate of this revolution (Borlaug, 2002). However, the real bases of GR were fertilizers, nitrogen in particular. The nitrogen is principal factor for crop productivity. Cheap nitrogen from industrial ammonium was available in great quantities after the WWII, since the explosives were not in high demand, any more. The synthetic fertilizer industry blossomed. Unfortunately, tall stature of landraces and "old" bred varieties were not suitable to withstanding large amount of nitrogen fertilizers application without logging. Beside N-fertilizers, density (the number of plants per unit area) was another corner stone of GR. The role of population significantly prevailed the role of an individual, in crops yield formation. But, the tall stature of plants could not follow the increment of sowing rate without logging. In order to solve the logging problem, new plant ideotype was required. Evolution had to be artificially accelerated. Newly designed GR plant ideotype had to be of shorter stature to overcome logging. Bread wheat and rice, are éclatant examples of these scientifically induced evolution in agriculture. Using genetic variation of wild relatives and landraces, as *Rht* (reduction of height) genes in wheat, new plant type was born. The type capable to put forward industrialization in agriculture.

The aim of this article is to summarize the impact of GR philosophy on wheat breeding program of the Institute of Field and Vegetable Crops in Novi Sad, Serbia, and to look at future trends.

Material and Methods

A research has been conducted on large sample of wheat genotypes consisting on varieties, landraces and wild relatives from the Wheat breeding program of the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS), and accompanying genetic collection listed in Dimitrijević (1987), and Dimitrijević (1997). A wide palette of results processing was applied, mainly statistical models and laboratory analysis after Steel and Torrie (1960), Singh and Shepherd (1985), Gauch and Zobel (1997), Ng *at al*. (1988).

Results and discussion

The Harvest index - Truth or Dare. Harvest index is a synthetic parameter representing the ratio of grain yield and total biomass yield. Introduced by Donald (1962), HI represents effectiveness of assimilative translocation from vegetative to generative plant parts, towards grain filling, and hence high grain yield. This parameter was paradigm of GR philosophical approach materialized in Donald's "communal" ideotype that performs poorly as individual, but gives its best in interplant competition provoked by crop population density (Donald, 1968). Hence, radically changed plant architecture, particularly the reduction of plant stature resulting in better sink to source ratio expressed through higher HI value is the main cause of 3 to 5 time higher grain yield due to the GR. The HI, as a selection criterion, was widely used in plant breeding and investigated in scientific papers, but rarely criticized. Ismail (1993) had a case contemplating usefulness and credibility of this parameter that relies on complex quantitative genetic influence. Moreover, analyzing data of 22 divergent wheat genotypes the trend could be denoted that HI appeared to be quite insensitive to reduction of plant height (Petrović, 2000; Petrović *et al.*, 2000), fig. 1. The sink to source ratio did not vary much, because the key seems to be overall robustness, and its proportional downscaling rather than height reduction *per se*. The previously stated trend was observed in investigation of very divergent wheat sample consisting of 41 genotypes, about half varieties and half wild relatives (Dimitrijević *et al*., 2001), fig. 2. The plant stature did not influenced average spike yield formation followed through grain weight per spike. Consequently, it seems that the dwarfing genes introduction brought by GR was not primarily for more efficient assimilative translocation to generative part of the plant, but mostly because of production intensification by fertilizers application increment, nitrogen in particular, and the plant ability improvement to withstand higher sowing density. The key words of GR were "chemistry" rather than "genetics", "logging", rather than "translocation", and "collectiveness", rather than "individuality".

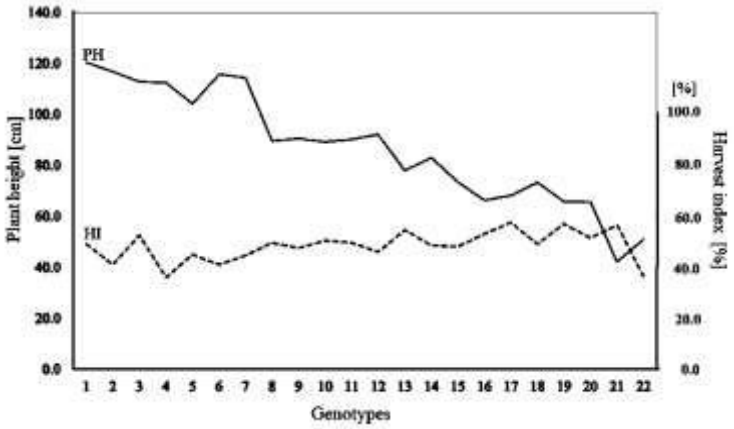

Figure 1. The relation of plant height (PH) and harvest index (HI) of 22 wheat genotypes from different foreign wheat breeding centers and IFVCNS, from tall old varieties, via semi-dwarf modern varieties to dwarf Aibian and Tom Thumb. Data from Petrović (2000)

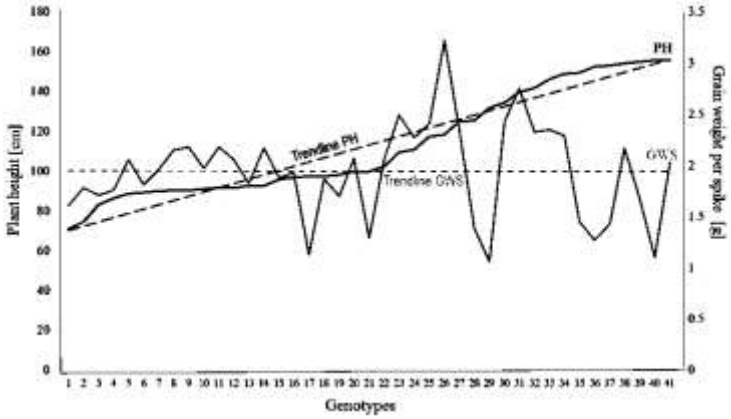

Figure 2. The relation of plant height (PH) and average spike yield given through grain weight pre spike (GWS) of 41 wheat genotypes of different ploidy level (4x=28 and 6x=42) and origin (modern varieties foreign and from IFVCNS, as well as, wild relatives). Data from Dimitrijević *et al*., 2001.

Green Revolution - Winners and losers. If yield was the winner of GR, biodiversity was definitely it's looser (Dimitrijević *et al.*, 2011). The pedigree of 23 bread modern wheat varieties from Yugoslavia (18) with 14 from IFVCNS, consisting of 123 varieties from all over the world illustrates that the vast number of heat varieties created within the GR philosophy, particularly in American, Australian and European wheat breeding centers originate from single cross of Squarehead, an early English cultivars, introduced in Holland around 1870 and Duch landrace Zeeuwse Witte. In 1886 Luitje Broekema, Director of the Government Agricultural School at Wageningen crossed Squarehead x Zeeuwse Witte, getting variety Spijk. In 1889, Broekema backcrossed a F_3 generation plant with maternal parent Squarehead obtaining the variety Wilhelmina, released in 1901. The general idea was to combine quality and good yield of Zeeuwse Witte and good spike architecture of Squarehead, as well as, it's good baking quality (de Haan, 1957). The real father of GR, Italian breeder Nazareno Strampelli crossed in 1913, Wilhelmna Tarwe with local landrace Rieti, mixing superior Dutch variety with rust resistant Rieti, and then top-crossing this combination with Japanese agronomically poor variety Akago-mughi of short stature up to 65cm tall (*Rht8*), therefore resistant to logging, and of early maturation (*Ppd1*) (Salvi *et al.*, 2013). The cornerstone of the GR was set. This combination played the fundamental role in Yugoslavian, Serbian and Novi Sad wheat breeding program, as well (fig. 3). Beside this crucial Anglo-Dutch cross combination, there were some other principal sources of genetic variation, so called, "common ancestors" in pedigree, like Barletta, landrace from Argentina, ancient Italian genotypes Gentil Rosso and Inallettabile, Japanese genotypes Akago-mughi, Soyiku-akage and Kaya-komughi *via* Saitama 27, English populations with a common name Prince Albert etc., but the role of landrace named "Banatka", after the Banat region divided between Serbia, Romania and Hungary. In Serbia, landrace Banatka (Old Banatka) was well spread, not only in Banat and Vojvodina, but also in Šumadija, Kosovo and Metohija, until the 30's of the XX century wheat landraces had been gradually replaced by "scientifically" bred varieties (Petrović and Dimitrijević, 2012). Beside Serbia, Banatka was grown in Hungary, Poland, Ukraine, Russia. In Ukraine, the landrace Banatka was the progenitor of variety Bezostaja 1, contributing 12.5% to its nuclear gene fund (fig. 4). Bezostaja 1 was a very important source of genetic variability for modern high yielding semi-dwarf varieties in wheat breeding programs in Europe, and beyond. However, following pedigrees of 139 wheat varieties and lines from comparative trials, from mid-60's to early 90's, the breeding source of variability changed, which could be denoted in studying IFVCNS wheat breeding program trends. As the domestic wheat breeding developed, the principal source of genetic variability has gradually shifted from foreign gene pool to newly formed domestic wheat varieties gene fund. By the end of 80's the novel advanced genetic variation

Figure 3. Combination Squarehead x Zeeuwse Witte, "Adam and Eve" of the most European, and even broader, wheat varieties. Akagomughi, Japanese wheat variety was donor of earliness and dwarfism (*Ppd1+Rht8*). The pedigree of a sample of Yugoslavian semi-dwarf varieties, in the background (Dimitrijević, 1987).

Figure 4. Landrace Banatka the progenitor of variety Bezostaja 1. Full Pedigree in Dimitrijević, 1987.

had been searched in fixation of small improvements obtained from recombination of domestic varieties. Having in mind decades of selection under the same criteria, the narrowing of genetic variability in producing new superior varieties, primarily in yield, was inevitable (data Dimitrijević, 1997). The advancement was slower and slower, and a new breakthrough is desperately needed.

Trying harder or the border is reached. The crucial question, facing the human population of 10 billion and rising, is are breeders trying hard enough or they just reached the limit of what vertical gene transfer could offer them? According to some data there is no place to put a question like this. Holt-Giménez *et al.* (2012), analyzing FAO 2009 reports state that "the world produces more than 1½ times enough food to feed everyone on the planet. That's already enough to feed 10 billion people". FAO (2017a) declares that "After a prolonged decline, world hunger appears to be on the rise again". The general cause is not in inadequate quantity of world's food production, but in poorness on the rise in some parts of the World. Anyhow, FAO estimates, relying on UN projection that the "world's population would reach 9.73 billion in 2050", "to meet demand, agriculture in 2050 will need to produce almost 50 percent more food, feed and biofuel than it did in 2012", cereals should increase production 43.4% by 2050 comparing to production 2005-07. The assumption is that 80% of this increment should come from yield increases meaning that yield should increase 35% by 2050, however, "yield increases are slowing, despite overall improvements in agricultural efficiency" (Alexandratos & Bruinsma, 2012; FAO, 2017b). The "contractor" of GR predicted that in the foreseeable future food security is to rely on plants, cereals in particular, estimating that world food production should increases 57% by 2025, comparing to 1990 production (Borlaug, 2002). So, following the domestic wheat breeding trends, as an illustration, it could be stated that the dramatically yield increment in wheat has been achieved by the introduction of *Rht-Ppd* genes combination. The smaller, but still remarkable, gran yield enhancements were achieved using chromosome engineering through translocations. The seed storage protein genetic variation of the representative sample of 139 wheat varieties of the IFVCNS indicates that by the end of 70's parental gene pool genetic variation should be renewed in order to continuing step by step yield improvement. At that time wheat rye translocation 1Rs.1Bl was introduced to wheat breeding program in Novi Sad through Soviet varieties Aurora, Kavkaz and Skorospelka 35. According to Dimitrijević (1997) and Dimitrijević *et al.* (2008), the novel variability brought by this chromosomal aberration enhanced potentials to rusts resistance, as well as hectoliter and absolute mass, and consequently the rain yield up to 10% comparing to averages of genotypes without this translocation. Negative effects were denoted in the higher susceptibility to powdery mildew and adverse effect on rheological dough properties. However, dough stickiness which is the main deleterious effect of 1Rs.1Bl was not observed. According to Dimitrijević (1997) results, there is a strong possibility of deletion in the 1Rs.1Bl translocation region that happened spontaneously in IFVCNS wheat breeding program, diminishing the dough

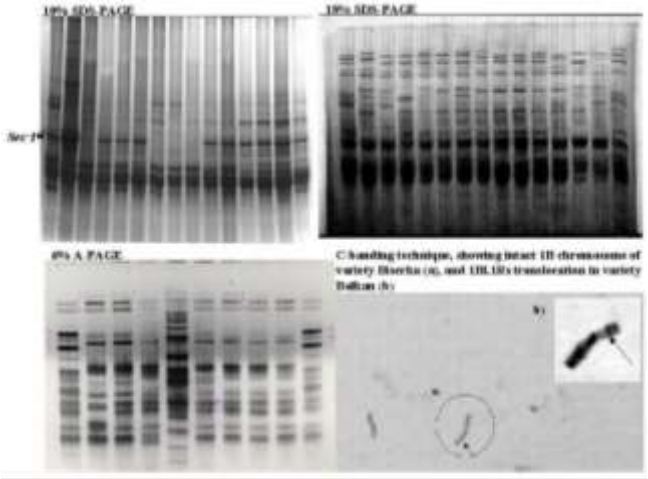

Figure 5. Seed storage protein and chromosomal aberration analysis illustrate the selection criteria of an advanced wheat breeding program based on the Green revolution philosophy (illustrations from Dimitrijević, 1997, given in Dimitrijević (2008).

stickiness as adverse effect. Moreover, there is another momentum concerning high molecular weight glutenin subunits genetic variation (HMW Glu) which made possible rather successful combination of high yield and good quality. The negative effect of 1Rs.1Bl translocation highly depends on genetic background, particularly on quality genetic background in which HMW Glu genes on 1D chromosome play an important role. The introduction of 1Rs.1Bl translocation was followed by favoring higher quality genetic background through selection of 5+10 HMW Glu subunits composition over 2+12. Hence in the representative sample of 139 about 40% had 1Rs.1Bl wheat-rye translocation and about 66% 5+10 HMW Glu subunits composition. Varieties that shared both genetic variations in the same time made about 50% of studied genetic variation, (fig. 5). This example shows that after reaching a satisfactory yield level, the main selection criterion was shifted from the "Stakhanov's grain yield" achievement to breaking a negative correlation between high grain yield, and the quality. Was it too soon? Was it a voluntary decision, or imposed by force of reaching the yield border, limitations of GR wheat ideotype? Anyway, the yield is a winner of GR, but overall genetic variability is a looser, plant breeding programs, included. To reach new heights in yield and food production, facing a human population growth challenges a novel genetic variability should be created.

Conclusions

Was the GR humanitarian effort or mammonic deed in its essence? Judging the results, after 60 years, there is a space for discussion. However, the fact is that hard working geneticists and plant breeders, all over the World, did their best to meet the demands for food. This is a gigantic task, and here is elaborated the tip of the iceberg. The swing of GR is weakening, going to halt. In the meantime demands for food are rapidly growing since the human population is still not at its peak. The Green revolution had the convenience not to take care about environment, but "Anyone who believes in indefinite growth in anything physical, on a physically finite planet, is either mad or an economist." (*cit.* Kenneth E. Boulding). The GR legacy is to continue food production increment without further environmental damages. The genetic variability meant to carry out the yield regardless of costs is exhausted. The trying in breeding for special purposes, like to prevail stress conditions and seize new areas for food production, or revive a branched wheat could appear insufficient. Are we to find new genetic variability capable to speed up food production, which is inadequately slow, now, making more turns. Are we to go NASA way, and try to avoid climate changes and field stresses in closed production systems? Does the vertical gene transfer reach the border of its potentials? Is the horizontal gene transfer, we still know so little about, the path of salvation? While vertical gene transfer has its limits relying on reproductive mechanisms and chromosomal compatibility in creating novel genetic recombination, horizontal gene transfer bypasses all these obstacles recombining at nucleic acid level, directly. Anyway, in order to prevail, it seems that new "revolution" is required, not only in doing, but also in thinking. The elephant is in the room, and we cannot ignore it, trying to solve the problem rearranging old furniture. Dramatic challenges require resolute response.

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INFLUENCE OF THE RECIPROCAL CROSSES ON GRAIN YIELD AND MORPHOLOGICAL TRAITS IN SINGLE-CROSS MAIZE HYBRIDS

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Abstract

Every single-cross maize hybrid can be produced in its original and reciprocal variant depending on the choice of the maize inbred line to serve as a maternal, or paternal component. Due to the manifestation of maternal effects, significant differences for various traits can be exhibited between the two variants of the same hybrid. The goal of this experiment was to examine a possible influence of reciprocal crosses on grain yield and some morphological traits in maize. Ten commercial ZP maize hybrids belonging to the maturity FAO 500-600 group were examined. All hybrids share a common parental line T1 which is used as a paternal line in original and as maternal in the reciprocal variants of investigated hybrids. The trial was set on two locations during 2015 and 2016. Grain yield, plant height , ear height and total number of leafs per plant were recorded. Location, year and reciprocal crosses were significant factors for all examined traits. Hybrids ZPH2, ZPH7 and ZPH8 had significantly higher grain yield in their reciprocal compared to the original variants with the difference ranging from 4.5 to 6.87%. Plant height was significantly higher in reciprocal variants of hybrids ZPH7 and ZPH9, and original variants of hybrid ZPH3 and ZPH6 (3,9 and 5,1% respectivily). Hybrid ZPH7 exhibited the highest difference in leaf number in reciprocal variant, compared to the original (11,6%). Hybrids ZPH1, ZPH4 and ZPH10 weren't influenced by the reciprocal crosses for neither of the investigated traits.

Key words: *maize, reciprocical effect, grain yield, morphological traits.*

Introduction

The choice of the maize inbred line to serve as a maternal, or paternal component in any specific maize hybrid combination can have a considerable impact on some phenotype characteristics of the hybrid. Due to the manifestation of maternal effects, significant differences for various traits can be exhibited between the two variants of the same hybrid. The maternal effect is in theory usually defined as the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from both parents. To understand the nature of maternal effect it is important to distinguish different classes of it. Roach and Wullf (1987) distinguish three different classes of maternal effects in plants: cytoplasmic genetic, endosperm nuclear and maternal phenotypic. Cytoplasmic genetic effect are based to the fact that maternal cytoplasm is transferred to the offspring including organelles such as plastids and mitochondrias containing genetic material. It's important to note that some research point the possibility of male cytoplasm to influence the hereditary expression in the progeny as a small quantity of male cytoplasm can be incorporated in the zygote from the male gamete or the male gametophyte during fertilization (Fleming, 1975). Endosperm nuclear effect is based to the fact that the maternal plant contributes with two nuclei in the formation of 3N endosperm. The larger proportion of maternal genes in endosperm which contains important enzymes for germination and is the source of nutrient for the future plant may greatly influence the development of the plant and the traits that it will exhibit.

Differences between reciprocal F1 crosses for important characters can influence decisions at many steps in a breeding program (Hansen and Baggett, 1977). Differences between reciprocal crosses for important agronomic traits of maize due to maternal effects were reported by many researchers (Garwood et. al., 1970; Kalsy and Sharma, 1971; Khehra and Bhalla, 1976; Pollmer et. al., 1978; Seka and Cross, 1995; Revilla et al., 1999; Gonzalo et. al., 2007; Pepo et. al., 2007).

Materials and methods

Ten commercial ZP maize hybrids belonging to the maturity FAO 500-600 group were examined (Table 1.). Ten inbred lines belonging to different heterotic and maturity groups serve as maternal component of each original hybrid variants. All hybrids share a common parental line T1 which is used as a paternal line in original and as maternal in the reciprocal variants of investigated hybrids. T1 inbred line belongs to the lancaster heterotic group and FAO 500 maturity group.

Table 1. Original and reciprocal variants of investigated inbred lines

ZPH1	ZPL1	x	T1	**ZPH6**	ZPL6	x	T1
ZPH1 R	T1	x	ZPL1	**ZPH6 R**	T1	x	ZPL6
ZPH2	ZPL2	x	T1	**ZPH7**	ZPL7	x	T1
ZPH2R	T1	x	ZPL2	**ZPH7 R**	T1	x	ZPL7
ZPH3	ZPL3	x	T1	**ZPH8**	ZPL8	x	T1
ZPH3 R	T1	x	ZPL3	**ZPH8 R**	T1	x	ZPL8
ZPH4	ZPL4	x	T1	**ZPH9**	ZPL9	x	T1
ZPH4 R	T1	x	ZPL4	**ZPH9 R**	T1	x	ZPL9
ZPH5	ZPL5	x	T1	**ZPH10**	ZPL10	x	T1
ZPH5 R	T1	x	ZPL5	**ZPH10 R**	T1	x	ZPL10

The trial was set on two locations (Zemun Polje-Institut and Zemun Polje- Skolsko dobro) during 2015 and 2016. The trial had an RCBD design in two replications for each original and reciprocal hybrid combination.. Grain yield (kg/ha), plant height (cm) , ear height (cm) and total number of leafs per plant were recorded. Grain yield was adjusted at 14% grain moisture. The data for vegetative traits were recorded on ten average plants for each replication. The statistical significance of the difference in traits values between original and corresponding reciprocal crosses were tested using ANOVA.

Results and discussion

The ANOVA results show that years, locations and genotypes were significant factors for most of examined traits (table 2.). Years and genotypes influenced all traits at 1% level of significance. Different locations didn't affect variations in plant and ear height. Year x Location interaction was signicficant for ear height and leaf number, whileyear x genotype interaction influenced all four examined traits. The variation coefficient ranged from 1.75% for plant height to 3.55% as recorded for ear height.

Table 2. Factor and their interactions significance according to analysis of variance for examined traits

	Factors and Interactions		df	Yield	Plant height	Ear height	Leaf number
1	Replication		1	ns	ns	ns	ns
2	Year	**A**	1	**	**	**	**
3	Location	**B**	1	**	ns	ns	**
4	Year x Location	**A x B**	1	ns	ns	*	*
5	Genotype	**C**	19	**	**	**	**
6	Year x Genotype	**A x C**	19	**	**	**	**
7	Location x Genotype	**B x C**	19	*	*	ns	**
8	Year x Locaton x Genotype	**A x B x C**	19	ns	ns	*	**
	Variation coefficient (%)			**3.18**	**1.75**	**3.55**	**2.33**

ns- not significant, *, **: Significant at the p< 0.05 and p < 0.01 level, respectively

Table 3 shows mean values for grain yield and the difference between the original and reciprocal variants of the same hybrids. Highest yield hybrid were the reciprocal ZPH 2 R (12665 kg/ha) and ZPH (12453 kg/ha) and ZPH 7 R (12453 kg/ha) hybrids. Smallest differences between original and reciprocal hybrids were recorded for ZPH 4 / ZPH 4 R and ZPH 9 / ZPH 9 R hybrids, 0.6 and 0.62% respectively. Significant reciprocal effects were recorded for hybrids ZPH2, ZPH7 and ZPH8 with the difference ranging from -4.5 to 6.87%.

Table 3. Grain yield mean values for original and reciprocal hybrids and their difference (kg/ha)

	ZPH 1	**ZPH 1 R**	**ZPH 2**	**ZPH 2 R**	**ZPH 3**	**ZPH 3 R**	**ZPH 4**	**ZPH 4 R**	**ZPH 5**	**ZPH 5 R**
	10635	10732	12119	12665	10676	10805	10565	10502	10926	10803
Δ	-97.4		-546*		-129.6		63.2		123.1	
%	-0.92		-4.51		-1.21		0.60		1.13	
	ZPH 6	**ZPH 6 R**	**ZPH 7**	**ZPH 7 R**	**ZPH 8**	**ZPH 8 R**	**ZPH 9**	**ZPH 9 R**	**ZPH 10**	**ZPH 10 R**
	12263	12134	11815	12453	10780	11521	10242	10306	11556	11663
Δ	128.6		638**		741**		63.9		106.8	
%	1.05		5.40		6.87		0.62		0.92	

*, **: Significant at the p< 0.05 and p < 0.01 level, respectively

Plant height mean values for original and reciprocal hybrids and their difference is presented in table 4. Six hybrids exhibited significant reciprocal effect for plant height . Plant height was significantly higher in reciprocal variants of hybrids ZPH7, ZPH8 and ZPH9, and original variants of hybrid ZPH3, ZPH5 and ZPH6. Highest reciprocal difference was exhibited in hybrid ZPH 6 and ZPH7 (5.13 and -5.68, respectivily). Reciprocal effect for maize plant height was Greatest ear height was recorded in reciprocal hybrid ZPH 6 R (127cm) and his original ZPH 6 (124cm). Significant reciprocal effect was noticed for hybrids ZPH 7 and ZPH 8. Original ZPH 7 hybrid had on average 9,5 cm higher positioned main ear compared to his reciprocal variant. Contrary to that reciprocal ZPH 8 R had significantly greater values for ear height compared to the original variant of the same hybrid.

Table 4. Plant height mean values for original and reciprocal hybrids and their difference (cm)

	ZPH 1	ZPH 1 R	ZPH 2	ZPH 2 R	ZPH 3	ZPH 3 R	ZPH 4	ZPH 4 R	ZPH 5	ZPH 5 R
	271	274	276	274	277	266	277	274	285	275
Δ	-2.25		1.73		10.88**		2.91		9.7*	
%	-0.83		0.63		3.93		1.05		3.41	
	ZPH 6	**ZPH 6 R**	**ZPH 7**	**ZPH 7 R**	**ZPH 8**	**ZPH 8 R**	**ZPH 9**	**ZPH 9 R**	**ZPH 10**	**ZPH 10 R**
	293	278	273	288	270	279	273	285	265	261
Δ	15**		-15.49**		-8.46*		-11.33*		3.85	
%	5.13		-5.68		-3.13		-4.14		1.45	

*, **: Significant at the p< 0.05 and p < 0.01 level, respectively

Table 5. Ear height mean values for original and reciprocal hybrids and their difference (cm)

	ZPH 1	ZPH 1 R	ZPH 2	ZPH 2 R	ZPH 3	ZPH 3 R	ZPH 4	ZPH 4 R	ZPH 5	ZPH 5 R
	111	110	110	110	110	112	120	121	119	123
Δ	0.19		0.1		-2.71		-1.11		-4.05	
%	0.17		0.09		-2.47		-0.93		-3.40	
	ZPH 6	**ZPH 6 R**	**ZPH 7**	**ZPH 7 R**	**ZPH 8**	**ZPH 8 R**	**ZPH 9**	**ZPH 9 R**	**ZPH 10**	**ZPH 10 R**
	124	127	112	103	111	119	120	121	105	102
Δ	-3.01		9.5**		-8.41**		-1.38		3.45	
%	-2.43		8.48		-7.60		-1.15		3.29	

*, **: Significant at the p< 0.05 and p < 0.01 level, respectively

ZPH7 is the only hybrid to exhibit a significant reciprocal effect for leaf number (table 6). The reciprocal variant ZPH7 R had 11.6% greater average leaf number compared to the original hybrid variant.

Table 6. Leaf number mean values for original and reciprocal hybrids and their difference

	ZPH 1	ZPH 1 R	ZPH 2	ZPH 2 R	ZPH 3	ZPH 3 R	ZPH 4	ZPH 4 R	ZPH 5	ZPH 5 R
	16.3	16.7	16.9	16.2	16.4	16.7	16.6	16.4	16.7	16.3
Δ	-0.44		-0.16		0.69		0.16		0.42	
%	-2.71		-0.95		4.21		0.97		2.51	
	ZPH 6	**ZPH 6 R**	**ZPH 7**	**ZPH 7 R**	**ZPH 8**	**ZPH 8 R**	**ZPH 9**	**ZPH 9 R**	**ZPH 10**	**ZPH 10 R**
	15.8	16.3	14.9	16.7	16.1	16.3	16.2	15.9	15.7	16.0
Δ	-0.53		-1.73**		-0.21		0.35		-0.28	
%	-3.36		-11.60		-1.31		2.16		-1.78	

*, **: Significant at the p< 0.05 and p < 0.01 level, respectively

Hybrids ZPH1, ZPH4 and ZPH10 weren't influenced by the reciprocal crosses for neither of the investigated traits.

Conclusion

The maternal effect is a complex phenomena and more research should be done to understand its nature. While some maize hybrids seems unaffected by the reciprocal effect, others exhibite significant differences between their original and reciprocal variants for important traits that can affect hybrid performances. Taken in consideration the correlations between

different traits of maize (morphological traits, yield components), the possible significant influence of maternal effect should be regarded as one of the important factors in the planning of maize hybrid seed production, together with yield potential of the mother inbred line, pollination capabilities of the father line and others.

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MORPHOLOGICAL AND PRODUCTION CHARACTERISTICS OF OATS CULTIVATED ON EUTRIC CAMBISOL, PRODUCTIVITY AND QUALITY OF OAT GRAINS (*Avena sativa* L.)

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Abstract

The aim of this research was to examine the impact of the application of different nitrogen amounts and the timing of nitrogen fertilizer applications on morphological characteristics, yield and yield components and grain quality of the winter oats. The research was performed in 2015/2016 production year, with the domestic variety Vranac, on the eutric cambisol soil type, according to the randomized block system with four replications. Two factorial trial was based on the following variants: A) Time of fertilization (1. February, 2. March, 3. February and March); B) Nitrogen content (1. Control (without fertilization), 2. 50 kg ha^{-1} N; 3. 100 kg ha^{-1} N; 4. 50 + 50 kg ha^{-1} N). Combinations i.e. variants were as follows: Control, February 50 kg N ha^{-1} (F_{50}); March 50 kg N ha^{-1} (M_{50}), February 50 kg N ha^{-1} + March 50 kg N ha^{-1} (F_{50} + M_{50}); February 100 kg N ha-1 (F_{100}) and March 100 kg N ha-1 (M_{100}). The highest values of plant height (82.6 cm), number of internodes (4.6), panicle length (20.8 cm), number of spikes in a panicle (30.2), number of grains (66.4) and grain weight (1.78 g) per panicle were obtained in the variant F_{50} + M_{50}. The highest values of the 1000-grain weight (32.4 g) and the hectoliter weight (51.6 kg) were measured when fertilization was carried out in March with 50 kg N ha-1. The largest grain yield (4.23 t ha^{-1}) and grain protein content (8.6 %) were observed for the variant F_{50} + M_{50}.

Keywords: *Oats, nitrogen, morphological and production characteristics, protein content*

Introduction

Oat (*Avena sativa* L.) is an annual field crop from the grass family (*Poaceae*). It is originated from Eastern and Southeast Asia. Thanks to the high energy and nutritional value of the grain, it has a great economic importance. Oats are used in the diet of humans, domestic and fattening animals (Glamočlija, 2012). Secondary metabolites of oat grain exhibit antioxidant properties (Rakić *et al*., 2014). Compared to other cereals, oats are better adapted to the production in less favorable, colder and humid conditions, as well as on less fertile soil (Hoffman, 1995). It is particularly important for growing in mountainous areas where growing of other field crops is less successful (Maksimović, 1998). Requirements of oats regarding soil quality are modest, compared to other cereals (except rye). Because of the good suction power of the root system, oats can perfectly use the remaining nutrients after first crop harvesting, and it is usually the last crop in the crop rotation, when the high grain yields can not be expected. However, although oats can be grown on less fertile soils, with somewhat reduced yields, better results are achieved on more fertile land (Reiner *et al.*, 1983; Malešević, 2008). In the past years areas under the oats in the world ranged between 10 and 14 million ha, with an average grain yield of around 2.3 t ha^{-1}. In Serbia, oats are grown on the area of about 35,000 hectares with the average grain yield of 2.2 t ha^{-1} (Glamočlija, 2012). Nitrogen is the key element for yield and most often is the limiting factor for achieving high yields. As the constituent of vitamins and chlorophylls, it indirectly participates in the photosynthesis process (Jevtić, 1986). Nitrogen

fertilization is specific because the mineral, accessible form of nitrogen can be decreased by washing out due to its mobility in the soil and denitrification, and on the other hand, it is increased by the mineralization of soil organic matter (Kastori, 2005). In order to determine the optimum amount of nitrogen from the fertilizer, it is necessary to know the dynamics of the nitrogen absorption by the crop and the N - NO_3 dynamics in the soil, as well as the time of application or the type of nitrogen fertilizer. Oat strongly responds to supplemental nutrition, especially with nitrogen. Nitrogen fertilization significantly increases the yield and quality of oats, as well as the production of overhead biomass, while phosphorus and potassium have significantly less effect on the yield increase. Halezov and Anisimov (1982) recommend from 30 to 60 kg N for oats fertilization ha^{-1}, Sawicki (1982) 60 kg N ha^{-1}, Hmurec and Markovec (1982) 90 kg N ha^{-1}. Based on the investigation of the influence of different amounts of nitrogen on the yield of three varieties of oats, Pržulj *et al.* (2010) found that the highest grain yield in all varieties was obtained with 30 kg N ha^{-1} while Burke *et al.* (2001) stated that the effects of different doses of nitrogen and density of the sowing have had a less effect on the yield and quality compared to the variety of oats. The same researchers pointed out that the yield of the oats increased by increasing the amount of nitrogen up to 160 kg N ha^{-1}. However, the percentage of lodging significantly increased in treatments with more than 100 kg N ha^{-1}. For agro-ecological conditions of Vojvodina Aćin *et al*. (2012) recommend 60 kg N ha^{-1} for oats fertilization, as they did not establish statistically significant differences between 60 and 90 kg N ha^{-1}. Accordingly, the amount of nitrogen in the oats fertilization depends on the resistance of the variety to the lodging and low temperatures, fertility and physical properties of the soil, the agro-ecological conditions of the area, the planned yield and the financial status of the producer (Maksimović, 1998). When planning this research, it was assumed that the investigated amounts of nitrogen and different fertilization timing would have different impact on morphological characteristics, yield components, yield and grain quality of the winter oats.

Materials and Methods

Testing of the winter oats productivity in the dependence from fertilization time and the amount of nitrogen was carried out on the private farm near Sopot (village Drlupa), Serbia, in 2015/16 production year. The field trial was performed on the eutric cambisol soil type, according to the randomized block system with four replications. At the profile depth of 0 - 30 cm the soil was characterized by the following agrochemical properties: pH in H_2O – 4.99; pH in KCl - 4.15; humus - 4.26%; nitrogen - 0.213%; P_2O_5 - 9.60 mg/100 g and K_2O - 26.60 mg/100 g of soil. The actual plot area for grain yield was 6.0 m^2 (3.0 x 2.0 m). Two factorial trial was established, comprising the following variants: A) Time of fertilization (1. February, 2. March, 3. February and ?. March). B) Nitrogen content (**1**. Control (without fertilization); **2.** 50 kg ha^{-1} N; **3.** 100 kg ha^{-1} N; **4.** 50 + 50 kg ha^{-1} N). Combinations i.e. variants were as follows: Control, February 50 kg N ha^{-1} (F_{50}); March 50 kg N ha^{-1} (M_{50}), February 50 kg N ha^{-1} + March 50 kg N ha^{-1} (F_{50} + M_{50}); February 100 kg N ha-1 (F_{100}) and March 100 kg N ha-1 (M_{100}). Crop previous to oats was winter wheat. After the harvest of wheat, the stubble field was ploughed down to the depth of 10 - 15 cm. The sowing of winter oat variety Kg - Vranac was performed in the second half of October 2015, according to the plan of the sowing, with 500 seedlings per square meter or about 160 kg ha^{-1}. Within the cultivation measures, the protection of the crop with the herbicide based on *Bentazone* and the protection of the grain from cereal leaf beetle with insecticide based on *Deltamethrin* were carried out. Nitrogen fertilizer AN (33.5% N) was applied in the second half of February and the second half of March. The harvest of the oats was done manually in physiological maturity, in the second half of July. Immediately after the harvest, necessary measurements of the oats yield basic indicators and productivity were made. The chemical composition (quality) of the grain

was determined on the apparatus DICKEY - JOHN, NIR Analyzer. Data on the weather conditions were obtained from Mladenovac meteorological station.

Meteorological conditions. Oats, as a plant of the northern chilly areas, does not have a great need for warmth, so the heat sum for winter varieties is about 1,900°C (Glamočlija, 2012). Average monthly air temperature, monthly and annual amount of rainfall for the vegetation period of winter oats in the year of the study, as well as the multi-annual average of air temperature and rainfall (2009 - 2014) are shown in Table 1.

Table 1. Average monthly air temperature (°C), monthly and annual sum of rainfall (mm) for the vegetation period of the winter oats and the multi-annual average (Mladenovac)

	Month									
	X	XI	XII	I	II	III	IV	V	VI	Average/ Sum
Air temperature (^{o}C) 2015/2016	11.2	6.9	3.3	0.2	7.3	7.8	14.1	16.1	21.4	9.8
Multi-annual average 2009-2014	11.8	9.7	2.8	2.0	1.2	7.5	12.5	16.4	20.7	9.4
Rainfall (mm) 2015/2016.	42.4	54.3	4.4	47.2	97.0	95.8	67.8	90.0	106.6	605.5
Multi-annual average 2009-2014	53.7	25.5	58.3	49.4	56.2	47.0	68.8	93.6	68.1	520.6

The average air temperature in the investigated year did not differ significantly compared to the multi-annual average of 9.8°C. The total sum of the precipitation for the vegetation of the winter oats in the year of investigation was significantly higher (by 84.9 mm) in comparison to the multi-annual period for Mladenovac area, and it was 605.5 mm, which certainly affected the obtained results, i.e. the nitrogen fertilizer had stronger effect.

Results and Discussion

Within the morphological characteristics of the oats, the influence of timing of fertilization and amount of nitrogen on the plant height and the number of internodes of the stem were examined. Average plant height for investigated variants was 70.9 cm (Table 2). The lowest plant height (50.8 cm) was noted in control (variants without fertilization) and the highest one (82.6 cm) was measured in variant where nitrogen was applied twice (February+March) in the quantity of 50 + 50 kg N ha^{-1}. Higher plant height was achieved by nitrogen fertilization in February compared with that in March. The average number of internodes was 3.8 (Table 2). Also, the smallest number of internodes was counted in the control variant (2.6), while the largest number of internodes was recorded on F_{50} + M_{50} (4.6), followed by the F_{50} variant (4.2).

Table 2. Influence of fertilization timing and nitrogen amount on the plant height (cm) and the number of internodes

Fertilisation variant	Plant height (cm)	Index (%)	Number of internodes	Index (%)
Control	50.8	100.0	2.6	100.0
F_{50}	72.9	143.5	4.2	161.5
M_{50}	68.6	135.0	3.7	142.3
F_{50} + M_{50}	82.6	162.6	4.6	176.9
F_{100}	75.7	149.0	4.0	153.8
M_{100}	74.7	147.0	3.9	150.0
Average	70.9	-	3.8	-

The average length of the panicle was 16.7 cm (Table 3). The minimum panicle length (11.7 cm) was detected in control, while the highest value (20.8 cm) of this parameter was registered in the combination F_{50} + M_{50}. The average number of spikes per panicle was 25.1 (Table 3). Fertilization in March, on average for nitrogen amounts, resulted in 1.2% higher number of spikes compared to the February fertilization. Furthermore, by using 100 kg of N ha^{-1}, a larger number of spikes (3.9) was found compared to the smaller amount of nitrogen (50 kg N ha^{-1}).

Table 3. Influence of fertilisation time and nitrogen quantity on the lenght of panicle (cm) and the number of spikes in panicle

Fertilisation variant	Lenght of the panicle (cm)	Index (%)	Number of spikes per panicle	Index (%)
Control	11.7	100.0	16.7	100.0
F_{50}	14.6	124.8	22.7	135.9
M_{50}	16.2	138.5	25.1	150.3
F_{50} + M_{50}	20.8	177.8	30.2	180.8
F_{100}	18.3	156.4	28.7	171.8
M_{100}	18.3	156.4	26.9	161.1
Average	16.7	-	25.1	-

The average number of grains in the panicle was 51.2 (Table 4). The smallest number of grains in the panicle (30.9) was counted in the control and the largest (66.4) in the variant F_{50} + M_{50}. The average grain weight per panicle was 1.38 g. The application of larger amounts of nitrogen (100 kg N ha^{-1}) caused the increase of grain weight per panicle by 16.4%, while fertilization in March, on average for nitrogen amounts, increased grain weight per panicle by 6.9% (Table 4).

Table 4. Influence of fertilisation time and nitrogen quantity on the number of grains per panicle and grain mass per panicle (g)

Fertilisation variant	Number of grains per panicle	Index (%)	Grain weight per panicle (g)	Index (%)
Control	30.9	100.0	0.85	100.0
F_{50}	48.8	157.9	1.20	141.2
M_{50}	50.8	164.4	1.42	167.1
F_{50} + M_{50}	66.4	214.9	1.78	209.4
F_{100}	55.0	178.0	1.54	181.2
M_{100}	55.2	178.6	1.51	177.6
Average	51.2	-	1.38	-

The average 1000-grain weight was 31.4 g (Table 5). The minimum 1000-grain weight (29.3 g) was detected in control and the largest (32.4 g) when fertilization was carried out in March with the amount of 50 kg N ha^{-1}. Average hectoliter weight was 49.9 kg. In this case, a higher hectoliter weight was achieved with a smaller amount of nitrogen (50 kg N ha^{-1}) both in February and March, while the greatest hectoliter weight (51.6 kg) was measured when fertilization was carried out in March with 50 kg N ha^{-1}.

Table 5. Influence of fertilisation time and nitrogen quantity on the 1000-grain weight (g) and hectoliter weight (kg)

Fertilisation variant	1000-grain weight (g)	Index (%)	Hectoliter weight (kg)	Index (%)
Control	29.3	100.0	48.2	100.0
F_{50}	31.9	108.9	51.2	106.0
M_{50}	32.4	110.6	51.6	107.1
$F_{50} + M_{50}$	30.7	104.8	49.1	101.9
F_{100}	31.7	108.2	49.9	102.9
M_{100}	32.1	109.6	50.2	104.1
Average	31.4	-	49.9	-

The average grain yield was 3.71 t ha^{-1} (Table 6). The lowest grain yield (2.77 t ha^{-1}) was achieved in the variant without nitrogen supplementation and the largest (4.23 t ha^{-1}) in combination $F_{50} + M_{50}$. The same tendency was observed for the protein content of the grain. On average for nitrogen amounts, the higher protein content in the grain (by 14.05 index points) was achieved in the variant of nitrogen utilization in March compared to the fertilization in February. On the other hand, by applying a larger amount of nitrogen (100 kg ha^{-1}) in March, the protein content in grains was higher by 6.2 index points compared to 50 kg N ha^{-1}.

Table 6. Influence of fertilisation time and nitrogen quantity on the grain yield (t ha^{-1}) and grain protein content (%)

Fertilisation variant	Grain yield (t ha^{-1})	Index (%)	Grain protein content (%)	Index points
Control	2.77	100.0	6.4	100.0
F_{50}	3.60	130.0	6.8	106.3
M_{50}	3.77	136.1	7.8	121.9
$F_{50} + M_{50}$	4.23	152.7	8.6	134.4
F_{100}	3.97	143.3	7.4	115.6
M_{100}	3.90	140.8	8.2	128.1
Average	3.71	-	7.5	-

Conclusion

On the basis of the obtained results the following conclusions can be drawn:

Meteorological conditions during the vegetation period were favorable for the growth and development of the winter oats. Both investigated factors - amount of nitrogen and the timing of nitrogen fertilizer application had an effect on the parameters involved in the research.

Regarding morphological characteristics of the oats, the plant height was more dependent on the amount of nitrogen compared to the timing of application, while the its influence on the number of internodes was opposite. By increasing the nitrogen amount from 50 to 100 kg N ha^{-1}, plant height increased by 6.2%. Fertilization in February resulted in increased plant height (by 3.3%) compared to March. There was no significant difference in the number of internodes for 50 and 100 kg N ha^{-1}, but earlier fertilization (in February) resulted in the higher number of internodes compared to nitrogen supplementation in March. Regarding yield components, by applying the larger amount of nitrogen (100 kg N ha^{-1}), a greater panicle length (by 18.8%) was measured and the greater number of spikes (by 16.3%) compared to the application of the amount of 50 kg N ha^{-1}. Fertilization in March resulted in higher panicle length (by 4.8%) and the greater number of spikes (by 1.2%) compared to the nitrogen utilization in February. The 1000-grain weight, as well as the hectoliter weight, showed low dependence, both from the timing of fertilization and the amount of nitrogen. The applied

nutrition with nitrogen had the strong influence on the yield and quality of oat grains. Compared to the non-fertilized variant, two-fold nitrogen utilization in the amount of 50 kg N ha^{-1} (variant F_{50} + M_{50}) increased grain yield by 52.7% or 1.46 t ha^{-1}, as well as protein content (34.4 index points or 2.2%). This is also a recommendation to the winter oats producers when deciding about timing of fertilization and the amount of nitrogen for the investigated area.

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WHEAT PLANT STATURE AND EAR PRODUCTIVITY PARAMETERS VARIATION IN MULTI-ENVIRONMENT TRIAL

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Abstract

The human population has increased more than 200% during the 20th Century. UN projection is 10 billion people in 2055 demanding food. To meet the challenge, contemporary agriculture is to using all its resources to increase the food production. In order to investigate the way to enhance productivity level of marginally suitable land multi-environment trials has been established. A portion of these multiyear trials is analyzed in this article. Six bread divergent wheat varieties were grown 4 environment trials (2 years x 2 localities). The first year was extremely humid and rainy, and the second could be considered as an average year. Two localities differed in soul type. One locality was characterized by chernozem, and the other by solonetz soil. The paper presents the results of the plant height (cm), the spike length (cm), the mass of the spike (g), the grain mass per spike (g), the number of grains per spike and the spike index of 6 bread wheat varieties (Renesansa, NSR5, Pesma, Banatka, Jugoslavia and Bankut 1205). The results are presented via the GGE biplot in order to analyze the common genotype effect and the genotype by environment interaction (G + GE). GGE biplotes were used to distinguish genotypes with the highest GGE effect. Graphs are constructed based on PCA results. For all tested traits, the first principal component (PC1) has explained more than 50% of the total variation of the trial. The genotype by environment interactions (GEI) for each examined variety varied depending on variety and trait.

Keywords: *wheat, MET, GEI, GGE, yield components.*

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most widespread agricultural crops. It represents a portion of human diet of about 25% in developed, and even up to 80% in developing countries comparing to all the cereals use being the main source of minerals and vitamins. According to UN data, human population is growing at the rate of 83 million individuals per year, and up to 2055, the projection is that it would be about 10 billion people on Earth (United Nations, 2018). General view is that the food production *per capita* of today is sufficient enough to feed the World, however the basic problem is unevenly distributed production and consumption. Hence, about 2.3 billion people in the World are not fed enough or intake less than required 2000kcal/day, while 1.9 billion people consume more than 3000kcal/day *(TEEB, 2015)*. Consequently, contemporary agriculture faces great challenges not only in increased requirement for food production, but also in finding and reaching for new agricultural resources.

Plant breeding, including the breeding of wheat, is of crucial importance in achieving high and stable yield, as well as, in searching for usable genetic variability for crop growing in limited conditions of less convenient, stressful environments. One of the important plant breeding directions is broadening production area by putting in good use less productive soil, by producing usable genetic variation that could be utilized in wide range of agro-ecological conditions including different soil types. In phenotypic variability components estimation the important is the role of genotype by environment interaction (GEI), not only in respect of

stable genotype reaction to environmental variation that is one of the selection criteria, but also in identifying the main sources of phenotypic variability in order to find optimal growing conditions where a given genotype is going to perform to the best of its genetic background.
The aim of the article is to estimate GEI of different wheat varieties in multi environmental trial (MET) seated on chernozem and solonetz soil in order to assesing grain yield performance and identifying the most valuable genetic variation, through grain yield component traits, using genotypes (G) and genotype/environment (GE) interaction biplot model (GGE).

Material and methods

The 2Lx2Y MET trial was set at two locations (2L), Rimski Šančevi, near Novi Sad (45.322°N and 19.839°E), on chernozem soil at the Experimental field of the Institute of Field and Vegetable Crops in Novi Sad (EFIFVC - NS), as control locality, and on the soil type solonec, in Banat region, in the village of Kumane - KU (45.539°N and 20.228°E), in two vegetation seasons (2Y - 2014/15 and 2015/16). The first season was characterized by exceptionally high averages of monthly rainfall and humidity, while the second season was within the average values for the observed region. In the study, six varieties of bread wheat (Renesansa, NSR 5, Pesma, Banatka, Jugoslavija and Bankut 1205) were investigated, in the full maturity phase, for the phenotypic variation of the following yield components: plant height (cm) - PH, spike length (cm) - SL, spike weight (g) - SW, grain mass per spike (g) - GMS, grain number per spike - GNS and the spike index - SI, defined as the ratio between the mass of grains per spike and the total mass of spike (Denčić and Borojević, 1992). Varieties Renesansa, NSR 5, Pesma, and Jugoslavija are of Green revolution (GR) ideotype, semi-dwarf wheat varieties, where NSR 5 (Novi Sad's early) is considerably earlier than other three. Variety Bankut 1205 is and pre GR bred variety of taller stature, showing 120cm of height on average. Finally, Banatka, or Old Banatka is a landrace from Banat area that had been grown in Banat region by small farmers before the introduction of Hungarian variety Bankut 1295. At both localities, labeled as NS and KU, varieties were sown in 6 rows of 2m length, with the raw spacing of 15cm. Before sowing, 130kg/ha of mineral fertilizer NPK (15:15:15) was applied, while 200kg/ha KAN mineral fertilizer was additionally applied.
The data were analyzed using the GGE biplot software (http://www.ggebiplot.com/software), and genotype (G) variation and GE interaction interpreted according to the model for the GGE biplot based on singular value decomposition (SVD) of first two principal components:

$$Y_{ij} - \mu - \beta_j = \sum_{k=1}^{K} \lambda_k \xi_{ik} \eta_{jk} + \varepsilon_{ij}$$

Where Y_{ij} = the mean yield of genotype i (= 1,2,…,g) in environment j (= 1,2,…e), μ = the grand mean, β_j = the main effect of environment *j*, ($\mu + \beta_j$) = mean yield of environment j, λ_k = the singular value (SV) of k^{th} principal component (PC), ξ_{ik} = the eigen-vector of genotype i for PC_k, η_{jk} = the eigen-vector of environment j for PC_k, K is the number of PC axes retained in the model ($K \leq \min(g,e)$ and $K = 2$ for a 2-dimensional biplot) and ε_{ij} = the residual associated with genotype i in environment j (Yan, 2001; Yan and Holland, 2010).

Results and discussion

Plant height. Variation for plant height highly depended as expected, on locality and on vegetation period. The season of 2015/16 (sowing 2015) was meteorologically more favorable for wheat than 2014/15

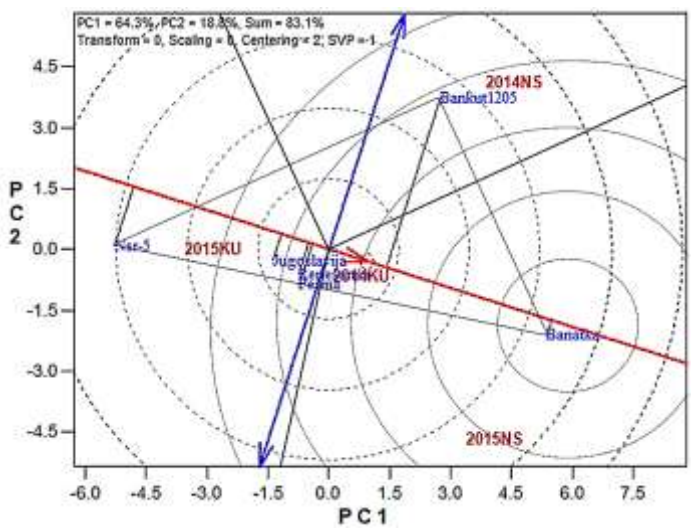

Figure 1. GGE biplot of two Principal Components (PC) based on 2Lx2Y MET trial with 6 bread wheat genotypes for plant height (cm). The GGE biplot showing genotype variability and GE interaction in each environmental variation.

(sowing 2014), where the weather was unstable with a lot of precipitations that followed dry period in spring. The complex nature of environmental variation caused that almost every environment represented a distinct environmental sector. The locality more suitable for agricultural production, EFIFVCNS (labeled as NS in the graph) exhibited higher level of GEI, meaning that in favorable agro ecological conditions varietal response to environmental variation is more prominent comparing to solonetz soil condition at locality Kumane (KU) combined with less favorable over all meteorological conditions. The smaller GEI variation in Kumane showed that, concerning PH variation, more abiotic stressful conditions lessen the differences between varietal reactions to environmental variation. Modern varieties, created after Green revolution projected ideotype in wheat expressed low level of GEI variation, three semi-dwarf varieties of medium maturity, particularly. As expected "ideal environment" was 2015/16 (2015NS). Landrace Banatka took the position of the most adaptable variety concerning PH, similarly behaved pre GR variety Bankut 1205 reacting with full PH performance on NS favorable environmental conditions, however the most stable modern varieties, Jugoslavija, Renesansa and Pesma were distributed near to average environment. NSR 5 appeared the best suited for unfavorable conditions both years and soil type, since this is the only variety bred for early maturity in the trial (fig. 1).

Spike length. Though the variability of SL and PH is influenced by the same gene system, in this case a cocktail of major *Rht* genes, and minor genes genotypes in MET exhibited different but expressly GEI variation for SL comparing to PH. According to sector division, as well as array of environments, locality was predominant variation source of SL. This could be as a consequence of selection criteria, where PH is commonly more in focus than SL, *per se*. Hence, GEI is more variable than PH, regardless of genotype origin. So, locally well adapted instinctively selected landrace Banatka, appeared to be the best suited for an average environment. Moreover, modern varieties Jugoslavija and NSR 5 exhibited potentially desirable variation of SL in the list favorable production conditions in this trial (2014KU), fig. 2.

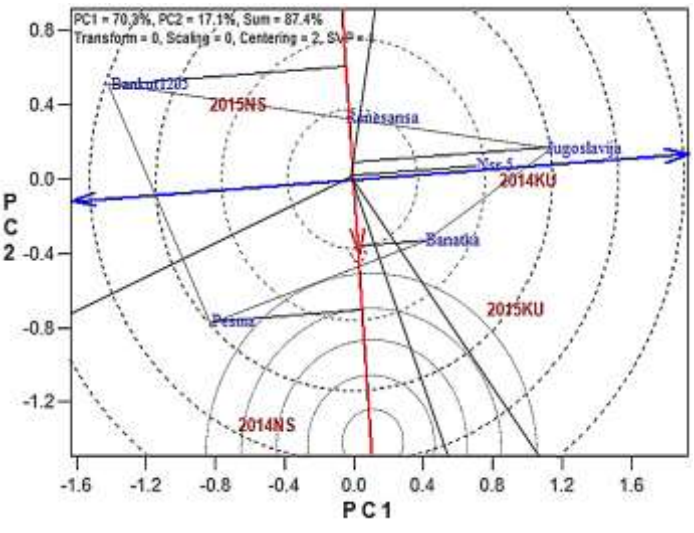

Figure 2. GGE biplot of two Principal Components (PC) based on 2Lx2Y MET trial with 6 bread wheat genotypes for spike length (cm). The GGE biplot showing genotype variability and GE interaction in each environmental variation.

Spike mass. This is the first of three of distinctively quantitative traits. The SM

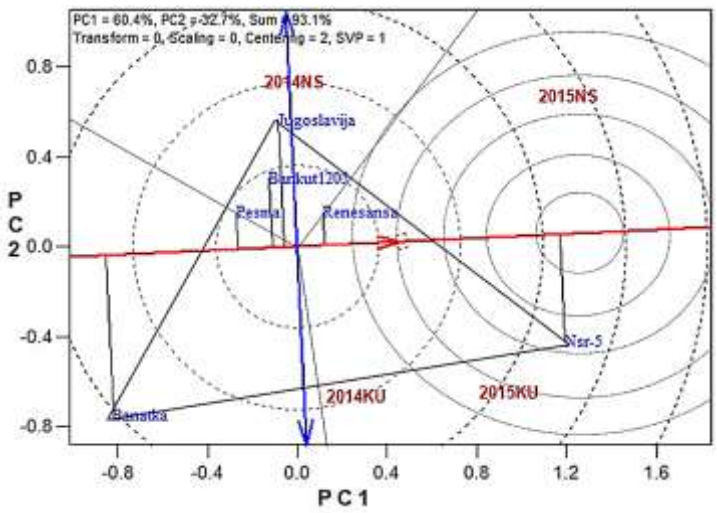

Figure 3. GGE biplot of two Principal Components (PC) based on 2Lx2Y MET trial with 6 bread wheat genotypes for spike mass (g). The GGE biplot showing genotype variability and GE interaction in each environmental variation.

polygenic genetic background caused more even distribution of variation percentage brought out of total trial variability by first two principal axes comparing to PH and SL. According to environmental array, the first variation source of SM variability was soil type, the second was variation of physical weather factors thru vegetation periods, where 2014/15 (2014KU, and 2014NS) where on unfavorable side for the SM formation. The position of EFIFVC (NS) locality in the upper part of the graph regardless of year witnessed that chernozem, as expected, was more productive substrate for SM formation in all varieties except NSR 5, where earliness helped to prevail unfavorable conditions of solonetz soil, but with a help of more convenient meteorological conditions in second season of the trial (2015KU). Landrace Banatka was the best adapted to unfavorable conditions both of year and soil type for SM (fig. 3).

Grain mass per spike. The similarity of gene system responsible for SM and GMS was the main cause of similar variation pattern of these two traits. The additive variation component brought out by PC1 reflected genotype differences in spike yield trough their GMS averages where the influence of the year dominated the effect of location. However, the environmental array along multivariate PC2 showed that GEI variation relied on the soil type variation (locality) rather than on yearly meteorological conditions variation. Bred varieties obtained in breeding institutions, behaved quite alike responding well to favorable production conditions, all except NSR 5, that was more resilient in somewhat less favorable conditions of solonetz soil, at the locality of Kumane. Landrace Banatka expressed the same GMS variation as in it was the case for previously reported SM (fig.4).

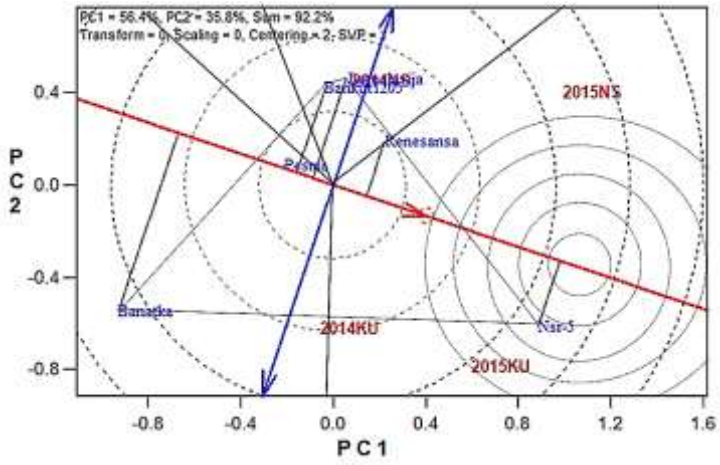

Figure 4. GGE biplot of two Principal Components (PC) based on 2Lx2Y MET trial with 6 bread wheat genotypes for grain mass per spike (g). The GGE biplot showing genotype variability and GE interaction in each environmental variation.

Grain number per spike. The individual plant traits, especially spike traits are fading in its importance in favorable agro-ecological conditions, where the population behavior has the decisive role in grain yield formation. However, in less favorable even stressful production conditions individual plant variation gains in significance. Consequently, GNS complements the triad of quantitative spike traits SM and GMS, expressing the phenotypic variation of the most

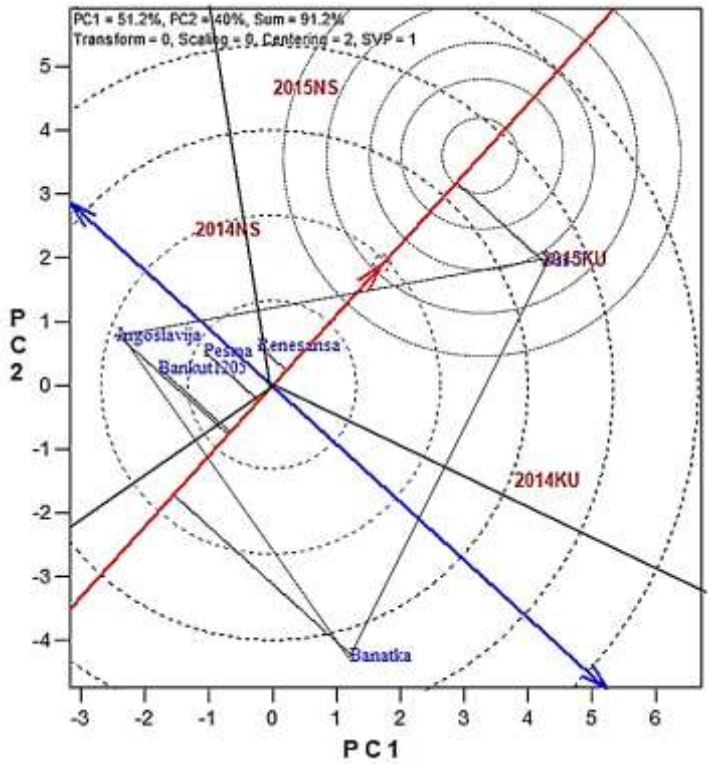

Figure 5. GGE biplot of two Principal Components (PC) based on 2Lx2Y MET trial with 6 bread wheat genotypes for the number of grains per spike. The GGE biplot showing genotype variability and GE interaction in each environmental variation.

complex nature. Though, the first two PCs explained up to 91.2% of total variation, the share of each PC was almost evenly distributed PC1 51.2% and 40% for PC2. This was the highest influence of multiplicative component of total variation denoted in this trial. Consequently, the influence of meteorological physical factors variation appeared to be more influential cause of phenotypic variation for GNS than soil type that should be taken into consideration when forming selection criteria to getting desirable genetic variation in wheat breeding aimed to overcome alkaline soil stress conditions. Bred varieties expressed similar variation pattern both in additive and multiplicative part of total variation. Early variety NSR 5, and the landrace Banatka differed in main, as well as, in multiplicative variation, Banatka performing better in less favorable locality and in less favorable year (2014KU), and NSR 5 expressing good use of better meteorological conditions at the solonetz soil locality in 2015 (2015KU), fig. 5.

Spike index. In modern bread wheat varieties SI is significantly positive correlated to harvest index that quantify the economic (grain) yield versus the total amount of produced biomass (total biological yield), representing sink to source ratio, hence, varietal productivity (Donald, 1962; Petrović *et al*., 2000). The most challenging environments for GEI variation of SI appeared to be 2015KU and 2014NS, but of quite different nature. Genotypes reacted positively to meteorological conditions improvement on solonetz soil in 2015, while less favorable weather conditions in 2014, provoked negative effect on GEI for SI of the genotypes in the experiment on chernozem. According to the environmental array along PC1 it could be observed that the most unfavorable environment (2014KU) provoked better relative efficiency in grain formations, particularly in a group of genotypes landrace Banatka. The perseverance of early maturity variety NSR 5 could be witnessed for SI, as well, while poor behavior of old variety Bankut 1205 for SI parameter variation could be a consequence of the tall stature

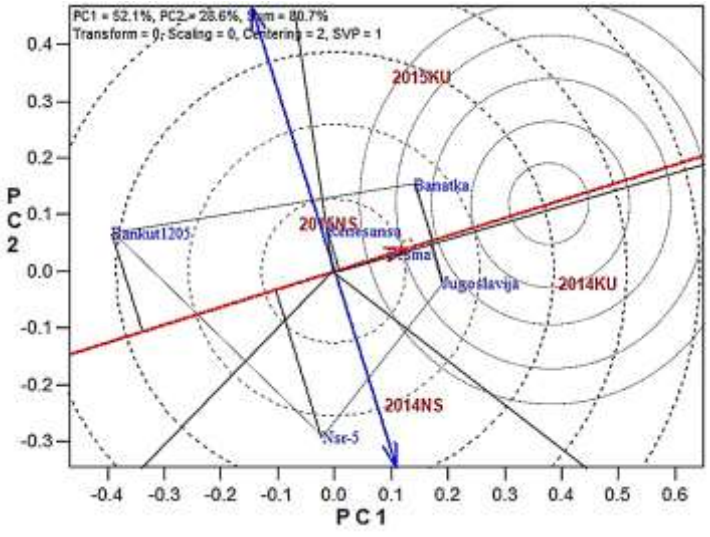

Figure 6. GGE biplot of two Principal Components (PC) based on 2Lx2Y MET trial with 6 bread wheat genotypes for spike index. The GGE biplot showing genotype variability and GE interaction in each environmental variation.

resulting in less efficient translocation of nutrients from sink to source (Dimitrijević *et al*., 2001; Petrović *et al*., 2011), fig. 6.

Conclusion

The investigation presented in this article relay on plant breeding strategy aimed to high and stable grain yield utilizing indirect selection for the traits of interest, the yield components. This approach could be quite efficient in an *in situ* breeding programs for getting usable genetic variability for agricultural production in stressful ecological conditions. For the effectiveness of indirect selection, one of the requirements is the simplicity of testing, accuracy of the results, as well as economical and rapid approach (Yan and Kang, 2001). The results of this trial led to conclusion that the GGE biplot analysis fulfils all the postulated requirements in handling experimental data and drawing in plant breeding applicable conclusions. Though, Kaya *et al*. (2006) commented that favorable environments were more representative of the overall environments and more powerful to discriminate genotypes than the unfavorable ones, according to this results the combination of unfavorable environments using favorable as a parallel control part of the trial, could be of use not only to study varietal behavior, but also to catch sight of usable genetic variation in breeding programs for special purposes. The sources of genetic variation for broadening genetic variability in selection of genotypes suitable for growing on solonetz soil could be found not only in existing commercial genetic variation, but also in landraces and old bred varieties.

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PRODUCTION AND GENETIC MAINTENANCE OF PURE QUALITY MAIZE SEEDS BY SMALLHOLDER FARMERS IN KARAMOJA SUB-REGION, UGANDA

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Abstract

Improving agricultural productivity in Karamoja, Uganda commenced with implementation of a comprehensive program for seed revitalization system through: creating a multi stakeholder innovation platform for commercial seed production, training and equipping farmers with practical grower knowledge, quality control and marketing of pure quality seeds in a sustainable venture, and increasing seed volumes through block farming. Successes achieved involved application of effective methodologies including: establishing an inter-linkage platform for sourcing production and marketing information used in seed system, application of nucleus and farmer field school (FFS) perspective for practical farmer training, application of isolations (time and distance) and half-sib methods for genetic purity maintenance, and establishing block fields for seed production. Four farmer groups were formed each comprising of 25 farmers across selected districts. Farmers started with foundation seed stocks and produced quality declared seed and finally pure quality seeds after three seasons. The project registered the following benefits including: platform for seed enterprise established and functionalized, favorable contract agreements for commercial seed scheme developed and operationalized, model for seed profit margin analysis for crop enterprise selection developed, 5 farmer groups under FFS made operational. Growers (62.5%) trained in production and marketing of maize seed systems, 80% of farmers applied half-sib method at various isolation perspectives, and 108MT of pure maize seeds were produced. Presently growers have knowledge of seed production and access to pure quality seeds for use in production.

Keywords: *seeds-purity, smallholder, half-sib, farmer, school.*

Introduction

Maize (*Zea mays*) is one of the predominantly grown cash crops in Uganda with average yield of 2.7MT Ha^{-1} (Molnes, 2014) whilst ranked 2nd world wide to sugarcane, rice and wheat among produced crops (FAOSTAT, 2014). Uganda, like many sub Saharan countries, depends on agricultural products to finance the country development programs. The sources of income are predominantly from crop products to which maize output is very crucial and 4th in Uganda total value production ($387m) (Molnes, 2014). Maize productivity is low but can increase under intensive use of factors of production. Intensive use of pure quality seeds and availability to the smallholder farmers can help increase productivity per unit area. Maize production is mostly concentrated in West and Eastern low land regions. The far Eastern sub-region of Karamoja, maize output is low at 1.6MTha^{-1} below the average yield of 2.7MTha^{-1}, attributing the cause in particular to use food grains sourced from mobile food grain selling markets, neighbors and harvest from previous seasons than using adaptable maize seed grains of pure quality. Karamoja is endowed with vast cultivable land (27,000 Km^2) for which about 145,391 households are employed through farming to feed the increasing population estimated at 0.97million people (NPHC, 2014). The seed sector is majorly informal, with few

Seed Companies involved in producing, processing and marketing of seeds. The baseline survey on seed system in Africa found the formal use of improved seeds very low at 2.1% (AGRA, 2014) with majority using home saved maize grains than pure quality seeds. Hitherto growers needed a constant and reliable system of seed supply especially at planting time. The knowledge to understand the values and benefits of using pure quality seeds and or producing the seeds for sustainable sources during the cultivation time is very important. It's anticipated that when pure quality seeds are used with good crop husbandry practices, yield can potentially improve by over 50% (Farmers hand book on basic agriculture; 2016). Therefore with crop transformation into a health, and highly vigorous plant with minimal character variation in growth gives an indication of pure seeds being used in production. Such values when are paired with grower knowledge of the seed variety, seed rates, and viability then are ascertained that growers know, understand and can grow seeds. In Karamoja, growers had very limited access to pure quality seeds and also lacked knowledge about value and benefits of using pure quality seeds. The little seeds provided were in some cases found to be actually food grains with poor genetic quality and viability. The potential for cultivation is high, combined with fertile soils under semi-bimodal rains but with no actual, reliable and sustainable source of seed inputs especially at a time of planting hence justification for a pure seed production system. Therefore this intervention involved setting a strong framework for enabling the adoption of technologies that suits production of pure quality seeds through creation of a multi stakeholder innovation platform for commercial seed production, training and equipping farmers with practical grower knowledge, quality control and marketing of pure quality maize seeds in a sustainable venture, and increasing maize seed volumes through block farming.

Materials and methods

Area description

Karamoja comprises of seven districts situated in the Northeastern corner of Uganda between the limits of 1^0 and 4^0 North and 33^0 and 35^0 East, measuring 1000 sq. miles (27,200 Km^2), with ephemeral rivers that flow only periodically after heavy rains. A large area is dry but notably the Western and Southern margins, receive considerably higher rainfall (600-890mm). The area also has 7 green belts for cultivation in during the second season. Therefore technologies for production of pure quality maize seeds were conducted in the green belts as target foundation for increasing maize productivity.

Materials

Resources identified and used included farmer farmland, production inputs (foundation maize seeds, fertilizers: Urea 45.6%N and pesticides etc) and market for commercial product sales. Engaged stakeholders in the platform included: Research Scientists, private sector seed companies, Agro-seed dealers, NGO's operating in project scope areas, National Seed Certification Services (NSC's) and selected farmers. Maize commodity was chosen for farmer training in seed production due to its complexity in genetic purity maintenance, whereby if mastered then other cereal cross pollinate crops can be easier to produce using the same protocol. Foundation stock of open pollinated maize varieties used in seed production included MM3, Longe5 and Vpmax (Maize), sourced from National Crops Resources Research Institutes (NaCRRI), while waterproof pollination bags were used in pollen collection and transfer during selfing and cross pollination for genetic purity assurance. In popularization of seed production, fliers, posters, leaflets, signposts, banners and brochures were developed and used for reference in production.

Multi stakeholder innovation platform

The Approach of integrated Agricultural Research for Development (IAR4D) and constituency Innovation Platform identified by Adekunle *et.al.* (2014), were adopted in developing a multi-stakeholder innovation platform (MSIP) for maize seed production and genetic purity maintenance. The platform oriented problems involved in accessing, production of quality maize seeds using the hard and soft science to identify solutions for maize seed quality control. A number of key stakeholders identified to serve on the platform included: Breeders, Agronomists, Pathologists, Socio-economists, private seed companies, non governmental organizations (ACDI-VOCA, FAO) and the Ministry of Agriculture Animal Industry and Fisheries (MAAIF) and farmers. The platform was systemically engaged in sourcing production and marketing information for effective maize seed system operation, establishing a business venture through forecasting and identifying seeds on seed demand, analyzing seed business profitability and drawing a plan for actual production through contract agreements with outgrowing scheme.

Maize seed grower training

An approach of farmer field school (FFS) perspective as cogitated effective and efficient in extending proven technologies to farmers (Braun *et.al*., 2000) was adopted in imparting practical skills of quality maize seed production to growers. FFS is a practical approach involving training and implementation of gained knowledge (Elske *et al.,* 2000) during Farmer-Research interventions for application in improving agricultural productivity. Four farmer groups (≤ 25 grower) with 50% gender consideration were established on basis of FFS in 4 districts of Moroto, Nakapiripirit, Amudat, and Napak in Karamoja. The total numbers of farmers to be trained were summarized in the following model: fx (4 FGs*25 F/Grp) for initial training in the first cropping year with each participative farmer cultivating 2.0 acres of land for seed production. Different field plots (1.0 acres size) for practical studies were identified and for practical training of farmers and thereafter applies the gained technology on own individual field. Study fields involved the practice of learning production activities ranging from field identification, siting, preparation, seeding, crop husbandry practices, breeding and harvesting and concurrently implement what has been learnt at own farm under maize grower group supervision.

Maize genetic purity maintenance

A strategic application of *half-sib* method in production of pure maize seeds from varieties with pure genetic control (Maize Program, 1999) was adopted and isolation method as applied by MacRobert (2009). Under half sib method farmers grew maize and were able to collect and pollinate developed silks of selected plants. If each farmer was capable of selecting and pollinating at least 300 plants ensuring enough pure seed are achieved for source of seed use in the next cropping season. Different isolation methods of field under full sib were established within 400m distances apart to avoid foreign pollen grain reception, and time of planting either delayed or applied 2 weeks in advance to avoid same field flowering synchrony. Growers were also introduced to the National seed legislation through the Seed and Plant act 2007 regarding production, policy, processing registration, marketing and quality assurance.

Seed marketing approach

The strategy for maize seed marketing involved two adopted schemes the *voucher* and *Matrix* system. A Seed *voucher* is a credit scheme where a small holder farmer is offered a voucher specifying the seed quantity, type and cost, to obtain seeds from a central community seller and re-payable after harvest. Farmers receiving the voucher could be registered for easy follow-up. The essence was to enhance easy availability and ease of access to pure quality seeds especially at planting time. The *matrix* (Fig.1) system involved seed business commercialization with 3fold of research-farmer private sector (seed company). Research

obtains information on maize seed demand from Seed companies and in return provides the required maize variety for multiplication. Through credit scheme growers obtains, grow and sell maize seeds under contract agreement to the seed company, with backstopping knowledge of production from research.

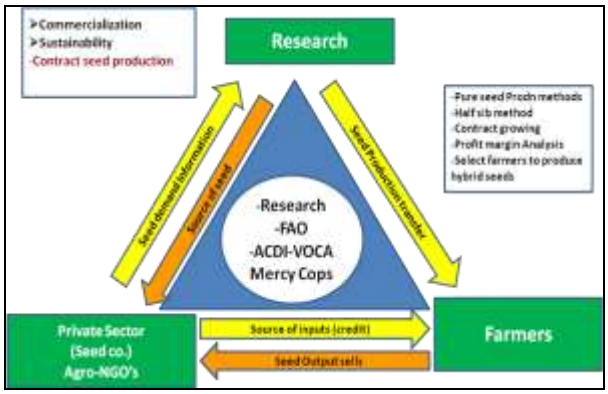

Figure 1: Showing matrix for seed business

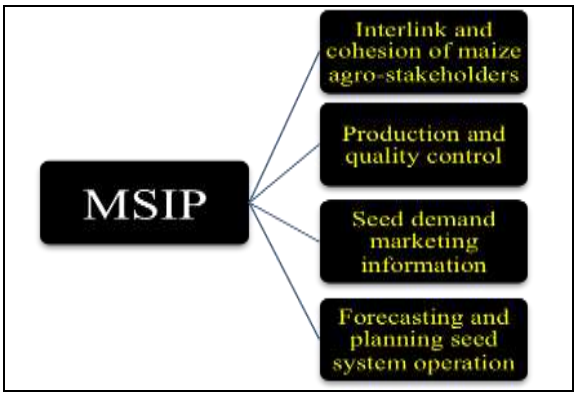

Figure 2: MSIP functional interaction

Block farming, popularization and promotion of maize seeds

Strategies for maize seed production and technology promotion were implemented through (i) *result* and *method* demonstration to enhance seed growers and other stakeholders an opportunity to observe, compare and evaluate the performance of the improved maize seed (ii) field day organization seed products exhibition and notion exchanges about venture success (iii) Awareness on radio talk shows (Karamoja 97.4FM) about the need for knowing maize seed production and accessibility through farmer production scheme (iv) development and availability of seed production reference materials such as fliers, poster and brochure. Maize fields in a homogenous cluster of seed growers under same variety were established in the community for production. This practice is relevant in avoiding foreign variety mixture from acting as source of contaminants thus increased productivity of good genetic purity.

Results and discussion

Using the integrated Agricultural Research for Development (IAR4D) approach, a strong Multi Stakeholder Innovation Platform (MSIP) was established with functional structures of interlink and cohesion of maize agro-stakeholders, production and quality control, marketing information, forecasting and planning seed system operations (Fig. 2). This structural function correlated positively with the stakeholder interaction in innovative platform identified by Adekunle *et.al.* (2012). The various portfolios on the MSIPs identified and placed include: Seed Companies, Farmers, Seed inspector (MAAIF) and Research and tutor in agricultural school. The platform identified lack of quality seeds of maize and production knowledge as cardinal problems in improving productivity of maize. Emphasis towards increasing productivity were geared to systematic cohesion and interlinks in sourcing production and marketing information for effective maize seed system operation, establishing a business venture through forecasting and identifying seeds on seed demand, analyzing seed business profitability and drawing a plan for actual production through contract agreements with outgrowing scheme.

Farmer training concentrated on grower practical learning of production field siting and preparation, seed sowing using dribble method, nutrient (Urea 46%N) application, variety descriptions and plant structure and weeding methods timing at respective crop growth stages for ensuring proper growth of pollinated plants. At least 8 farmer field schools (FFS) were formed and 62.5% (1148 farmers) of farmers trained to become maize seed growers (Tab. 1).

Table1. Showing number of trained farmers in production of pure quality maize seeds

Season	Farmers Trained	Cropping year 2017	2016	2015
A	206	104 (50.5%)	82 (39.8%)	20 (9.7%)
B	72	52 (72.2%)	10 (13.9%)	08 (11.1%)
A	150	116 (77.3%)	25 (16.7%)	09 (6.0%)
B	60	45 (75%)	9 (15.0%)	6 (10.0%)
A	201	120 (59.4%)	62 (30.9%)	19 (9.4%)
B	56	24 (42.9%)	21 (37.5%)	11 (19.6%)
A	315	210 (66.7%)	93 (29.5%)	12 (3.8%)
B	88	46 (52.3%)	22 (25.0%)	20 (22.7%)
	1148	**717 (62.5%)**	**324 (28.2%)**	**105 (9.2%)**

Table 2. Pure quality seed produced from study plots

Grower Group	Acreage	WAP	Numbers			Aspect		Husk Cover	Yield (THa^{-1})
			Tassels Covered	Silk sets Pollinated	% Off Types	Plant	Cob		
Naitakwai	5	12	5330	1821	<5	1	2	2	2.87
Apeitolim	5	12	4900	1644	<5	1	2	2	2.46
Kawachi	5	12	4500	1590	<5	1	2	2	2.47
Lobulio	5	12	6000	1962	<5	1	2	2	3.28
Kolir	5	12	5120	1880	<5	1	2	2	2.89
Nabuin	5	12	4980	1497	<5	1	2	2	2.46

**WAP=Weeks after planting; Yield model: ((10000)/area)*((100-MC)/85)*(fwt*0.8)*

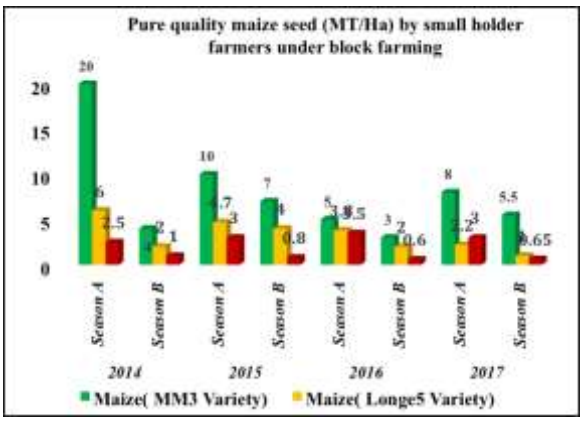

Figure3. Shows maize productivity under block farming

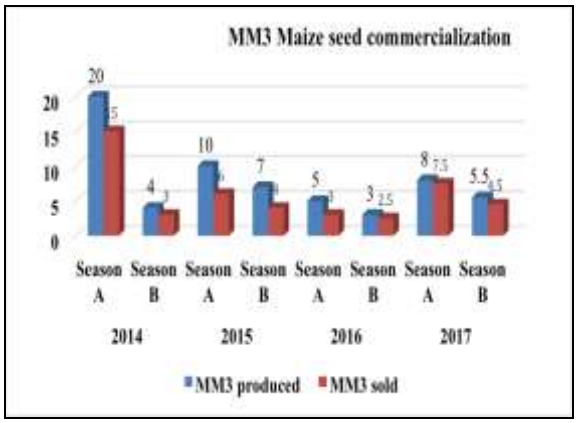

Figure 4. Maize (MM3) seed venture under nucleus farms

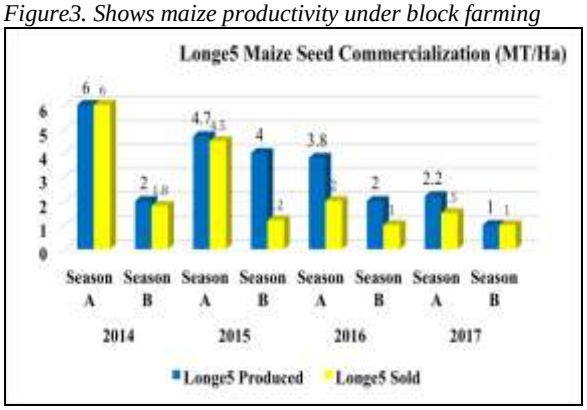

Figure 5. Maize (Longe5) seed venture under nucleus farms

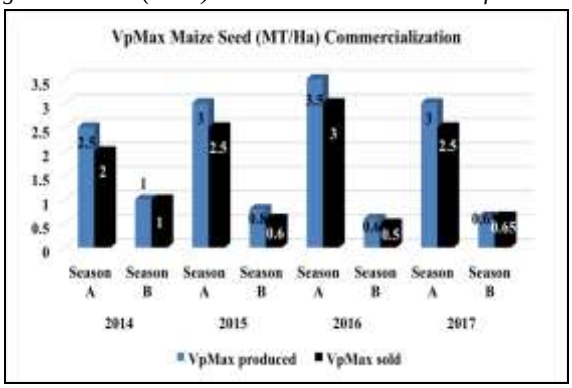

Figure 6. Maize (VpMax) seed venture under nucleus farms

In genetic purity maintenance, 60% of growers learnt and practiced the protocol of varietal purity control where maize tassels and silks were covered for pollen collection and protection of silks from foreign pollen grains respectively (Tab 1. and 2.). Growers used the same knowledge to identify off types prior to flowering, de-tassled and or rogued to maintain the genetic purity of same maize variety. The success of this technology enhanced growers to own implement the half sib technology where 98% of pollinated plants produced pure quality seeds hence preferred as quality declared seed (QDS). Similarly maize fields isolated at 400m from other maize plots were found uniform in tassel and silk structural appearance hence conformation of morphological plant purity, and harvested cobs appeared uniform hence purity assurance. The achieved results of genetic purity were in correlation with observation of MacRobert (2009) on maintaining and production of breeder seed of self-pollinated maize crops. Thus maize seeds produced under block farming system (Fig. 3) exhibited purity with 80% of MM3, longe5 and VpMax seed variety highly demanded by agro-stakeholders (Fig. 4-6) hence commercialization with cost benefit ratio of 0.55 under smallholder farmer management.

Conclusion

Equipping smallholder farmers with practical knowledge of production and genetic maintenance of pure quality maize seeds were and still remains one of the viable forms enhancing increased maize productivity within the confines of smallholder farmers under a commercialized seed system. Strengthening the venture with the MSIP platform helped to plan and implement with guided protocol for quality control assurance. 80% of smallholder farmer are in position to sustainably produce pure quality maize seeds and it's a stimuli for increase food production in Karamoja. Intensive seed system development is anticipated to stimulate more demand hence enhancing commercialization and ultimately leading to improved livelihood. However emphasis needs to be put on seed legislation for implementation under provided seed act.

Acknowledgement

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EFFECT OF GIBERELLIC ACID ON BERRY QUALITY OF SEEDLESS TABLE GRAPES

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Abstract

In Lebanon, it has been shown that about 50% of table grapes productions are not exportable or marketable due to small berry size. Giberellic acid (GA3) is known as plant growth regulator used in vineyard in order to enhance berry quality, especially berry size. Therefore, investigating the effect of GA3 on berry quality of the seedless ARRA 15 table grapes variety was the objective of the study. The applied treatments were: T1: thinning treatment (1.5ppm GA3), T2: thinning treatment (1.5ppm GA3) + sizing treatment (20ppm GA3), T3: thinning treatment (1.5ppm GA3) + sizing treatment (40ppm GA3), T4: thinning treatment (1.5ppm GA3) + sizing treatment (40/20 ppm GA3), T5: thinning treatment (1.5ppm GA3) + sizing treatment (20/40/60ppm GA3), T6: thinning treatment (1.5ppm GA3) + sizing treatment (20ppm GA3 + 2ppm Synthetic Cytokinin), T7: control (without GA3 or Synthetic Cytokinin application). During growth cycle, the highest berry diameter and weight were obtained in T5 (19 mm, 5.3g), while at harvest berry diameter was the highest in T5 and T6 (≈6.5g). T6 gave the crispiest aspects with the lowest brix index (11.16°) while it reached 14° in T1 and in the control. T6 delayed the date of reaching brix 16° by 10 days compared to control. Finally, T6 was the best way to reach a better quality. But, in Lebanese conditions it is recommended to apply T5 because Synthetic Cytokinin should be used at low concentration to be effective without side effect on clusters which could be hard to some growers to regulate it and this products are almost unavailable in Lebanese market.

Keywords: *ARRA 15, Lebanon, Synthetic Cytokinin*

Introduction

The adaptation to the requirements of the markets is one of the basic characteristics of modern table grape production aiming to improve grape quality such as berry size. In, addition, the seedlessness represent an important attribute of the grape berry quality while seedless cultivars are characterized with small grains and required management for improvement of their size (Dimovska et al., 2014). The application of plant growth regulators represents a way to face this problem (Nampila, 2010). Giberellic acid (GA3) is one of plant growth regulators used extensively to increase berry size of seedless cultivars (Korkutal et al., 2007; Dimovska et al., 2011) as it stimulates parthenocarpic fruit development of grapes (Casanova *et al.*, 2009). Exogenous application of GA3 promotes cell division, stimulates the earlier flowering, increases the size and yield of fruits, and induce seedlessness in the seedless cultivars (Khan, 2009; Dimovska et al., 2006). It has been reported the GA3 was effective in improving berry size of table grapes such as Black Corinth, Red Malaga, and Thompson Seedless 51 (Christodolou et al. 1968, Singh et al. 1978, Weaver 1957, Weaver and McCune 1959).

In fact, Lebanese farmers are seeking to meet market demand for new seedless table grapes variety while it has been shown that about 50% of table grapes productions are not exportable or marketable in Lebanon due to small berry size. ARRA 15 variety is a new seedless variety introduced in Lebanon but it is still under experiment and not used for commercial aim yet. Without hormonal addition berry size did not required the need of local and exported market.

Consequently, the objective of this study was to investigate the effect of exogenous Giberellic acid on berry size, weight and Brix index of the seedless ARRA 15 table grapes variety.

Materials and methods

The variety used was ARRA 15 seedless table grapes and it was planted in normal conditions required for vine development. Seedlings were grafted on rootstock 1103P. Vines were trained by pergolas system, irrigated by drip irrigation and grown with a distance of 3 x 3 m. Several agricultural practices were also adopted (Pruning, thinning, fertilization…).

Seven treatments were applied: T1: thinning treatment (1.5ppm Giberllic acid (GA3)),T2: thinning treatment (1.5ppm GA3) + sizing treatment (20ppm GA3), T3: thinning treatment (1.5ppm GA3) + sizing treatment (40ppm GA3), T4: thinning treatment (1.5ppm GA3) + sizing treatment (40/20 ppm GA3), T5: thinning treatment (1.5ppm GA3) + sizing treatment (20/40/60ppm GA3), T6: thinning treatment (1.5ppm GA3) + sizing treatment (20ppm GA3 + 2ppm Synthetic Cytokinin (S.C)), T7: control (without GA3 or Synthetic Cytokinin application). The rates were chosen according to several references (Domingosa et al., 2016; Mohsen 2015; Abu Zahra, 2013 and Strydom 2013). The first application was at 120% bloom and the second one when the berry diameter reached 4-7mm. For T4, T5 and T6 the third application was done one week after the second application. The fourth application in T5 was also done one week after the third application. Spraying bunches was adopted for thinning application while bunch dipping was adopted for all other applications.

The experimental plot contains 126 vines of ARRA 15. The Complete Randomized Bloc Design was adopted with three blocks (repetitions). Seven treatments were applied in each block and each one is consisted on 6 plants. Preventive steps were also adopted to prevent contaminations between treatments.

In each block 6 berries were randomly collected from 3 clusters per treatment. Berry diameter and weight were measured during growth cycle and at harvest. Brix index was studied at 21th July and at 30th August using refractometer.

Results and Discussion

Berry diameter during growth cycle

Table 1 below showed that T5 increased berry diameter by around 5% compared to T2, T3, T4 and T6 and by around 10% and 15% compared to T1 and T7 (control) respectively.

Table 1: berry diameter during growth cycle

Treatments	Berry diameter during growth cycle (mm)
T1 (thinnhig)	17.0 c
T2 (20 ppm GA3)	18.0 bc
T3 (40 ppm GA3)	18.0 ab
T4 (40/20 ppm GA3)	18.0 bc
T5 (20/40/60 ppm GA3)	19.0 a
T6 (20 ppm GA3 + 2 ppm S.C)	18.5 ab
T7 (control)	16.0 d

In fact, during growth cycle, the application of GA3-S.C, single or double GA3 and thinning treatment increased slightly berry diameter while the triple application of GA3 as in T5 achieved a constant stimulation of cell size growth by a residual activity of GA3 resulting in highest berry diameter.

Berry diameter at harvest

Berry diameter results were changed at harvest. The highest berry diameter was recorded in T5 and T6 where it was higher by around 10% compared to all other treatments (Table 2).

Table 2: Berry diameter at harvest

Treatments	Berry diameter at harvest (mm)
T1 (thinnhig)	18.1 b
T2 (20 ppm GA3)	18.3 b
T3 (40 ppm GA3)	18.2 b
T4 (40/20 ppm GA3)	18.3 b
T5 (20/40/60 ppm GA3)	20.4 a
T6 (20 ppm GA3 + 2 ppm S.C)	20.2 a
T7 (control)	17.8 b

The only difference between T6 and T2 was the presence of S.C. in the first one. Consequently, the results observed in T6 could be due to the synergetic effects of GA3 and S.C. Those results confirm the finding of Dokoozlian (1995), Strydom (2013) and Mohsen (2015) on 'Sugraone' table grapes.

Berry weight

During growth cycle, T5 recorded the highest berry weight where it increased this parameter by around 39% and 19% compared to the control and to all other treatments respectively (Table 3).

Table 3: Berry weight during growth cycle

Treatments	Berry weight during growth cycle (g)
T1 (thinnhig)	3.8 bc
T2 (20 ppm GA3)	4.2 abc
T3 (40 ppm GA3)	4.4 ab
T4 (40/20 ppm GA3)	4.4 ab
T5 (20/40/60 ppm GA3)	5.3 a
T6 (20 ppm GA3 + 2 ppm S.C)	4.6 ab
T7 (control)	3.2 c

At harvest, as berry diameter, berry weight was the highest in both treatments T5 and T6 (around 6.5 g) while all other treatments were almost similar (around 4.5 g). The results obtained could be due to the fact that the application of GA3 in seedless table grapes compensates the low gibberellins berry concentrations (Pérez *et al*., 2000), which is correlated with seed number (Lavee and Nir, 1986). In fact, it is well established that GA3 promote cell expansion (Ubeda-Tomás *et al.*, 2008) and increase cell size by taking place to a higher accumulation of sugars and water (Casanova et al., 2009) leading to increase berry size and weight. In addition, Abu-Zahra (2010) had obtained an increase in berry weight after treating seedless table grapes with GA3 and S.C products.

Regarding berry diameter and evolution in time, it was remarkable that T5 and T6 had a similar constant growth during the growth stage until harvest. In addition, although T2, T3 and T4 had a higher berry size than T7, all had an immediate acceleration in growth but berries almost never grew at harvest. Moreover, T1 and T7 had a similar constant growth

during the growth cycle until harvest with superiority in berry diameter in T1 and this due to the fact that berry thinning means less berry number per cluster with a bigger size.
Even though the last T5 application was done 50 days before harvest and taking into consideration that the residual activity of GA3 after single application lasts up to 6 days, the increased dose of GA3 application (20-40-60 ppm) have managed to constantly stimulate cell size growth; hence, increasing the berry size and eventually it ranked the highest.

Brix index on July 21
T6 had the lowest Brix index (11.2°), while T1 and T7 recorded the highest one (14°) (Table 4).
Those results could be due to the fact that in general S.C delayed harvest date while GA3 delayed fruit maturity reducing berry color (Dokoozlian, 1998). On the other hand, the natural growth and therefore natural accumulation of sugar content led to the highest index brix recorded in T1 and T7. Actually, those results confirmed the findings of Reynolds et al. (1992) where they studied the effect of GA3 and S.C on harvest delay of Seedless table grapes.

Table 4: Brix index on July 21

Treatments	Brix index on July 21 (Brix°)
T1 (thinnhig)	14 a
T2 (20 ppm GA3)	12.7 b
T3 (40 ppm GA3)	13 ab
T4 (40/20 ppm GA3)	13 ab
T5 (20/40/60 ppm GA3)	13 ab
T6 (20 ppm GA3 + 2 ppm S.C)	11.2 c
T7 (control)	14 a

Brix 16° achievement in number of days

Table 5: Brix 16° achievement in number of days

Treatments	Days
T1 (thinnhig)	241 a
T2 (20 ppm GA3)	246 b
T3 (40 ppm GA3)	247 ab
T4 (40/20 ppm GA3)	246 ab
T5 (20/40/60 ppm GA3)	246 ab
T6 (20 ppm GA3 + 2 ppm S.C)	249 c
T7 (control)	239 a

The table 5 above showed that T6 had the highest number of days needed to reach Brix 16° where it need 10, 2, 8 and 4 days extra than the control, T3, T1 and the other treatments respectively. Those results also agree the findings of Reynolds et al. (1992).
Finally, berries of T6 have the crispiest aspects. As for control grapes, they had limp and a kind of dry aspect which confirm the results of Ben-Arie et al. (1997) and Dokoozlian (1998). The last author affirmed that in some cases S.C showed to increase firmness and reduce juiciness of fruits. In fact, the juiciness was not experimented in this study while no reduction in juiciness was remarked.

Conclusion

In conclusion, triple treatments of GA3 and the mixture of GA3-S.C where the most efficient was to reach the objective of this study where they almost have a similar effect on all parameters. On the other hand, the crispiest aspect provided by the mixture is mostly demand in markets. Consequently, it is recommended to use T6 if S.C is easily accessible which not the case in Lebanon. Therefore in Lebanon T5 is recommended especially for beginners or ignorant growers because S.C should be use carefully at low concentration to prevent cluster damage.

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APPLICATION OF GIBBERELLIC ACID ON THREE SEEDLESS TABLE GRAPES GROWN UNDER GREENHOUSE OR IN OPEN-FIELD

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Abstract

Table grapes grown in Lebanese coast especially under greenhouse are faced by lack of enough chilling hours which affect negatively bud-break, some phenological stages and consequently yield. Few studies reported the beneficial effect of Gibberellic acid (GA) as plant growth regulator used in vineyard in order to regulate bud dormancy release in table grapes. Consequently, the objective of this study was to investigate the effect of gibberellic acid on budbreak, flowering and fruiting of three seedless table grapes varieties: ARRA 15, ARRA 18 and ARRA 19 grown under greenhouse and in open-field conditions in Lebanese coast. Therefore, Four treatments were the subject of this study: greenhouse/GA-, open-field/GA- (control), greenhouse/GA+ and open-field/GA+. Three parameters will be discussed in this paper: number of bursted bud per main shoot, number of flowers and number of clusters per vine. Giberellic acid did not have any effect in open-field conditions. However, under greenhouse, Giberellic acid induced full budburst compared to 35 % and 25 % in the control and the treatment greenhouse/GA- respectively. Number of flowers and clusters were higher in the treatment greenhouse/GA+ by around 67 and 80 % for ARRA 19 and ARRA 18 respectively compared to all other treatments. A full fruit set was observed in all treatments and regarding all varieties except on treated ARRA 15 under greenhouse where it was 54%. Thus, greenhouse cultivation created a suitable microclimate compared to open-field and could help Giberellic acid to be effective on resolving the disturbance of bud-break on vine in Lebanese conditions.

Keywords: *ARRA variety, bud-break, Dormex, Lebanon.*

Introduction

Cultivation of table grapes under greenhouse started arising Lebanese coast as experimental trials due to the need of local and exported markets for new seedless varieties and extra-seasonal products. In fact, this type of cultivation in the Lebanese coast was faced by the lack of enough chilling hours which affected negatively bud-break and consequently reduced yield. Recent study was conducted on the effect of Dormex application on the performance and productivity of three table grapes varieties grown under greenhouse and in open-field conditions in Lebanese coast (El Masri *et al.,* 2018). The results showed that the application of Dormex under greenhouse induced more uniform and full budburst, enhanced fruiting and flowering status and had a great effect on other parameters. However, this product was prohibited in many countries and recently in Lebanon. Therefore, finding an alternative was the main objective of this study. Actually, the alternative should be a plant growth regulator that could help vine to function normally. Several studies have reported an increased level of endogenous Giberllic acid (GA) in grapes, or an up-regulation of GA biosynthesis genes, following exposure of perennial endodormant buds to chilling, or fluctuations in the levels of GA during the natural bud endodormancy cycle (Zheng *et al.,* 2018). Larrouy *et al.* (2016) also reported that grapes require a cold period of several weeks to induce germination and the release from dormancy is associated with a quick increase in endogenous gibberellins. In

addition, the period of dormancy induction was accompanied by a gradual decrease in transcript levels of genes coding for GA biosynthetic enzymes, and the levels were lowest during deepest dormancy. Reduced GA biosynthesis capacity and the maintenance of stable GA inactivation ability during dormancy induction of grapevine mature buds correlate with the decrease in level of endogenous active GA1 following the period of dormancy induction, and its lower level during dormancy maintenance. Therefore, during the period of gradual dormancy release, expression levels of the GA biosynthesis genes generally increased (Zheng *et al.,* 2018). Few references indicated the role of exogenous GA on regulating bud dormancy in grapevine. In fact, both negative and positive effects of GA were reported on outgrowth of paradormant buds which are carried on actively growing shoots and are under the control of apical dominance (Zheng *et al.,* 2018) in various crops other than grapevines. For instance, in hybrid aspen, decreased bioactive GA levels in the apex and young tissues resulted in loss of apical dominance and increased branching of lateral buds (Mauriat *et al.*, 2011). GA has also been reported to induce lateral bud outgrowth in papaya. In addition, GA biosynthesis inhibitors inhibited cytokinin (CK)-mediated outgrowth of axillary buds of *Jatropha curcas*, whereas GA promoted it (Ni *et al.*, 2015). Consequently, the current study was conducted in order to investigate the effect of giberellic acid as Dormex alternative on bud, flower and fruits of three seedless varieties grown under greenhouse and in open-field conditions.

Materials and Methods

The experimental site was located at a village in Byblos (Jubeil) in the Lebanese coast. The lowest temperature recorded there was 5 degree and detected only in early January. Fluctuating temperature during the year and between days and nights were also detected there. Three seedless table grapes varieties: ARRA 15, ARRA 18 and ARRA 19 were planted under greenhouse and in open-field conditions. Each cultivation system occupied 0.3 hectares where each variety occupied 0.1 hectares. The seedlings were grafted on rootstock 1103P and cultivated with a space of 3 meters between plants as well as 3 meters between rows. The 4 years old plants were the target of the current study, were trained with pergolas system and were irrigated with drip irrigation. The work started in December by pruning the vine leaving 6 buds per main shoots (per cane). After Several trials, Giberellic acid (GA) was applied as follow: thinning treatment (1.5ppm GA) + sizing treatment (20/40/60ppm GA). At 120% bloom the canopy was sprayed with a 1.5ppm of GA as first application. The Second one was adopted when the berry diameter reached 4-7mm (20ppm). The last 2 applications were applied with an interval of 1 week. Bunch dipping for 2 seconds was adopted in the last three applications. Four treatments were the subject of this study: greenhouse/GA-, open-field/GA- (control), greenhouse/GA+ and open-field/GA+. And each sub-treatment consisted on 9 plant of each variety grown under the same experimental conditions. Statistical analysis was done using Spss program where ANOVA was applied. The three parameters discussed in this paper were taken by daily observation: number of bursted buds per main shoot, number of flowers per vine and number of fruits per vine.

Results and Discussion

Table 1 below showed that the non-interactive effects of factors cultivation system and Giberellic acid and the interactive effects of those factors had significant differences on all parameters. On the other hand, the non- interactive effects of the factor ARRA varieties and the interactive effects between this factor and the others had only significant differences on number of flowers and fruits per vine. It should be noted that Pvalue ≤ 0.05 was adopted for analysis of results.

Table 1: LSD table (parameters: yield, date of fruiting and date of harvest)

	Number of bursted/main shoot	Number of flowers/vine	Number of fruits/vine
ARRA varieties	.536	.000	.000
Cultivation system	.000	.000	.000
Giberellic acid (GA)	.000	.000	.000
ARRA*System	.298	.000	.005
System*GA	.000	.000	.000
ARRA*GA	.975	.000	.001
ARRA*System*GA	.975	.000	.001

In fact, GA did not have any effect in open-field conditions regarding the studied parameters. In addition number of bursted buds was almost similar among all varieties regarding all treatments (Figure 1). Under greenhouse, GA increased significantly the average of bursted buds from around 1.5 buds per main shoot to reach 6 buds which represent a full budburst. The number of bursted buds in the treatment greenhouse/GA- was slightly lower than in the control, this fact could be due to the higher air temperature under greenhouse which reduced chilling hours and affected negatively bud-break.

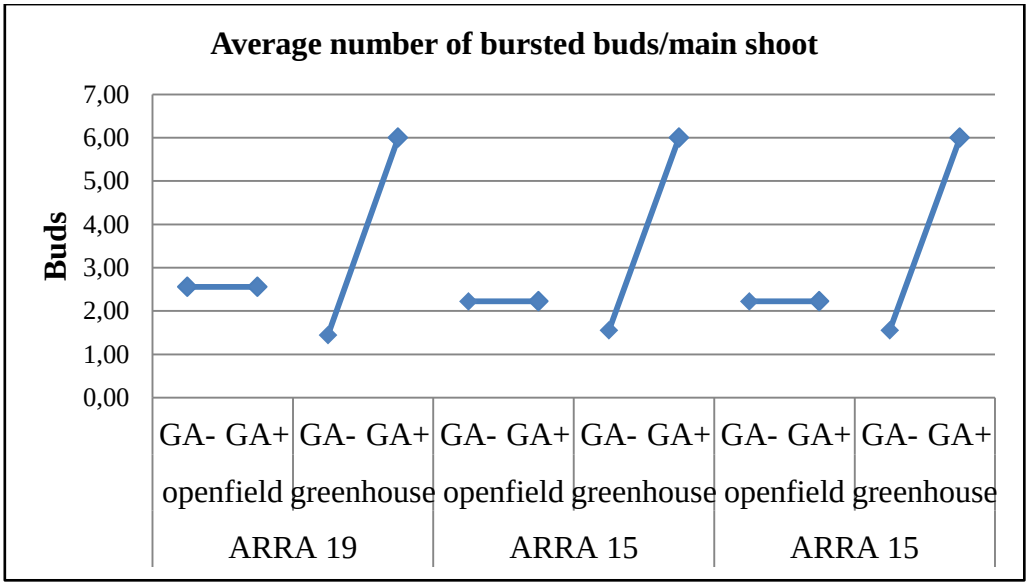

Figure 1: Average number of bursted buds/main shoot in each treatment.

When comparing all treatments and regarding all varieties, a full fruit set was observed. The only exception was observed for ARRA 15 where average number of flowers per vine was around 11 flowers and number of fruit was around 6 fruits per vine (54% fruit set) in the treatment greenhouse/GA+ (Figure 2 and Figure 3). This could be due to varietal effect were Giberellic acid effects is variety dependent (Khan, 2009; Dimovska *et al.*, 2006). In addition, this result confirmed the findings of Dokoozlian 2001. Moreover, number of flowers and fruits was almost similar (around 2 flowers or fruits/vine) in the three treatment open-field/GA-, open-field/GA+ and greenhouse/GA-. GA increased significantly those 2 parameters under greenhouse regarding all varieties reaching around 6 and 9 flowers or fruits per vine for ARRA 19 and ARRA 18 respectively.

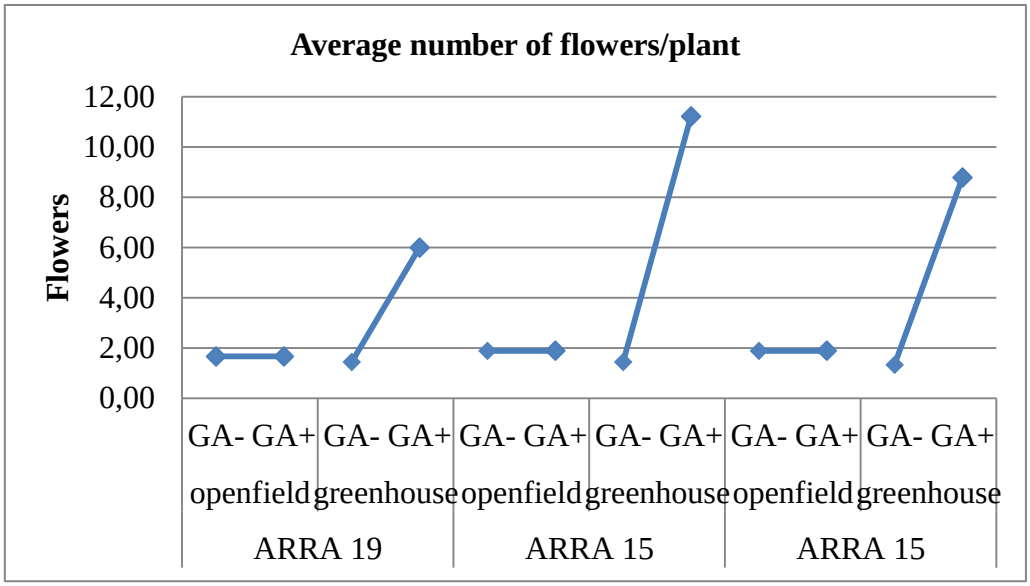

Figure 2: Average number of flowers per vine in each treatment.

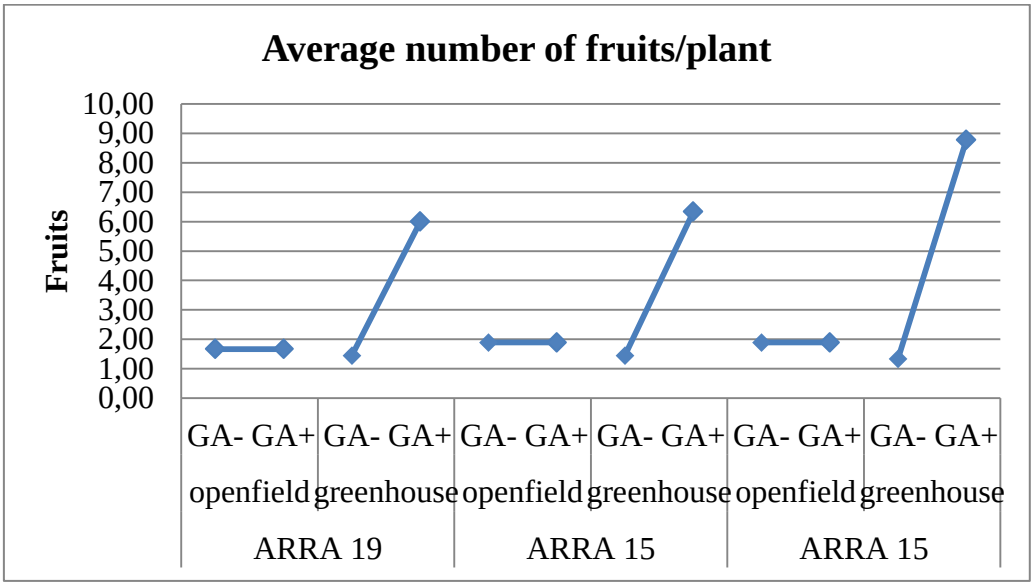

Figure 3: Average number of fruit per vine in each treatment.

In fact, it was mentioned by EL Masri *et al*., 2018 that greenhouse cultivation created more suitable microclimate with more stable conditions which could be a good reason to explain the beneficial effect of GA under greenhouse than outdoor where the climate is known by the fluctuating temperature especially between days and nights. On the other hand, those plant were in the last year treated by Dormex (3.5% Hydrogen Cyanamid) which also enhanced budburst, flowering and fruiting (El Masri *et al.,* 2018) and could be the reason behind the success of Giberellic acid effects where Dormex help vines to function normally as if the plant did not suffered from lack of enough chilling hours (El Masri *et al.,* 2018). Consequently, it is recommended to retry this experiment for more than one year to known the reality behind those behaviors where in fact treatment with GA improved embryo germination only when it was used in combination with sub-optimal cold treatments (Larrouy *et al.*, 2016).

Conclusion

Further studies should be carried out to ensure if Giberellic acid help vine to function normally in warm winter region as Dormex do. In addition, it is recommended to conducted studies that help knowing if Dormex had a residual effect on vine for several seasons.

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COMPARING THE PERFORMANCE OF TWO PASSION FRUIT (PASSIFLORA EDULIS) CULTIVARS UNDER LEBANESE CLIMATE CONDITIONS

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Abstract

Passion fruit is a woody perennial vine that bears a delicious fruit which occurs in purple and yellow colors. Although in Lebanon passion fruit has not yet reached commercial volumes, however there is an increasing market demand for such fruit. In the current study, two cultivars of the purple passion fruit (*Passiflora edulis*) known as Perfecta and Black Knight were grown in Ghazir, Lebanon that is characterized by an altitude of 539 meters above sea level; latitude of 34°1'6 N and longitude of 35°39'58 E. This site was selected for being a suitable site that agrees with the minimum climatic requirements of purple passion fruit. The objective was to compare the behavior of both cultivars under local soil and climate conditions. Results indicated that the Perfecta was more adapted to clay soil and prevailing weather conditions than Black Knight. It showed a better vegetative growth that was expressed through greater averages of plant height (by 2.1 cm), leaf number (1 leaf), leaf nodes (by 11 nodes) and a higher accumulation of dry matter in leaves, shoots and roots. However, there was no significant difference at the level of leaf area, Specific Leaf Area (SLA), and Leaf Area Ratio (LAR) among both cultivars. Moreover, Perfecta plants flowered earlier and produced more flowers compared to Black Knight (94 and 4 flowers, respectively). Perfecta plants fruited while those of Black knight did not. Consequently, Perfecta cultivar showing a better adaptation is recommended for Lebanese growers seeking to diversify their income by growing alternative crops.

Keywords: *Passion fruit, adaptation, soil, climate.*

Introduction

Passion fruit belongs to the genus *Passiflora* (Pongpan *et al*., 2007). There are many varieties of passion fruit with clearly distinctive characteristics; however, the most-known two varieties are the bright yellow flavicarpa variety, also known as golden passion fruit (*P. edulis var.flavicarpa)* and the dark purple edulis variety (*P.edulis var.edulis*) (CRFG, 1996). Recent hybrids and cultivars of passion fruit are *P. edulis* 'Perfecta', and *P. edulis* 'Black Knight'. *P. edulis* 'Perfecta' offers large purple fruits that are eaten fresh (Chloé, 2014). Passion fruit is inhabitant of the tropical and semi-tropical zones of North, Central, and South America (Pongpan *et al*., 2007) where the climate is hot and humid (Joy, 2010) and could grows in warm to cool climates within altitudes ranging from 1200-2000 m above sea level (Mbora *et al*., 2008). The temperature range between 21 and 25°C is considered as the most favorable for the growth of the plant, being best between 23 and 25°C, but passion fruit is being successfully cultivated in temperatures between 18°C and 35°C (Joy, 2010). Cool temperatures are favorable for flower initiation and fruit set (18-23°C), while relatively high temperatures seem necessary for promoting juice production (23-33°C) and improvement in quality (Sema and Maiti, 2006). They are sensitive to severe frost (especially the purple granadilla). Additionally, Passion fruit vines are grown on many soil types but light to heavy sandy loams, of medium texture and at least 600 mm deep are most suitable. PH should optimally be from 5.5-6.5. Passion fruits are sensitive to excessively wet soil conditions, and

it is better to avoid clay soils (NDA, 1999). Such plant requires frequent application of balanced fertilizer during the growing season (Cooper *et al.*, 2011). In Lebanon, passion fruit has not yet reached commercial volumes. A limited number of trees are planted in private forests and gardens. Since the crop is still new, farmers are not familiar with its climatic requirements, as well as it cultivation techniques and the cultivars which are suitable for local climate conditions. Therefore, a research study was carried out in the field of the Ghazir research center, in the year 2015, which sought the comparison of the vegetative and reproductive development of two cultivars of purple passion fruit: "Perfecta" and "Black Knight" under the climate conditions of Ghazir, Mount Lebanon the assumption which of the purple passion fruit cultivars is the mostly adapted.

Materials and methods

Experiment

The experiment was conducted in Ghazir is a region located in Mount Lebanon mainly in Kesrouane with an altitude of 539 m above sea level; latitude of 34°1'6 N and longitude of 35°39'58 E. It was considered as a suitable site that agrees with the minimum climatic requirements of purple passion fruit. In fact, calculations has shown that in July the average temperature was 22.7 °C, beyond this month the temperature fluctuated between 24.2 °C - 20.06 °C and decreased to 15.2 °C in November. In addition, the average relative humidity was 72.5% in July, it fluctuated between 68.6 - 67.8% during August and September and was the highest during October 75%. The relative humidity became 69.3% in November. Precipitation was of 2.04 mm/day in October and then increased to 4.84 mm/day in November. Moreover, soil analysis has shown a clay soil that contained moderate amounts of phosphorus; it was poor in nitrogen, iron, and organic matter and rich in magnesium and potassium.

The two wire fence trellis and four shoots training methods were adopted.

Seedlings of 1.5 months old at the stage of four leaves were cultivated after being hardened in greenhouse with a distance of 170 cm between plants. Seedlings were irrigated twice per day and every 15 days, 6 g of the fertilizer N-P-K (12-11-18), 50 mL of iron (33.3g/L), and 50 mL of ammonium nitrate (166.6 g/L) were provided to each plant.

Data recording

Plant height, leaf number, number of leaf nodes, flower number, and fruit number were continuously recorded starting one week after the date of transplantation on the total number of plantsA destructive sampling was done where plant height, leaf area, fresh and dry weights of leaves, shoots, and roots were assessed. The Specific Leaf Area (SLA) and the Leaf Area Ratio (LAR) were calculated using the following formulas: SLA = LA/DML ($cm^2.g^{-1}$) and LAR = LA/DMP ($cm^2.g^{-1}$). LA is the total leaf area, DML is the total dry mass of leaves and DMP is the total dry mass of plant.

Statistical analysis

Two types of statistical analysis tests were adopted: the Independent-samples t test and the repeated measures ANOVA test. $P \leq 0.05$ was adopted for the statistical tests.

Results and discussion

Perfecta plants grew faster and reached an average height of 23.2 cm in October, while Black Knight reached an average height of 12.6 cm in the same month. This difference in growth behavior of plants was observed until the end of the experiment. Leaf nodes that produce both a flower and a vegetative lateral meristem (Chayut *et al.*, 2014) were observed starting from mid-September on plants of both cultivars simultaneously. In mid-September, Perfecta plants recorded a total of 24 leaf nodes while plants of Black Knight recorded a total value of 13 leaf nodes. Plants of the cultivar Perfecta produced the highest number of leaf nodes with the

quickest ramification where 129 leaf nodes in late-October was recorded compared to 46 leaf nodes in Black Knight plants. In July, leaf production by both cultivars was almost; it was about 6 leaves for Perfecta plants and 5 leaves for Black Knight plants. However, in the following months the average number of leaves started to differ; in late-September 40 leaves were registered for the first cultivar while 16 leaves were recorded as average for the second one. This tendency continued in the following months where leaf number of the first cultivar exceeded that of the second cultivar (63 leaves and 22 leaves respectively). Flowering was observed first on Perfecta plants which produced more flowers than those of Black Knight that started to flower later; on the 29th of September, the total number of flowers for the first cultivar was 42 while that of the second was only 1 flower. The total number of flowers for Perfecta cultivar increased largely where it reached 94 flowers in October while the total number of flowers of Black Knight cultivar reached only 4 flowers in the same month. In general, successful flower development of passion fruit requires certain environmental conditions; photoperiod influences flower induction and long days are necessary for intact flower development (Chayut *et al.*, 2014). In addition, the cold weather is not conducive to good flower production and fruit set and high levels of moisture can destroy the pollen (McCarthy and Dick, 1995). The average air temperature that was 24.1 °C in September in Ghazir decreased to reach an average of 20.1 °C in October. In addition, in September and October, days were becoming shorter. On the 20th of October, the total number of flowers recorded on Perfecta plants decreased to a total of 21 flowers while the one recorded Black knight plants which were 4 on the 13th of October reached only 7 flowers on the 20th of the same month. Thus, weather conditions at the field could have induced the drop in the total number of flowers on plants of the Perfecta cultivar. On the other hand, the lower flower number observed on plants of Black Knight may be due to the effect of photoperiod on the flower induction. In general, it seemed that flower induction of Perfecta cultivar was less affected by the photoperiod factor and the effect of temperature variation than Black Knight plants. An early fruiting was observed on plants of Perfecta (2 months after transplanting) in comparison to Dirou (2004) findings that had recorded fruit production at six months of age and Mbora *et al.* (2008) who indicated that plants of purple fruit bear fruits within 7 to 9 months after transplanting. In October, Perfecta cultivar started to give fruits; on the contrary, no fruits were produced by plants of the Black Knight during the whole duration of the experiment. In fact, the average fresh weight recorded for fruits of Perfecta was of 46.5 g while the average dry weight was of 6.64 g where the fresh weight was almost similar to Joy (2010) indications. Passion fruit plant yield is related to the growth of the branches because flowering starts in the axils of these parts of the plant (Gomes *et al.*, 2006). As it was observed plants of the cultivar Perfecta grew longer and prior to those of the cultivar Black Knight and produced a higher number of leaf nodes in the same period of time. Therefore, a general tendency for an earlier and heavier fruit production for plants of the cultivar Perfecta could be due to earlier and stronger vegetative development in comparison with plants of the cultivar Black Knight. Although both cultivars of passion fruit were grown in same soil conditions, the root system of Perfecta plants seemed heavier, thicker and more ramified as compared to the one of Black Knight plants. According to NDA (1999), growing granadillas requires the avoidance of clay soils, and soil test revealed that it was a clay one; therefore, the lower development of roots of Black Knight plants indicated that this cultivar may have a low tolerance to clay soils. On the contrary, Perfecta plants showed a higher tolerance to clay soils since root development was less affected, thus a greater root biomass was formed which was translated by a high fresh weight of roots (Figure 1a). In addition, plants of both cultivars were supplied by the same type and amounts of fertilizers that were provided simultaneously to all plants, however, they developed different fresh weight of roots, with a superiority for Perfecta cultivar which could explained by a higher absorption of nutrients from soil. Black

Knight cultivar may have not been able to absorb same amounts of nutrients from soil and consequently they developed a smaller, less ramified, and a lighter root system compared to the one developed by plants of the other cultivar. Dry matter accumulation in roots was significantly higher in Perfecta cultivars and this assumed the difference in nutrient absorption among plants of both cultivars and could explain the fact that Perfecta plants grew longer than Black Knight (Figure 1b). Furthermore, dry matter was more allocated to the upper parts of plants rather than to the root system.

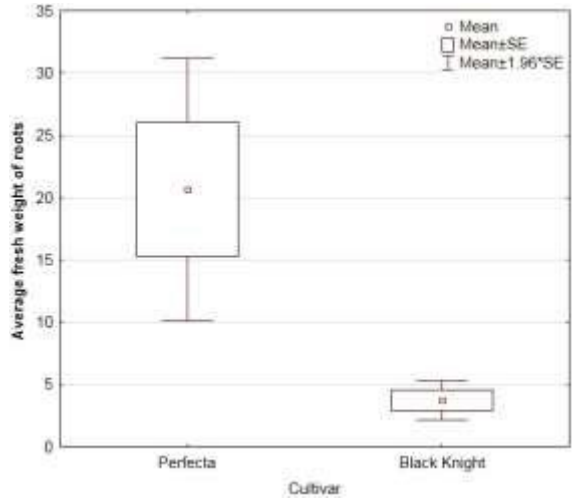

Figure 1a: Mean fresh weight (g) of roots.

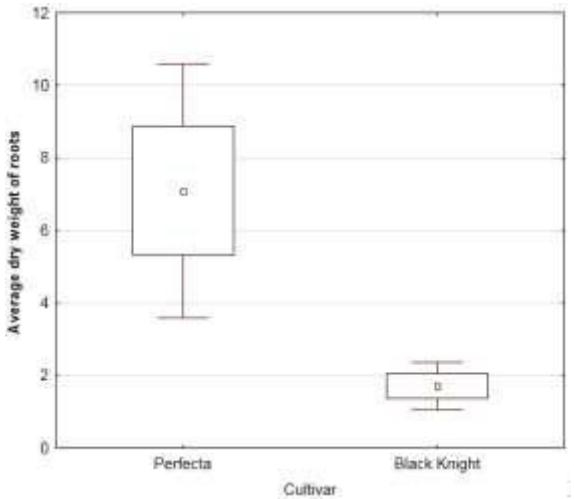

Figure 1b: Mean dry weight (g) of roots.

The higher nutrient absorption and translocation to the upper parts of plants that was observed on Perfecta cultivar resulted in longer plants and led to the development of a higher number of leaf nodes in Perfecta plant compared to Black Knight. Although the fresh biomass of shoots produced didn't significantly differ among plants, the high absorption of nutrients by plants of Perfecta cultivar has led to the accumulation of higher amounts of dry matter in their shoots compared to Black Knight plants (Figure 2a, b). Perfecta produced a significantly higher number of leaves than that of plants of Black Knight. Consequently, plants of the first cultivar showed a higher level of adaptation to the soil and climatic conditions of the experimental field as compared to the level of adaptation of Black Knight plants.

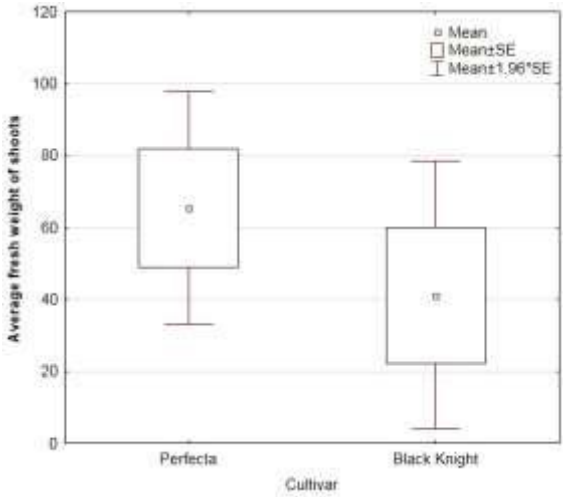

Figure 2a: Mean fresh weight (g) of shoots.

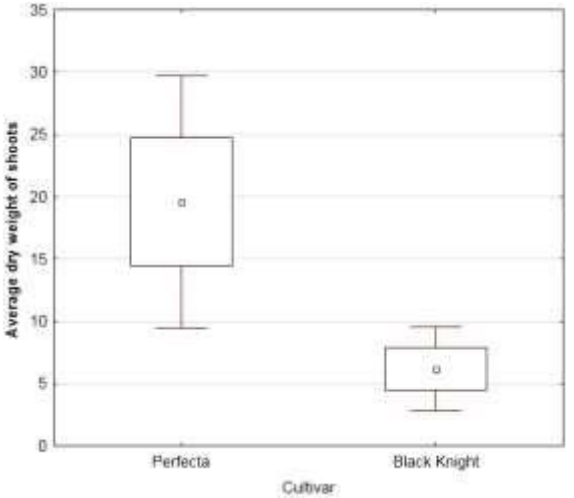

Figure 2b: Mean dry weight (g) of shoots.

During September and October, the weather at the orchard in Ghazir was cloudy sometimes and plants were shaded in a good part of the day. It seems that plants of both varieties of passion fruit had a similar behavior in compensating such deficiency in light by increasing the surface area of leaves (Gratani, 2014). The high number of leaves produced in Perfecta plants consequently the high photosynthetic activity may have allowed a high biomass accumulation in leaves that was sensed through a greater fresh weight and dry matter content in leaves compared to the one of Black Knight cultivar (Figure 3a,b). In fact, the difference of dry weight of leaves of both cultivars may have induced the difference of dry weight of roots (Bouteillé, 2011; Lizarazo, 2013). The high dry accumulation in leaves could also be due to the heavy fertilization that was adopted based on ammonium nitrate (46%) together with the complex fertilizer N-P-K (12-11-18) (Lizarazo *et al.*, 2013).

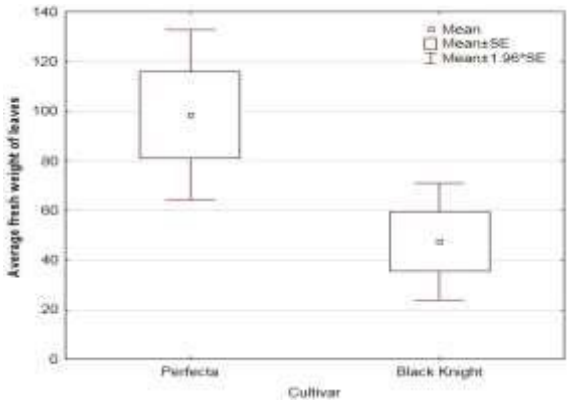

Figure 3a: Mean fresh weight (g) of leaves.

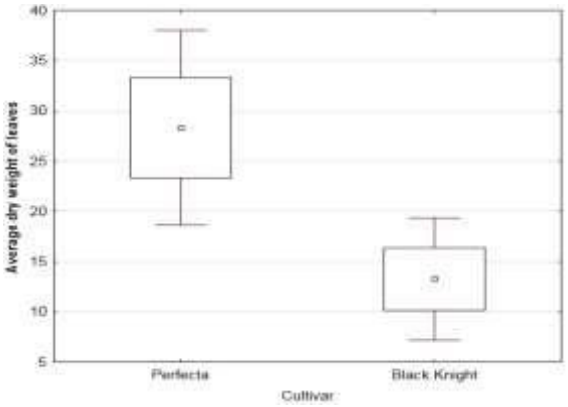

Figure 3b: Mean dry weight (g) of leaves.

Moreover, the high values of SLA (Figure 4a) and LAR (Figure 4b) could be attributed to the effect of cloudy weather t that has promoted an increase in leaf area and a decrease in dry matter accumulation in plants (Utsunomiya and Higuchi, 1996; Castro *et al.*, 2000). Regarding the cultivar Perfecta SLA (323.01cm^2/g) was higher than one of *Passiflora mucronata* (227cm^2/g) (Junior *et al.*, 2013). LAR values of both studied cultivars were higher by around 40%. However, the mean SLA and LAR values did not differ significantly among both cultivars.

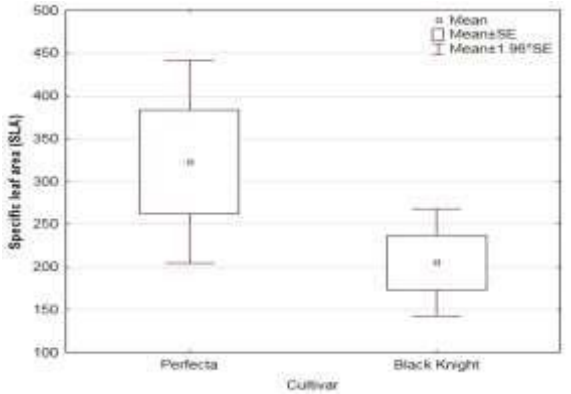

Figure 4a: Mean fresh weight (g) of leaves.

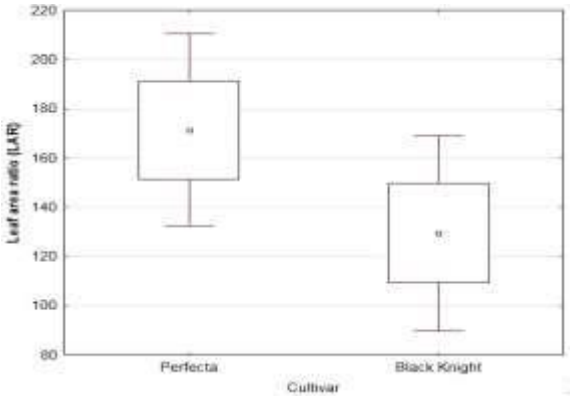

Figure 4b: Mean dry weight (g) of leaves.

Conclusion

Growing two cultivars of the purple passion fruit Perfecta and Black Knight and the comparison of their vegetative and reproductive development revealed a higher level of adaptation for the first cultivar to the climatic conditions of Ghazir. Therefore, the cultivar Perfecta can be considered as a promising cultivar at the agronomical level in Lebanon; it can be successfully cultivated.

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EFFECT OF GIBERELLIC ACID AND SYNTHETIC CYTOKININ ON CLUSTER QUALITY AND YIELD OF SEEDLESS TABLE GRAPES VARIETIES

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Abstract

In order to meet local and export markets demand, seedless table grapes should require the visual and standard demands of those markets. Then several studies reported the beneficial effect of Giberellic acid (GA3) and Synthetic Cytokinin (S.C) on table grapes quality and yield. Consequently, the current study was conducted to investigate the effect of those plant growth regulators on cluster quality and yield of ARRA 15 seedless table grape variety in Lebanese cost. Seven treatments were applied: T1: thinning treatment (1.5ppm GA3), T2: thinning treatment (1.5ppm GA3) + sizing treatment (20ppm GA3), T3: thinning treatment (1.5ppm GA3) + sizing treatment (40ppm GA3), T4: thinning treatment (1.5ppm GA3) + sizing treatment (40/20 ppm GA3), T5: thinning treatment (1.5ppm GA3) + sizing treatment (20/40/60ppm GA3), T6: thinning treatment (1.5ppm GA3) + sizing treatment (20ppm GA3 + 2ppm S.C), T7: control (without GA3 or S.C application). T5 and T6 increased yield by around 38% where the highest cluster length (≈26 cm), the most compact cluster and some rotten berries were recorded compared to control. Cluster weight was the highest in T5 (780g) and the lowest one was in T7 (306g). Harvest was delayed in T5 and T6 by 3 and 7 days respectively compared to control. The control clusters were sold at 0.7$/kg compared to 1$/kg for clusters of T5 and T6. Since S.C is hard to obtain in Lebanon, the application of T5 is preferred which also has an acceptable delay in harvest date.

Keywords: *ARRA 15, Lebanon, market prices.*

Introduction

Table grape is an important agriculture product covering 15% of total Lebanese fruit production (MoA, 2012). Nowadays international table grapes varieties are being introduced and planted by Lebanese commercial farmers (USAID, 2014). It has been always known that local production of table grapes faces problems concerning marketability and exportation norms. In addition, the main challenge in the local production is the consistency of selling the product at valuable prices. This can be ensured if the production complies with the visual demands and standards of local markets and visual and sanitary standards of the importing country. In fact, big and homogenous clusters represent some of those characteristics. Farmers are overhauling and planting new vines of new variety especially seedless table grapes (USAID 2014) where ARRA varieties are newly introduced in Lebanon but they are under experimental trials. To meet market demand for new seedless varieties, plant growth regulators or plant hormones during the development cycle should be used to enhance ARRA grape quality in Lebanese conditions, since Dokoozlian (1998) reported that usage of plant growth regulators could increase berry size leading to better quality and therefore to high yield and expressed as a bigger part of quality production. Cytokinin is presented in plant as Kinetin and other forms and produced where cell division occurs. As exogenous application, it is also promoting cell division. In addition, Gibberellic acid (GA3) is also found in the plant where it promotes cell division and internodes elongation and enhances seed germination and flowering (Harms and Oplinger 1990). According to Durner (2013) Gibberellin and Cytokinin

induce a normal fruit growth in seedless fruits where in general seedless fruits tend to be smaller than seeded. Normal fruit set in table grapes is excessive, leading to high number of berries per clusters and thus to a potentially smaller size of berries (Di Lorenzo et al., 2011). Domingosa et al. (2016) succeeded in reducing the number of berries in seedless table grapes varieties via GA3 by spraying it on the vine canopy. Additionally, Williams and Roper (1989) were found that GA3 application increases berry size in table grapes where GA3 is commonly used for this purpose (Dimovska et al., 2011). In order to obtain optimal results from applying GA3, time of application, concentration and the part of vine must be carefully studied (Mullins et al., 1992) since GA3 effect is variety dependent (Khan, 2009). Moreover, Synthetic Cytokinin increases also berry size and weight (Dokoozlian, 1998).
Consequently, the objective of the current study was to investigate the effect of Giberellic acid and Synthetic Cytokinin on the yield and quality improvement of ARRA 15 seedless table grapes for better marketing and export.

Materials and methods

4 years old vines of the seedless table grapes ARRA 15 variety were studied. 126 vines were planted with a distance of 3 meters between rows as well as 3 meters between plants. The drip irrigation was adopted and training as pergolas system was applied. The Seedlings were grafted on rootstock 1103P. Giberellic acid (GA3) and Synthetic Cytokinin (S.C) were used at several doses and rates resulting in seven treatments represented in the table 1 below:

Table 1: Dose and rate of treatments

Treatments	Thinning application		Sizing application	
	Dose	Time	Dose	Time
T1			-	-
T2			20 ppm of GA3	4-7 mm berry diameter
T3			40 ppm of GA3	4-7 mm berry diameter
T4			20 ppm of GA3	4-7 mm berry diameter
			40 ppm of GA3	One week after the first sizing application
T5	1.5 ppm of GA3	120% bloom	20 ppm of GA3	4-7 mm berry diameter
			40 ppm of GA3	One week after the first sizing application
			60 ppm of GA3	One week after the second sizing application
T6			20 ppm of GA3	4-7 mm berry diameter
			2 ppm of S.C.	One week after the first sizing application
T7	-	-	-	-

The rates were chosen according to several references (Domingosa et al., 2016; Mohsen 2015 and Abu Zahra, 2013). Thinning application was applied by spraying the canopy (Domingosa et al., 2016) while sizing treatments was applied by bunch dipping for 2 second. The Complete Randomized Bloc Design was adopted with three repetitions. The seven treatments were studied in each repetition where 6 plants were selected randomly from each treatment. To avoid the contamination between treatments, preventive methods were used. 3 clusters per treatment were also randomly selected. At harvest, cluster weight and length were measured and cluster compactness was evaluated following international compactness grade scale:

grade1: rigid, unable to move berries on cluster; grade 2: some movement of berries; grade3: able to manually separate berries …. Finally, yield was calculated by multiplying the number of clusters in vine by the mean cluster weight in the same vine.

Results and Discussion

Length of cluster was similar between T5 and T6 (26 cm) and it was the highest. No significant difference was recorded between T2, T3 and T4 where length of clusters were higher than in T1 and T7 which had the lowest length (Figure 1). Considering T5 and T6 results, many studies obtained almost the same results (Dimovska *et al.* 2011).

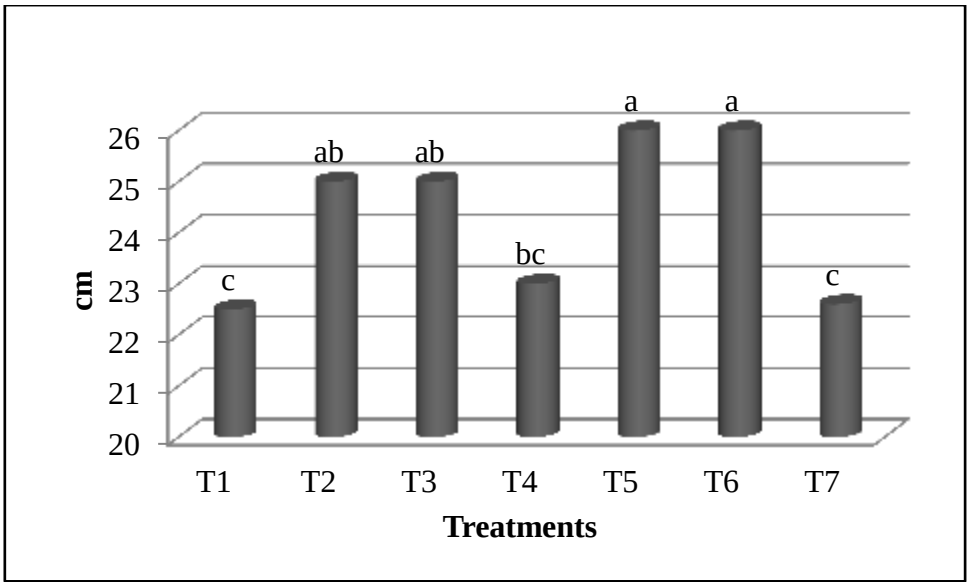

Figure 1: Cluster length at harvest

T5 increase significantly cluster weights compared to all other treatments (Figure 2). Whereas, cluster weight in the treatment T5 was higher by 46, 37, 34, 29, 12 and 60% than in the treatments T1, T2, T3, T4, T6 and T7 (control) respectively. In addition, Abu Zahra (2010) recorded an increase in cluster weight after applying those Plant growth regulators.

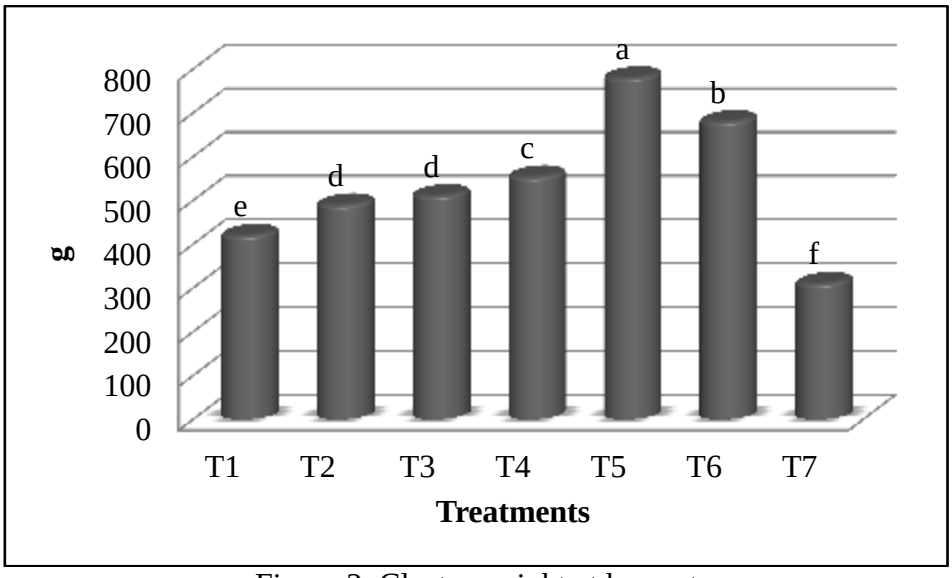

Figure 2: Cluster weight at harvest

The residual activity of GA3 after a single application lasts up to 6 days (Dokoozlian, 1998); thus for T5 the last 3 consecutive applications that were applied with an interval of one week between each one had led to achieve a constant stimulation of cell size growth resulting in a high cluster weight and length. In addition, the synergetic effect between GA3 and S.C. (Dokoozlian, 2001) could be the reason behind the increase of clusters length and weight in T6.

Moreover, the most compact clusters with grade 1 were obtained with T5 and T6, followed by T2, T3 and T4 with grade 2 and T1 and T7 with grade 3. Those findings were almost disagreeing with the results of Dokoozlian (1998). Although 1.5 ppm of GA3 was applied as thinning treatments, compact cluster were still obtained due to the sizing applications of plant growth regulators. Consequently, when certain sizing applications were applied, the dose of GA3 thinning application should be higher than 1.5 ppm to be effective avoiding the presence of rotten berries especially in the heavily compacted clusters as obtained in T5 and T6. Those results agreed with the findings of Domingos et al. (2016) while they reached the sufficient thinning doses. Furthermore, the yields obtained with T5 and T6 were significantly higher than all other treatments (Figure 3). They increased yield by around 15% compared to T1, T2 and T4 and by around 26% compared to T3 and the control. It is logical after obtaining the highest cluster weight in T5 and T6.

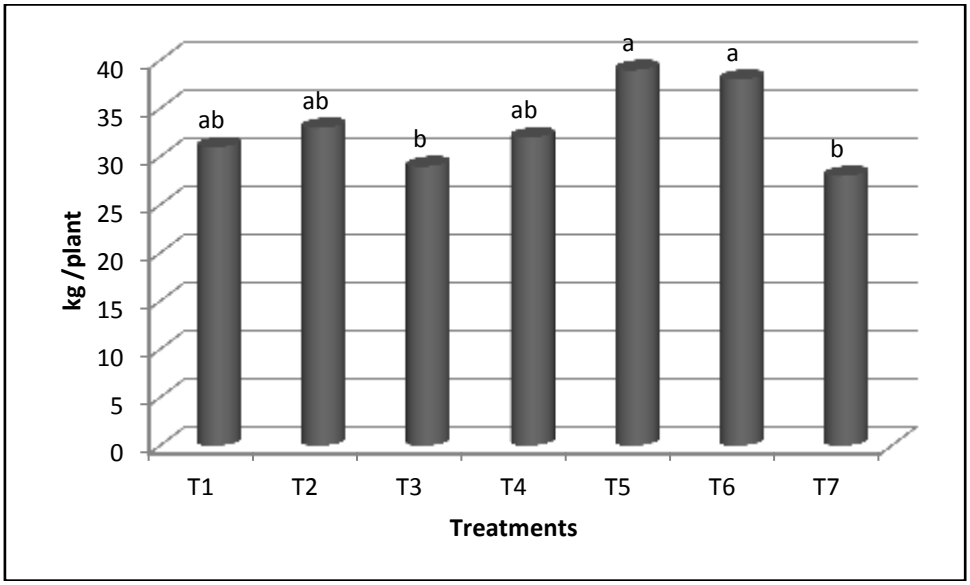

Figure 3: Total yield at harvest

As visual comparison, T7 developed small berries with poor uniformity overall as well as empty spaces within the cluster. On the other hand, T5 clusters exhibited big berry size with a good cluster symmetrical form. Additionally, T6 had decent oval berries with big dimensions as well as good berry uniformity; therefore the clusters had a big size with symmetrical shape. Finally, the clusters were sold by 0.7 and 1 $/kg for T7 (control) and T5 respectively. It should be noted that T5 delayed the date of harvest by 3 days compared to the control. Although T6 also delayed harvest by 1 week, its clusters prices were also 1 $/kg. Dokoozlian (1998) was found also that GA3 delayed fruit maturity and S.C delayed harvest date. A quick and mini feasibility study recorded that T6 cost less than T5 while it increased yield as much as T5 did. In fact, bunch dipping methods required a big amount of labors so a new technique should be tried in order to reduce the cost of production.

Conclusion

As conclusion, T5 and T6 increased yield and cluster length and weight with compact aspect, while the second one delayed harvest for 1 week which is not preferred by Lebanese farmers. It is recommended to apply T5 in Lebanese conditions since S.C. is less available in market. In addition, the dose of thinning should be adjusted to obtain a good berry quality.

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DETERMINATION OF YIELD AND BIOFUEL POTENTIAL OF SOME EARLY SWEET SORGHUM (*SORGHUM BICOLOR VAR. SACCHARATUM* (L.) MOHLENBR.) GENOTYPES

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Abstract

Sweet sorghum is used for the production of biofuels. The stalk and seed are used directly for biomass energy and lignocellulosic biofuel production. Sweet sorghum biomass is burned for getting energy. And also sweet sorghum biomass is burned by fast pyrolysis to produce syngas, bio-oil, and charcoal. One of the main products of sweet sorghum is its sweet juice which is obtained from stems of plant. The stalks are pressed and juice obtained. Sweet sorghum juice contains 15-20% sugar. High sugar content of juice allows to fermentation to make ethanol. The juice is fermented and distilled for the production of ethanol and biofuels. The residual fiber (bagasse) from sweet sorghum can be used to feed livestock or pelletized to burn for heat in buildings, produce electricity, paper, and cattle fodder. The objectives of the study were to determine yield and biofuel potential of some early sweet sorghum genotypes.
This research was carried out during the second crop growing season of 2016 in Sanliurfa, Turkey. The experiment was designed as complete randomized blocks design with four replicates, with 12 early sweet sorghum genotypes. In the study, flowering duration, plant height, stem yield, plant juice yield, water soluble dry matter ratio and ethanol yield properties were investigated. Significant differences were found between the genotypes for tested characteristics ($P \leq 0.01$). Flowering duration values ranged from 57.5 to 72.5 day, plant height from 252.25 to 340.75 cm, stem yield from 7440 to 13950 kg da^{-1}, plant juice yield from 3525 to 6225 L da^{-1}, water soluble dry matter ratio (brix) from %13.50 to %18.25 and ethanol yield from 214.3 to 464.5 L da^{-1}. It was determined that UNLY-hybrid-4, Blue Ribben, Rex and Colman sweet sorghum cultivars gave higher values than others in terms of stem yield, plant juice yield, water soluble dry matter ratio and ethanol yield.

Keywords: *Sweet sorghum, stem yield, plant juice yield, brix, ethanol.*

Introduction

Fossil energy resources are limited in the world and is depleting dramatically day by day to meet the ever-increasing energy demands globally. Also fossil fuels are causing environmental pollution. For this reason, it is necessary to focus on clean, environmentally friendly, renewable biofuels sources. Biofuels are sustainable and renewable source of energy derived from organic matter in the form of biomass. Biofuels can be derived from plant as well as animal biomass. Plants grown for biofuel purposes have potential to reduce the net greenhouse gas emissions. Crop plants are one of the best sources of renewable energy which can be used as feedstock for biofuel production. Sweet sorghum has a very good potential as an alternative feed stock for ethanol production.
Sorghum (*Sorghum bicolor* (L.) Moench) is a cereal grain. It is widely considered the most important cereal crop in the world. Sorghum ranks fifth among the most important cereal crops of the world, after wheat, rice, corn, and barley in both total area planted and production (Anonymous, 2018a). Sweet sorghum is well adapted to environmental conditions ranging from tropical to temperate conditions within 40°N and 40°S of the equator (Dogget, 1988). It tolerates drought and high-temperature stress better than many crops and has the capability of remaining dormant during the driest periods (Gnansounou et al. 2005). Its waxy leaves and

deep roots are better suited for dry and hot climates. It also has a high tolerance to salt, biotic and abiotic stresses (Bitzer, 2009). Sweet sorghum requires less fertilizer and water to produce significant biomass.

Sweet sorghum is a multipurpose crop providing food, feed, fiber and biofuel across a range of agroecosystems (Kilambya and Witwer, 2013). Sweet sorghum is also considered to be a significant crop for animal feeds used as animal feed (Maunder, 2018). The grain can be used as a gluten-free human food product. Sorghum is also used for human nutrition all over the world (Carter et al. 2018). Sorghum grain is higher in protein and lower in fat content. Grain sorghum is used for flours, porridges and side dishes, malted and distilled beverages, and specialty foods such as popped grain (Anonymous, 2018b). Sweet sorghum biomass is burned by fast pyrolysis to produce syngas, bio-oil, and charcoal. Also biomass can be used for lignocellulosic biofuel production.

The stalks of sweet sorghum has a high concentration of fermentable sugars. Sweet sorghum juice contains 15-20% sugar such as sucrose, fructose, and glucose which can easily be made into ethanol. The stalks are pressed, and the juice is fermented and distilled for the production of bioethanol. When the 10% bioethanol mixed to fuel, biofuels can be obtained. Sweet sorghum-based ethanol is sulfur-free and cleaner than molasses-based ethanol, when mixed with gasoline. The residual fiber (bagasse) from sweet sorghum can be used to feed livestock or pelletized to burn for heat in buildings, produce electricity, paper, and cattle fodder.

The objectives of the study were to determine yield and biofuel potential of some early sweet sorghum genotypes.

Materials and methods

This study was conducted as second crop conditions in 2016, Sanliurfa, Turkey. The experimental field is located in Harran Plain (36^o 42' N and 38^o 58' E) where the climate varies from arid to semi-arid. Table 1. provides the climatic data obtained from Sanliurfa City Meteorological Station. As can be seen from Table 1. that the weather is hot and dry in the months of June, July and August where maximum temperatures were all above 40 ^{o}C while the relative humidity was below 50%. Rainfall was very low from June to August in 2016.

Table 1. Monthly some climatic data during 2016 sweet sorghum growth period in Sanliurfa[†]

Meteorological observations	Months							
2016	May	June	July	August	September	October	November	December
Min. Temp. ^{0}C	9.30	13.1	15.4	16.2	14.0	9.2	1.7	- 0.8
Max. Temp.^{0}C	35.5	40.5	41.8	42.2	36.2	31.0	22.4	18.0
Av. Temp.^{0}C	22.3	28.2	31.9	31.2	26.8	20.2	12.7	7.5
Av. Humidity (%)	45.0	42.1	40.5	49.8	48.1	60.0	56.8	55.6
Rainfall (mm)	16.4	0	0	0	1.0	15.8	26.4	63.8

[†]Data collected from the Sanliurfa Meteorological Station (Anonymous, 2016).

Table 2. Some physical and chemical properties of research soil

Deep (cm)	Organic Matter (%)	Total Salt (%)	pH	$CaCo_3$ (%)	P_2O_5 (kg da^{-1})	K_2O (kg da^{-1})	N (%)	Texture (%)		
								Sand	Clay	Silt
0-15	0.66	0.098	7.65	48.17	3.6	99.3	0.06	2.42	15.12	82.46
15-30	0.68	0.086	7.64	46.88	3.9	93.8	0.06	5.50	8.41	86.08

The soil of the research field was clay, slightly alkaline, high in lime and very low in salt contents. Field capacity of the soil was 33.8% on dry basis, permanent wilting point was 22.6% and bulk density was 1.41 g cm^3. Some physical and chemical properties of research

soil were given in Table 2. Twelve early sweet sorghum genotypes (*Sorghum bicolor* (L.) Moench *ssp. saccharatum*) were used as crop material.

Land was ploughed and cultivated then prepared for planting with a single pass of a disk-harrow. The experiment was laid out in a randomize block design with four replications. Each plot area was 14 m^2 (5 m x 2.8 m) and consisted of four rows of 5 m in length. The plants were grown 70 cm apart between the rows with 15 cm spacing in each row. The seeds were sown in second part of June at a 50-60 mm depth. At sowing, 50 kg ha^{-1} of pure N, P and K, as a 15-15-15 composed fertilizer, was applied to each plot; this was followed by 50 kg ha^{-1} of N as urea when the plants reached 30-40 cm in height.

Irrigation water was first applied to all the plots using a sprinkler irrigation system. After the emergence of plants, plots were irrigated equally by the furrow irrigation system. All tested characteristics were measured on randomly selected 10 plants in the center of each plot. An analysis-of-variance (ANOVA) was performed using Jump statistical package program to evaluate statistically differences between results. Means of the data obtained from research were compared using Duncan test at $P \leq 0.05$.

Results and discussion

Flowering duration (day)

According to variance analyses, flowering duration were significant ($P \leq 0.01$). As seen from table 3. that flowering duration values were ranged from 57.5 day to 72.5 day. The highest flowering duration value was found at Rex genotype whereas the lowest flowering duration value was seen at Simon genotype. It was found 15.0 day duration between the highest and the lowest flowering value. Some of the genotypes such as Rex, Colman, UNL-Hybrid-4, H.Sugarcane and Honey gave higher flowering duration value than other genotypes. Generally stem yield, plant juice yield and ethanol yield were lower at the early flowering genotypes.

While Rex, Colman and UNL-Hybrid-4 genotypes' flowering duration values were over 70 day, Rox, Orange, Norkan and Simon genotypes' flowering duration were below 60 day. Some researchers reported different flowering duration values such as 55-72 day (Gul et al., 1999) and 69-88 day (Reddy et al., 2005) in sorghum plant.

Plant height (cm)

Variance analyses results show that sweet sorghum genotypes were significant at plant height ($P \leq 0.01$). Plant height values varied between 252.25 and 340.75 cm. The longest plant height value was found at UNL-Hybrid-4 genotype whereas the shortest plant height was seen at Norkan genotype (Table 3). Average plant height value was found as 307.41 cm. Most of tested genotypes were over average plant height value. Plant height values of Blue Ribben, Colman, Honey, Early Folger, Rex, Rox Orange, UNL-Hybrid-4 and Waconia-L genotypes were over 300 cm whereas plant height values of H. Sugarcane, Mennonita, Norkan and Simon genotypes were below 300 cm.

Similar results were obtained by Clegg et al. (1986), Saglamtimur (1988) and Avcıoglu et al. (2009). These authors observed that plant height values were between 355, 370 and 394 cm, respectively. Some researchers reported lower plant height values such as 249 cm (Yılmaz and Hosaflioglu, 2000), 231 cm (Ozbilen, 1991), 226 cm (Acar and Akgun, 2009), 198.2 cm (Iptas, 1993) and 184.9 cm (Sevimay et al., 2001). It was found that early flowering genotypes gave lower plant height values.

Stem yield (kg da^{-1})

According to variance analyses, differences among genotypes were significant ($P \leq 0.01$) at stem yield. Stem yield values ranged from 7440.0 to 13950.0 kg da^{-1} (Table 3). The highest stem yield value was seen at UNL-Hybrid-4 genotype whereas the lowest stem diameter value was found at Norkan genotype. Stem yield values were dramatically different among tested

sweet sorghum genotypes. It was found 5385.0 kg da^{-1} differences between the highest and the lowest stem yield value. Stem yield values were over 10000 kg da^{-1} at UNL-Hybrid-4, Blue Ribben, Rox Orange, Colman and Mennonite genotypes. Some researchers reported lower stem yield values than our findings. Different stem yield values were stated by other researchers such as 4790-6593 kg da^{-1} (Tsuchihashi and Goto, 2004), 4100-5200 kg da^{-1} (Reddy et al., 2005), 2276-4485 kg da^{-1} (Chavan et al., 2009) and 2150-2660 kg da^{-1} (Geren et al., 2011).

Table 3. Flowering duration, plant height, stem yield, plant juice yield, brix and ethanol yield

Genotypes	Flowering duration (day)	Plant height (cm)	Stem yield (kg da^{-1})	Plant juice yield	Brix (%)	Ethanol yield (L da^{-1})
Blue Ribben	66.50 cd	316.50 abc	12750.0 a	6225.0 a†	15.88 bcd	446.7 ab
Colman	70.50 ab	323.50 abc	12262.5 ab	6150.0 a	16.75 abc	464.5 a
Early Folger	63.25 de	327.75 ab	8863.5 d	3637.5 e	14.50 cde	238.1 de
H.Sugarcane	68.25 bc	287.25 bcd	7642.5 d	3712.5 de	14.25 de	237.1 de
Honey	67.50 bc	322.00 abc	9855.0 bcd	4762.5 b-e	14.50 cde	309.9 cde
Mennonite	63.25 de	284.50 bcd	11715.0 abc	4237.5 cde	17.25 ab	330.9 cd
Norkan	58.25 f	252.25 d	7440.0 d	3525.0 e	13.50 e	214.3 e
Rex	72.50 a	313.00 abc	7905.0 d	4275.0 b-e	18.25 a	353.2 bc
Rox Orange	59.00 f	329.00 ab	12825.0 a	5025.0 a-d	16.50 a-d	369.9 abc
Simon	57.50 f	277.50 cd	7912.5 d	3862.5 cde	15.75 b-e	275.1 cde
UNL-Hybrid-4	70.25 ab	340.75 a	13950.0 a	5625.0 ab	17.50 ab	445.3 ab
Waconia-L	63.00 e	315.00 abc	9435.0 cd	5175.0 abc	15.50 b-e	361.3 abc
Average	64.97	307.41	10213	4684.4	15.84	337.2
LSD	3.29	47.53	2585.38	1363.21	2.34	104.02
F	**	**	**	**	**	**
CV (%)	2.043	6.228	10.196	11.721	5.959	12.43

†There is no statistical difference among values annotated with the same letter in a column according to Duncan test
** : denotes P≤0.01

Plant juice yield (L da^{-1})

According to variance analyses, sweet sorghum genotypes were significant for plant juice yield (P≤0.01). Plant juice yield values were varied from 3525.0 L da^{-1} to 6150.0 L da^{-1}. The highest plant juice yield value was obtained from Colman genotype whereas the lowest values were seen at Norkan genotype. There was a big differences at plant juice yield among tested sweet sorghum genotypes. Differences were 2625.0 L da^{-1} between the lowest and highest plant juice yield values. Blue Ribben, Colman, UNL-Hybrid-4, Waconia-L and Rox Orange genotypes gave higher plant juice yield than others. Rains et al. (1993) reported that plant juice yield ranged from 135.8 to 964.5 L da^{-1}.

Water soluble dry matter ratio (Brix) (%)

As seen from Table 3 that water soluble dry matter ratio (Brix) value were significant (P≤0.01). Brix values varied between 13.50% and 18.25%. The highest brix value was seen at Rex genotype whereas the lowest brix value was found at Norkan genotype. The highest brix values obtained from Rex, UNL-Hybrid-4 and Mennonite genotypes. It was seen 4.75% differences between the lowest and highest brix values. Our findings were in accord with other researchers' results. Similar brix values were reported as %8-17 (Maarouf and Moataz, 2009), % 6.2-20.7 (Subramanian, 2013) and %12.5-17 (Prasad et al., 2007).

Ethanol yield (L da^{-1})

Significant differences (P≤0.01) were seen among genotypes for ethanol yield. Ethanol yield values ranged from 214.3 to 464.5 L da^{-1} (Table 3). Similar founding reported by Grassi (2001). It was seen big differences about ethanol yield among tested sweet sorghum genotypes. There was 250.4 L da^{-1} between the lowest and highest ethanol yield.

The highest ethanol yield value was seen at Colman genotype whereas the lowest ethanol yield value was found at Norkan genotype. Some of the genotypes such as Colman, Blue

Ribben, UNL-Hybrid-4, Rox Orange and Waconia-L genotypes gave higher ethanol yield than other genotypes (Figure 1).

Different ethanol yield values were reported by some researchers. Smith and Buxton (1993) stated that 600 L da^{-1} ethanol yield was found in Iowa. Ethanol yield values reported as 125-562.5 L da^{-1} by Li (2003) and 81.16-345.85 L da^{-1} by Teetor et al. (2011).

As seen from Table 3 that ethanol yield values were higher at some genotypes which has long flowering duration. Dogget (1988) reported that late maturated genotypes were high yielding than earlier sweet sorghum genotypes.

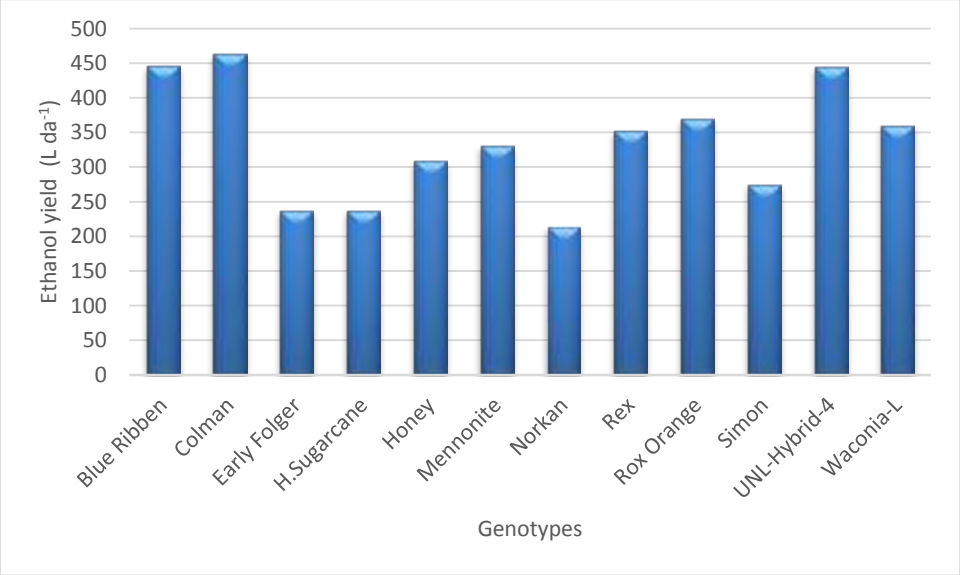

Figure 1. Ethanol yield values of sweet sorghum genotypes

Conclusion

As a result, it was determined that UNLY-hybrid-4, Blue Ribben, Rex and Colman sweet sorghum cultivars gave higher values than other ones in terms of stem yield, plant juice yield, water soluble dry matter ratio and ethanol yield. Ethanol yield values ranged from 214.3 to 464.5 L da^{-1}. Colman, Blue Ribben and UNL-Hybrid-4 genotypes gave higher ethanol yield than other genotypes.

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FRUIT SET, YIELD AND SOME QUALITY TRAITS OF DIFFERENT FOREIGN ALMOND CULTIVARS GROWN SANLIURFA PROVINCE

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Abstract

This study was conducted on the 4th and 5th leaf old Ferragnes, Ferraduel, Lauranne, Bertina and Felisia cultivars which are growing at the area of private sector at Bozova Province in Sanliurfa (Turkey). In the experiment, 9 fruiting trees were used as plant materials. Fruit samples were taken during harvesting time of the trees. Fruit set, yield and some quality traits were analyzed. According to two years results the highest (29,23 %) fruit set was determined Ferragnes cultivar while the lowest (11.57 %) was Bertina. According to yield per tree Ferragnes cultivar is the best average of two years. Pomological analyzes were done such as weight and dimensions of fruit and kernels. The obtained results show that the biggest fruit was Bertina cultivar. So the kernel and dimensions of fruit and kernel was higher than the others.

Keywords: *Prunus amygdalus, Almond, fruit set, yield, quality.*

Introduction

Almond is one of the most important nut crops worldwide, whose fruits are widely used in food production. In the viewpoint of total production around 3 000 000 tons in the world. Main producer country is USA. Second is Spain, according to four years average of FAO records. Turkey's production is over 83 000 tons. The largest almond plantations in Spain but it was second country by means of production. There are suitable areas to grow almond trees (Ak, 2016). Spain, historically the second leading region, produced approximately 7% of World production but used a cultivated area of over 436,500 ha. Australian production has recently surpassed that of Spain, being slightly more than 7% of total. The remaining world production comes from about 20 countries including Morocco, Iran and Turkey (~4% each), Italy (3%), Tunisia (2%) and Chile (1%). The yield and income is not satisfactory for farmers (Socias y Company and Gradziel, 2017). The edible (sweet) varieties of almonds are consumed whole as a snack food. Apart from its snack value, almond kernels are limitedly used in the confectionary industry as a topping item placed on cakes, Turkish Delights, ice creams and other traditional Turkish sweets. Bitter varieties of almonds, however, are used as supplements and odor additives in the cosmetics industry (Ozudogru, 2003). There are wild named almond species i.g. *Amygdalus orientalis*, *A. turcomonica* etc. common in Turkey (Ak et.al., 2001). Almond culture is possible at South East Anatolia because: (i) there is enough high temperature and low relative humidity in summer time during maturation and harvesting time; (ii) winters are cold enough to provide chilling requirement; (iii) trees can be grown in soil with high lime content; and (iv) trees are resistant to dry or o reduced water conditions (Ak et al., 2005). South East Anatolia is mainly covered with pistachio trees. But almond is an alternative crop to pistachio (Kuden, 1998; Ak and Sakar 2012). Farmers prefer to grow almond instead of pistachio under irrigated areas (Kaska et al., 1999) because: (i) juvenile period is very short; (ii) there is no alternate bearing habit; (iii) harvesting is easy; and (iv) fruits can be stored easily. The main aim of this research is determination on yield and quality of some foreign almond cultivars which started to plant as new large plantations by farmers at Southeast Anatolian region.

Materials And Methods

This experiment was conducted on the four and five years old Ferragnes, Ferraduel, Lauranne, Bertina and Felisia cultivars, which are grown at the area of private sector at Bozova District of Sanliurfa. They were grafted on almond (*Amygdalus communis*) rootstocks. They are France and Spanish cultivars. In the experiment, nine fruiting trees were used as plant materials. Fruit samples were taken during harvesting time of the trees. The trees are irrigated subsurface irrigation methods from April to October (Ak, 2004). Some meterological data were given Table 1.

Table 1. Some metrological data for Bozova District

Months	Minimum temperature (^oC)		Maximum temperature (^oC)		Average temperature (^oC)		Relative humidity (%)		Precipitation (mm)	
	1th Year	2th Year	1th Year	2th Year	1th Year	2th Year	1th Year	2th Year	1th Year	2th Year
January	-3.4	-3.4	17.0	17.0	7.1	7.1	73.3	73.3	52.8	52.8
February	-2.6	-2.6	16.4	16.4	6.3	6.3	73.0	73.0	88.2	88.2
March	-3.0	-3.0	21.6	21.6	9.6	9.6	63.4	63.4	52.0	52.0
April	1.6	1.6	28.0	28.0	15.9	15.9	60.5	60.5	27.6	27.6
May	7.0	7.0	37.0	37.0	23.8	23.8	36.9	36.9	16.4	16.4
June	14.2	14.2	41.0	41.0	29.2	29.2	27.6	27.6	1.0	1.0
July	16.8	16.8	46.0	46.0	31.8	31.8	32.4	32.4	-	-
August	14.9	14.9	45.4	45.4	32.0	32.0	33.1	33.1	-	-
September	8.3	8.3	42.2	42.2	24.8	24.8	34.0	34.0	-	-
October	0.4	0.4	37.0	37.0	20.3	20.3	43.2	43.2	20.1	20.1
Novmber	-1.7	-1.7	27.3	27.3	11.3	11.3	64.4	64.4	45.8	45.8
December	-4.2	-4.2	16.8	16.8	6.6	6.6	74.1	74.1	35.8	35.8

Source: Meteorology Directorate of Bozova District

Pomological analyses were applied on nut and kernel. The traits analysed were weight (g), length (mm), width (mm), thickness (mm), double kernel rate (%) and shelling percentage (%).

Results and Discussion

The yield of different almond cultivars, which are grown in Sanliurfa-Turkey, were given Table 1. According to Table 1, the yield of per tree were changed of their ages and among the cultivars. But when it is considered as averagely, the highest (8.22 kg/tree) yield is obtained from Ferragnes cultivar while the lowest (4.53 kg/tree) yield from Felisia cultivar. The others also changed between this figures.

Table 2. Yield of different foreign almond cultivars (kg/ tree)

Cultivars	**4th leaf**	**5th leaf**	**AVERAGE**
Ferragnes	8.04	8.40	8.22
Ferraduel	8.16	7.60	7.88
Lauranne	4.56	9.85	7.20
Bertina	5.49	10.8	8.14
Felisia	2.79	6.27	4.53
AVERAGE	5.81	8.58	7.19

The yield efficiency that means of the yield per Cross Sectional Area were calculated as well. The results of this were given Table 2. The highest yield 136.21g/cm2 with Bertina cultivar.

Table 3. Yield efficiency in some almond cultivars (g/cm2)

Cultivars	**Yield Efficiency (g/cm2)**
Ferragnes	99.18
Ferraduel	99.11
Lauranne	99.44
Bertina	136.21
Felisia	57.65
AVERAGE	98.34

Fruit setting rate is given Table 3. As it is seen in the table, The highest (29.23 %) fruit set obtained from Ferragnes. The lowest (11.57 %) rate was obtained Bertina cultivar.

Table 4. Fruit set of different foreign almond cultivars (%)

Cultivars	**fruit set rate (%)**
Ferragnes	29.23
Ferraduel	13.05
Lauranne	22.47
Bertina	11.57
Felisia	26.04
AVERAGE	29.47

Some pomological analaysis were done such as fruit and kernel weights, dimensions, and shelling percentages were given Table 4. According to Table 4, Bertina is large and heavy nuts and kernels but shelling percentage is not same paralell. This means that the endocarp is thick and heavy. According to two years average, lenght, widith and thickness are very similar although statistically different.Nut weight is also heavy in Bertina cultivar when compared the others. But fruit set rate was low. Shelling percentages of the cultivars which used at the experiment is very important to determine of yieldness. Felisia cultivar is highest (38.09) when compared the other cultivars.

Table 4. Some dimensions, weights and shelling rate of different foreign almond cultivars

	NUT			
THE AGE	**Cultivars**	**Length (mm)**	**Width (mm)**	**Thickness (mm)**
4th leaf	Ferragnes	33.537 b	22.374 b	14.779 c
	Ferraduel	35.082 b	23.632 b	16.253 ab
	Lauranne	32.925 b	22.300 b	16.724 a
	Bertina	40.689 a	27.939 a	15.431 abc
	Felisia	30.492 c	19.288 c	15.275 bc
	AVERAGE	**34.54**	**23.10**	**15.68**
	LSD (% 5)	**2.26**	**1.69**	**1.43**
5th leaf	Ferragnes	31.59 b	22.61 b	15.73 c
	Ferraduel	31.78 b	22.66 b	15.77 bc
	Lauranne	29.01 c	22.13 b	16.55 b
	Bertina	37.13 a	28.07 a	17.53 a

	Felisia	26.29 d	19.56 c	13.46 d
	AVERAGE	**31.16**	**23.00**	**15.80**
	LSD (% 5)	**1.35**	**0.76**	**0.79**
2 YEARS AVERAGE	Ferragnes	32.56 b	22.49 bc	15.25 a
	Ferraduel	33.43 ab	23.14 b	16.01 a
	Lauranne	30.96 b	22.21 c	16.63 a
	Bertina	38.9 a	27.99 a	16.48 a
	Felisia	28.39 b	19.42 d	14.36 a
	AVERAGE	**32.84**	**23.05**	**15.94**
	LSD (%5)	**6.31**	**0.86**	**2.42**

Table 4. Some dimensions, weights and shelling rate of different foreign almond cultivars (Cont.)

		KERNEL			
THE AGE	**Cultivars**	**Length (mm)**	**Width (mm)**	**Thickness (mm)**	**Shelling (%)**
4th leaf	Ferragnes	25.33 bc	13.64 c	7.31 b	1.15 ab
	Ferraduel	22.96 c	22.32 a	14.98 a	1.04 b
	Lauranne	29.42 ab	16.27 bc	11.43 a	1.71 a
	Bertina	33.81 a	18.24 b	6.57 b	1.99 a
	Felisia	21.15 c	12.66 c	6.70 b	0.84 b
	AVERAGE	**26.53**	**16.62**	**9.39**	**1.34**
	LSD (% 5)	**1.57**	**0.98**	**0.57**	**0.17**
5th leaf	Ferragnes	24.9 b	13.72 b	8.23 b	1.2 b
	Ferraduel	25.31 b	13.85 b	8.43 ab	1.27 b
	Lauranne	22.09 c	14.36 b	9.16 a	1.18 b
	Bertina	30.32 a	16.63 a	7.14 c	1.64 a
	Felisia	19.96 d	12.65 c	6.83 c	0.76 c
	AVERAGE	**24.51**	**14.24**	**7.95**	**1.21**
	LSD (% 5)	**0.94**	**0.68**	**0.85**	**0.2**
2 YEARS AVERAGE	Ferragnes	25.1 bc	13.59 b	7.77 a	1.16 b
	Ferraduel	25.72 bc	13.84 b	7.83 a	1.22 b
	Lauranne	27.17 ab	13.9 b	8.45 a	1.16 b
	Bertina	32.02 a	17.43 a	6.88 a	1.79 a
	Felisia	20.49 c	12.29 b	6.88 a	0.79 c
	AVERAGE	**26.1**	**14.21**	**7.56**	**1.22**
	LSD (%5)	**5.92**	**1.74**	**1.62**	**0.28**

Conclusions

Almond husbandry in Turkey has not yet reached the point desired, because the infrastructure necessary to commercially maintain almond trees is not adequate. Being not capable of producing almonds in the desired quantity and quality makes our country a net almond importer although Turkey has the capacity to do better as far as almonds production are concerned. To break this vicious circle in the almond sector, efforts directed towards enhancing the area, yield, and production of almonds must be accelerated through preventing growers from using almond trees as bordering materials in orchards rather than using them in commercial production (Isgin and Ak, 2011). Some of the cultivars were obtained by breeding end of the long researches (Kester and Assay, 1975). Southeast Anatolian Region has started to be irrigated. These areas were covered by pistachio trees that are very highly resistant to

drought conditions. But new areas started to turn for almond orchards. Due to: (i) availability of high quality of irrigation water; (ii) existence of fertile and deep soils; (iii) productive late flowering and early bearing cvs; (iv) dry and hot summers with no rains at harvest times; (v) almost no frost damage; and (vi) high profits in comparison to field crops (cotton, corn,wheat, lentil, etc.), almond is going to be the major alternative nut to field crops in the GAP Region of Turkey. The obatined results showed that especially late flowering cultivars are suitable to establish as an economical orchards. If modern almond growing techniques such as pruning, harvesting, cracking, etc. are applied, Turkey can be at least self- sufficient in almond (Kaska, et al., 2005). It appeared that 'Ferragnès' and 'Ferraduel' are the most promising cvs for the region. It was determined many years before and these cultivars are commonly growing in nowadays.

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INVESTIGATIONS ON STOMATA OF SOME OLIVE VARIETIES GROWING IN SANLIURFA PROVINCE IN TURKEY

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Abstract

This research estimated changes in the number of stoma and types found on different types of olive and olive plant leaves. It also investigated different types of olives adaptation to low moisture levels in the hot and dry region of Sanliurfa (Turkey) from a stoma density point of view. Different cultivars were examined and no significant statistical differences were found on the three types of leaves found in the region. Stoma numbers varied from region to region in the same cultivar on the leaf. The changes are random, and for every cultivar the result varied. Negatif statistical results were found between stoma sizes and numbers. On the same type of leaves differences were noticed between different cultivars after examination on the middle, upper and lower part of the stem. Leaves ranging in size and length were examined from different regions for different olive types. Leaves found on the south regions were larger and longer in size.

Keywords: *Olive, Stoma, Density, Stoma Size, North, South.*

Introduction

A plant gives its water to the air as water steam by transpiration which is known to be carried out mostly by stomata in leaves. Pores on the epidermis of leaves which achieve the evaporation and the exchange the gases between outside and results from physiological activities of plant is named as stomata. When the stomata are open; oxygen, carbondioxide and water steam exchange between the intercellular cavities of the leaves and the atmosphere. This exchange is mostly prevented when stomata are closed (Sahin and Soylu, 1991; Olmez et al., 2001). Transpiration constant shows the amount of water necessary for the production of soluble solids and is an indicator how effective the plant uses the water (Secer 1988). Antitranspirant substances have been shown to increase the largeness of fruit and yield of tree in apricot (Ben-Porath, and Greenblat,1994). In addition, density and morphology of stomata in leaves are known to affect the amount of transpiration (Blanke,1987; Olmez, et al., 2001) . The mechanisms developed by plants as a response to water stress may be found in adaptations by roots, stems, leaves and fruits; the leaf modifications include the size and number of stomata (Kozlowski, 1976; Palasciano et al., 2005). Stomata are the morphological structures which control photosynthesis and transpiration. In general, there is an inverse relationship between the stomata number and size, characteristics which vary greatly among species and genotypes Moreover, the stomata frequency and size can be significant both in plant genetics and ecology (Fregoni and Roversi, 1968; Palasciano et al., 2005).

Requirements of the plant are provided by stomata (Karamanoglu, 1983; Resh, 1981). 90% of the water taken by plant is lost as water steam from the surface of the leaves. This phenomenon is called as transpiration and carried out mostly by stomata (Olmez et al., 2001).

Olmez et al., (2001) the variations of stomata numbers in leaves of apricots (*Prunus armeniaca*) related to the cultivars and regions and if these variations of stomata numbers are related with fruit characteristics (fruit and stone weight, acidity and soluble solid percentage) were investigated. They founded that a positive and statistically significant correlation between fruit weight and stomata numbers, the correlation coefficiency (r) was found as 0.295 meaning that the more stomata numbers are, the more fruit weight is. There was similar

correlation (r=0.194) between stone weight and stomata numbers, too. A negative but statistically non-significant correlation (r=0.127) was determined between stomata numbers and soluble solids percent. Available cultivars appropriate for drying process and fresh consumption were compared in three different region and interestingly, the stomata numbers of the cultivars appropriate for drying process were found less than that for fresh consumption. The results of this investigation demonstrated that stomata numbers in leaves of apricot cultivars appropriate for drying process were different from that for fresh consumption. In addition, we showed a relationship between stomata numbers and fruit weight.

The aim of this study was to investigate the variations of stomata numbers in olive leaves in related to the cultivars which are used as table and oil consumptions. Whether stomata numbers vary according to the part of the leaves (basal, middle and distal parts) and the leaves situation on the shoots in which the stomata were counted and evaluated.

Materials and Methods

The samples of leaves were taken from 9 or 10 years old olive trees which planted Akcakale Reasearch Institute. The samples were taken both north and south sides of the trees. Two annual shoots in each tree were used. Leaf samples were taken from tip, middle and basal of the shoots. Stomata counts were made on the tip, middle and base portion of leaves. For the counting of stomata, the epidermis layer was pulled out by nail polish from the abaxial side of leaves (Elci, 1994). Then, stomata were counted by using a microscope with a 10x40 magnification in 0.0625 mm^2 area(Gulcan and Mısırlı, 1990; Ozeker and Misirli, 2001). The obtained values were calculated as stomata numbers per mm^2. The countings were made 5 different leaves (6x5 = 30 replications) .Each leaf three part (30x3=90countings) and one shoot three part (tip, middle, basal leaves) (90 x 3= 270 countings) counted. So two sides (north and south) considered. That means 540 (270x2=540) countings were applied each cultivar (Figure 1).

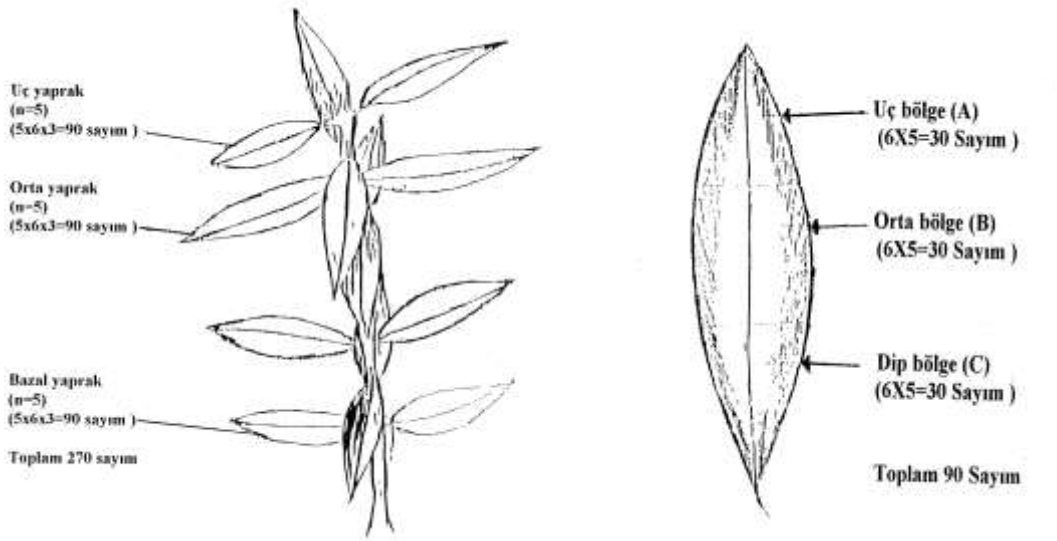

Figure 1. Leaf sampling and counting stomata on the shoot and each leaf.

Results and Discussions

Stomata numbers (per mm^2) were determined according to leaves positions (Table 1). The number of stomata were changed according to cultivars as well. According to average of stomata; the highest (464.467) in Nizip yaglik cultivar while the lowest one (388.242) Gemlik cultivar. Number of stomata were found less in the tip parts of the shoots (410.320) than the base (426.284) and middle (420.817) of the shoots.

Table 1. Stoma numbers of the different cultivars state of shoot (n/mm²)

CULTIVARS	SHOOT			AVERAGE
	TIP	MIDDLE	BASE	
Gemlik	369.381 c	416.200 b	379.144 c	388.242 b
Manzanilla	446.114 a	461.410 a	476.952 a	461.492 a
Nizip Yaglik	450.101 a	471.209 a	472.091 a	464.467 a
Domat	392.486 bc	379.111 c	398.272 bc	389.954 b
Memecik	393.517 b	376.155 c	404.962 b	391.554 b
AVERAGE	410.320 b	420.817 ab	426.284 a	

$LSD_{0.01}$(Cultivar): 13,83 $LSD_{0.01}$(Shoot): 10,72
$LSD_{0.01}$ (Cultivar x Shoot):23,96

Stomata number also shows the drought resistancy. The *Quercus coccifera* (433 adet/mm²) more resistant to drought conditions than *Arbatus andrachne* (364 adet/mm²) (Olmez et al., 2001). The results of according to leaf states of the stomata numbers of leaves in different olive cultivars were given at Table 2.

Table 2. Stoma numbers of the different cultivars state of leaf (n/mm²)

CULTIVARS	LEAF			AVERAGE
	TIP	MIDDLE	BASE	
Gemlik	381.597 b	393.425 b	389.703 b	388.242 b
Manzanilla	464.468 a	465.027 a	454.981 a	461.492 a
Nizip Yaglik	461.883 a	469.391 a	460.793 a	464.023 a
Domat	391.072 b	386.440 b	392.357 b	389.956 b
Memecik	385.437 b	394.608 b	395.317 b	391.787 b
AVERAGE	416.891	421.778	418.630	

$LSD_{0.01}$ (Cultivar): 15,20; $LSD_{0.01}$ (Shoot): NS
$LSD_{0.01}$ (Cultivar x Shoot):26,33

The highest stomatae number was found 469.391 with Nizip Yaglik at middle of the leaf. The lowest (381.597) in Gemlik cultivar. When it compared amond the tip' midlle and base of leaf' averagely the highest number (421.778) of stomata were obtained from middle of the leaf. The lowest number was obtained from tip of the leaf.
In this experiment the number of stomate side was compared(Table 3). Generally north side of leaf stomata number was found higher than south side of the taken leaves.

Table 3. Stoma numbers of the different cultivars state of the sides (n/mm²)

CULTIVARS	CARDINAL POINTS OF TREES		AVERAGE
	NORTH	SOUTH	
Gemlik	357.696 b	418.799 ab	388.247 b
Manzanilla	488.570 a	436.640 a	462.605 a
Nizip Yaglik	497.093 a	431.841 a	464.467 a
Domat	385.284 b	394.457 b	389.871 b
Memecik	383.873 b	399.449 b	391.511 b
AVERAGE	422.443	416.237	

$LSD_{0.01}$ (Cultivar): 20,86; $LSD_{0.01}$ (Side): NS.; $LSD_{0.01}$ (Cultivar x Side):29,50

The stomata sizes(length and width) were given Table 4, 5 and Figure 2. As the sizes were seen in these tables, sizes are changed according to cultivars.

Table 4. Determination of stomata sizes and differences between the North and South sides.

CULTIVARS	SIDES	LENGHT (µm)	AVER.	WIDTH (µm)	AVER.	WIDTH / LENGHT	AVER
Gemlik	North	11.4 ± 1.17		6.8 ± 1.23		0.596	
	South	10.8 ± 0.62	11.1	7.3 ± 1.20	7.05	0.675	0.635
Nizip Yaglik	North	10.7 ± 0.67		5.9 ± 0.87		0.551	
	South	10.9 ± 0.74	10.8	6.6 ± 0.96	6.25	0.605	0.578
Manzanilla	North	10.3 ± 0.82		6.2 ± 1.23		0.601	
	South	10.4 ± 0.96	10.35	6.6 ± 0.27	6.40	0.634	0.617
Domat	North	10.6 ± 1.17		6.3 ± 0.82		0.594	
	South	11.5 ± 0.85	11.05	6.9 ± 1.91	6.60	0.60	0.597
Memecik	North	10.8 ± 1.03		6.6 ± 0.96		0.647	
	South	11.0 ± 0.66	10.9	7.3 ± 1.49	6.95	0.663	0.637

Stomata bigness of the sampled cultivars were changed (Table 5). The bigger (78.25) was Gemlik cultivar, while the smallest (66.24) was Manzanilla. It can be seen in Figure 2 as well. This situation may affect to photosynthesis. Paul and Eagles (1988) reported a positive correlation between the stomata numbers of leaves and the rate of photosynthesis in four different Brassica varieties.

Table 5.The bigness of olive stomata rate according to side average.

CULTIVARS	WIDTH X LENGHT(µM²)
Gemlik	78.25
Memecik	75.75
Domat	72.93
Nizip Yağlık	67.50
Manzanilla	66.24

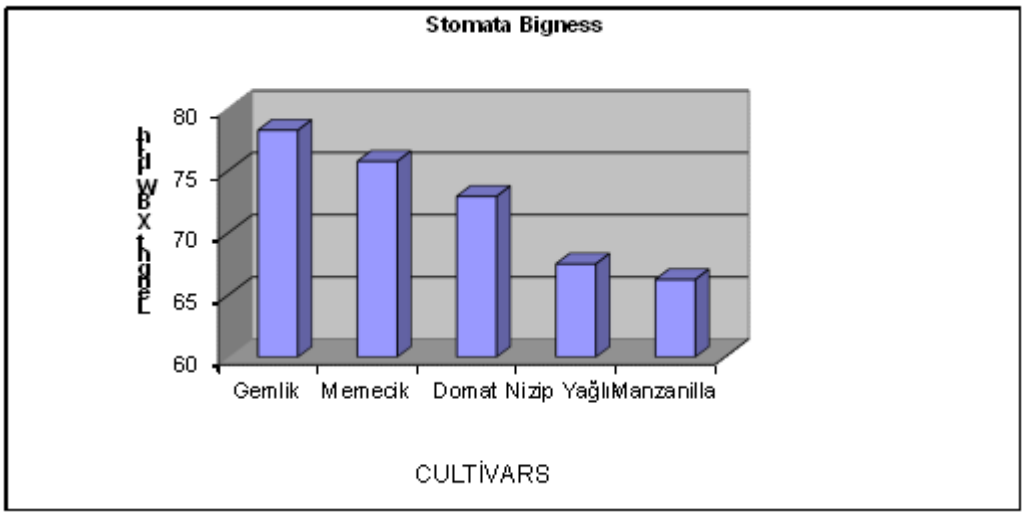

Figure 2. The changes of bigness (Lenght x Width)according to size of stomata in some olive cultivars.

Conclusions

The results of this study confirm significant differences among genotypes of olives cultivars about stomata numbers and sizes. It was understand that stomata number can be changed according to leaf position on the shoot, leaf part and side. But generally stomata number is changing according to olive oil contents. Because olives which is used to extract for oil' such as Nizip Yaglik the number of stomata is changing, that Gemlik cultivar which has been used for table consumption. In conclusion, it was demonstrated that stomata numbers in the leaves of olive cultivars maybe changed according to cultivars.

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PISTACHIO PRODUCTION IN THE WORLD AND SOME NEW PROBLEMS RELATED TO IRRIGATION IN TURKEY

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Abstract

Pistachio can be grown in only some part of the world due to special climatic requirements. The most important pistachio producer countries in the world are Iran, U.S.A., Turkey and Syria, respectively. Growing and processing techniques after harvest in Iran and U.S.A differ from those in other countries. Modern growing and processing techniques are performed in only U.S.A. In this presentation, the production values, growing problems and solutions, harvest and post-harvest processing techniques are compared that pistachio producer countries. In Turkey, growing of pistachio is intensified to the Southeast of Anatolia. After irrigation facilities start to be used in this area with GAP Project, it is expected to be important changes in new and old orchards. The farmers know the effect of water for pistachio trees. They are trying irrigation methods. An additional to this growing system, post harvest processing is start to be modernized during last decades.

Keywords: *Pistachio, production, yield, quality, irrigation.*

Introduction

Some of the species play an important role in vegatation at the Mediterranean and Asian regions and most of them have proved successful as rootstocks for top working the cultivated pistachio nut. Except *Pistacia vera* the other ones are not economical. They are called wild pistachio. In Afganistan, Iran, Pakistan, Turkey and all The Mediterranean countries there are millions of wild pistachio trees or bushes which belong to different *Pistacia* species. Pistachio is grown most intensively in Iran, Syria, Turkey and U.S.A. The other pistachio producing countries are in the Near East, North Africa and the Southern Europe. According to four years avarega (2013-2016), the production values in Iran is over 352 000 tons which is half of the world production (Table 1). The second country, USA, produces approximataly 300 000 tons covers 32 % and next one is Turkey with yield of 120 000 tons with 13.24 % of total world production.

Table 1. Pistachio production in the World

Country	2013	2014	2015	2016	Average	Rate %
Iran	225 001	440 814	430 000	315 151	352 742	38.70
USA	213 188	233 146	313 811	406 646	291 698	32.01
Turkey	88 600	80 000	144 000	170 000	120 650	13.24
China	74 000	75 554	79 455	83 310	78 080	08.57
Syria	54 516	28 786	32 574	56 833	43 177	04.74
Greece	7 122	8 566	5 540	6 338	6 892	00.76
Italy	3 227	3 555	3 868	3 649	3 575	00.39
Afganistan	2 769	2 784	2 799	2 814	2 792	00.31
Tunusia	2 100	2 500	3 000	3 400	2 750	00.30
Spain	2 489	2 428	2 423	2 418	2 440	00.27
Madagascar	1 900	1 821	1 506	1 730	1 739	00.19
Australia	1 334	1 304	1 343	1 378	1 341	00.15

Others	--	---	---	---	---	00.67
WORLD	679 556	884 654	1 023 865	1 057 566	911 410	100.00

Source:FAO web page

Pistacia vera is grown 30-40 altitude and suited in microclimate areas in the world. The biggest (420 000 ha) production areas belong to Iran. Second country is Turkey. But its yield is very low when compared the production areas (Arpaci et al., 2005). This situation makes the scientist to think about the problems and solutions.

Pollination

Pollination occurs by the transport of pollens from male to female trees by wind. Pistachio flowers have no petals which attract insects. Therefore pistachio orchards must contain male trees and ratio of male to female should be 1/8 or 1/11 (Kaska, 1990, Ak,1992). It is necessary to have enough male trees to insure adequate pollination. Male and female flowers of pistachio are on separate trees. This situation has been recognized since 1697, (Whitehouse and Stone, 1941) that means pistachio trees (*Pistacia vera* L.) are dioecious. However male tree number can be enough in orchard. But, pollen shedding time should be with female flowering period. (Ak et al., 1998; Ak, 2001).

Rootstocks

In Turkey, there are four common *Pistacia* species (*P.vera, P.atlantica, P.khinjuk, P.terebinthus)* used as rootstocks. Except *P. vera* the others are not used commonly as seedling because of their low percentage of seed germination. According to observation, Siirt cultivar seeds have better growth and development than the other cultivars.

P.vera seedlings are stronger and more homogenous than the other rootstocks. Budded trees'on this rootstock grow slowly during the first year but growth becomes faster in the following years. There is no incompatibility in budding with cultivars of pistachio. In the budded trees the juvenility period lasts long; therefore, the trees bear fruit quite late. In arid zones the trees reach the bearing stage 15 to 20 years after planting. They can withstand drought, high lime content and salinity in the soil but are sensitive to high soil moisture. The trees of *P.terebinthus* are grown generally as bushes. The plant has a strong and deep root system. Therefore it can be grown in poor rocky and stony soils. It is a kind of dwarfing rootstock for *P.vera,* L. so the trees budded on this rootstock can be easily recognized. *P.terebinthus* is as hardy as *P.vera* but hardier than *P.atlantica.* Therefore it is preferred to *P.atlantica* in cold areas. In Turkey it can be grown successfully at elevations up to 1200 m. *P.terebinthus* is suitable for planting in calcareous soils along the Mediterranean coast. It grows very well in places where the annual precipitation is about 400 to 600 mm (Kaska, 1995).

Irrigation

James (1993) stated that between 60 and 95% of living plant biomass is water. Although plants need water primarily for transpiration, it also require water for other processes including photosynthesis, transport of minerals, structural support, and growthPistachio orchards were established in dry and unirrigated lands in Turkey besides the soils are very bad, unfertile and calcareous. The farmers believe in that pistachio should be grown without irrigation so far. This idea comes from their grandfathers. These old men have a bad experience about this matter. Because they were irrigated the result pistachio trees were died. And they teach this knowledge to their sons. But this situation had been occurred by wrong irrigation method. However last decades Research Institutes which are depend on Ministry of Agriculture, Universities start to make experiment on irrigation researches of Pistachio trees. Now, the positive effects of irrigation are told to farmers. The region where pistachio is

growing areas have not irrigation facilities until 1980's. Now in Southeast Anatolia project (GAP) provide to irrigation facility. The water is distributing by control. In the very near future new and irrigated pistachio nut orchards in the Southeast Anatolia Project (GAP) region will expand Turkey's pistachio nut areas. It is expected that when the project is completed the Turkish pistachio nut production will be at least doubled. As it is well known that irrigation is a very important factor in obtaining high yields on good quality.

In order to preserve the water from the winter and spring rains and snows, the soils are ploughed in Autumn. On the other hand in order to prevent evaporation, the soils are cultivated by chisel ploughs in whole spring and summer months. This type of soil workings destroy the weeds as well (Kaska, 1995).

As it is well known, some expected changes by irrigation, leaf size, number of current year's shoot and length of shoot will be increased. The result with irrigation increasing the yield, bigger nut size, high splitting percentage, low blank nut rate and lesser alternate bearing will be obtained. Arpaci et al., (1995) determined that in irrigated conditions with Siirt cultivar's yield 262 kg/da while 60-80 kg/da in dry conditions. This experiment shows that yield will be increased at least three or four times when pistachio orchards were irrigated.

As it is mentioned above pistachio orchards established under dry and very bad soil conditions. But, high yielding can be obtained irrigated and fertile soil conditions. According to Ak and Agackesen (2003) reported that pistachio trees should be established under irrigated and fertile or good soil conditions to get high yield and quality. The yield is compared. The yield of Kirmizi pistachio cultivar either fresh weight or dried weight in good conditions that means irrigated and fertile soil is higher than other conditions. As it is seen fertile soil without irrigation is better than poor soil with irrigation. The very important obtained results as below: (i) Fresh fruit weight was determined in arid soil irrigated conditions 21.80 kg, unirrigated conditions 15.50 kg. The yield were obtained fertile soil irrigated conditions 31.00 kg, unirrigated conditions 25.87 kg, respectively. 1 kg fresh fruit was determined 559.63 g after sun dried. According to this calculation, the highest dry weight was determined 17.043 kg per tree grown under fertile soil at irrigated conditions. (ii) The shell splitting rate was determined analyzing 300 fruits each tree. According to results of arid soil irrigated conditions 58.89 %, unirrigated conditions 37.45 %, at fertile soil 61.43 % obtained irrigated conditions, 61.10 % unirrigated conditions. The blank fruit rate was lowest, shell splitting and total filled fruit number rate increased with irrigation. (iii) 100 fruit weight determined the highest value under fertile soil irrigated conditions in splitted fruits. 100 fruit weight was determined statistically different from each others. Fruit weight and kernel weight were increased with irrigation. General average were obtained 86.64 g in nut, 44.37 g in kernel.

Blank or empty nut can be occurred by lack of pollination and fertilization. But mainly irrigation can effect to fill in. Irrigated condition blank fruit rate was 17 % when unirrigated trees were 30 % averagely. Likewise poor soil was 29.71 % while fertile soil 17.70 %.

Irrigation has been started but to apply suitable irrigation method is very important. Generally it should be drip irrigation system. This may be lying on soil surface or lying or buried sub soil sytem. There are advantages and disadvantages both systems. The amount of water is the key factor to prevent the trees from diseases.

Irrigation by underground drip systems are being used on olive plantations in Spain. This system has been started to be used for pistachio and almond orchards in Turkey. However farmers are not confident with this method. The advantages of underground drip irrigation system are mentioned as follows: lower water consumption, better water distribution, greater uniformity, waste water can be used, less evaporation, greater transpiration, location of fertilizer, less calcification, less diseases, possibility of tillage, The depth of the watering line is at approximately 20-25 cm deep, under the soil surface. This sytem is applying in Iran

because of lack or due to limited water. Exceesive water or weak dranage sytems or very high watertable kill the trees (Figure 1).

Figure 1. Excessive water killsthe pistachio trees.

Fertilization

The soil in area under pistachio plantation is mostly inadequate to N, P, K and organic matter. A survey conducted in 30 pistachio orchards in Southeast Anatolia, Tekin et al., (1985) revealed that in many orchards the trees were markedly deficient in phosphorus and zinc and slightly deficient in nitrogen, iron and manganese. The level of potassium was found adequate in many orchards though there were some districts where the trees showed slight deficiencies. In this region pH of the soils varies between 7.5 and 9.3 and the organic matter content is very low. Especially zinc deficiency effects on fruit set (Ak and Parlakci, 2006).

Crane and Maranto (1988) claimed that pistachio is not a luxury N consumer when it is abundantly available in the soil. Chemical form of nitrogenous fertilizers should be chosen depending on the soil pH. For instance, in the GAP (Southeast Anatolia Region) area only ammonium sulfate is recommended as a source of N because of the alkaline soils. Nitrogenous fertilizers should be given at the end of February or the beginning of March at the rate of 1.5 to 4 kg per tree (Kaska, 1995). In U.S.A. boron plays a unique role in pistachio. Responses to foliar B application include increased pollen viability and germination rate, increased fruit set and yield, decreased blanking percentage and increased leaf B concentration (Brown, 1995a). Generally nutrient statutes of pistachios should be critisize according to leaf analyzes. Leaf samples should be taken 16-31 July by Tekin et al. (1990).

Cultivars And Harvesting

The most important characteristics of the pistachio nuts desired by the markets are the should be; large size, high percentage of shell splitting, low percentage of blank nuts, high oil and protein content, .regular bearing, high percentage of green kernels. In pistachio green kernel is one of the most desired characteristics. Green kernel nuts are always at a premium. Though the green kernel is a varietal characteristic it is also related to altitude and harvest time. Generally the nuts of early harvested trees and plants grown on high plateaus produce greener kernels than those of late harvested and low land grown plants. On the other hand greenish is affected by pollen source. According to observation *Pistacia terebinthus* pollens causes metaxenia and xenia (Ak, 1992)

There are major cultivars each main producing countries. According to Sheibani (1995) there are 60 named cultivars in Iran. But main production cultivars are Ohady and Kaleh Ghochi. In Turkey, Kirmizi, Uzun, Halebi and Siirt are the major cultivars.

Alternate bearing is one of the important features of pistachio. Alternate bearer cultivars produce heavy crops in "on" years and little or no crop in the "off" years. In contrast to other fruit species such as olive, apple etc (Ak and kaska 1992). This situation can be change from

one cultivar to another one. For example Siirt variety is less alternating one (Ak, 1998). This means the tendency of alternate bearing of cultivars different. This can be decreasing by irrigation and fertilization.

Pistachio is harvested from late August to late October depending on cultivar maturation. During maturation the under colour of exocarp changes from a light green to pale straw or whitish, opaque appearance. The hulls can be removed from the fruits by being squeezed between the fingers. Another sign of maturity is splitting of shell. Decreasing in fruit removel force. Kernel dry weight and crude fat content are increase (Ak, 1998).

Conclusions

Pistachio nut culture is centuries old in Iran, Turkey and Syria. In this country the area under pistachio nut is the largest and the number of trees is the highest in the world. In spite of these facts the production is very low in Turkey and Syria. But the taste is good but the nuts are small and their splitting percentage is low. However, one should bear in mind that Turkey is the only country in the world that pistachio nuts are grown in such marginal lands with dry climate and poor, rocky and calcareous soils (Ak, 2002).

Turkey is full of other types of *Pistacia spp* called as wild pistachios. The topworking has done in 1980's. These trees are on the hill or mountains as irregular orchards. Some of them are very small, some of them not applied cultural practices. This situation makes the total yield very low. But These areas are not suitable for other agricultural crops. Because of this reason this type of areas gain as valuabled. Except this situation the regular orchards will be supported by government of Turkey and growing techniques will change to modern system. On the other hand, Turkey has very important advantage about aflatoxin. Because of low humidity and high temperature during growing and harvest season, the fruits are free from aflatoxin. It can be seen or contaminate during processing. Processing systems are also modernizing.That means Turkish pistachios has no aflatoxin and high quality about flavour and taste. Turkish cultivars are preferred in many European and U.S.A. markets due to their good taste and uniformly green kernels (Ak et al., 2016).

The researches had been showed that effects of irrigation 70 %, fertilization 50 %, variety 45 % and light pruning 17 % on yield of the pistachio trees . Pest and desiaese problems should be solved using chemicals wisely. The ecology of the tree is changing by irrigation so the pest which economically important types is chaning as well. Near future, when the problems are solved pistachio will gain big importance such as hazelnut in the world. Turkey's production will be increased three or four times when irrigation system established all the pistachio orhards either old trees or young new plantations.

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DETERMINATION OF THE EFFECTS OF LESS AND EXCESSIVE LEAF REMOVAL ON CLUSTER CHARACTERISTICS IN "TRAKYA ILKEREN" GRAPE VARIETY

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Abstract

In Turkey, stuffed vine leaves is one of the main traditional Turkish foods. Because of this, consumption of vine leaves are quite high. The "Trakya Ilkeren" grape variety is an early ripening and high quality dark red colored table grape which is also use for brine. Leaf removal is made intensively for brine leaf production in Turkey. Also leaf removal is made for green pruning during the summer season in vineyards. This study was carried out at the research and application vineyard of Ondokuz Mayis University in 2017 growing season. The aim of the study was to determine the effects on the cluster properties of less and excessive leaf removal in "Trakya Ilkeren" variety. In the study no leaf removal, less and excessive leaf removal applications were carried out between May and June. Leaf removal application had significant positive effect on TSS, titratable acidity, bunch width, berry width and length. Less leaf removal had a positive effect on pH, berry weight, bunch weight and bunch length. Hue and Chroma values were found to be significantly high in the control, b (-) and *L* values were found to be considerably high in the less leaf removal. In the study, less leaf removal application had positive effect on cluster characteristics in "Trakya Ilkeren" grape variety.

Key words: *leaf removal, grape, brine leaf, cluster, berry.*

Introduction

The leaf is one of the important main organs of the plant body. The primary function of the leaf is to perform photosynthesis and respiration. Photosynthesis decrease by continuous (Caspari et al., 1998) or 90% leaf removal (Chaumont, 1995; Ollat, 1997). Since the photosynthesis products cannot be transported completely from the leaf throughout the day, they are also important as nutritive organ (Downton et al. 1987, During 1988, Roper and Williams 1989). Grape leaf consumption has a significant potential in Turkey. The composition of the grape leaf contains sugars, organic and amino acids, phenolic compounds and some vitamins (Ribereau and Reynold, 1971). On the other hand, crude cellulose, which is found in plant-based foods, was reported to reduce the formation of arterial stiffness, spinal disease and colon cancer (Gürses, 1980). However, an excessive removing of the leaves prevents the growth of the vines and the ripening of the fruits well. For this reason, it should be avoided excessive leaf removing during growth season. In order to produce brined leaves, it is necessary remove clusters to provide for leaf formation and development. Leaf removal provides better light penetration, good ventilation and disease control (Cangi and Yağcı, 2017).

In spring, vine leaves which is collected during periods when the shoots are still fresh, evaluated for making brined. Wrapping leaves is preferred with thin, hairless, fiber free, thin veined, small slices and having sour taste. The most important wrapping leaf in terms of these characteristics is "Sultani Seedless". For this reason, brine leaf production is an important income for the Aegean region. In this region, most of the grape fields are planted with dried seedless grapes. "Sultani Seedless" grapes are also regarded as brine leaves at the same time. This situation is the same for the Tokat region. In fact, "Narince" is the most important grape variety evaluated as wine and table grapes beside its leaves for brined for grape grower in

Tokat province and around. Beside these varietis, "Yapıncak" grape variety which is grown especially in Trakya region, is also widely evaluated for this purpose (Çelik et al. 2005). Ağaoğlu et al. (1988), stated that leaf picking begins on shoots when they reached to 15-20 cm length in Tokat region between mid-May to the beginning of August. In vineyards for planted brine leaf, about 600-700 kg of leaf is pickled from the acre without paying attention to the flower cluster. There are 45 brine companies registered in Turkey, about 30 company in the Aegean region and 15 company in Tokat province. The financial value of brined leaf in these companies is estimated; 40.000.000 Turkish Liras (Anonymous, 2014). Grape leaf picking for brine in the early vegetation period caused the vines weaken and the quality of the grape berry decreased in the late period (Göktürk et al., 1997). In particular, when the excess leaf picking done, grape bunches are not able to feed adequately and thus yield and quality are reduced. Some researchers have suggested that leaf picking should be done in the period when they take 1/3 to 2/3 of the full size of the leaves (Göktürk et al., 1997; Sat vd., 2002). The harvest of grape leaves for pickled are usually done in May and June, but leaves should not be taken after the veraison period so that grape yield and quality are not adversely affected. In viticulture for grape production, due to the leaf picking at different times cause decreased total assimilation and undesirable reductions may occur in yield and quality (Mullins ve ark., 1992). Grape cultivation for brine leaf; number of vine cluster negatively affect grape leaf area and vegetative development. For this reason, it is suggested that remove the clusters they are still in the flower stage and apply a regular fertilization in new establish vineyards for brine leaf production (Odabaş ve ark., 1995).

Flower abscission affected by solar radiation (Ferree et al., 2001) and leaf area (Coombe, 1962; Koblet, 1966; Caspari *et al.*, 1998). Reduction of leaf area during flower formation leads to a decrease of nutrient availability for developing organs and flower abscission (Candolfi-Vasconcelos and Koblet, 1991). However, when the leaf removal reaches 90%, plant photosynthesis is so much reduced (Caspari et al., 1998; Duchêne et al., 2003). Grape leaves are capable of storing solar energy by converting it into chemical energy by chlorophyll. With the removal of vine leaves, grape quality decrease and acidity increases (Ruckenbauer and Traxler, 1975). In order to obtain high quality grape leaves in vineyards which are established to produce brine leaves, regular and necessary fertilization should be done. In this regard, particular attention should be paid to nitrogen fertilization (Odabaş et al., 1992).

Uslu (1980) found that leaf picking reduce the total assimilation surface, but increase the photosynthetic activation of the leaves on the vines and increase carbon dioxide absorption. Uslu (1980) found that 4.51-7.12 cm^2 leaf area is sufficient for ripening of 1 g grape. Currle et al. (1983) calculated that the leaf area required for maturation of a cluster or 1 g of berry should have a 1.6-2.8 m^2 leaf area and it should be avoided excessive leaf picking. When this value falls below, insufficient photosynthesis cause delay ripening of grapes due to the lack of organic matter. Candolfi and Koblet (1990) reported that sugar accumulation in berry seems to depend on active leaf surface between veraison and harvest period. Leaf removal at the early stage of berry growth stages leads to both millerandage and decreased bud fertility in the following season. This critical period is limited from full bloom to 2-3 weeks after. The leaf area of the vine has a great influence on the bunch quality (Drissi et al., 2009). There is a positive relationship between leaf area per vine and fruit quantity per vine while a negative relationship between bunch number per vine is reported (Edson et al., 1995). Schultz (1995) found that young leaves have as high photosynthetic rate. Increasing leaf area per vine provides increase in photosynthesis and dry matter (Campo et al., 2002).

Materials and Methods

The research was conducted in the experimental vineyard of Ondokuz Mayıs University, Turkey in 2017. The vineyard located at 41°21'52 N latitude and 36°11'29 E longitude with an altitude of 195 m, and a distance of about 2.8 km from the Black Sea coast of Turkey. The research vineyard has 62.2% clay soil, pH 7.1 and 4.33% organic matter content. In the study; 10-year-old "Trakya Ilkeren" grape variety grafted on 5C rootstock was used. Spur pruned grapevines were trained onto double cordon with 3x1.5 m spacing. Grapevines were not irrigated, not fertilized and the only supplementary water received from rainfall. In the study, control, less and excessive leaf removal applications were carried out. Grape leaves removed when the size reach enough to wrapping.

Applications

1) Control (no leaf removal),

2) Less leaf removal: Between May 15, 2017 and July 15, 2017, at intervals of 10 days, 7 times per shoot were applied. Two mature leaves just above the flower cluster have been removed in each term. As long as the shoots are extended, 2 mature leaves in the middle of the shoot are removed. The secondary shoots are removed with the leaves.

3) Excessive leaf removal: From the flower cluster, all the leaves were picked except for the last two small leaves at the top of the shoot between May 15, 2017 and July 15, 2017, at intervals of 10 days with 7 times. The secondary shoots are removed with the leaves.

Leaf removal applied from 15 May 2017, 25 May 2017; 05 June 2017; 15 June 2017; 25 June 2017; 05 July 2017 and 15 July 2017 dates. In the vines, the veraison started in 15 July 2017 and leaves did not picking after this date.

The fruits were harvested on 22 August 2017. In clusters; TSS (Brix %), titratable acidity (%), pH, maturity index, cluster weight (g), berry weight (g), berry width (mm), berry length (mm), cluster width (cm) and cluster length (cm) were investigated. The berry skin color is measured with a Minolta CR-300 colorimeter. TSS (total soluble solid content) of the juice was measured with a digital refractometer (Atago PAL-1, McCormick Fruit Tech., Yakima, Wash.) and expressed as percentage. The pH of grapes was measured by pH meter (Hanna, model HI9321). Titratable acidity (%) was measured by titrimetric method. Skin color characteristics for all replication were measured in 10 grape berries using a colorimeter (Minolta, model CR–400, Tokyo, Japan). Chromatic analyses were conducted in accordance with the CIE (Commission Internationale de l'Eclairage) system of 1976. The L∗, a∗, and b∗ values explain a 3-dimensional color space. The L∗ value is the vertical axis and defines the lightness, and a∗ and b∗ values are perpendicular horizontal axes and define red-to-green and blue to-yellow, respectively. In addition, hue angle (H) and chroma (C∗) can be calculated from a∗ and b∗. H is distributed in the 4 quadrants of the a∗ and b∗ plane, and C∗ is higher the further it is from the origin of the coordinate (Bakker and others 1986). The color measurements of berry samples were computed as the means of each application. The hue angle (h°), calculated as $h^o = \tan^{-1} \times b^*/a^*$ formula. The chroma (C*), obtained as $C^* = (a^{*2}+b^{*2})^{1/2}$, is a measure of chromaticity, which defines the purity or saturation of the color (McGuire, 1992).

Statistical Analysis

The mean measurements were evaluated with complete randomized block design in the study. Cluster characteristics were measured in four vines and 3 replications per treatment. The data were analyzed by two-way analysis of variance (ANOVA) to test main effects and interactions between for treatments. Data analysis was performed using SPSS 16.0 for Windows. Results studied were presented as means and a pooled standard error of mean (SEM). Differences among means were detected using Duncan's multiple range tests at significance levels of ($p<0.05$).

Results and Discussion

The effects of less and excessive leaf removal on cluster characteristics of "Trakya Ilkeren" grape variety are given in Table 1. There were found statistically difference between applications in terms of cluster characteristics. Berry width, berry length, cluster length, cluster width, cluster weight and berry weight values was found significantly higher in less leaf removal. Excessive leaf removal application was decreased all cluster characteristics (Table 1).

Table 1. The effects of less and excessive leaf removal on cluster characteristics of "Trakya Ilkeren" grape variety

Leaf removal levels	Berry width (mm)	Berry length (mm)	Cluster width (cm)	Cluster length (cm)	Cluster weight (g)	Berry weight (g)
No- removal	19.07 b	18.20 b	14.13 ab	23.00	360.17 b	4.46
Less removal	19.46 a	18.54 a	15.20 a	23.00	431.32 a	4.59
Excessive removal	18.96 b	18.12 b	12.97 b	21.23	352.43 b	4.27
SEM	0.053	0.063	0.326	0.463	13.168	0.112

In the research, there were not statistically difference in cluster weight and berry weight in terms of cluster characteristics. Nevertheless, cluster length (23.0 cm) and berry weight (4.59 g) was found in less leaf removal application. The leaf area of the vine has a great influence on the bunch quality (Drissi et al., 2009). According to Winkler et al. (1974), there is no negative effect on the vine making green pruning when leaf picking done at on time and the adequate level. Palliotti et al. (2012) determined that the grapevine leaves removed before flowering were formed smaller berries and thin clusters than no leaf removed. Gomez del Campo et al. (2002) reported that dry matter production with increased leaf area in vines was in the same range as stressed and stress-free conditions.

Table 2. The effects of less and excessive leaf removal on berry characteristics of "Trakya Ilkeren" grape variety

Leaf removal leves	TSS (Brix %)	Titratable acidity (%)	Maturity index	pH
No-removal	15.8 b	0.61 a	26.5 b	3.38
Less removal	16.4 a	0.55 b	29.5 a	3.41
Excessive removal	15.2 b	0.57 b	27.2 ab	3.42
SEM	0.180	0.560	0.566	0.011

The effects of less and excessive leaf removal on berry characteristics of "Trakya Ilkeren" grape variety are given in Table 2. There were found statistically difference between applications in terms of berry characteristics. While total soluble solids and maturity index values was found higher in less leaf removal, titratable acidity (0.61 %) was determined high in no- leaf removal application. While TSS was obtained as 16.4%, maturity index was determined as 29.5 in less leaf removal. The least TSS (15.2%) was measure at excessive leaf removal vines. While pH is close together, the highest pH (3.42) was obtained in excessive leaf removal. Poni et al. (2009), determined that leaf removal application in pre-bloom stage increased berry set, and thus yield per vine. Unlike Alessandrini et al. (2018) stated that, leaf removal in pre- bloom stage is an effective practice for modifying canopy microclimate, vine yield, grape composition and wine quality. Pre- bloom leaf removal enhanced canopy porosity, total soluble solids and reduced bunch thickness. Similarly Pallliotti et al. (2012) in Ciliegiolo wines; found that wines obtained from leaf removal applications in the early period

were better in terms of aroma and taste than wines of non-leaf removal applications. They determined that because of the higher amount of polyphenols and anthocyanins, the structure of the wines were enhanced, resulting in better color intensity.

Table 3. The effects of less and excessive leaf removal on berry skin color of "Trakya Ilkeren" grape variety

Leaf removal leves	*L**	*a**	*b**	*C**	*h°*
No- removal	34.53a	2.06a	-3.70b	4.42a	298.54a
Less removal	33.55b	1.40b	-4.01a	4.40a	293.66b
Excessive removal	33.43b	1.38b	-3.77b	4.11b	290.23b
SEM	0.064	0.036	0.032	0.029	0.535

The effects of less and excessive leaf removal on berry skin color of "Trakya Ilkeren" grape variety are given in Table 3. It was determined that there are statistically difference between applications in terms of berry skin colour. *L**, *a** and *h°* values was highest in no-leaf removal vines (Table 3). *L**, *a**,*C** and *h°* values was measured in no-leaf removal vines as 34.53, 2.06, 4.42 and 298.54, respectively. In the present study, the highest *b** and *C** values was determined in less leaf removal as 4.01 and 4.40, respectively. In the study, less leaf removal was favorable in terms of b* and c* value. Berry skin coloration was found higher in less leaf removal application due to high sun light exposure.

Conclusion

Leaf picking is made intensively for brine leaf production in Turkey. In the present study, excessive leaf picking was led to reduction in cluster and berry weight. Less leaf picking was found more successful in terms of cluster and berry characteristics. However, brine leaf growers attach more importance to leaf production rather than grape. Therefore, they do not give much importance to grape yield. Also, it is necessary to apply leaf removal from fruit set to veraison more ventilation and to benefit from sunlight.

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HERBAL MEDICINE IN HYPERTENSION TREATMENT

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Abstract

World health Organization defined Hypertension (also known as high or raised blood pressure) as a condition in which the blood vessels have persistently raised pressure. Hypertension is an important public health problem worldwide due to the high prevalence and the increased risk of accompanying disease. Hypertension is prevalent all over the world and has many diseases. So, for centuries, there have been medical developments for the treatment of this disease, as well as the use of herbal products. Herbal products used vary from country to country. However, some herbal products in countries are more known and used in hypertension. The aim of this study is to investigate the herbal products used in hypertension in the world and studies on possible side effects.

Keywords: *Herbal medicine, hyperetension.*

Introductıon

Hypertension, also known as high or raised blood pressure, is a condition in which the blood vessels have persistently raised pressure (WHO, 2018). Hypertension is an important public health problem worldwide due to the high prevalence and the increased risk of accompanying disease (Kearney et al., 2004). The prevalence is rapidly increasing due to increased lifetime and increased prevalence of contributory factors such as unhealthy nutrition, obesity and physical inactivity (Cappuccio and Miller, 2016). Untreated hypertension has been shown to increase cardiac failure, coronary heart disease, hemorrhagic and thrombotic stroke, renal failure, peripheral arterial disease, aortic dissection and mortality (TEMD, 2018; Pierdomenico et al., 2009). Raised blood pressure affects 1.13 billion people worldwide (WHO, 2017). There are about 970 million people all over the world suffering from hypertension. The prevalence of hypertension is approximately 2/3 in developing countries and 1/3 in developed countries (WHF, 2012). Low-income countries have a higher prevalence of high blood pressure. In the WHO African region, it is estimated that in many countries more than 30% of adults have high blood pressure and this rate is increasing. Also, the average blood pressure levels in this area are much higher than the global average (WHO, 2015).

BLOOD PRESSURE CATEGORY	SYSTOLIC mm Hg (upper number)		DIASTOLIC mm Hg (lower number)
NORMAL	LESS THAN 120	and	LESS THAN 80
ELEVATED	120 – 129	and	LESS THAN 80
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 1	130 – 139	or	80 – 89
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 2	140 OR HIGHER	or	90 OR HIGHER
HYPERTENSIVE CRISIS (consult your doctor immediately)	HIGHER THAN 180	and/or	HIGHER THAN 120

Figure 1. Blood Pressure Categories according to the American Heart Association (AHA/ASA)

(AHA/ASA, 2017)

Hypertension treatment

Both dietary and lifestyle changes as well as medicines can improve BP control and decrease the risk of associated health complications (Tabassum and Ahmad, 2011).

To Make important changes in the treatment of hypertension: a balanced diet having a low salt rate, regular physical activity, limited alcohol, stress management, healthy weight management, quit smoking, regular drug use, act with the doctor in disease management (AHA/ASA, 2017). Also; The benefits of the DASH diet on blood pressure have been demonstrated in many studies (Moore et al., 2001; Craddick et al., 2003; Salehi-Abargouei et al., 2013; Fung et al., 2008).

Currently, there are various antihypertensive drugs with different mechanisms of action. Although these medicines are effective, many of them may have very annoying side effects. It is generally stated that the use of herbal medicine is high for such reasons. In addition to these studies, the antihypertensive therapeutic effects of local herbs on hypertension have been investigated and some of them have been proven to be effective and some need further investigation.

Herbal medicines in the treatment of hypertension

An herb is a plant or plant part that is used for its odor, flavor or therapeutic properties. Herbal remedies are a kind of food supplements. They are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants. People can choose herbal remedies to protect and improve their health. Most people think that products with a "natural" label are always safe and good (OMICS, 2014). The use of herbal products is regulated by the Food and Drug Administration (FDA) as a special food category and classified as "food supplements" according to the 1994 Dietary Supplement Health and Education Act (DSHEA) (Sellami et al., 2018). Herbal medicines are often used for clinical purposes. Ayurveda is a medical system practiced in India, especially since 5000 years. It includes diet and herbal medicines, while emphasizing body, mind and spirit to prevent and treat the disease (OMICS, 2014).

Traditional herbal medicine use is high among adults in Subh Sahra Africa (38.5%-90%) (Eddouks et al., 2002; Oreagba et al., 2011). In a study conducted in Palestine, this rate was about 59%. In 2000s, 20% of the population over 16 years of age in Germany used "natural medicines", and they stated that they were the first choice for the treatment of minor diseases or disorders. 1.8% of respondents in a study conducted in Turkey reported using herbal medicine due to high blood pressure (Soner et al, 2013).

Herbal products used in the world vary according to the vegetation, cultures and beliefs of the living geography. The most commonly used herbal product in Ghana is bitter leaves grown in the tropical climate, while Tİan Ma Gou Teng Yin believed to be a healing effect in Taiwan, gamat in Malaysia, garlic in Nigeria and Palestine, while lemon and garlic in Turkey (Kes et al., 2016).

It is also noted that the herbal medicines used in some studies may cause adverse reactions (Mahomoodally et al., 2018; Olisa and Oyelola, 2009; Liperoti et al., 2017). Depending on the quality of the product or when used with other medicines, traditional medicines can cause harmful side effects (Soner et al., 2013).

Table 1. The Most Common Herbal Medicines And Possible Side Effects In Hypertension

Herbel Medicines	Yan etkileri
Citrus Limonum (Açıkgöz et al., 2014; Adıbelli et al., 2009; Toprak and Demir, 2007; Biçen et al., 2012; Bahar et al., 2013)	Any side effects haven't been specified.
Allium sativum L (Açıkgöz et al., 2014; James et al., 2018; Liperoti et al., 2017; Bahar et al., 2013; Biçen et al., 2012)	The most common side effects are breath and body odor, heartburn, nausea and vomiting. People treated with antiplatelet or anticoagulant medications should be cautious.
Petroselinum sp. root (Açıkgöz et al., 2014)	Any side effects haven't been specified.
Garcinia cola (Olisa ve Oyelola, 2009; James et al., 2018)	Palpitations and heartburn
A. vera (Olisa and Oyelola, 2009; James et al., 2018)	Skin reactions (contact dermatitis) Kan şekerini düşürebilir (Latif Ahmad at all., 2015)
Matricaria recutita (James at all, 2018)	
Crataegus sp. (Liperoti et al., 2017)	In clinical trials, side effects are rare and light. Nausea, dizziness, gastrointestinal complaints, headache, and palpitation can be observed.

Conclusıon

Although different crops are used in different societies, it is also seen that some herbal products are common. Most hypertensive patients using herbal products do not inform their doctors that they use herbal products (Biçen et al., 2012; Bahar et al., 2013; Mahomoodally et al., 2018). Having the antihypertensive effect of the plants and knowing their toxicity will be useful for safe use (Neamsuvan et al., 2018). Unconsciousness and frequent use of herbal products can lead to disruption of the patient's actual treatment and side effects on these products (Biçen et al., 2012). The Ministry of Health, doctors, herbalists, dietitians and the media have important duties to direct the society in the right direction.

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DETERMINATION OF COMPATIBILITY OF TRITICALE TO DIFFERENT SOIL CONDITIONS AND COMPARISON OF YIELD WITH COOL CLIMATE CEREALS

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Abstract

In the study, 7 triticale, 3 bread wheat, 2 barley, oats and 1 rye varieties were used as material. Experiments were established in four different locations in terms of soil properties as a randomized block design with three replications. Sowing was made by hand on a length of 6 m to 1 m wide (17 cm row spacing and 6 row) 500 seeds per square meter plots. In the study, grain yield and green grass yield and hay yield were investigated. According to the results of average grain yield, ITYN 818, TT 201 and ITYN 819 triticale varieties had the highest grain yield while Presto 2000 and Tatlıcak 97 triticale varieties had the lowest yield. According to the results of two year grain yield, TT 201 and ITYN 818 triticale varieties in Agriculture Faculty location, TT 201, ITYN 818 and TR 2201 triticale varieties in Ortaca location (water holding land), TT 201, ITYN 818 and ITYN 819 triticales in Kılavuzlu location (arid and slope land) and TT 201 and Tatlıcak 97 varieties of triticale in Selçuklu location gave the highest yield. According to average green grass yield, Tatlıcak 97 and Karma 2000 triticale varieties had the highest, while TT 201 and ITYN 819 triticale varieties the lowest yield values. In terms of the average hay yield, the ITYN 819, Karma 2000 and TR 2201 were found to have the highest value while the TT 201, Presto 2000 and Tatlıcak 97 triticale varieties gave the lowest values.

Key words : *triticale, line, different soil conditions, grain yield, forage yield and hay yield*

Introduction

Triticale (X*Triticosecale*Wittmack) is an annual C3 cool-season grass within the Poaceae (Graminae) family (Kavanagh and Hall 2015). Triticale is an amphiploid species stably baring the genomes of wheat (*Triticum* sp.) and rye (*Secale*sp.) (Ammar et al. 2004). Triticale is an established small grain cereal crop that combines the productivity of wheat with the hardiness of rye. Triticale may be octoploid (2N = 56; AABBDDRR) or hexaploid (2N = 42; AABBRR) depending on which wheat is crossed with rye (RR), i.e., common wheat (AABBDD) or durum wheat (AABB). The large genome of octoploid is less stable and with the exception of Asia cultivars; most cropping triticale is hexaploid type (Ammar et al. 2004). As triticale cultivation areas in the world are analyzed considering the last 10 years' data, it can be determined that while it is approximately 3.2 million ha in 2002, it has raised to 4.16 million ha in 2016 with 30% increase (FAOSTAT, 2017). 80% of the world's triticale cultivation area is used for winter, and 20% for summer. While Poland has the widest cultivation area among the countries producing triticale with 1.4 million ha, Germany follows it with 396 thousand ha, and France with 334 thousand ha (FAOSTAT, 2017). For the fact that the ecological conditions in our country are quite suitable for other crops and the alternative products are easily cultivated, triticale production could not reach to the desired level. In our country since 2017, triticale has been sown on 37.6 thousand ha area and 125 thousand tons of grain product has been obtained (FAOSTAT, 2017).

The average grain yield of triticale (332 kg/da) in our country is higher than the average grain yield of wheat (270 kg/da), rye (263 kg/da), barley (248 kg/da) and oat (226 kg/da). Trakya region has quite suitable characteristics considering the cool climate grain production. The average yield of the cool climate grains produced in the region is much higher than the

country average. Sowing, production and yields of cool climate grains cultivated in our country and Trakya region are presented (TÜİK, 2017).

When the average grain yield of cool climate grains cultivated in our country is examined, it is obvious that triticale has higher yield with 332 kg/da compared to other cool climate grains. Korkut et al. (2009) in their study which is conducted with 9 triticale genotypes, 3 bread wheat cultivars, 3 durum wheat cultivars, 3 barley cultivars and 1 rye in three years and 3 different locations of Trakya region, revealed that triticale genotypes have average grain yield 30 kg/da higher than bread wheat cultivars, 95 kg/da higher than barley cultivars, 63 kg/da higher than durum wheat and 121 kg/da higher than rye cultivar. They also found out that the fodder yield of triticale genotypes is 523 and 106 kg/da higher than bread wheat cultivars, 1183 and 381 kg/da higher than durum wheat, 1749 and 409 kg/da higher than barley cultivars. Triticale is the most important candidate plant against both biotic stress factors such as disease and pests, and abiotic stress factors such as drought, high temperature and cold. The study was conducted at four locations as Ortaca village (water holding), Klavuzlu village (sloped), Selcuklu village (productive land) and Agricultural Faculty experimental area. The aim of the study is to compare triticale with other cool climate cereals in terms of grain yield and grass yield in different soil structures.

Material and method

The study was conducted by using 7 triticale, 3 bread wheat, 2 barley, 2 oat and 1 rye type in 4 different locations in the first year considering the soil characteristics, and 3 different locations in the second year. Some agricultural features of 7 triticale (Karma 2000, Tatlıcak 97, Presto, ITYN 818, ITYN 819, TT 201 and TR 2201), 3 bread wheat (Flamura-85, Pehlivan and Yunak), 2 barley (Sladoran and Epona), 2 oat (Yeşilköy 330 and TT 301) and 1 rye (Aslım-95) cultivars are presented in the Table 1.

Table 1. Genotypes used in the study

Genotypes	Species	Maturing	Institution/origin
Flamura-85	Bread wheat	Medium	Tareks-Ankara
Pehlivan	Bread wheat	Medium	Trakya Tar. Res. Ins.
Yunak	Bread wheat	Early	Bit. ve Gen. Kay. Ens. Sadova – Bulg.
Sladoran	2 rowed barley	Early	Trakya Tar. Res. Ins.
Epona	6 rowed barley	Early	FranceNationalAgricultureRes. Ins.
Aslım 95	Rye	Early	BahriDağdaşUlus.Tar. Res. Ins.
TT 301	Oat	Early	Trakya TarımVe Vet. Tic. Ltd. Şti
Yeşilköy 330	Oat	Early	Trakya Tar. Araş. Enst.
Tatlıcak-97	Triticale	Early	BahriDağdaşUlus.Tar.Araş. Ens.
Karma-2000	Triticale	Early	Anadolu Tar. Res. Ins.
Presto-2000	Triticale	Early	Anadolu Tar. Res. Ins.
ITYN 818	Triticale	Early	Namık Kemal University
ITYN 819	Triticale	Early	Namık Kemal University
TT 201	Triticale	Early	Trakya TarımVe Vet. Tic. Ltd. Şti
TR 2201	Triticale	Early	Trakya TarımVe Vet. Tic. Ltd. Şti

The study was conducted according to the randomized block experiment design with 3 replicates. Sowing was made manually by using marker to the parcels 6 m length and 1 m width (17 cm row distance and 6 rows) as 500 seeds in per square meter. Some visuals of the trial field are given in Figure 1. Four kg/da pure nitrogen (20-20-0) for per decare with sowing, compound fertilizer, 6,9 kg urea (46%) as pure nitrogen in tillering-bolting period, and 5 kg ammonium nitrate fertilizer (33%) as pure nitrogen before heading were given.

Thus, 15.90 kg nitrogen (N) and 4 kg (P2O5) were applied per decare during vegetation. Pesticides were applied against grass weeds in tillering period.

Picture 1. Some images from trial areas

Grain yield: 5 m^2 parcel field was obtained by disposing of the plants at 0.50 m from the beginning and the end as edge effect in the parcels planted as 6 rows, and the grain yield values gained from these parcels were found as kilograms by transforming them into the yield per decare. Fresh yield: Fresh yield was found as gram by reaping the plants in 1.0 square meter in each parcel from 8-10 cm height in their milk stage, and this value was transformed into kg/da later. Hay yield: The dry matter ratio was found as gram by drying fresh plant samples taken from each parcel at 105 C^o for 24 hours, and this value was transformed into kg/da later.

Results and Discussion

According to the results in 2008 and 2009 years, location and genotypes on grain yield is considered as significant statistically (Table 2). While the highest grain yield was obtained from Selçuklu location experiment field with 583.00 kg, the lowest values were observed in Kılavuzlu location which is inclined and infertile with 394.62 kg.

Table 2. Average grain yield and significance groups at 4 different locations during 2 years

Locations	Grain Yield	Locations	Grain Yield
Selçuklu	583.00 a	Faculty	522.933 a
Faculty	531.889 ab	Selçuklu	479.533 b
Ortaca	505.578 b	Klavuzlu	437.356 c
Klavuzlu	394.622 c		
HKO	3146.770		228.465

The highest grain yield in 2009-2010 growing period was gained from faculty location with 522.93 kg/da, and the lowest results were observed in Kılavuzlu location with 437.36 kg. The results reveal that the soil structure of the cultivation location is very effective in grain yield.

The grain yield of 15 genotypes in two cultivation years and significance groups are given in Table 3. The grain yield values of 15 genotypes in 2008-2009 cultivation period change between 646.08-255.00 kg. While the highest grain yield was obtained from TT 201 triticale genotype with 646.08 kg, it was followed by ITYN 818 with 644.167 kg, TR 2201 with 637.75 kg, ITYN 819 with 608.33 kg and standard Tatlıcak 97 with 608.00 kg. Bread wheat, barley, rye and oat are not in the same statistical group with triticale. The lowest grain yield was obtained from Yeşilköy oat cultivar with 255.00 kg, and it was followed by TT 301 with 275.417 kg and Aslım rye cultivar with 362.58 kg.

Table 3. The significance groups for the grain yield in 2009 and 2010

2008-2009		2009-2010	
Genotypes	Grain yield	Genotypes	Grain yield
TT 201	646.083 a	ITYN 818	546.667 a
ITYN 818	644.167 a	ITYN 819	543.000 ab
TR 2201	637.750 a	TT 201	538.444 abc
ITYN 819	608.333 ab	Karma 2000	524.667 bcd
Tatlıcak 97	608.000 ab	TR 2201	522.000 cde
Karma 2000	575.167 bc	Tatlıcak 97	516.778 def
F – 85	535.833 cd	Pehlivan	509.556 d-g
Pehlivan	525.583 cd	Yunak	506.333 efg
Presto	517.500 d	F – 85	500.444 fg
Yunak	510.000 d	Presto	495.222 g
Sladoran	429.500 e	Epona	430.889 h
Epona	425.667 e	TT 301	417.556 hi
Aslım-95	362.583 f	Aslım-95	414.000 hi
TT 301	275.417 g	Sladoran	410.444 i
Yeşilköy 330	255.000 g	Yeşilköy 330	323.111 j
HKO	3146.770		228.465

The grain yield values in 2009-2010 growing year changed between 546.67-323.11 kg. The highest grain yield was obtained from ITYN 818 genotype with 564.67 kg, and it was followed by ITYN 819 with 543.00 kg and TT 201 genotype with 538.24 kg.

Table 4.Significance results of location x genotype interaction for grain yield in 2008-2009

Faculty	Avarega		Ortaca	Avarega		Kılavuzlu	Avarega		Selçuklu	Avarega	
TT 201	686.67	a	ITYN 818	683.33	a	ITYN 819	549.00	a	Tatlıcak 97	780.00	a
Tatlıcak 97	680.00	a	TR 2201	661.00	a	TT 201	538.67	a	TT 201	726.67	ab
ITYN 818	680.00	a	TT 201	632.33	ab	ITYN 818	533.33	a	TR 2201	706.67	abc
TR 2201	673.33	ab	ITYN 819	611.00	ab	TR 2201	510.00	ab	Karma 2000	680.00	a-d
ITYN 819	633.33	abc	Tatlıcak 97	605.67	ab	Karma 2000	427.33	abc	ITYN 818	680.00	a-d
F – 85	626.67	abc	Karma2000	583.33	abc	Presto	400.00	bc	F – 85	666.67	a-d
Karma2000	610.00	abc	Pehlivan	578.00	a-d	Sladoran	389.00	c	Yunak	646.67	b-e
Pehlivan	573.33	abc	Presto	543.33	b-e	F – 85	383.33	c	ITYN 819	640.00	b-f
Yunak	560.00	bc	Yunak	489.00	c-f	Tatlıcak 97	366.33	c	Pehlivan	606.67	c-f
Presto	546.67	c	F – 85	466.67	d-g	Pehlivan	344.33	cd	Presto	580.00	def
Sladoran	416.67	d	Epona	444.33	efg	Yunak	344.33	cd	Sladoran	540.00	ef
Epona	408.33	d	Aslım-95	383.33	fg	Aslım-95	333.67	cd	Epona	533.33	f
Aslım-95	366.67	de	Sladoran	372.33	gh	Epona	316.67	cd	Aslım-95	366.67	g
TT 301	275	ef	TT 301	268.33	h	TT 301	250.00	d	TT 301	308.33	g
Yeşilköy330	241.67	f	Yeşilköy330	261.67	h	Yeşilköy330	233.33	d	Yeşilköy330	283.33	g

The lowest grain yield was obtained from Yelişköy oat cultivar as in the first year, and it was followed by Sladoran barley and Aslım rye. The average values and significances of location x genotype interaction are presented in Table 4.

In the first year, location x genotype interaction was found as significant statistically. In the first year, the highest grain yield was gained from TT 201, Tatlıcak 97, TR 2201 and ITYN 818 genotypes in the Faculty of Agriculture location, ITYN 818TTR 2201, TT 201, ITYN 819 and Tatlıcak 97 in Ortaca location, ITYN 819, TT 201, ITYN 818 and TT 2001 in Kılavuzlu location, Tatlıcak 97, TT 201, TR 2201, Karma, ITYN 818 in Selçuklu location.

Table 5. The average values and significances of location x genotype interaction for grain yield in 2009-2010

Faculty	Avarega		Ortaca	Avarega		Kılavuzlu	Avarega	
TT 201	686.67	a	ITYN 818	683.33	a	ITYN 819	549.00	a
Tatlıcak 97	680.00	a	TR 2201	661.00	a	TT 201	538.67	a
ITYN 818	680.00	a	TT 201	632.33	ab	ITYN 818	533.33	a
TR 2201	673.33	ab	ITYN 819	611.00	ab	TR 2201	510.00	ab
ITYN 819	633.33	abc	Tatlıcak 97	605.67	ab	Karma 2000	427.33	abc
F – 85	626.67	abc	Karma2000	583.33	abc	Presto	400.00	bc
Karma2000	610.00	abc	Pehlivan	578.00	a-d	Sladoran	389.00	c
Pehlivan	573.33	abc	Presto	543.33	b-e	F – 85	383.33	c
Yunak	560.00	bc	Yunak	489.00	c-f	Tatlıcak 97	366.33	c
Presto	546.67	c	F – 85	466.67	d-g	Pehlivan	344.33	cd
Sladoran	416.67	d	Epona	444.33	efg	Yunak	344.33	cd
Epona	408.33	d	Aslım-95	383.33	fg	Aslım-95	333.67	cd
Aslım-95	366.67	de	Sladoran	372.33	gh	Epona	316.67	cd
TT 301	275	ef	TT 301	268.33	h	TT 301	250.00	d
Yeşilköy330	241.67	f	Yeşilköy330	261.67	h	Yeşilköy330	233.33	d

The data reveal that the highest grain yield values were obtained from triticale genotypes in three locations. The lowest grain yield was observed in Yeşilköy and TT 301 oat in 4 locations. These genotypes were followed by Aslım 95 rye and other barley genotypes. The average values and significances of location x genotype interaction in 2009-2010 cultivation period are presented in Table 5. Location x genotype interaction in 2009-2010 growing year was found as significant statistically. The highest grain yield was obtained in ITYN 818, ITYN 819, Karma 2000 and TR 201 genotypes at Faculty location, ITYN 818, ITYN 819, and TT 201 genotypes at Kılavuzlu location, ITYN 819, ITYN 818, Tatlıcak 97, Yunak bread wheat and TT 201 genotypes at Selçuklu location. The lowest grain yield was observed in Yeşilköy cultivar at 3 three locations, and it was followed by rye and barley genotypes. In the second year, triticale genotypes gave the highest grain yield values in those three locations.

Green Grass and Hay Yield

Fresh and hay yields were examined in the obtained data in Selçuklu location in both two years. As the fresh yield was examined, it was observed that in the first year 3.88 ton of yield and in the second year 3.36 ton of yield were gained in Table 6.

Table 6. Fresh and hay yields in both years and significance groups

Year	Fresh yield
2008-2009 years	3.878 a
2009-2010 years	3.364 b
HKO	0.040

Fresh yields of the genotypes changed between 4.37-2.43 ton/da in Table 7. The highest fresh yield was obtained from TT 2201 genotype with 4.37 ton, and it was followed by Tatlıcak 97 and Karma triticale genotypes with 4.20 ton.

Table 7. Fresh yield obtained from fifteen genotypes and significance groups

Genotypes	Fresh yields
TR 2201	4.367 a
Tatlıcak 97	4.200 ab
Karma 2000	4.200 ab
Yeşilköy 330	4.167 ab
ITYN 818	4.083 ab
TT 301	4.017 bc
ITYN 819	4.017 bc
TT 201	3.733 cd
Presto	3.550 de
Pehlivan	3.350 ef
Epona	3.167 fg
Yunak	3.083 fg
Aslım-95	3.050 g
Sladoran	2.900 g
F – 85	2.433 h
HKO	0.040

The lowest fresh yield was observed in bread wheat Flamura 85 with 2.43 ton. It was followed by Sladoran barley cultivar with 2.90 ton and Aslım rye cultivar with 3.05 ton. Average hay yields of the fifteen genotypes in both years were found as significant considering years and genotypes in Table 8.

Table 8. Average hay yield values and significance groups

Year	Hay yield
2008-2009	1.271 a
2009-2010	1.011 b
HKO	0.012

When the hay yields are examined according to years, it can be observed that while the hay yield is 1.27 ton/da in the first year, it becomes 1.011 ton in second year. The hay yield of the genotypes changes between 1.50-0.867 ton in Table 9.

Table 9. Hay yields from fifteen genotypes and significance groups

Genotypes	Hay yield (ton/da)
ITYN 819	1.500 a
Karma 2000	1.433 ab
TR 2201	1.400 ab
ITYN 818	1.317 bc
Tatlıcak 97	1.217 cd
Aslım-95	1.183 cde
Pehlivan	1.150 def
Yunak	1.100 d-g
Yeşilköy 330	1.033 e-h

Presto	1.017	f-i
Epona	1.017	f-i
Sladoran	1.000	f-i
TT 201	0.950	ghi
TT 301	0.933	hi
F – 85	0.867	i
HKO	0.012	

The highest hay yield was gained from ITYN 819, Karma and TR 2201 triticale genotypes with 1.50, 1.43 and 1.40 ton. The lowest hay yield was observed in Flamura 85 bread wheat cultivar with 0.87 ton, and it was followed by TT 301 and TT 201 oat cultivars with 0.93 and 0.95 ton. When the data are examined, it is revealed that triticale genotypes have higher fresh and hay yield compared to other cool climate cereals.

Conclusion

According to the results of the study which was conducted in at 4 different locations in the first year and 3 different locations in the second year, triticale genotypes gave higher grain yield, fresh and hay yield values compared to other cool climate cereals. While triticale genotypes gave higher values in infertile soil conditions, they have higher values in fertile soil conditions as well compared to other cool climate cereals. These results reveal that triticale should be preferred in every kind of soil conditions especially in the regions in which culture animal husbandry is densely made.

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PLANT AND PRODUCTIVITY CHARACTERISTICS OF SOME POMEGRANATE GENOTYPES IN GAZIANTEP CONDITIONS (TURKEY)

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Abstract

In the study, plant and productivities of six pomegranate varieties and types ('Kışnarı', 'Kirli Hanım', 'Nuz Ekşi', 'Çekirdeksiz', 'Hicaz' and 'Oğuzeli Çekirdeksiz'') were evaluated. The experiment was designed according to completely randomized design with five replications having single tree in each replication. In this study, the plant specifications such as tendency suckers from the base, branching condition, spiny situation, canopy width and height, and productivity such as yield per plant (kg/plant), yield per unit canopy volume(kg/m^3), yield per shoot length (kg/cm), yield per canopy geometric projection (kg/m_2) were determined for pomegranate varieties and types. Fifteen and more suckers from the base were observed for all varieties and types. Kışnarı and Kirli Hanım varieties had often branching while others had mid often branching. Hicaz and Kirli Hanım had medium spiny while the others had more spines. Canopy width and heights of Kışnarı and Kirli Hanım cultivars were higher than those of other genotypes. Kışnarı, Nuz Ekşi and Hicaz varieties had more yield per plant (14.8 kg,13.7 kg ve 13.3 kg, respectively) than the others. Oğuzeli çekirdeksiz and Nuz ekşi had higher yield per canopy volume (5.85 kg/m^3 and 4.81 kg/m^3 , respectively) than the others while Nuz Ekçi and Hicaz had higher value than the other types and varieties for yield per canopy geometric projection (4.74 kg/m2 and 4.49 kg/m^2, respectively).

Keywords: *Punica granatum, suckers, spiny situation, yield.*

Introduction

The pomegranate belongs to the *Punica* genus of the *Punicaceae* family, whose better known species is the *Punica granatum L*. The pomegranate is one of the oldest known species of fruit and its cultivation dates back to 3000 BC. It is native to the regions between the South Caucasus, Iran, Afghanistan, South Asia, Western Asia, Anatolia and the Mediterranean (Vardin and Abbasoğlu, 2004). The pomegranate is one of the fruits delivering the highest harvest per unit area. It is easy and cheap to produce its saplings and keep it maintained. It delivers produce shortly after being planted. Turkey lies within the pomegranate's native region. It has very favorable conditions enabling its cultivation. This explains the ever-increasing production of pomegranate (Özkan, 1993). The total production amounted to about 2.5 million metric tons, according to the USAID Report. The same data showed that India is the world's biggest pomegranate producer (1,773.66 million metric tons for 2015; Anonymous, 2017) followed by Iran (about 1,000.00 million metric tons for 2015; Anonymous, 2015a). Among other countries engaging in pomegranate production are Turkey (465,200 metric tons for 2016; TÜİK, 2018), the USA (the total production amounted to 282.532 metric tons from 2012-2013; Anonymous 2015b), Spain, Iraq, Afghanistan, Azerbaijan, Uzbekistan, Israel, Tunisia, Morocco, China, Cyprus, Egypt, Peru, Chile, Argentina and South Africa (Kahramanoğlu and Usanmaz, 2016). Turkey is home to eleven agricultural regions where pomegranate cultivation is carried out. An examination into pomegranate cultivation based on these areas shows that the highest amount of production is carried out in the Mediterranean, the Aegean, and Southeastern Anatolia.

This study aims to define the productive and botanical properties of some of the pomegranate types and varieties grown in Gaziantep, Turkey.

Materials and Methods

Material

The study was carried out at a pomegranate orchard containing six different types and varieties of pomegranates belonging to the Pistachio Research Institute (Gaziantep/Turkey) between 2011and 2012. The experiment orchard is 705 m above sea level and 38^{o} 57' north longitude. The Kış Narı, Kirli Hanım, Nuz Ekşi, Çekirdeksiz VI, Hicaznar and Oğuzeli Çekirdeksiz pomegranates in the experiment orchard were planted at intervals of 1x5 m in 2006. The plants were irrigated by drip irrigation since their transplantation. Pomegranate plants were formed as shrubs form with 3-4 trunks. The experiment was designed according to completely randomized design with five replications having single tree in each replication.

Fruit Productivity Elements of the Varieties

With the yield (kg) per tree in all varieties in mind along with productivity, the fruit count was calculated by counting every single fruit seen on the tree in July and considering the yield per crown unit volume (Westwood, 1988). The yield per canopy projection area was designated using the formula πr^2.

Plant Properties

Suckers springing from the root collar were assessed based on a scale of 1 to 5: (Mars, 1995).

Suckering tendency	Scale Value
Extremely low	1
Low	2
Medium	3
High	4
Extremely high	5

Level of branching is designated based on a scale of 1 to 3:

Level of Branching	Scale Value
Frequent	1
Mean	2
Rare	3

Thorniness is designated based on a scale of 1 to 5:

Thorniness	Scale Value
Without thorns	1
Few	2
Mean	3
Many	4
Too Many	5

Result and Discussion

The findings related to the yield elements of the pomegranate types and varieties are given in Table 1.

Table1. Fruit yield components of genotypes

Genotype	Yield per tree (kg/tree)	Yield per shoot length (kg/cm)	Yield per unit canopy volume (kg/m^3)	Yield per canopy geometric projection (kg/m^2)	No. of fruits/tree (piece/tree)	
					July Count	Harvest Count
Kış Narı	14.82 a[(x)]	2.01 ab	2.61 bc	4.04 a	66 a	69 a
Hicaznar	13.33 ab	3.12 a	3.20 bc	4.49 a	61 ab	39 ab
Çekirdeksiz VI	7.15 b	2.14 ab	3.50 abc	3.98 a	18 c	22 b
Oğuzeli Çekirdeksiz	6.66 b	1.08 b	5.85 a	3.17 a	32 abc	49 ab
Kirli Hanım	9.22 ab	1.15 b	1.53 c	2.74 a	48 abc	50 ab
Nuz Ekşi	13.69 ab	1.10 b	4.81 ab	4.74 a	29 bc	27 b
Significance	*	*	*	NS[(y)]	*	*

[(x)]Means within a column followed by different letter are significantly at the 5% level by Tukey's HSD test.

(y): No significant.

Yield

The Kış Narı provided the highest yield per tree (14.82 kg/tree). This was followed by the Hicaznar (13.33 kg/tree) and Nuz Ekşi (13.69 kg/tree) varieties. The lowest yield was delivered by the Oğuzeli Çekirdeksiz (6.66 kg/tree) and Çekirdeksiz VI (7.15 kg/tree) varieties. Difference between the yield of the types and varieties per tree was found to be statistically significant at %5 level. A selection study carried out to determine the pomegranate varieties appropriate for the Aegean region designated the average yield of 13 pomegranate types as 10-29 kg/tree (Ercan et al., 1991). Yılmaz (2005) determined the average yield values as being between 6.16 kg/tree and 10.6 kg/tree in a trial carried out on seven pomegranate varieties. Among the other determinations are Hernandez *et al*. (2012), 26.11kg/tree in Spain; Korkmaz (2013), 29.20 kg/tree in Muğla; El-Sayed *et al*. (2014), 21.83 - 40.50 kg/tree in Egypt; Parvizi *et al*. (2014), 73.6 kg in Iran; Ibrahim and El-Samed (2009), 23.35 kg/tree in Egypt; Bakeer (2016), 39.64 kg/tree in Egypt; Davarpanah *et al*. (2016), 16.00 kg/tree in Iran; Selahvarzi *et al*. (2017), 24.88 kg/tree in Iran. Our research findings are consistent with the literature values.

Hicaznar cultivar had the highest value (3.12 kg/cm) in terms of the yield per shoot length . This was followed by the Çekirdeksiz VI (2.14 kg/cm) and Kış Narı (2.01 kg/cm) varieties. Three other genotypes were obtained 1kg/cm yield. The yield per unit canopy volume of the types and varieties varied between the Oğuzeli Çekirdeksiz (5.85 kg/m^3) and Kirli Hanım (1.53 kg/m^3). In Egypt, Ibrahim and El-Samed (2009) found the Manfalouty pomegranate variety's yield per unit canopy volume to be 3.00 kg/m^3 and in Iran, Selahvarzi *et al*. (2017) found the Shahvar variety's yield per unit canopy volume to be 2.88 kg/m^3 as the average of various applications. Difference between the varieties in terms of both the yield per shoot length and the yield per unit canopy volume was found to be statistically significant at %5 level. The yield of the types and varieties per canopy geometric projection was found to be between 4.74 kg/m^2 for the Nuz Ekşi and 2.74 kg/m^2 for the Kirli Hanım. The difference between yield per canopy geometric projection was not found to be statistically significant. Onur and Tibet (1991) reported that yield per m^2 of canopy projection of the Hicaznar variety in Antalya conditions is 22.78 kg/m^2. Zhu *et al*. (2005) stated in a study carried out in China

that the Hongyushizi variety's yield per canopy geometric projection was 3.31 kg/cm^2. Our research study findings are consistent with the literature values.

Number of Fruits/ Tree

Fruit counting was carried out in two different periods for different types and varieties. The counting performed in July revealed that the Kış Narı produced the highest number of fruits (66 pcs/plant) and the Çekirdeksiz VI produced the lowest number of fruits (18 pcs/plant). The counting performed in the harvest season also revealed a similar result with the Kış Narı giving the highest value (69 pcs/plant) and the Çekirdeksiz VI giving the lowest (22 pcs/plant). Difference shown by the varieties in both of the counting periods was found to be statistically significant at 5% level. Hernandez et al. (2012) stated that the number of fruits per tree was 86.04 pcs/tree in their study carried out in Spain; Bakeer (2016) stated that the number of fruits per tree for a 13-year-old Manfalouty variety in Egypt was 100.85 pcs/tree; Davarpanah *et al*. (2016) stated that the number of fruits per tree for an 8-year-old Ardestani pomegranate variety in Iran was 57.4 pcs/tree; Marathe *et al*. (2017) gave the value of 18.9 pcs/tree for the Bhagwa variety in India; and Selahvarzi *et al*. (2017) provided the average value of 101.17 pcs/tree for the Shahvar variety in Iran. It can be stated that the average values we obtained are within the data ranges stated by our country's researchers.

Plant Properties

The findings related to the botanical properties of the genotypes can be found in Table 2.

Table 2. Tree properties of genotypes

Genotype	Tendency to sucker	Branching state	Thorn volume	Width of canopy (m)	Canopy height (m)
Kış Narı	Too many (5)	Frequent (1)	Many (4)	2.19 a$^{(x)}$	2.23 a
Hicaznar	Too many (5)	Mean (2)	Mean (3)	2.09 ab	2.19 a
Çekirdeksiz VI	Too many (5)	Mean (2)	Many (4)	1.51 c	1.53 c
Oğuzeli Çekirdeksiz	Too many (5)	Mean (2)	Many (4)	1.48 c	1.69 bc
Kirli Hanım	Too many (5)	Frequent (1)	Mean (3)	2.41 a	2.12 a
Nuz Ekşi	Too many (5)	Mean (2)	Many (4)	1.65 bc	2.05 ab

$^{(x)}$Means within a column followed by different letter are significantly at the 5% level by Tukey's HSD test.

It was determined that all of the genotypes have too many tendency to bottom branches (15 and above). It was observed that the Kirli Hanım and Kış Narı have a frequent branching structure, while the other varieties have a branching structure of medium frequency. It was also found that the Kış Narı, Çekirdeksiz VI, Oğuzeli Çekirdeksiz and Nuz Ekşi are too thorny while the Hicaznar and Kirli Hanım have medium thorniness. In a study on Hicaznarin tree properties in Antalya(Turkey) conditions; Onur and Tibet(1991) determined base shoot growth as being too high, level of branching as being medium, and thorniness as being of mid-frequent structure. Our study is also consistent with such data.

Canopy width and heights

The canopy height of the genotypes was determined as being between 2.41 m for the Kirli Hanım and 1.51 m for the Çekirdeksiz VI. The width of canopy values were measured between 2.23 m(for the Kış Narı) and 1.53 m(for the Çekirdeksiz VI). Difference shown by the varieties in terms of both the width of canopy width and height was found to be statistically significant at 5% level. Onur and Tibet (1991) determined the four-year average value of canopy height in Antalya as being 2.43 m and width of canopy as being 2.36 m; Abdelaty and Gamal (2009) determined the tree canopy height of the Manfalouty variety in Egypt as being 2.45 m; and Bakeer (2016) found the tree canopy height for the 13-year-old Manfalouty variety in Egypt to be 3.18 m. In Egypt, Ibrahim and El-Samed (2009) stated the Manfalouty pomegranate variety's width of canopy as being 2.45 m. Our study also generated similar results.

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PHENOLOGICAL AND POMOLOGICAL CHARACTERISTICS OF VARIOUS POMEGRANATE GENOTYPES IN GAZIANTEP ECOLOGICAL CONDITIONS

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Abstract

This study was carried out at the pomegranate experimental orchard of Pistachio Research Institute in Gaziantep, Turkey. The objective of the study was to determine phenological and pomological characteristics of various pomegranate cultivars and types. Altitude of the experimental site was 705 m a.s.l. (38° 57' N). In the experiment, six pomegranate types and cultivars ('Kışnarı', 'KirliHanım', 'Nuzekşi', 'Çekirdeksiz', 'Hicaz', 'Oğuzeli Çekirdeksiz') were used. The experimental cultivars were planted on 1 x 5 m in 2006 as one-year-old plants. The plants were irrigated by drip irrigation since their transplantation. Pomegranate plants were formed as shrubs form with 3-4 trunks. The experiment was designed according to completely randomized design with five replications having single tree in each replication. In the study, some phenological observations (first flowering, full flowering and as well as fruit maturation date) fruit pomological analyses [such as fruit weight (g), fruit size (mm), seed weight (g), soluble solid (SS) (%), titratable acidity (%), pH, and fruit cracking ratio (%)] were determined. Fruits were harvested between 5 October and 11 November. The cultivars and types had a range of 168-453 g for fruit weight, 52-61% percent arils, 30-49% juice rate, 13.8-16.5% total soluble solids (TSS), 0.21-1.23% titratable acidity and 17-56% cracking rate.

Keywords: *Punica granatum, fruit characteristics, phenological stage.*

Introduction

The pomegranate is a tropical and sub-tropical plant grown in the Mediterranean, the Aegean and Southeastern Anatolia, up to 1000 m above sea level as a characteristic plant of the Mediterranean climate (Kurt and Şahin, 2013). Turkey had large swathes of land where pomegranates can be cultivated, adding that there was now an even greater capacity in Southeastern Anatolia with the completion of the GAP project and the increase of irrigation facilities. There is no precise data on pomegranate production areas and production quantities in the world. According to the USAID Report, total pomegranate production in 2008 equaled around 2.5 million metric tons (Kahramanoğlu and Usanmaz, 2016). Today, pomegranates are grown in the USA, Afghanistan, China, Morocco, Palestine, India, Iraq, Iran, Israel, Italy, Cyprus, Egypt, Syria, Saudi Arabia, Thailand, Tunisia, Turkey and some other countries. The global pomegranate trade is conducted on a small scale. Spain, Tunisia and Turkey are exporters of pomegranates. Pomegranate production in Turkey was 465,200 metric tons in 2016. Pomegranate cultivation is most widespread in the Mediterranean Region, followed by the Aegean and Southeastern Anatolia (TUİK, 2018). The aim of the study is to determine the phenological and pomological properties of the pomegranate varieties and types included in the experiment.

Materials and Methods

The study was carried out at a pomegranate orchard containing six different types and varieties of pomegranates belonging to the Pistachio Research Institute (Gaziantep/Turkey) between 2011 and 2012. The Kış Narı, Kirli Hanım, Nuz Ekşi, Çekirdeksiz VI, Hicaznar and Oğuzeli Çekirdeksiz pomegranate cultivars in the experiment orchard were planted at

intervals of 1x5 m in 2006. The plants were irrigated by drip irrigation since their transplantation. Pomegranate plants were formed as shrubs form with 3-4 trunks. The experiment was designed according to completely randomized design with five replications having single tree in each replication. In the study, some phenological observations (first flowering, full flowering and as well as fruit maturation date) fruit pomological analyses [such as fruit weight (g), fruit size (mm), seed weight (g), soluble solid (SS) (%), titratable acidity (%), pH, and fruit cracking ratio (%)] were determined. Flowering observations for varieties and types were made according to Özgüven (1997).

For the pomological analyzes, a total of 15 fruits were randomly selected from each variety at each maturity stage, and the analyzes were conducted according to completely randomized design with three replications having five fruits in each replication. The average fruit weight was determined by weighing the fruits one by one on a digital scale sensitive to 0.01g and classified into five categories [Very Small (<100g); Small (100-200g); Medium (200-300g); Large (300-400g); Very Large (> 400g)] (Mars, 1995). The fruit width, fruit length, and the skin thickness were measured using a Mitutoyo digital caliper sensitive to 0.01mm.

Total Soluble Solids (TSS %): The pomegranates were separated into four pieces with a steel knife and manually husked so as not to damage the texture of the arils. The juice from the arils was obtained by filtration through a cheesecloth. The total soluble solids was measured from this filtered juice using an Atago branded digital refractometer (NAR-1T, Atago, Japan). The results were expressed in percentages.

Titratable Acidity (%): Ten ml were taken from the above juice samples and 90 ml of distilled water was added to the 100 ml test tube. Acidity percentage was calculated in terms of citric acid type from the value determined by titration of this solution with 0.1N NaOH, according to Karacali (1990). Titration was determined with a Hanna branded pH meter (pH 211 RI, USA).

Aril Juice Content (%) and Skin Water Content (%): After the arils and skins of 10 cracked and uncracked fruits from each variety were separated after the harvest, they were dried in a drying oven until reaching their fixed weight and the obtained dry aril and skin weights were compared to the weights of the fresh arils and peels to determine the aril juice and peel water content (Yılmaz, 2005).

Results and Discussion

Phenological Observations

The phenological analysis results of the genotypes in the trial are given in Table 1. As seen in the Table 1, differences were observed between genotypes. Wood bud burst started between 21-23, March. Similarly, wood buds were observed to sprout between 28-30 March. The date of the first appearance of flower buds was between 25 April and 1 May. The first flowering was occurred between 19-30 May. Full blooming of the genotypes occurred between May 31 and June 7. When reviewed in general, both in terms of the appearance and sprouting dates of the buds, as well as the first flowering and the full flowering dates, the Hicaznar and Kirli Hanım were found to be early bloomers in comparison to other genotypes.

The sprouting of the Hicaznar buds was found to start on March 2, the first appearance of the flower buds on April 9, the first flowering on May 5, maximum flowering on May 24, fruit ripening on October 15, yellowing of the leaves on November 25 and the falling of the leaves on December 13, under Antalya ecological condition, Turkey (Onur and Tibet, 1991). The difference in timings is due to the different ecological characteristics of the regions.

Pomological Analyzes

The 2011 and 2012 results from the pomological analyzes of the varieties and types included in the experiment are are given in Table 2. The highest values of fruit weight (454.71g), fruit width (93.40 mm) and fruit length (80.31 mm) were obtained from the Nuz Ekşi variety,

followed by the Hicaznar and Çekirdeksiz VI. The lowest values in terms of all three properties were obtained from the Oğuzeli Çekirdeksiz variety. In terms of varieties and types, these properties were considered statistically significant at the level of 5%. Polat et al. (1999) determined the fruit weight of five different types of pomegranates grown in the region of Hatay/Kırıkhan to be 250.00-461.00g; Korkmaz (2013) found the average fruit weight of the pomegranate type Hicaznar grown in Muğla to be 454.32-557.51g according to different application results; El Sayed et al. (2014) found the average fruit weight of the Manfalouty pomegranate grown in Egypt to be 339-413g according to different application results; and Dandachi et al. (2017) found that fruit weights ranged between 43.15-358.60g in a study conducted in Lebanon. The average fruit weights of the varieties and types in this study had fruit weights ranging between 173.09-454.71g showing a large variation similar to the previous studies conducted in our country.

The highest value was obtained from the Hicaznar type (671.72 pieces/fruit) in terms of the number of fruit seeds, followed by the Nuz Ekşi (564.50 pcs/fruit) and Çekirdeksiz VI (531.49 pcs/fruit). The lowest value was obtained from the Kirli Hanım variety (374.00 pcs/fruit). Differences between the numbers of arils in the genotypes were found to be statistically significant at the level of 5%. In a trial conducted on seven types of pomegranates, Yılmaz (2005) found that the type containing the highest number of arils was the Hicaznar (594.6 pcs), and that the type containing the least number of arils was the İzmir 16 (295.2 pieces). Korkmaz (2013) found that the number of arils ranged between 606.807-713.002 pcs/fruit in different trials conducted on the Hicaznar in Muğla, while Tapia-Campos et al. (2016) found the least number of arils in the Manzanita (414.8 pcs), and the highest number of arils in the Chichona (823.9 pcs) in a trial conducted on 14 different types of genotypes in Mexico. In this study, the highest number of arils among all the varieties and types was obtained from the Hicaznar. The peel percentage of varieties and types ranged from 36.71%-39.41%; aril ratio from 55.68%-59.67%; while fruit juice yield ranged between 45.51%-48.8%. However, such differences between the types and varieties were not found to be statistically significant. Tehranifar et al. (2010) found that the average peel ratio of 20 types of pomegranates in Iran ranged between 32.28-59.82%, the aril ratios between 37.59-65.00% and the fruit juice yield between 26.95-46.55%. Korkmaz (2013) found that the Hicaznar had a peel ratio of 50.70% and a aril yield ranging between 45.41-51.82 % in the first year of the trial and 40.69-50.11% in the second year of the trial in Muğla, while she found that the fruit juice yield ranged between 35.20-40.83% in the first year and between 32.19-37.94% in the second year. Bakeer (2016) found that the Manfalouty had a peel ratio of 42.70% and aril yield ratio of 57.3% and Selahvarzi et al. (2017) found that the Shahvar had a fruit juice yield of 36.47% in Iran. It can be stated that the mean values we obtained are within the data ranges found by other researchers. The peel thickness was the highest (4.25 mm) in the Hicaznar and the lowest (2.87mm) in the Oğuzeli Çekirdeksiz. The peel thickness of the other types and varieties was found to lie between these two values. Differences found in the peel thickness of the types and varieties were found to be statistically significant at 5%. The total soluble solids (TSS) of the varieties and types varied between 16.48% (Hicaznar) and 14.03% (Nuz Ekşi) and the titratable acid amount varied between 1.31% (Nuz Ekşi) and 0.24% (Çekirdeksiz VI). The differences between the types and varieties in terms of total soluble solids and titratable acidity values were statistically significant. In a study covering six types of pomegranates in Morocco, Martínez et al. (2012) found that the total soluble solids ranged between 15.3-17.6% °Brix, and the acidity value between 2.1-4.7g/l; Korkmaz (2013) found that the total soluble solids in the Hicaznar ranged between 13.35-14.80% and the titration between 1.66-2.14% in different trials conducted in Muğla; Bakeer (2016) found that the Manfalouty had a total soluble solids of 16.29% and a titratable acidity rate of 1.34%; and Marathe et al. (2017) found that the Bhagwa had a total soluble solids of 15.21% and a

titration rate of 0.42% in India. Our research findings are consistent with the literature values. Tehranifar et al. (2010) reported peel thicknesses of 3.13-5.36 mm in Iran; El Sayed et al. (2014) found that the Manfalouty in Egypt had a peel thickness of 0.31-0.40 cm; and Dandachi et al. (2017) reported peel thickness as ranging between 0.10-0.34cm. Our research findings demonstrate a compliance with the values found in other studies.

While the aril juice content of varieties and types showed significant difference in physically uncracked fruits, they yielded similar values in cracked fruits. Yılmaz (2005) determined that there was no significant difference in aril juice content between cracked fruits and uncracked fruits when studying the average cracking ratio of pomegranate varieties. This study also showed similar results. On the other hand, the fruit peel juice content showed statistically significant differences in both types of uncracked and cracked fruits. Yılmaz (2005) reports that the cracking of pomegranate varieties and the average fruit peel juice differences between types of uncracked fruits are statistically significant and that the aril juice content amount of cracked fruits (65.87%) is lower when compared to uncracked fruits (69.04%). This study also showed similar results. The cracking rates of different varieties and types ranged from 57.05% (Kış narı) to 24.50% (Çekirdeksiz VI). Differences found in the cracking rates of the types and varieties were found to be statistically significant at 5%. In a study conducted in Izmir, Ercan et al. (1991) found that the cracking rates of types ranged between 1.41 and 26.64%; Özgüven et al. (1997) found that the cracking rates ranged between 0.9-24.7% in adaptation trials conducted between 1993-1997 for different types of pomegranates in Southeastern Anatolia; and Yılmaz (2005) found the rate of cracks to be 22.8% in the first year on average and 13.0% in the second year, tantamount to a decrease of almost half, and 5% in the third year in a study showing rates of cracks in fruits over the years.

Table 1. Mean phenological properties of genotypes

Genotype	Date of bud burst	Sprouting date of the bud	Date of the first appearance of flower buds	Beginning of flowering	Full blooming	A type flower	B type flower	Appearance date of first fruit	Fruit ripening dates	Foliage of leaves	Falling leaves		Date of Rest
											% 50	% 80	
Kışnarı	22 March	30 March	1 May	24 May	4 June	109	74	30 May	3 Nov.	4 Nov.	22 Nov.	24 Nov.	4 Dec.
Çekirdeksiz VI	22 March	29 March	25 April	26 May	4 June	59	23	25 May	17 Oct.	6 Nov.	22 Nov..	26 Nov.	2 Dec.
Oğuzeli Çekirdeksiz	23 March	29 March	25 April	30 May	7 June	83	49	26 May	23 Oct.	16 Nov.	21 Nov.	24 Nov.	5 Dec.
Kirli Hanım	21 March	28 March	27 April	21 May	31 May	88	54	3 June	27 Oct.	2 Nov.	24 Nov.	27 Nov.	30 Nov.
Hicaznar	21 March	29 March	30 April	19 May	31 May	88	55	28 May	16 Oct.	1 Nov.	20 Nov.	24 Nov.	3 Dec.
Nuz Ekşi	22 March	29 March	2 May	26 May	5 June	64	31	28 May	16 Oct.	24 Oct.	11 Nov.	19 Nov	26 Nov.

Table 2. Mean pomological properties of genotypes

Genotype	Fruit average weight (g)	Fruit diameter (mm)	Fruit length (mm)	Number of arils per fruit (pieces/fruit)	Peel percentage (%)	Peel thickness (mm)	Aril ratio (%)	Juice ratio (%)	TSS (%)	Titrable acidity (%)	Aril juice content (%)		Fruit peel juice content (%)		Fruit cracking ratio (%)
											Uncracked fruit	Cracked fruit	Uncracked fruit	Cracked fruit	
Kış narı	310.97 b[x]	83.09 ab	74.61 a	471.44 bcd	39.41	3.37 bc	56.68	46.25	14.74 bc	0.38 c	76.98 c	77.36 a	69.10 c	66.30 ab	57.05 a
Hicaznar	350.24 ab	87.14 ab	77.95 a	671.72 a	37.78	4.25 a	55.68	45.51	16.48 a	0.91 ab	78.29 bc	79.84 a	73.33 a	65.56 ab	49.53 ab
Çekirdeksiz VI	335.91 ab	85.75 ab	78.31 a	531.49 abc	38.20	3.49 bc	59.01	48.80	15.31 b	0.24 c	81.22 ab	81.04 a	77.23 ab	68.41 ab	24.50 c
Oğuzeli Çekirdeksiz	173.09 c	68.02 c	61.82 b	387.04 cd	36.71	2.87 c	59.67	47.24	15.44 b	0.61 bc	80.92 abc	81.20 a	76.45 abc	69.44 ab	26.43 bc
Kirli Hanım	262.66 bc	79.39 bc	71.71 a	374.00 d	38.28	3.26 bc	57.39	46.70	14.76 bc	0.42 bc	78.98 bc	78.91 a	70.31 bc	60.21 b	31.38 bc
Nuz Ekşi	454.71 a	93.40 a	80.31 a	564.50 ab	37.73	3.65 ab	59.35	46.62	14.03 c	1.31 a	84.98 a	79.62 a	75.59 abc	73.54 a	25.19 c
Significance	*	*	NS[y]	*	NS.	*	NS.	NS.	*	*	*	NS.	*	*	*

[x] Means within a column followed by different letter are significantly at the 5% level by Tukey's HSD test. [y] Not significance

Conclusion

The fact that the cracking ratios of varieties and types examined in this study are high compared to similar studies may be due to the fact that the day and night temperature difference of the region was high on the date of fruit ripening, that the upkeep of the orchard had been neglected for many years, that there was rainfall during harvest time, that the orchard was founded on rocky and stony terrain and that the plants were planted at close intervals.

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THE EFFECTS OF DIFFERENT POTATO GENOTYPES YIELD CHARACTERISTICS OF DIFFERENT ALTITUDES

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Abstract

This research was carried out in Tokat-Erbaa (230m), Tokat-Kazova (640m) and Tokat-Artova (1200m) in 2017 in Turkey. In different maturity groups 20 potato genotypes were used in the study. Fifteen (15) of these genotypes were advanced breeding clones and it was aimed to determine the responses of these clones to different altitudes. The experiment was established in the randomized complete block in a split plot design with three replications. According to the results of the research, Agria variety (4142.25 kg/da) and GOU-7/12 clone (4093.25 kg/da in Tokat Erbaa, Agata variety (3928.4 kg/da) and GOU6/28 clone (3927.7 kg/da) in Tokat Kazova and PAI-8.16 (4930.6 kg/da), PAI-8.8.57 (4695.7 kg/da) in Tokat Artova were determined as higher yielding other genotypes. It has been determined that the tuber yields per decare from the early season to the high altitude towards the late season increases from the low altitude to the late season.

Keywords: *Potato, tuber yield, altitude.*

Introduction

Potato, which is second main nutritional product of human nutrition after wheat, is orginated from high plateau of Ant mountain of South America Continent. It has been cultivated for 8000 years in the world. There are 2000 species of genus *Solanum* including potato and 160-180 species of them produced tuber. Eight species were used as food source in human nutrition. *S. tuberosum* is the most common species growen in the world (Rowe 1993). Potatoes are grown in a wide range of environments (Haverkort, 1990). The highest potential and actual tuber dry matter productions and yield are obtained in areas with temperate climates in North-western Europe and the North-west of the United States (Van der Zaag, 1984; Stol et al., 1991), thanks to long growing seasons combined with adequate cropping practices. The potential production and the ratio between actual and potential production are lower in tropical and subtropical climates. Part of this difference originates from the use of cultivars that are high yielding in temperate regions but are not adequately adapted to the high temperatures and short day length of tropical and subtropical climates. Another cause of the lower yields may be the shorter growth cycles due to unsuitable weather conditions in these climates. Some of the factors effecting high quality seed potato production are as follows; healthy beginning materials, altitute of production area and free of diseases and pests, experience level of growers, and production techniques (Yılmaz 2003). For healty seed tuber production, growing area should be free of pathogens or effective disease management programs should be performed (De Bokx and Mooi 1974; O'brien and Allen 1986) additionaly soil preparation, planting, cultivation, pest management, vine killing, harvest, and storage techniques also important for healthy seed tuber production(Sahtiyancı 1990; Tugay et al. 1995). The objective of this research was to test performance, yield and dry matter content of different potato genotypes and cultivars in various environments at different altitudes. A set of fifteen promising clones and five commercially available cultivars was tested in field trials at Tokat-Erbaa, Tokat-Kazova and Tokat-Artova conditions in 2017.

Material and Methods

This research was carried out in Tokat-Artova (1189 m), Tokat-Erbaa (276 m), Tokat-Kazova (571 m) and Tokat-Artova conditions in the 2017 vegetation periods. The material used in the experiment is composed of 16 promising clones and 5 commercial varieties (Agata, Alegria, Agria, Lady Claire, Lady Olimpia). The experiment was laid out in Randomized Complete Block Design, with three replications. The planting was carried out in Erbaa in the second half of April in Kazova in early March and in the first half of May in Artova. Potato tubers were planted at spacing of between rows and plants 70 cm and 30 cm respectively. The trials were fertilized 12kg/da 15:15:15 (N:P:K) at the time of planting in both locations. 8 kg/da N was applied 45 days after planting (Tugay et al., 1995). The plants were irrigated as required to maintain adequate moisture levels by drip irrigation. When cultural and chemical methods are needed to combat diseases and pests, necessary applications have been made.

Table 1. Plant material used in the experiment

Number	Clones	Number	Clones
1	PAİ-8-1-6	11	GOU 3/110
2	PAİ-8-3-15	12	GOU 4/4
3	PAİ-8-5-34	13	GOU 6/28
4	PAİ-8-6-35	14	GOU 7/12
5	PAİ-8-7-49	15	GOU 10/15
6	PAİ-8-8-57	16	*Agata*
7	PAİ-8-9-63	17	*Alegria*
8	PAİ-8-11-79	18	*Agria*
9	PAİ-8-12-86	19	*Lady Claire*
10	PAİ-8-15-138	20	*Lady Olimpia*

Results and Discussion

Total Tuber Yield (kg/da): Total tuber yield were significantly affected by genotypes and various locations (Table 1). The Total tuber yield of genotypes were ranged between 908.1 kg/da and 4142.3 kg/da in Erbaa, between 1776.9 kg/da and 3772.4 kg/da in Kazova, and between 744.0 kg/da and 4930.6 kg/da in Artova (Table 1). The highest total tuber yield was obtained from Agria in Erbaa. The highest total tuber yield was obtained from Agata and GOU 6/28 in Kazova. The highest total tuber yield was obtained from PAİ-8-1-6 in Artova.

Table 1. Total Tuber Yield (kg/da) of clones and varieties in all locations

Genotype	Erbaa		Kazova		Artova		Average
Agata	2468.3	c-g	3928.4	a	2515.6	cd	2970.8
Alegria	3907.5	ab	3772.4	a	4434.7	ab	4038.2
Agria	4142.3	a	2620.3	de	3655.3	b	3472.6
Lady Claire	3014.0	b-e	2580.0	de	2191.3	cd	2595.1
Lady Olimpia	2076.7	efg	3660.3	ab	4315.6	ab	3350.9
PAİ-8-1-6	1910.3	fgh	3337.1	abc	4930.6	a	3392.7
PAİ-8-3-15	3027.3	b-e	3125.7	bcd	2631.1	c	2928.0
PAİ-8-5-34	3094.8	bcd	3407.9	abc	4431.8	ab	3644.8
PAİ-8-6-35	3374.0	abc	3061.3	bcd	3733.6	b	3389.6
PAİ-8-7-49	1609.0	ghi	3382.5	abc	4245.1	ab	3078.9
PAİ-8-8-57	3849.5	ab	3748.9	a	4695.7	ab	4098.0
PAİ-8-9-63	1091.8	hi	2307.3	ef	744	e	1381.0
PAİ-8-11-79	2792.0	c-f	3648.9	ab	3770.4	b	3403.8

PAİ-8-12-86	3449.8	abc	2752.4	de	4115.2	ab	3439.1
PAİ-8-15-138	2316.0	dg	3454.9	abc	1617.7	de	2462.9
GOU 3/110	1985.4	fgh	2956.8	cd	1726.5	cde	2222.9
GOU 4/4	2364.8	d-g	929.9	g	948.5	e	1414.4
GOU 6/28	3307.0	a-d	3927.6	a	4661.7	ab	3965.4
GOU 7/12	4093.3	a	2718.1	de	1082.5	e	2631.3
GOU 10/15	908.1.0	i	1776.9	f	4071.3	ab	2252.1
Average	**2739.1**		**3054.9**		**3225.9**		
F Value	5.9**		4.1**		19.5**		

Mean comparison among tuber yield indicated that PAİ-8-8-57 and Algeria consisted of higher tuber yield than other genotypes. The highest total tuber yield was obtained of respectively Artova, Kazova and Erbaa. Results of total tuber yield showed that differences among different genotypes were significant (P<0.01). **Dry Matter Content (%):** In this study, the dry matter contents of the tubers were given in Table 2. Accordingly, the overall average of the tubers in terms of the dry matter content % 20,7 in Erbaa, %18.7 in Kazova, % 18.2 in Artova.

Table 2. Dry Matter Content (%) of clones and varieties in all locations

Genotypes	**Erbaa**		**Kazova**		**Artova**		**Average**
Agata	16.9	f	19.0	a-d	14.9	f	16.9
Alegria	18.4	ef	20.1	a-d	18.6	cd	19.0
Agria	19.8	c-f	19.0	a-d	17.4	de	18.7
Lady Claire	20.6	a-e	21.5	ab	19.0	bcd	20.4
Lady Olimpia	20.9	a-e	19.5	a-d	19.0	bcd	19.8
PAİ-8-1-6	19.1	ef	15.6	cde	16.1	ef	16.9
PAİ-8-3-15	23.0	ab	18.0	a-d	19.4	bc	20.1
PAİ-8-5-34	19.9	cde	16.6	b-e	17.8	cd	18.1
PAİ-8-6-35	22.4	a-d	20.7	abc	19.1	bcd	20.7
PAİ-8-7-49	22.4	a-d	22.9	a	21.1	a	22.1
PAİ-8-8-57	23.4	a	19.0	a-d	18.3	cd	20.2
PAİ-8-9-63	20.6	a-e	20.7	abc	20.5	ab	20.6
PAİ-8-11-79	18.8	ef	20.5	abc	19.0	bcd	19.4
PAİ-8-12-86	20.5	a-e	17.8	a-d	15.3	f	17.9
PAİ-8-15-138	21.3	a-e	20.6	abc	19.2	bc	20.4
GOU 3/110	20.0	b-e	17.4	a-d	17.9	cd	18.4
GOU 4/4	21.4	a-e	12.3	e	19.5	bc	17.7
GOU 6/28	22.8	a-d	17.8	a-d	17.5	de	19.4
GOU 7/12	22.6	abc	19.1	a-d	15.2	f	19.0
GOU 10/15	19.5	def	15.1	de	19.4	bc	18.0
Average	**20.7**		**18.7**		**18.2**		
F Value	16.7**		29.3**		33.1**		

Conclusion

Dry matter contents of the 15 advanced clones and standard varieties differed from each other as well as from each other. In this study, the dry matter content of the genotypes ranged from 16.9 to 22.1 %. in all locations. The highest dry matter content was obtained from clone PAI-8-7-49. The dry matter content was determined significant in the all locations.

Acknowledgments

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THE STABILITY OF SOME QUALITY CRITERIA IN POTATO GENOTYPES AT DIFFERENT LOCATIONS

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Abstract

This study was done as a part of the studies carried out on breeding potato varieties adaptable to central-north intersectional region. This study started in 2007, with about 20 thousand hybrid potato seedlings and as a result of clonal selection. Twenty clones, performing excellent characteristics in terms of yield and quality characteristics, were subjected to the analysis of stability in terms of genotype x environment interactions. The study was conducted under Tokat-Niksar, Tokat-Kazova (2012-2013) and Tokat-Artova (between 2011-2012 and 2012-2013) conditions as a randomized complete block design with 3 replications in 8 different environments, as the years and locations were accepted as an environment. In the study, four commercial varieties were tested as standards beside white, cream, light yellow and dark yellow clones. The data were subjected regression analysis. Based on the Finlay-Wilkinson regression coefficients, some of the clones (A7-12, T5-4, T11-10, T5-14, A3-15, A3-167, and T10-8) which had better yield and quality characteristics than commercial varieties, showed good adaptation to specific environments while the clones (T6-28 and A3-234) had good adaptation to all the environments (Tokat-Niksar, Tokat-Kazova, and Tokat-Artova). Also in terms of dry matter, A13-1 was identified as a clone with higher (28.4%) dry matter contents.

Keywords: *Potato, Clone Selection, Solanum tuberosum, Breeding, Stability Analysis.*

Introduction

Successful new varieties must show high performance in respect to yield and other essential agronomic traits. Moreover, their superiority should be reliable over a wide range of environmental conditions. The basic cause of differences between genotypes in their yield stability is the wide occurrence of genotype-environment interactions (GxEinteractions) i.e. the ranking of genotypes depends on the particular environmental conditions where they are grown. These interactions of genotypes with environments can be partly understood as a result of a differential reaction to environmental stress factors like drought or diseases, and consequently resistance breeding is of significance in improving yield stability (Becker and Leon, 1988). As a matter of fact, studies were carried out by different researchers in different agro ecological regions of the world have resulted in significant differences between genotypes in terms of yield and quality in the conditions of the region and it has been concluded that the most suitable genotypes should be determined for regional conditions [Caesar et al., 1978; Yılmaz and Tugay, 1999]. Potato is an important source of food which contains high levels of carbohydrate, protein, vitamins and minerals. It is also a source of income and employment opportunity in developing countries (FAO, 2016). In addition to high yields, the dry matter content of the tubers is considered an important quality criterion. This property is more important in terms of obtaining economically processed products and nutrient content of the industry. Potato tubers contain between 18 and 28% dry matter, depending on the variety and environment. The most important part of dry matter is starch. According to cultivars and growing conditions, 70-80% of dry matter is starch. On the other hand, 1.7-2.5% of protein is found in potato tubers and about 8% of dry matter is protein. Potato is the fourth most important plant used as a carbohydrate source in human nutrition. Varieties containing more than 16% starch are evaluated in the starch industry (Er and

Uranbey, 1998). The process of starch accumulation is expected to be profoundly dependent on the genotype and environmental conditions. Similar to other important agronomic traits, genotype (G), environment (E), and genotype by environment interaction (GEI) effects can be important to understanding the biochemistry and physiology behind starch development. GEI are common for multigene traits, such as starch content, and are important to understanding underlying sources of variation. Temperature, pH, and nutrient levels are important for maintaining plant health and subsequent starch accumulation (yield) throughout the growing season. The objective of this research was to test performance, stability of starch and dry matter content of different flesh color tubers in various environments. A set of 16 promising clones and four commercially available cultivars and one local genotype was tested in field trials at Tokat-Niksar, Tokat-Kazova (2012 - 2013) and Tokat-Artova (between 2010-2011 and 2012-2013) conditions. In order to determine genotypes with stable expression of traits and to examine possible differences in stability, analysis of yield, quality, and GEI was undertaken (Finlay and Wilkinson, 1963).

Material and Methods

This research was carried out in Tokat-Artova (1189 m) during the 2011 and 2012 vegetation periods and Tokat-Niksar (276 m), Tokat-Kazova (571 m) and Tokat-Artova conditions in the 2012 and 2013 vegetation periods. The soil reaction of the trial field is mildly alkaline. The material used in the experiment is composed of 16 promising clones and 4 standard varieties (Başçiftlik Beyazı, Slaney, Hermes and Agria) with different flesh colors selected as a result of the studies carried out within the scope of TUBITAK project 106 O 626 initiated in 2007 (Table 1). The experiment was laid out in Randomized Complete Block Design, with three replications. The planting was carried out in Niksar in the second half of April in Kazova in early March and in the first half of May in Artova. Potato tubers were planted at spacing of between rows and plants 70cm and 30 cm respectively. The trials were fertilized 12kg/da 15:15:15 (N:P:K) at the time of planting in both years and locations. 8 kg/da N was applied 45 days after planting (Tugay et al., 1995). The plants were irrigated as required to maintain adequate moisture levels by drip irrigation. When cultural and chemical methods are needed to combat diseases and pests, necessary applications have been made. After harvest, the tubers were stored for 8 weeks. Dry matter content was assessed as underwater weight (UWW), which can be converted into specific gravity (SG) by the formula: SG = 5000/(5000-UWW) (Meijers & Van Veldhuisen, 1972). Underwater weight was determined for each experimental unit, standardized to an underwater weight of 5 kg tubers in air.

Table 1. Flesh colors and pedigrees of clones used in the experiment

Number	Clones	Pedigree	Flesh Colour	Number	Clones	Pedigree	Flesh Colour
1	*T5/4*	*Serrana x DTO-33*	*White*	9	*T4/4*	*Granola x TS-2*	*Light yellow*
2	*A13/1*	*Pentland Crown x TS-2*	Cream	10	*T6/28*	*Serrana x LT-7*	
3	*T5/14*	*Serrana x DTO-33*		11	*A2/11*	*MF-1 x TS-4*	*Dark yellow*
4	*A7/12*	*Serrana x TS-4*		12	*A3/110*	*Serrana x TS-9*	
5	*T11/10*	*Granola x Huincul*		13	*A3/15*	*Serrana x TS-9*	
6	*A3/234*	*Serrana x TS-9*	*Light yellow*	14	*A3/142*	*Serrana x TS-9*	
7	*A3/29*	*Serrana x TS-9*		15	*A3/368*	*Serrana x TS-9*	
8	*A3/167*	*Serrana x TS-9*		16	*T10/8*	*MF-1 x LT-7*	

It was determined the regression coefficient by regressing variety mean on the environmental mean, and plotting the obtained genotype regression coefficients against the genotype mean

yields [6]. According to Finlay and Wilkinson (1963), regression coefficients approximating to 1.0 indicate average stability. When the regression coefficients are approximating to 1.0 and are associated with high yield mean, genotypes are adapted to all environments. When associated with low mean yields, genotypes are poorly adapted to all environments.

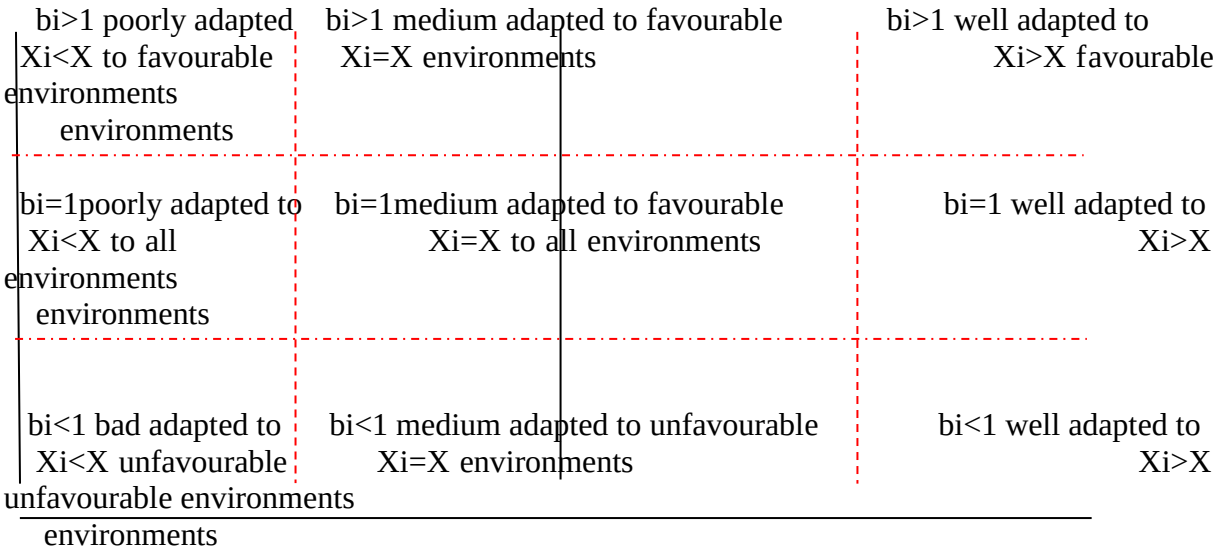

Figure 1. A generalized interpretation of the genotypic pattern obtained when genotypic regression coefficients are plotted against genotypic mean adapted from Finlay and Wilkinson, 1963.

Results and Discussion

Dry Matter Content (%)

In this study, the dry matter contents of the tubers were given in Table 2. Accordingly, the overall average of the tubers in terms of the dry matter content % 21,65, average of commercial cultivars were % 21,42 and average of advanced clones were % 22,03. Dry matter clones of the 16 advanced clones and standard varieties differed from each other as well as from each other. In this study, the dry matter content of the clones ranged from 17.2 to 28.4%. The highest dry matter content was obtained from clone A13-1(flesh color white).

According to flesh colors, the highest dry matter content was obtained from A13-1 clone (%28,4) in whites for all colors. Also the highest dry matter was obtained from A3-167 (% 24.50) in light yellows, the highest dry matter content was obtained from A3/368 (% 24.60) in the dark yellows. The dry matter content of the cream colors was lowest (17.2-20.2%) in all clones in this study. As for the locations, when ranking in terms of yield it was determined that Artova (22,86 %), Kazova (21,99 %) and Niksar (20,40%).

The highest dry matter content was determined from A13/1 in all locations and environments. It has been reported that the content of dry matter and starch varies not only with the genetic characteristics of the variety but also under the effect of environmental practices during production. It has been reported that late maturity varieties produce higher dry matter and starch content in cool climatic conditions and high altitude locations. In addition differences in quality of seed tubers affect the content of dry matter and starch (Yılmaz and Karan, 2011).

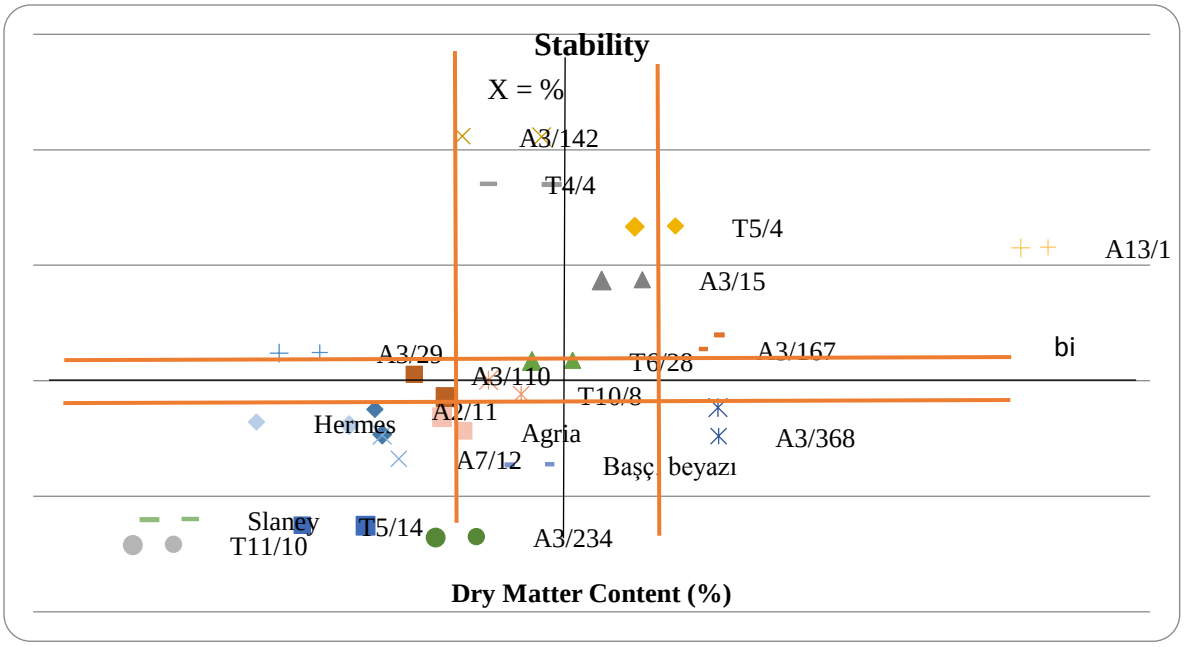

Figure 2. Adaptation classes of clones and varieties dry matter content (%)

Meanwhile, when all the environmental were examined separately, it was seen that some clones could have a dry matter content of over 23%. These clones have the potential to be evaluated as clones with high dry matter content. For example, These are A13-1, A3-368 (24,6%), A3-167 (24,5%), T5-4 (23,5%) and A3-15 (23,1%). All of the clones had higher dry matter content than standard commercial varieties in all environments (Table 3).

When the dry matter contents obtained from this study were examined in terms of genotype x environment interaction and stability, the regression coefficients ranged from bi = 0.29 to 2.06. T6-28 clone had better compatibility than other clones in terms of dry matter content. That is, as it was above the average dry matter content in different environmental conditions, it was the genotype that underwent minimal change. The average dry matter content of this clone (T6-28) in all environmental was above the general average and the regression coefficient was closest to bi = 1 (Table 2 and Figure 2). When the adaptation in terms of dry matter content was investigated, T5/4, A13/5 and A13/1 had high dry matter content in the favorable condition, A3/142 and T4/4 showed medium adaption in the favorable condition, A3/29 had low dry matter content in the all environmental. A2/11, A3/110, T10/8 and T6/28 were medium adapted in the all environmental. The A3/167 clone was a clone that can be regarded as a stable material, such as T6-28, in terms of dry matter content when confidence limits were taken into consideration. Slaney, Hermes varieties and T5/14 and T11/10 clones were bad adapted to unfavorable environmental. Agria, Basciftlik Beyazi cultivars and A7/12 and A3/234 clones showed medium adapted to unfavorable environmental. The A3/368 clone has been identified as a well clone that is it had high dry matter content even in unfavorable environmental.

Starch Content (%)

In this study, the dry matter contents of the tubers were given in Table 3. Accordingly, the overall average of the tubers in terms of the starch content % 15,88, average of commercial cultivars were % 14,38 and average of advanced clones were % 16,03. Starch content of the 16 advanced clones and standard varieties differed from each other as well as from each other. In this study, the starch content of the clones ranged from 11.7 to 22.6%. The highest starch content was obtained from clone A13-1(flesh color white).

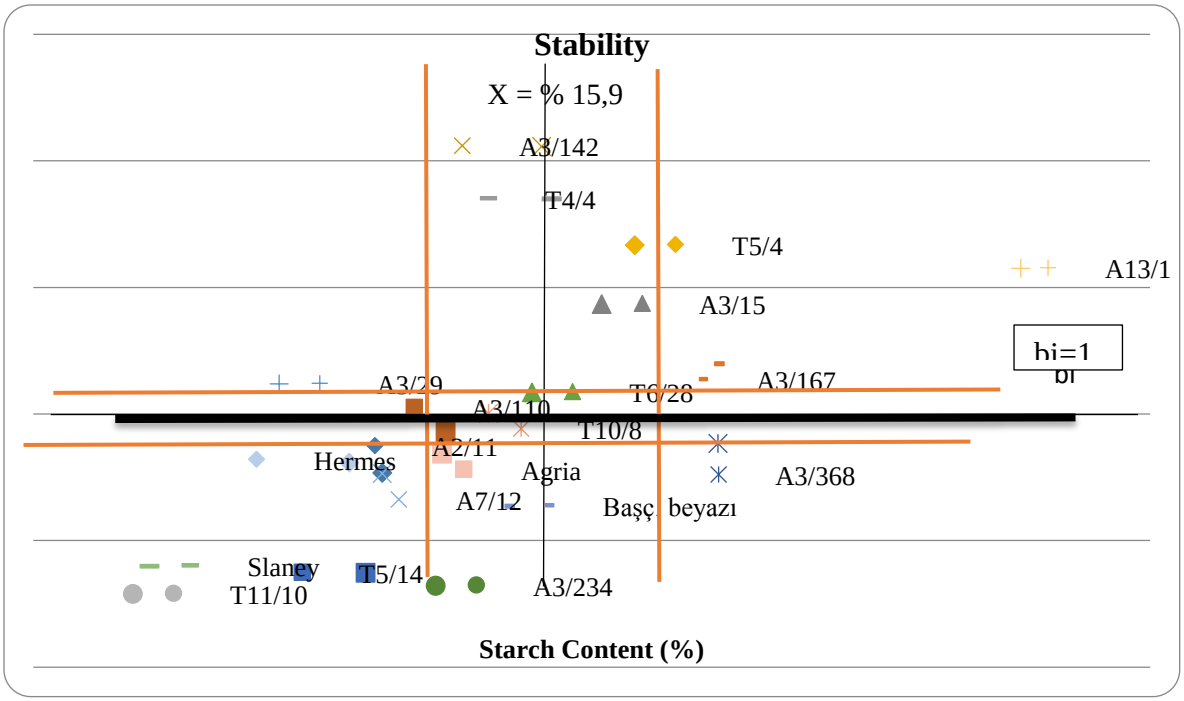

Figure 3. Adaptation classes of clones and varieties starch content (%)

The highest starch content was determined from A13/1 in all locations and environments. It has been reported that the content of dry matter and starch varies not only with the genetic characteristics of the variety but also under the effect of environmental practices during production. It has been reported that late maturity varieties produce higher dry matter and starch content in cool climatic conditions and high altitude locations. In addition differences in quality of seed tubers affect the content of dry matter and starch (Yılmaz and Karan, 2011). When the starch contents obtained from this study were examined in terms of genotype x environment interaction and stability, the regression coefficients ranged from bi = 0.70 to1.36. T6-28; T10/8; A7/12; A3/110 clones had better compatibility than other clones in terms of starch content. That is, as it was above the average starch content in different environmental conditions, it was the genotype that underwent minimal change. The average starch content of these clones in all environmental was above the general average and the regression coefficient was closest to bi = 1 (Table 3 and Figure 3).

Table 2. Total Dry Matter (%) and regression coefficients (bi) of clones and varieties in all locations.

Clone Name	2011	2012	2012			2013			Genotype Average	Effect of Genotype (g_{ii})	bi
	Artova	Artova	Niksar	Kazova	Artova	Niksar	Kazova	Artova			
A2-11	19,67 cde**	21,00 c*	18,33 hi**	20,67 ghi**	20,67 ijk**	19,33 efg**	21,33 de**	22,00 efg**	20,4	-1,2	0,77
A3-110	19,67 cde	21,33 bc	19,67 e-h	20,67 ghi	23,33 fg	20,33 def	21,67 de	22,67 efg	21,2	-0,4	0,93
A3-15	20,33 bcd	22,33 bc	21,00 cde	23,33 de	25,00 de	21,67 cd	24,67 c	26,67 bc	23,1	1,5	1,43
A3-142	20,33 bcd	23,33 bc	19,33 fgh	20,00 g-j	27,33 b	21,33 cd	20,33 ef	27,00 b	22,4	0,8	2,06
A3-368	22,33 ab	23,33 bc	23,67 b	25,00 bc	26,67 bc	24,00 b	25,00 bc	26,67 bc	24,6	3,0	0,88
A3-234	21,00 bcd	21,67 bc	20,33 d-g	20,67 ghi	22,00 ghi	20,68 de	20,67 ef	21,33 g	21,0	-0,6	0,32
A3-29	19,33 cde	22,33 bc	16,67 j	17,67 klm	19,33 k	17,00 h	18,67 gh	21,67 fg	19,1	-2,5	1,12
A3-167	22,67 ab	24,00 b	22,00 c	25,67 b	26,67 bc	22,67 bc	26,33 b	26,33 bc	24,5	2,9	1,20
T4-4	20,33 bcd	22,67 bc	19,33 fgh	22,67 def	25,33 cd	19,33 efg	24,33 c	26,00 bc	22,5	0,9	1,85
T5-4	22,33 ab	24,00 b	21,33 cd	24,00 cd	26,33 bcd	19,33 efg	24,33 c	26,67 bc	23,5	1,9	1,67
T5-14	20,67 bcd	21,33 bc	19,33 fgh	18,67 jkl	20,33 jk	20,00 d-g	19,67 fg	21,33 g	20,2	-1,4	0,37
T6-28	21,67 bc	22,33 bc	20,67 c-f	21,67 efg	23,67 ef	20,33 def	22,67 d	25,00 cd	22,3	0,7	1,09
A7-12	21,33 bcd	22,33 bc	18,33 hi	19,67 hij	20,33 jk	18,33 gh	20,67 ef	22,00 efg	20,4	-1,2	0,76
T10-8	21,00 bcd	22,67 bc	19,00 gh	21,33 fgh	23,33 fg	21,33 cd	21,33 de	23,67 de	21,7	0,1	1,00
T11-10	17,33 ef	18,00 de	16,67 j	16,33 m	17,33 l	17,33 h	16,67 i	18,33 h	17,2	-4,4	0,29
A13-1	24,33 a	27,67 a	26,67 a	28,67 a	32,00 a	27,33 a	28,67 a	31,67 a	28,4	6,8	1,58
Clone Average	**20,90**	**22,52**	**20,15**	**21,67**	**23,73**	**20,65**	**22,31**	**24,31**	**22.03**		
Bb	21,00 bcd	22,66 bc	20,67 c-f	21,33 fgh	22,33 fgh	21,33 cd	22,67 d	23,33 def	21,9	0,3	0,64
Slaney	16,67 f	17,33 e	17,00 ij	17,33 lm	17,67 l	17,00 h	18,00 hi	18,67 h	17,5	-4,1	0,40
Hermes	19,00 def	20,67 c	18,67 h	19,33 ijk	21,67 hij	18,67 fgh	20,67 ef	21,00 g	20,0	-1,6	0,81

		d									
Agria	21,33 bcd	22,67 bc	19,33 fgh	21,00 f-i	22,33 fgh	19,33 efg	20,33 ef	22,67 efg	21,1	-0,5	0,84
Cultivar Average	**19,50**	**20,83**	**18,92**	**19,75**	**21,00**	**19,08**	**20,42**	**21,42**	**General Average**		
Environmental Average	**20,6**	**22,2**	**19,9**	**21,3**	**23,2**	**20,3**	**21,9**	**23,7**	**21,65**		**1,00**
Effect of Environmental	-1,0	0,5	-1,7	-0,4	1,5	-1,3	0,3	2,1	**Confidence Interval**		
LSD (Genotype)	2,13	2,42	1,22	1,51	1,27	1,51	1,28	1,49	1,19		0,24

Table 3. Total Starch Content (%) and regression coefficients (bi) of clones and varieties in all locations.

Clone Name	2011	2012	2012			2013			Genotype Average		Effect of Genotype (g_{ii})	bi
	Artova	Artova	Niksar	Kazova	Artova	Niksar	Kazova	Artova				
A2-11	13,9 cde**	15,2 c**	12,6 hi**	14,9 ghi**	14,9 ijk**	13,6 efg**	15,5 de**	16,2 efg**		14.6	-1,2	0,77
A3-110	13,9 cde	15,5 bc	13,9 e-h	14,9 ghi	17,5 fg	14,5 def	15,9 de	16,9 efg		15.4	-0,4	0,93
A3-15	14,5 bcd	16,5 bc	15,2 cde	17,5 de	19,2 de	15,9 cd	18,9 c	20,9 bc		17.3	1,5	1,43
A3-142	14,5 bcd	16,5 bc	13,6 fgh	14,2 g-j	21,5 b	15,5 cd	14,5 ef	21,2 b		16.4	0,8	2,06
A3-368	16,5 ab	17,5 bc	17,9 b	19,2 bc	20,9 bc	18,2 b	19,2 bc	20,9 bc		18.8	3,0	0,88
A3-234	15,2 bcd	15,9 bc	14,5 d-g	14,9 ghi	16,2 ghi	14,9 de	14,9 ef	15,5 g		15.3	-0,6	0,32
A3-29	13,6 cde	16,5 bc	11,2 j	12,1 klm	13,6 k	11,5 h	13,0 gh	15,9 fg		13.4	-2,5	1,12
A3-167	16,9 ab	18,2 b	16,2 c	19,9 b	20,9 bc	16,9 bc	20,5 b	20,5 bc		18.8	2,9	1,20
T4-4	14,5 bcd	16,9 bc	13,6 fgh	16,9 def	19,5 cd	13,6 efg	18,5 c	20,2 bc		16.7	0,9	1,85
T5-4	16,5 ab	18,2 b	15,5 cd	18,2 cd	20,5 bcd	13,6 efg	18,5 c	20,9 bc		17.7	1,9	1,67
T5-14	14,8 bcd	15,5 bc	13,6 fgh	13,0 jkl	14,5 jk	14,2 d-g	13,9 fg	15,5 g		14.4	-1,4	0,37
T6-28	15,9 bc	16,5 bc	14,9 c-f	15,9 efg	17,9 ef	14,5 def	16,9 d	19,2 cd		16.5	0,7	1,09
A7-12	15,5 bcd	16,5 bc	12,6 hi	13,9 hij	14,5 jk	12,6 gh	14,9 ef	16,2 efg		14.6	-1,2	0,76
T10-8	15,2 bcd	16,9 bc	13,3 gh	15,5 fgh	17,5 fg	15,5 cd	15,5 de	17,9 de		15.9	0,1	1,00
T11-10	11,9 ef	12,3 de	11,2 j	10,9 m	11,9 l	11,7 h	11,2 i	12,6 h		11.7	-4,4	0,29
A13-1	18,5 a	21,9 a	20,9 a	22,9 a	26,2 a	21,5 a	22,9 a	25,9 a		22.6	6,8	1,58
Clone Average	**15,1**	**16,7**	**14,4**	**15,9**	**18,0**	**14,9**	**16,5**	**18,5**	**16,3**			
Bb	15,2 bcd	16,9 bc	14,9 c-f	15,5 fgh	16,5 fgh	15,5 cd	16,9 d	17,5 def		16.1	0,3	0,64
Slaney	11,2 f	11,9 e	11,5 ij	11,7 lm	12,1 l	11,5 h	12,3 hi	13,0 h		11.9	-4,1	0,40
Hermes	13,3 def	14,9 cd	13,0 h	13,6 ijk	15,9 hij	13,0 fgh	14,9 ef	15,2 g		14.2	-1,6	0,81
Agria	15,5 bcd	16,9 bc	13,6 fgh	15,2 f-i	16,5 fgh	13,6 efg	14,5 ef	16,9 efg		15.3	-0,5	0,84

Cultivar Average	**13,8**	**15,2**	**13,3**	**14,0**	**15,3**	**13,4**	**14,7**	**15,7**	**General Average**		
Environmental Average	**14.9**	**16.4**	**14.2**	**15.5**	**17.4**	**14.6**	**16.2**	**18.0**	**15.9**		**1,00**
Effect of Environmental	-1,0	0,5	-1,7	-0,4	1,5	-1,3	0,3	2,1	**Confidence Interval**		
LSD (Genotype)	2,13	2,42	1,22	1,51	1,27	1,51	1,28	1,49	1,19		0,24

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PLANT PROTECTION AND FOOD SAFETY

MONITORING OF AFLATOXINS IN DIFFERENT PHASES OF CORN AND IN CONCENTRATED FOOD OF DAIRY COWS

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Abstract

Some cereals and concentrated animal food are affected by mycotoxins which are produced from molds (genus of *Aspergillius, Fusarium, Penicillium*etc*)* in the different stages of grain growing and storage, being affected by atmospheric conditions such as humidity, temperature differences, drought and invasion of different insects.The purpose of this study was to determine the presence of aflatoxin in maize in the harvest phase, storage phase and in concentrated food for dairy cows. The determination of aflatoxins was carried out with rapid semi-quantitative methods and were confirmed by quantitative methods of ELISA's.The results of the study showed that 3 out of 10 samples of maize in the harvest phase and in storage were invaded by aflatoxins above the permissible norms for use in cows for milk production. Two samples obtained from concentrated food prepared with 50% from the above maize, resulted in permissible norms.From the above findings as well as from other authors, we can conclude that mycotoxins are a serious problem that can bring not only lower animal production but can also create disturbances in people. The monitoring of mycotoxin contamination in animal food, especially for dairy cows and using of various methods for preventing and absorbing mycotoxins if they are evident is a necessary and ongoing requirement. This monitoring and prevention process can be carried out in a cooperative way, where veterinarians, zoo techniciansagronomists and other scientists should be part of it.

Key words: *Mycotoxins, aflatoxins, cereals, animal food.*

Introduction

Many cereals and concentrated food of animals are affected by mycotoxins produced mainly by the molds of genus *Aspergillius, Fusarium, Penicillium* in the different stages of grain growing and storage, being affected by atmospheric conditions such as humidity, temperature differences, drought and invasion of different insects(Charmley et al, 1995; Ioannou-et al 1999;Creppy et al 2002; Biba and Mavromati 2014).

From the different authors are mention that the mycotoxins are a serious problem that can bring not only lower animal production and different pathologies (Helferich et al 1986; Diekman and Green 1992, Krogh et al 1992; Fernandez et al 1997), but can also create disturbances in people (Madsen et al 1982; Neal et al 1998; Biba 2007).

The purpose of this study was to determine the presence of aflatoxin in maize in the harvest phase, storage phase and in concentrated food for dairy cows. The determination of aflatoxins was carried out with rapid semi-quantitative methods and were confirmed by quantitative methods of ELISA's.

Material and Methods

The study was conducted based on the following methodology:

Sampling.

a . 10 samples of maize (1,5kg) during corn harvest. The farmer produced maize for their needs for the dairy farm

b. 10 samples of maize (1,5 kg) stored in their storage places for use in dairy farm.
c. 10 samples of 1.5 kg concentrated food for consumption of lactation cows of the farm. Concentrated food contained about 50% of the maize produced and stored by the farmer himself, as well as other protein elements (soy beans) or vitamins and microelements.

Rapid test sampling analyses.
The samples were analyzed by the rapid semi-quantitative test for the detection of total aflatoxins in corn and processed food.
TAFT-P Tests (Total Aflatoxin - Precise Rapid Test).

Instruction for use.
The rapid precision test is a competitive immunoassay for semi-quantitative detection of aflatoxin residues in cereals, livestock feed, and so on. Enables the detection of over 5ppb of aflatoxins in agricultural and livestock food. Time of proof: 5-10 min. Samples: Maize and concentrated food.

Results and discussion

The table below provides the TAF-P quick-release kit for samples taken during corn harvesting.

Table No. 1. The results of sample analysis are taken during corn harvesting.

Samples Nr	Dates of taking	Time	Parcel	Characteristics of plants of the parcels	Quantity of sampling	Dates of analysis	Results
01	25/9/2017	Dry	Nr 1	Healthy plants	1,5 kg maize	25.09.17	-
02	25.09.2017	Dry	Nr 1	Healthy plants	1,5 kg maize	25.09.17	-
03	25.09.2017	Dry	Nr 1	Healthy plants	1,5 kg maize	25.09.17	-
04	30.092017	Moisture	Nr 2	Not so healthy plants and invaded by insects	1,5 kg maize	30.09.17	+
05	30.092017	Moisture	Nr 2	Not so healthy plants and invaded by insects	1,5 kg maize	30.09.17	+
06	30.092017	Moisture	Nr 2	Not so healthy plants and invaded by insects	1,5 kg maize	30.09.17	+
07	30.092017	Moisture	Nr 2	Not so healthy plants and invaded by insects	1,5 kg maize	30.09.17	+
08	04.10.2017	Dry	Nr 3	Healthy plants	5 kg maize	04.10.17	-
09	04.10.2017	Dry	Nr 3	Healthy plants	5 kg maize	04.10.17	-
10	04.10.2017	Dry	Nr 3	Healthy plants	5 kg maize	04.10.17	-

Sampling took place on parcels during corn harvest. The corn was harvested on different dates. In parcel no.1, the harvest was done in dry weather. Plants in the parcels were uniform and with good performance. There was no invasion from different insects during the growing season and corn was clean.
The macroscopic examination for molds (by eyes) did not result infestation of corn. The results of the control for mycotoxin with the rapid test were negative.

In parcel No. 2 harvesting was carried out after a 3-day rainfall period. Plants in the parcels were not uniform and with low yields. There seemed to be invasion from different insects and the maize was not clean.
The macroscopic examination for molds (by eyes) of maize resulted with infestation of molds. The results of the rapid mycotoxin control test were positive.
In parcel No. 3 the harvest was done in dry weather. Plants in the parcels were uniform and above average. There was no visible invasion by different insects during the growing season and corn was relatively clean.
Eye examination for molds did not result in pronounced infestation of corn grain. The results of the control for mycotoxin with the rapid test were negative.

Table No. 2. The results of sample analysis are taken in storage.

Samples Nr	**Dates of taking**	**Time of storage**	**Origin of warehoused place of maize**	**The characteristics of storage place**	**Quantity of the samples**	**Date of analyses**	**results**
01	05.01.2018	3 months	Silos	Very good	1,5 kg maize	05.01.18	-
02	05.01.2018	3 months	Silos	Very good	1,5 kg maize	05.01.18	-
03	05.01.2018	3 months	Silos	Very good	1,5 kg maize	05.01.18	-
04	10.01.2018	3 months	Nr 1	Good	1,5 kg maize	10.01.18	-
05	10.01.2018	3 months	Nr 1	Good	1,5 kg maize	10.01.18	-
06	15.02.2018	4,5 months	Nr 2	Warehouse with humidity	1,5 kg maize	15.02.18	+
07	15.02.2018	4,5 months	Nr 2	Warehouse with humidity	1,5 kg maize	15.02.18	+
08	25.02.2018	5 months	Nr 3	Good	1,5 kg maize	25.02.18	-
09	25.02.2018	5 months	Nr 3	Good	1,5 kg maize	25.02.18	+
10	25.02.2018	5 months	Nr 3	Good	1,5 kg maize	25.02.18	-

From the data in Table 2, it turns out that maize storage is affected by the storage location, the quality of the storage environment and the length of the storage of maize in the werhouse.
In places with adequate ventilation, moist and dry places have the premise not to affect the quality of the product.
In storage No. 2 where storage conditions are not good, the level of mycotoxins in corn is above the permissible norms. 2 samples in this warehouse was positive in the TAF-P rapid analysis.
It draws attention to the three positive samples, which are 2 in warehouse no. 2 and one in warehouse no. 3.

Table no 3 The result of sample analyzes of concentrated food.

Smples Nr	Date of taking	Origin of warehoused place of maize	Quantity of concentrated food	Date of analyse	Results
01	15.01.2018	silos	1,5 kg	15.01.2018	-
02	15.01.2018	silos	1,5 kg	15.01.2018	-
03	15.01.2018	silos	1,5 kg	15.01.2018	-
04	15.01.2018	Nr 1	1,5 kg	15.01.2018	-
05	15.01.2018	Nr 1	1,5 kg	15.01.2018	-
06	20.02.2018	Nr 2	1,5 kg	20.02.2018	+
07	20.02.2018	Nr 2	1,5 kg	20.02.2018	+
08	28.02.2018	Nr 3	1,5 kg	28.02.2018	-
09	28.02.2018	Nr 3	1,5 kg	28.02.2018	-
10	28.02.2018	Nr 3	1,5 kg	28.02.2018	-

In table no. 3 are presented the results of sample analyzes of concentrated foods.
Grinding and mixing of foods are done on the cattle farm. There was a simple evidence of supply of maize from the respective warehouses and food preparation.
From prepared mixer food from storage No. 2 we have 2 positive samples that pass the permissible limits of mycotoxins.
All other samples including samples of concentrated foods prepared with maize from magazine no. 3 (see sample No. 9 in table No. 2) are negative. The mixture of concentrate is made by placing 50% maize and adding other elements like soy beans, vitamin, macro and micro element. From this mixture the level of mycotoxins decreases and the samples are negative.
In the case of positive samples of concentrated food, it was used the physicochemical methods by using bentonite, sodium-calcium aluminosilicatefor mixing in the food (Patterson and Young et al 1993, Ioannou et al 1999).
The other new biological methods usin glactic acid bacteria to bind aflatoxins and other methods,which provides the possibility of absorbing mycotoxins and their level at permissible for animal consumption(El-Nezami et al 1998; Pierides et al 2000; Peltonen et al 2001; Diaz DE 2002).

Conclusions

From theoretical information and research through rapid tests, it turns out that mycotoxins are a real and serious problem that should be taken into account for the damage that can bring not only lower animal production but what is more important, can create disturbances in people. Their continuous control and careful monitoring of the feeding of animals that produce livestock products for public consumption is a necessary and continuous demand. This monitoring and prevention process can be carried out in a cooperation way, where veterinarians, zootechnicians agronomists and other scientists should be part of it.
Relying on the potential contamination of agricultural and livestock products consumed by the public, we recommend continuous control of them with quantitative methods to determine the amount of mycotoxins in different products.
We recommend the use of rapid tests as the first fast opportunity (5-10 min) and practical for field use. Rapid tests are semi-quantitative method and can oriented to performing other quantitative analyzes more accurate such as ELISA or HPLC methods.

Will be much better not using corn for concentrated foods if they have amounts of mycotoxins above the permissible limits.

In case of the detection of amounts above the permissible norms of mycotoxins in concentrated foods for livestock consumption, use absorbent substances with bentonite or other substances that absorb mycotoxins for reduction their levels without risking the animals, their products and public health.

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ANTIFUNGAL ACTIVITY AGAINST PATHOGENIC FUNGI AND PGPR TRAITS OF RHIZOSPHERIC ACTINOBACTERIA

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Abstract

All species of plants, wild and cultivated, are subjected to diseases, although each species is susceptible to characteristic pathogens. Plant diseases cause a significant loss, it is 40% in industrialized countries and over 50% in developing countries. The occurrence and prevalence of plant diseases vary from season to season, depending on the presence of pathogens, environmental conditions, and crops varieties grown. The rhizosphere is rich in actinobacteria. This bacterial group has strong biocontrol capacity against plant diseases. In this study, isolation of plant pathogenic fungi was conducted on infested vegetable plants and cereal crops. The work was focused on the evaluation of antifungal capacity of actinobacteria against pathogenic fungi and their PGPR traits. Six strains of actinobacteria (*Streptomyces griseus* Lac1, *S. rochei* Lac3, *S. anulatus* Pru14, *S. champavati* Pru16, *Nocardiopsis dassonvillei* Vic8 and *N. alba* Pin10) isolated from rhizospheric soils of the semi-arid region in Northeast Algeria were used. These strains were tested for their ability to produce auxin (indole-3-acetic acid: IAA) in the presence of L-tryptophan (L-Trp) as a precursor, to hydrolyze phosphate and to produce siderophores. Thus, three phytopathogenic fungi were isolated and identified, namely: *Fusarium culmorum* TRI-1, *F. oxysporum* CIT-4, *Botrytis cinerea* SL-1. The *in vitro* antagonism of the actinobacteria strains against the phytopathogenic fungi showed that, except *N. alba* Pin10, the other actinobacteria inhibited the growth of all tested fungi, and *S. griseus* Lac1 presented the most significant activity. IAA was detected in all strains with L-Trp as a precursor and the quantities varying from 2.57 to 12.10 µg/mg DW. Four strains produced siderophores and only two hydrolyzed phosphate. This study revealed the antifungal activity and some PGPR traits of actinobacteria belonging to the genera *Streptomyces* and *Nocardiopsis*.

Key words: *Actinobacteria, Botrytis, Fusarium, IAA, phosphate, siderophores.*

Introduction

Phytopathogenic fungi significantly reduce crop productivity worldwide and cause significant economic losses (Prapagdee *et al.,* 2008). *Fusarium* wilt is one of the fungal diseases encountered in Algeria. It attacks several crops such as lentils (Belabid *et al.,* 2004), date palm (Sabaou *et al.,* 1983) and chickpeas (Larbi, 1991). Pathogens that are responsible for this disease belong to the genus *Fusarium*. Pesticides are broadly used to protect crops from plant diseases. Besides the emergence of pesticide-resistant insects and fungi, the agrochemical treatments may have a harmful effect on the environment, as well as human and animal health (Reddy *et al.,* 2016). Therefore, the biological control appears as a safe alternative method for replacing chemical treatments, has therefore attracted much interest and it is increasingly used (Tanaka and Omura, 1993; de Oliveira *et al.*, 2010). Among soil microorganisms, certain rhizobacteria are considered to be an effective biocontrol agents against plant pathogens (Mohandas *et al.*, 2013).

The current study aims to characterize six rhizospheric actinobacteria (*Streptomyces griseus* Lac1, *Streptomyces rochei* Lac3, *Streptomyces anulatus* Pru14, *Streptomyces champavati* Pru16, *Nocardiopsis dassonvillei* Vic8 and *Nocardiopsis alba* Pin10 for their antagonism against phytopathogenic fungi (*Fusarium culmorum* TRI-1, *Fusarium oxysporum* CIT-4, *Botrytis cinerea* SL-1). As the biocontrol agents also often promote plant growth, these strains were tested for traits typically found in PGPR: indole-3-acetic acid (IAA) production, phosphate solubilization and siderophore production.

Materials and methods

Collection of samples, and isolation of pathogenic fungi

Samples of durum wheat, tomato and watermelon which showed the wilt or rot disease symptoms were used for isolation of fungal pathogens. Durum wheat (*Triticum durum* Desf.) ears were collected from Ben Badis locality (Constantine). Infested tomato (*Solanum lycopersicum* L.) fruits were sampled in Biskra region, while infected seedlings of watermelon (*Citrullus lanatus* Thunb, Matsum & Nakai) were taken in the locality of Ben Badis (Constantine) (Figure 1). All samples were collected in sterile bags and transported directly to the laboratory for analysis.

Except the fungal colony collected from the tomato fruits, the other samples of grains, stems and roots (Table 1) were first washed with tap water then deceived in a 1% sodium hypochlorite solution for 10 minutes, then rinsed in sterile distilled water and dried on filter paper (Zehhar *et al.*, 2006). The culture of the different samples was carried out on potato dextrose agar medium (PDA) and Sabouraud amended with chloramphenicol. Plates were incubated for 7 days at 20°C (Rémi, 1997).

Figure 1: Collected plants (a) tomato fruit, (b) watermelon seedlings, (c) durum wheat ear

Table 1 : Origins and fungal diseases of collected plants

Localities	**Plants**	**Samples**	**Fungal disease**
Constantine (Ben badis)	Watermelon	Roots, stems	Fusariosis
Constantine (Ben badis)	Durum wheat	Ears (grains)	Fusariosis
Biskra	Tomato	Fruits	Grey rot

Identification of fungal isolates

The identification at the genus level was based on the fungi determination keys described by Botton *et al.* (1990), Messiaen (1991) and Rémi (1997). Species determination was carried out at the National Institute of Agricultural Research of Algeria (INRAA, Constantine, Algeria).

Antagonistic activity of actinobacteria against phytopathogenic fungi

Antagonistic actinobacteria used in this study are listed in Table 2. These strains were isolated from different rhizospheric soils collected in Algeria.

Table2: Origin of antagonistic actinobacteria

Strains	**Rhizospheric soil**	**Site**
Streptomyces griseus Lac1	*Lactuca sativa* L.	Cultivated plots, Batna
Streptomyces rochei Lac3		
Streptomyces anulatus Pru14	*Prunus domestica* L.	ITCMI, Oum el Bouaghi
Streptomyces champavati Pru16		
Nocardiopsis alba Pin 10	*Pinus halepensis* Mill.	Forest reserve, Khenchela
Nocardiopsis dassonvillei Vic8	*Vicia faba* L.	Cultivated plots, Batna

Antagonistic activity of actinobacteria against the fungal pathogens was evaluated according to Soares *et al.* (2006) with modifications. Each strain of actinobacteria was streaked as a straight line (7 mm wide) across the centre of PDA plates (145 mm diameter) and incubated at 28°C for 5 days. Two 7-mm diameter discs from an 8-day-old fungal culture were transferred at a distance of 1.5 cm from the edge of both halves of the plates. Plates were incubated at 28°C for 5 days and antagonistic activity was assessed by measuring fungal growth from the disk to the edge of the mycelium, perpendicularly to the bacterial line. Control treatments were prepared without actinobacteria.

Indole-3-acetic acid (IAA) production

The production of IAA by the six actinobacterial strains was assessed and quantified by spectrophotometric analysis. Flasks containing 50 mL of minimum starch medium (Legault *et al.*, 2011) supplemented with L-Tryptophan (10 mM, final concentration), were inoculated with 200 µL of bacterial inoculum from a 48h pre-culture. Cultures were incubated in the dark with shaking at 30°C for 6 days. Cells were then removed by centrifugation (3450 × g for 20 min) and dried to determine dry weight. One mL of the supernatant was mixed with 2 mL of Salkowski reagent (Gordon and Weber, 1951) in a test tube and kept at room temperature in the dark for 30 min. The development of a reddish to pinkish color in the solution indicated the IAA production which was measured at 530 nm (Tsavkelova *et al.*, 2007).

Siderophores production

Glassware was treated with 3 M HCl and rinsed with deionized water to remove iron contamination (Sridevi and Mallaiah, 2008). The six actinobacterial strains were grown for 48 h in ISP4 broth at 30°C with shaking (Lee *et al.*, 2012). The cells were harvested by centrifugation at (3450 × *g* for 10 min) and 1 mL of supernatant was mixed with 1 mL of chromo-azurol S (CAS) reagent prepared according to Schwyn and Neilands (1987). The color change from bluish to reddish-brown indicated the presence of siderophores. Un-inoculated ISP4 broth was used as a negative control and remained blue in the assay. This experiment was carried out three times in duplicate.

Microorganisms usually produce two types of siderophores: hydroxamate-type and catechol-type. Hydroxamate-type siderophores are detected by the ferric perchlorate assay (Atkin *et al.*, 1970). The isolates that tested positive with CAS were grown on medium composed (per liter) of 4.25 g Na_2HPO_4, 30 g KH_2PO_4, 0.95 g NH_4Cl, 0.35 g KCl, 0.65 g NaCl, 0.9 mL of trace elements solution, 1.3 mL of succinic acid solution (0.1 g L^{-1}), 1 mL of $MgSO_4$-$7H_2O$ (0.1 g mL^{-1}) and 3.7 mL of 1 M KOH (pH adjusted to 7.25). Cultures were incubated at 28°C with shaking (250 rpm) for 6 days. The colorimetric assay consisted of mixing 0.5 mL of culture

supernatant with 2.5 mL of ferric perchlorate reagent (5 mM Fe $(ClO_4)_3$, 0.1 M $HClO_4$) (Atkin *et al.*, 1970).The color change to purple was indicative of the presence of hydroxamate siderophores (Payne, 1994).
Detection of catechol-type siderophores was assayed by Arnow's reagent. Actinobacteria (200 µL of pre-culture) were grown in modified Fiss glucose medium (Barbhaiya and Rao, 1985). Fiss glucose medium was supplemented with 1% sucrose and 0.1% $(NH4)_2SO_4$ (Sridevi and Mallaiah, 2008). Cultures were incubated at 30°C for 6 days and cells were removed by centrifugation. One mL of sterilized supernatant was mixed with equal volumes of 0.5 M HCl, nitrite/molybdate reagent (10% $NaNO_2$ and 10% NaMo, wt/vol) and 1 M NaOH in a clear microcentrifuge tube, mixing vigorously between the addition of each reagent. The presence of catechol siderophores was indicated by a pink color change; the mixture remained clear if catechol siderophores were absent (Machuca *et al.*, 2001).

Screening for phosphate solubilization

The ability of the six actinobacterial strains to solubilize phosphate was determined on Pikovskaya's agar (Pikovskaya, 1948). After 5 days at 30°C, the presence of a clear zone around the colonies, revealed phosphate solubilization (Mohandas *et al.*, 2013).

Statistical analysis

The data were analyzed with analysis of variance (ANOVA), followed by the least significant difference (LSD) test using SAS 9.1 statistical software (SAS Institute Inc., Cary, NC).

Results and discussion

Isolation and identification of phytopathogenic fungi

Eight fungal isolates were obtained from samples. Durum wheat grains provided three fungal isolates belonging to *Fusarium*, *Alternaria* and *Penicillium* genera. Wilted roots and stems of watermelon provided four fungal isolates assigned to *Fusarium*, *Aspergillus*, *Rhizopus* and *Alternaria*. The grey rot tomato sample yielded a single isolate of the genus *Botrytis*. Considering their role in phytopathogenecity, three isolates that belong to genera *Fusarium* and *Botrytis* were selected and confirmed as pathogens. Their identification at the species level was carried out at National Institute of Agronomic Research of Algeria (INRAA). These isolates, that were obtained from watermelon, wheat and tomato were designated as follows: *F. oxysporum* CIT-4, *F. culmorum* TRI-1, and *Botrytis cinerea* SL-1, respectively (Figure 2, 3, 4).

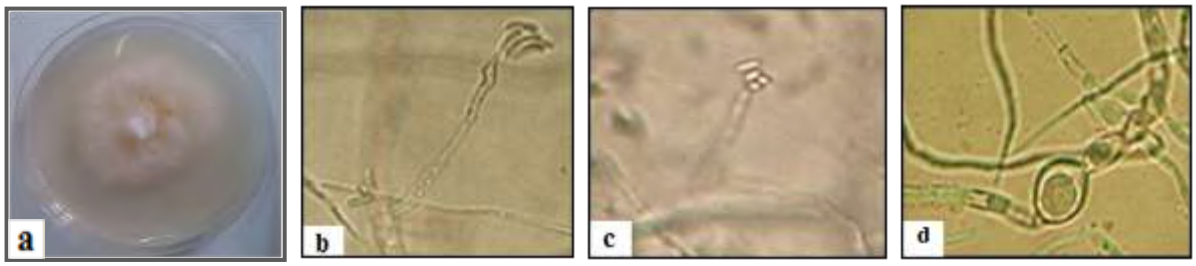

Figure 2: F. oxysporum CIT-4 (a) colony morphology on PDA (b) macroconidia; (c) microconidia; (d) chlamydospores.

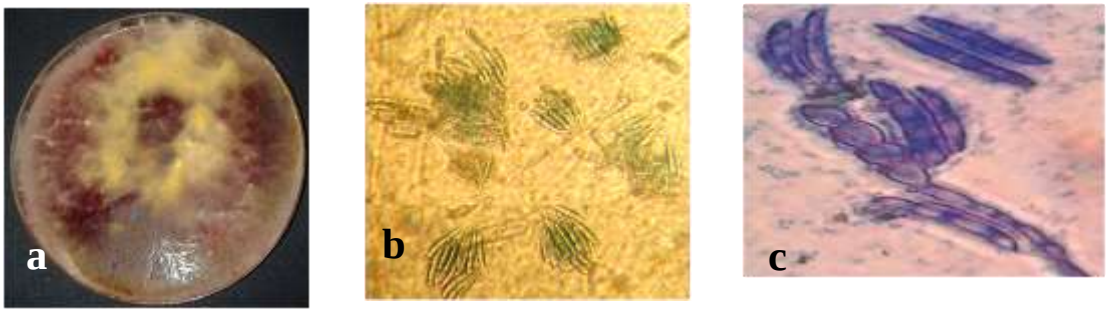

Figure 3 : *F. culmorum* TRI-1, (a) colony morphology; (b) sporodochia; (c) macroconidia

Figure 4 : *B. cinerea* SL-1, (a) colony morphology on PDA; (b) sclerotia (c) conidia

Screening of antagonistic actinobacteria against phytopathogenic fungi

All of the antagonistic actinobacteria reduced the *in vitro* growth of *Fusarium oxysporum* and *Fusarium culmorum*. The most significant antifungal activities were that of *S. griseus* Lac1, *S. anulatus* Pru14 and *S. champavati* Pru16 strains. Thus, *S. griseus* Lac1, *S. rochei* Lac3, *S. anulatus* Pru14, *N. dassonvillei* Vic8, and *S. champavati* Pru16 strains were found to be effective in reducing the growth of *Botrytis cinerea* mycelium rather than *N. alba* Pin 10 (Figure 5 C).

Production of indole-3-acetic acid (IAA)

The quantificative IAA assay was carried out with spectrophotometer at 530 nm according to the Salkowski reaction, a standard curve with commercial auxin was performed. The colorimetric assay revealed that in the presence of L-Trp, all strains of actinobacteria were able to produce IAA.

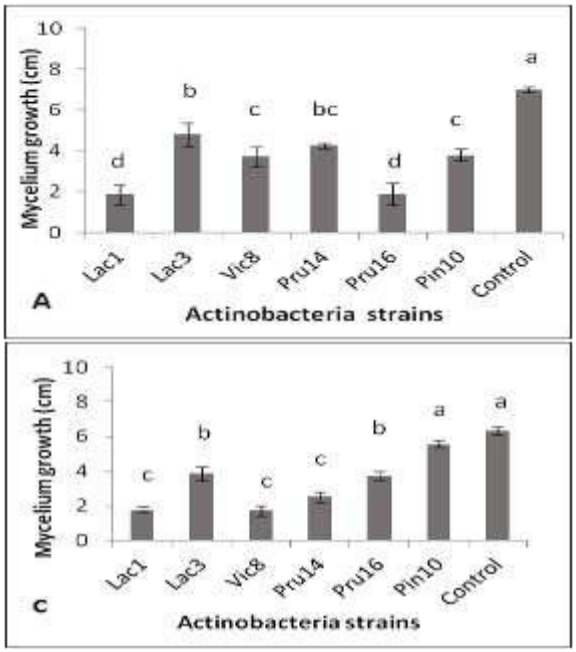

Figure 5: Effect of antagonistic actinobacterial strains on mycelium growth of phytopathogenic fungi. Results are the means of triplicates. Values with the same letter do not differ statistically (LSD). (A) *Fusarium culmorum* TRI-1, (B) *Fusarium oxysporum* CIT-4, (C) *Botrytis cinerea* SL-1.

However, there was no production of IAA when the growth medium was not supplemented with L-Trp (data not shown). The IAA amounts varied from 2.57 to 12.10 µg/mg bact. DW^{-1} (or 1.44 to 8.45 µg mL^{-1}). Strain *S. champavati* Pru16 was the highest IAA producer (Figure 6).

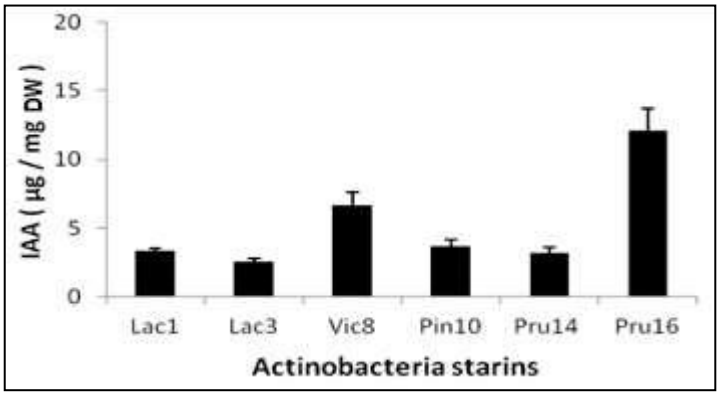

Figure 6: Indole-3-acetic acid production of actinobacterial strains

Solubilisation of phosphate and siderophores production

Among the six tested actinobacterial strains, two of them solubilized phosphate. After 5 days of incubation, diameter of clear zone was observed for two strains, *S. rochei* Lac3 (19.7 ± 0.6 mm) and *S. champavati* Pru16 (12.0 ± 1.0 mm). According to the CAS assays, four strains showed ability to produce siderophores. Specific detection revealed that three of them (*S. anulatus* Pru14, *S. champavati* Pru16 and *N. alba* Pin10) produced hydroxamate-type siderophores, while the strain *S.griseus* Lac1 produced catechol-type siderophores.

Among the eight isolated fungi, only three of them were retained since they are considered as pathogens. According to Breault *et al.* (2009), *Alternaria* and *Rhizopus* are saprophytic fungi which cause weakner diseases at the end of the growing season when the dried leaves are

covered with black pigmentation. Also, Patkowska (2002) reported that *Penicilium* sp. isolated from wheat, roots and necrotic stem bases are considered as saprophytes.

The results of this study coroborate with those obtained by Ajouz (2009) who isolated *Botrytis cinerea* from the tomato grown in France. The isolation of *F. culmorum* from lesions at the base of the stems of wheat seedlings has been already reported by Gindrat and Frei (2004). Patkowska (2002) analyzed the infected wheat samples and found that *Fusarium* species are the dominant phytopathogenic fungi, especially *F. culmorum* and *F. oxysporum*. *Fusarium culmorum* is the most phytopathogenic fungi, producing trichothecenes and it is the main agent of *Fusarium* wilt described in the world.

Fusarium oxysporum is found in soils all over the world, where it can behaves as a parasite or saprophyte. It attacks the vegetable crops (Messiaen *et al.* 1991). *Fusarium oxysporum* can be incriminated as the pathogen because *Rhizopus*, *Aspergillus* and *Alternaria* also isolated from watermelon roots do not cause dwarfism. The latter is due to vascular fusariosis. According to Larkin *et al.* (1996), *F. oxysporum* var. *niveum* is known to be a watermelon pathogen that causes vascular fusariosis which results in dwarfism. Corresponding to Rémi (1997), this pathogen has many specialized forms that attack several plants (peas, tomatoes, watermelons, melons, etc.). In this study, the percentage of actinobacterial strains active against *F. oxysporum* was also higher than what was obtained by Kitouni *et al*. (2005) with non-rhizospheric soil samples from a semi arid region in North-Eastern Algeria.

Actinobacteria are well known to produce secondary metabolites and extracellular enzymes (Khamna *el al.,* 2009). It has been reported that nearly 5% of rhizobacteria can promote growth of plants and protect them against pathogens such as bacteria and fungi (Suslow, 1982). According to Beauchamp (1993), PGPR stimulate indirectly plant growth by their antagonistic effect on detrimental microorganisms (antibiosis). PGPR are also beneficial to plants directly by increasing plant nutrients uptake via siderophores production and phosphate solubilization, producing plant growth regulators or triggering plant resistance mechanisms.

The production of IAA is a characteristic commonly found in PGPR. In the presence of L-Trp, the six actinobacterial strains tested produced appreciable amounts of IAA (from 2.57 to 12.10 µg/mg bact. DW), comparable to those found in the literature (Khamna *et al.*, 2009 ; Chaiharn and Lumyong, 2011). However, IAA amounts are significantly higher than those found by Mohandas *et al.* (2013). IAA production was higher for *S. champavati* Pru16 compared to the other strains.

Among the six actinobacterial strains explored in this study, three of them produced hydroxamate-type siderophores, while one produced catechol-type. In a study on siderophore-type production by *Streptomyces* strains, Lee et *al.* (2012) found that only 20% of isolates produced catechol, while all produced hydroxamates. Our study revealed catechol production by *S. griseus* Lac1, which is newly reported in *S. griseus* strains, previously shown to produce only hydroxamate-types siderophores (Yamanaka *et al.*, 2005 ; Macagnan *et al.*, 2008).

Phosphorus (P) is one of the most essential nutrient requirements in plants. In the soil, P is mainly found in insoluble forms that are not accessible to plants. The ability to solubilize phosphate is therefore an important trait of PGPR for increasing plant yields. In the present paper, only two strains (*S. rochei* Lac3 and *S. champavati* Pru16) were found able to solubilize phosphate, while strain *S griseus* Lac1 with the highest biocontrol potential, showed no abilities to solubilize phosphate. Franco-Correra *et al.* (2010) have isolated thirty rhizospheric actinobacteria, they found that twenty one of them exhibited phosphate solubilization ability. Among the several potential mechanisms for phosphate solubilization those involving the production of chelating compounds, like organic acids or by means of a modification of pH of the medium by the secretion of organic acids or protons are the more often described in the literature (Pikovskaya, 1948; Richardson *et al.*, 2009).

Conclusion

In the present study, the isolation of three phytopathogenic fungi was reported. Six antagonitic actinobacteria were tested against these fungi. These actinobacteria are of interest for the biocontrol of fungal plant pathogens since they were not only produced antifungal molecules, but they also showed PGPR traits. Interestingly, the present results showed that *Nocardiopsis* species possess the ability to produce IAA and siderophores *in vitro*. However, none of the two *Nocardiopsis* strains solubilized phosphate. There is scarce information in the literature on phosphate solubilization, IAA and siderophore production by non-*Streptomyces* genera and, to our knowledge, this is the first study focused on the *Nocardiopsis* species to evaluate their potential PGPR abilities. However, the apparently efficient biocontrol abilities of actinobacteria strains need to be examined under field conditions.

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CONTENT OF Pb, Fe, Cu AND Zn IN THE FIG AND HIP FRUIT ON DIFFERENT AREAS

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Abstract

In everyday diet, fruits and vegetables are used more and more because of their well known beneficial effects on human health. Population is mostly oriented on consummation of greater amounts of fruits that are planted in their surroundings. The Herzegovinian area is well known after figs and hips, during the history. These kinds of fruits are common for this area, and they are available for all population layers such as poor ones and rich ones as well. Both cultivations are available during the whole year and they can be consumed both as fresh ones and processed as well. This research had an aim to establish, on the science base, the way that metal went from the ground to the fig and hip fruit and their accumulation in these fruit cultivation. We chose different locations all around Mostar city area and its suburb. Our research showed that certain fig fruits, with or without peel, had different contents of researched elements, but content in all fruits was under permissible level. It was very interesting that all fig samples with peel showed higher level of iron presence, when we compared them with samples without peel. The presence of iron, copper and zinc was shown in the hip fruit, with or without mesocarp, and the levels were under permitted ones. Generally, we could say for the hip fruit that zinc, iron and copper were mostly accumulated in samples of grain with mesocarp instead of pure grain.

Keywords: *fig, hip, ground, heavy metals*

Introduction

The fig and the hip are plants and they are not demanding of land quality, therefore they are planted in barren soil, soil degraded from nutrients, they are also very persistent when they do not have enough water (Džubur, 1999). Nutrients from the soil influence on plant (Greaf, 2001). Plants cannot recognize nutrients they need and those that are not important for them, and that is the reason they take all of them (Blagojević *et al*., 2009). The intensity of accumulation depends on nutrient, its influence on biochemical reaction, its content in outdoor area and so on (Ćatović, 2007). Heavy metals are the most polluted elements, they pollute both environment and plant as well, and they cause oxidation stress. It is very important to find out mechanisms of their harmful influence, and tolerance of plants on presence of heavy metals in environments (Goyer *et al*., 1995). The accumulation of heavy metals is in the parts of plants, it is mostly accumulated in fruits and it is mostly taken from the root and from leaves (Borić-Šaćiragić, 2010). High content of heavy metals in the soil causes also high accumulation of heavy metals in plants (Yanyu *et al*., 1996).

Material and Methods

Through chemical analysis of adult plants, fig plant and hip plant, researched the quality of soil, presence of metals and their influence on development of plant that take all components from the soil. Transport of harmful matters is possible if they are present in larger quantities.
Researched locations were in the city of Mostar (Entity of Federation of Bosnia and Herzegovina), the center of the town and suburb area. Chose three locations: the area of Dubravska plateu, located 40 km away from the city of Mostar, on the south, private property,

at about 200m air distance from the highway. Small place called Blagaj, in the suburb of Mostar, at about 10 km away from the city centre. This location is 3km air distance away from highway, it is close to the place called „Zalik" on the north of Mostar city area. This place has large content of researched metals. Researched areas are far away from each other, and they can give us relevant data about presence of researched metals. We supposed that these locations had different quality of soil.

We passed through following phases of our research: choosing location, taking samples of the soil, taking samples of fruits (fig and hip), preparing of samples for analysis, lab analysis of soil and fruits. We used standard methods with chromed probe we took 5 individual samples by the diagonal of the parcel. We collected all of those samples into one average sample, it weights at about 2 kg. We had two different depths of the soil, one of them was from 0 to 30 cm and the other was from 30 to 60 cm. We prepared six samples of the soil, and they would be researched in order to show the quality of the soil, especially the content of following metals: Pb, Fe, Cu, Zn. We did this during vegetation period, but before the fruit ripening, during September and October in 2012.

Figs were ripe earlier, because of hot weather during the summer in 2012, so we started our research from 2nd to 4th of September. We took at about 1 kg of healthy fruit from all trees, from the average branches in all three locations, and we formed one sample. Fresh figs from all locations are separated in two different groups: one group was figs with skin and the other group was peeled figs. This is a period when the figs are dried in order to consume them during the whole year. We collected **six** different samples. Our samples were put into plastic boxes and they were frozen at -20^{o}C, in that way we protected our samples till analysis.

Hip was taken from 15th to 25th of October in 2012, hip fruits were ripe at that time, and its consummation was intensive at that time. We picked hips up for few different trees in different areas, and we took at about 30 healthy fruits, and after that we prepared one sample. From every picking fruits we prepared two samples: one sample was just grain of the hip and another was grain of the hip with mesocarp, because while we prepare the juice of the hip we also use one part of mesocarp. We prepared **six** samples. We put our samples into plastic boxes and we froze them at -20^{o}C, and we kept them at that temperature till our analysis.

Results and Discussion

Results of physical- chemical analysis are shown in the following table 1.

Table 1. Physical - chemical parameters of the soil

Location	Depth (cm)	pH values (±0,01)		% The content of nitrogene	% The content of phosphorus/ 100g of the soil	% The content of potassium/ 100g of the soil	% The content of humus	% Higroscopic humidity
		in H_2O	in n-KCl					
Dubrave	0-30	7.50	6.70	0.21	6.50	10.4	1.24	2.38
	30-60	8.12	7.20	0.23	9.15	14.8	1.20	2.21
Blagaj	0-30	7.70	6.90	0.25	9.45	12.1	3.18	2.37
	30-60	7.96	7.10	0.26	7.80	11.5	1.46	2.01
Zalik	0-30	8.06	7.15	0.18	6.40	12.5	3.62	3.33
	30-60	7.70	6.80	0.24	8.45	15.8	2.49	4.24

From the table, we can see that according to Ph values in the water, we can realize that it is slightly alcaline soil, and pH values are normal from 7.50 to 8.12. pH values in n-KCl-u are also neutral from 6,70 to 7,20, and all three researched locations are neutral. The result of

nitrgene in all three researched areas is balanced and it is at about 0,2 mg/100 g of the soil, and we can conclude that all three researched areas are rich in this element. The content of phosphorus is poor, because the content of this element is under 15 mg/100g of the soil. The content of potassium is very low from 10.4 to 14.8 mg/100g of the soil. All three researched soil are categorised as average as far as quality of soil is concerned. According to nutrients in the soil, all three researched soil can be categorised as barren soil. In the table 2, there are results of chemical analysis of the content of metal in the samples of researched locations.

Table 2 The content of researched metals in the soil

Location	Depth (cm)	Pb (mg/kg) MDK 50,00	Fe (mg/kg) MDK 30.000,00	Cu (mg/kg) MDK 50,00	Zn (mg/kg) MDK 100,00
Dubrave	0-30	16.36	13.774,85	32.56	90.20
	30-60	16.82	19.529,71	25.97	78.71
Blagaj	0-30	23.42	13.068,13	22.87	64.35
	30-60	17.18	13.999,21	21.74	54.31
Zalik	0-30	94.93	29.717,17	51.42	140.33
	30-60	76.29	35.346,06	53.07	191.40

The content of researched metals in the samples of the soil is very different in different locations. Values of permitted concentration go over maximal values. The presence of lead in the location of Zalik is high, and its value is 94,93 mg/kg (in the depth from 0 to 30 cm) and 76,29 mg/kg (in the depth from 30 to 60 cm), that is 89,86 %, or 52,58 % higher when we compare it with MDK. The lowest values were in the area of Dubrave, in the depth from 0 to 30 cm it is 16,36 mg/kg. In this area we expected low content of Pb, because it is far away from all potential pollutant. The content of iron in the location of Dubrave and Blagaj,in both depth, and in the location of Zalik in the first depth is under permitted values, while the concentration of this element is higher than permitted values in the second depth in the location of Zalik. The presence of copper is in permitted values in the location of Dubrave and Blagaj, while its presence in the location of Zalik is above permitted values. Higher values of copper are in other depth of the same location. In the location Dubrave and Blagaj, the content of present zinc is under MDK, while in the location of Zalik, this element is above permitted values. In the first depth, in the location of Zalik its presence is higher for 40,3%, while it value is higher for 91,4% than permitted value.

Table 3 shows data about content of the most relevant values that show the quality of fig fruit.

Table 3 Physical-chemical analysis of fig fruit

Location	Shape	pH-of fruit (±0,01)	% Dry matter	The level of acidity g/100ml	% total sugars
Dubrave	With the skin	5.16	35.95	0.21	23.40
	Without the skin	5.11	30.99	0.21	24.00
Blagaj	With the skin	4.79	34.69	0.18	23.00
	Without the skin	4.72	32.20	0.19	18.40
Zalik	With the skin	4.95	39.13	0.56	29.40
	Without the skin	4.86	30.21	0.42	17.00

The results of pH-values show that the fig fruit is acid taste. From the table we can see that the samples of fig fruit without of skin had lower pH-value. The percent of dry matter is between 23.54 and 39.13. The level of acidity shows that all researched samples have acidity very close to lower value, and we can explain that with dry climate conditions during the time of ripening the fruit. The level of acidity had an example from the location of Blagaj, and it was the fig with the skin ant its value was 0.18, while the highest level of acidity was in the fig with skin from the area of Zalik and it was 0.56. According to the content of total sugars, the sample of peeled fig from the location of Zalik had the lowest value, it was just 17.00, and the highest content of total sugars, in the fig with the skin, was in the location of Zalik, and it was 29.40.
In the table 4, there are data about the content of researched metals in the fig fruit.

Table 4 The content of researched elements in the samples of fig

Location	Depth (cm)	Pb (mg/kg) MDK 0.10	Fe (mg/kg) MDK 30.00	Cu (mg/kg) MDK 10.00	Zn (mg/kg) MDK 150.00
Dubrave	With the skin	It was not found	2.39	0.30	1.06
	Without the skin	It was not found	1.62	0.26	0.97
Blagaj	With the skin	0.004	2.78	0.59	1.14
	Without the skin	0.002	2.48	0.67	1.45
Zalik	With the skin	0.012	3.44	0.40	1.32
	Without the skin	It was not found	1.78	0.31	1.15

The presence of all researched elements in the samples of fig fruit, both peeled and unpeeled, was in the permitted values.
The content of lead was not found in 50% of samples, while in the rest of samples it was found under MDK. Amounts of present iron were also under permitted values, and the highest values were found in the sample of fig with skin in the area of Zalik. The content of copper and zinc were very low.
Parameters of basic values of the hip samples are shown in the table 5.

Table 5. Physical-chemical analysis of hip sample

Location	Shape	pH-fruit (±0.01)	% Dry matter	The level of acidity g/100ml	% Total sugars	The content of vitamin C mg/100gr
Dubrave	Grain	4.01	24.00	1.00	12.00	7.55
	With mesocarp	3.88	23.68	1.50	11.60	7.80
Blagaj	Grain	3.04	27.47	2.02	9.60	8.20
	With mesocarp	3.13	25.96	2.11	9.20	10.20
Zalik	Grain	3.18	26.29	2.42	10.60	10.50
	With mesocarp	3.06	26.33	2.64	8.20	5.60

According to pH-values, hip belongs to acid fruit, because the values of acidity are between 3.04 and 4.01. The content of dry matter is between 23.68 in the sample with mesocarp in the

location Dubrave, it is up to 27.47 in the location of Blagaj and in the sample of pure grain. The level of acidity is pretty low, the same as the level of total sugars.
This is acid fruit, and the proof for that is the content of vitamin C, and its content had the highest values in the area of Zalik, Those values were 10.50 mg.
The presence of researched metals in the samples of hip is shown in the table 6.

Table 6. The content of researched elements in the sample of hip

Location	Depth (cm)	Pb (mg/kg) MDK 0.10	Fe (mg/kg) MDK 30.00	Cu (mg/kg) MDK 10.00	Zn (mg/kg) MDK 150.00
Dubrave	Grain	Wasn't found	1.532	1.163	0.548
	With mezocarp	Wasn't found	2.180	1.293	0.573
Blagaj	Grain	Wasn't found	2.738	1.186	1.638
	With mezocarp	Wasn't found	1.856	0.765	0.641
Zalik	Grain	Wasn't found	2.735	1.111	1.207
	With mezocarp	Wasn't found	2.850	1.398	1.607

The results of chemical analysis of the hip sample showed presence of iron, copper and zinc and their values were under permitted. The content of lead was not found in the samples. In the areas of Dubrave and Zalik values of iron were higher in the samples with mesocarp, while in the area of Blagaj its presence was higher in the sample with pure grains. The presence of copper was found in all samples. The lowest values were found in the area of Blagaj in the sample with mesocarp, and highest value was found in the sample with mesocarp from the area of Zalik. The content of present zinc was pretty lower, when we compared it with permitted content. The lowest values were found in the area of Dubrave, and highest values were found in the sample with pure grain in the area of Blagaj.

Conclusions

In the area of Zalik in the samples of the ground, we found increased content of all four researched elements, when we compared them with MDK. The content of zinc was above normal values for 91,1%, the content of lead for 89,86%, iron for 16,7% and copper 3,1%. According to results of our research we can see that this area is the most polluted. Other two locations did not contain higher concentration of these elements.
Individual fruits of fig, with skin or peeled one, had different contents of researched elements. It is important to mention that all values in whole samples were under permitted values. All samples of figs with skin had higher value of iron, when we compared them with peeled ones. The content of zinc and copper in two samples with skin was higher when we compared them with peeled samples. In general, we can conclude that presence of researched metals is higher in samples with skin. The highest level of lead (0.012 mg/kg) was found in the sample of fig with skin in the area of Zalik, at the same place, there was the highest level of lead (94.93 mg/kg). The presence of iron, copper and zinc was found in all samples of hip fruit, with or without mesocarp, and values were under permitted values. Lead was not found, not even in one sample therefore we can conclude that hip is a plant that does not accumulate this element from the ground. The higher value of zinc and iron in the samples of hip with mesocarp was found in two samples, and only one sample of pure grain had increased content of these two elements, and this was in the area of Blagaj. The values of copper were higher in the samples with mesocarp in two locations, while it was vice versa in the area of Blagaj. For the hip fruit we can also conclude that iron, zinc and copper are more accumulated in the samples grain with mesocarp when we compared it with pure grain.

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ANALYSIS OF THE PRODUCTION OF SEED POTATOES IN REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA)

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Abstract

In Entity of Republic of Srpska (Bosnia and Herzegovina), average annual production of potatoes is produced on an area of about 15.000 ha, and the average yield is 11.9 t ha^{-1}, while the seed potato production represents about 80 ha, which is only about 8% of the total needs for Republic of Srpska. The largest part of seed production is located in the municipality of Rogatica, about 54 ha. This is influenced by agroecological conditions, but also by the tradition of potato production in this area. The analysis of seed potato production was conducted in the association of farmers „Solanum produkt", where the seed potatoes were grown on 30 hectares, and mercantile potatoes on about 8 hectares. A two-year study (2014 and 2015) was performed on 5 varieties (Agria, Faluka, Kennebec, Kurada and Desiree) which were grown on the larger areas on "Solanum produkt." In the two-year study the highest yield was found in Desiree variety with an average yield of 18.6 t ha^{-1}, while Kurada variety had the lowest average yield of 14.05 t ha^{-1}. In 2014, the average potato yield was 21.4 t ha^{-1} and in 2015 average potato yield was 27.9 t ha^{-1}. For all varieties we noticed a large variation in yield due to agro-ecological conditions, and lack of moisture in the embryo phase and in the filing of tubers. Since this is a seed potato production, it is necessary to take care of the size of tubers because the large tubers are not desirable. In 2014, there was a higher percentage of seed tubers because of the influence of weather conditions and the timely interruption of vegetation, while percentage of small tubers increased in 2015. In the case of seed production, it is necessary to stop the vegetation earlier, in order to obtain a greater percentage of seed tubers.

Keywords: *seed potatoes, variety, yield, tuber yield structure.*

Introduction

According to RZS data, the average perennial production of mercantile potato takes place on the surface of about 15,000 ha, and the average yield is 11,9 t ha^{-1} (*Milic and Bogdanovic*, 2009; *Govedarica et al*, 2016), while the seed production of potatoes is represented on about 80 ha, which is only about 8% of the total needs for the Republic of Srpska (http://www.rzs.rs.ba). The largest part of seed production was organized in the area of Rogatica municipality, about 54 ha. This was influenced by agroecological conditions, but also the tradition of potato production in this area. In the association of farmers "Solanum produkt" seed potatoes are grown on 30 hectares and mercantile on about 8 hectares.

Potatoes have high economic significance for the mountainous region of Republika Srpska. This significance arises from the surfaces on which it is grown, the wide use and the nutritional and physiological values *(Djordjevic*, 2000a and b; *Cota*, 2000; *Milic et al*., 2012; *Milic et al*., 2006a; *Milic et al*., 2000a, *Milic et al*., 2000b, *Milic et al*., 2006b). In addition to favorable agroecological conditions for the production of potatoes, the yields per unit area are still small and fluctuating by age (*Milic et al*., 2000c, *Milic et al*., 2016). Small average yields are the result of extensive production, which is reflected in the use of poor planting material, inadequate sorting and inadequate agro-technology.

Although in the Republic of Srpska there are constant needs for pre-basic and basic seed potatoes, as well as the center for its production, the basic planting material (elite) is imported from the Netherlands and other countries of Western Europe. The need for pre-basic or basic planting material (elite) is much higher than the current consumption. In the eighties of the last century a modern center was established in Sokolac with the aim of producing pre-basic seed potatoes, i.e. elite and certified planting material. Although well equipped, the production of a virus-free potato was suspended. Today, the production of seed potatoes in the Republic of Srpska deals with the private sector, and one of the most important domestic producers is "Solanum produkt" from Rogatica.

The aim of this paper is to analyze the seed production of potatoes in the "Solanum produkt" of Rogatica in two years of research.

Material and methods

A two-factorial experiment was placed on a random block system in four repetitions. Basic soil cultivation was carried out in autumn by destroying natural meadows at a depth of 30 centimeters. 450 kg ha^{-1} NPK 8:16:24 and 200 kg ha^{-1} NPK 9:18:27 were entered into the land. Planting was carried out at an interval of 70 cm and 24 cm in length, giving a set of 59.523 plants per hectare. Planting material was used in the category of elites purchased in the Netherlands. Planting was carried out in the period from May 15 to May 25. During the experiment, care measures were taken (hoeing, 300 kg ha^{-1} 27% cannabis and dressing), as well as protection (fight against weeds, insects and causative agents) according to needs. The interruption of vegetation was carried out at the end of August with Reglon in the amount of 5 l ha^{-1}.

Factor A - variety (experiment included 5 varieties): Agria (a_1), Faluka (a_2), Kenebek (a_3), Kuroda (a_4), Desire (a_5). The experiment was set in 2014 (b_1) and 2015 (b_2).

The experiment was set up at Mountain Borika, at an altitude of 980 meters, 43 ° 53'16 "SGS; 19 ° 06'34" IGD. Borika Mountains have a mountain climate with large snow, which lasts for a long time, until late spring. Maximum temperatures reach 30 °C, minimum -28 °C, and medium 15 °C. The annual rainfall reaches 1500 mm, while the amount of atmospheric precipitation (distributed over the seasons) is even the most accurate in Europe. Data on temperatures and precipitation in the biennial experiment were monitored at the registered meteorological station (Sokolac).

To determine the average weight and number of tubers, we took samples with twenty fruits in each basic parcel, while yield was obtained by weighting the yield of each plot and calculating the yield per hectare.

All data was statistically processed using Sigma Plot Windows 2000 (*Jandel Scientific, Erkhart, Germany*) and Statistica for Window. The obtained differences for each tested parameter were tested with the LSD test.

The average daily temperatures in 2014 and 2015 were higher compared to the perennial average (Table 1). During vegetation of potatoes (May-September), the average daily temperatures were 14.38 °C, which is more than 0.38 °C compared to the perennial average (May-September). In 2014 the amount of precipitation was significantly higher (1218.9 mm) than the perennial average. In the vegetation period, potatoes fell to 765.9 mm, which is sufficient to obtain higher yields of the tin of the corresponding fractional structure. Frequent shifts in sunny and rainy days affected the development of the disease, which often led to the protection of fungicides.

Table 1. Meteorological conditions for 2014-2015 and perennial averages for Sokolac (Sokolac Weather Station)

Month		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Average (1961 1990)	Temp. (°C)	-4.2	-2.6	2.2	5.7	11.5	14.6	16	15.7	12.2	7.7	2.8	-1.8
	Precipi. (mm)	51	45	63	71	81	96	67	76	75	86	80	57
2014	Temp. (°C)	2	4.7	4.8	7.6	10.7	15.2	17	16.8	12.2	9.1	6	-0.5
	Precipi. (mm)	37	11	63.6	153.9	260.1	170	76.2	112.6	147	42.9	56.6	88
2015	Temp. (°C)	-2.5	-1.3	2.1	6.2	13.4	15.3	19.8	18.9	14.5	8.4	3.2	-1.6
	Precipi. (mm)	89.9	56.1	74.7	53.2	75.4	113.3	25.2	85.7	84.3	106.9	71.2	0.3

In 2015, higher daily temperatures were recorded compared to perennial averages and temperatures in 2014. In 2015 there were 836.2 mm of precipitation, and during the vegetation it fell 383.9 mm of rain. The lack of precipitation in July (25.2 mm) did not compensate for the precipitation in August, which significantly influenced the yield reduction and the formation of minor troughs that are of no more significance. Potatoes are best grown in deep, fertile and loose soil. In such a root environment, the plant system can be developed more rapidly at greater depths and, if necessary, from these layers, the moisture necessary for normal growth and development. Potatoes do not tolerate heavy, wet and water-impermeable soils because they do not provide the aeration needed for breathing of the root of the plant. Potatoes are well grown on poorly acidic soils (pH/H_2O 5.5 to 6.5) (*Jakovljevic*, 1994). However, on extremely acid and alkaline soils (pH 4.5 and 7.5), poor yields are produced and the product is of poorer quality (*Maksimovic*, 1996). An increase in c. above-ground parts of the plant, it is best at a pH value of 7.40 to 8.27. (*Jakovljevic*, 1994; *Lazic et al.*, 1998). Table 2 gives the chemical characteristics of the land on which the tour was carried out.

Table 2. Chemical characteristics of the soil in the test field (Borike)

Depth (cm)	pH/H_2O	pH/KCl	Humus	N	Soluble mg/100g	
			%	%	P_2O_5	K_2O
0-30	5,02	3,85	2,55	0,16	8,98	25,38

The experiment was set on sour-brown soil (distric cambisol). After the chemical analysis of the soil sample, we determined that this is a highly acidic soil (pH/KCl analysis), i.e. a poorly acidic reaction (pH/H2O). The land is humus, provided with nitrogen, poorly secured with phosphorus and well secured with potassium. Potatoes are poorly developed in heavy, humid and cold soils, they are late to trowle, grow old and remain small and irregular. In such soil conditions, potatoes often also form air tusks in the lower leaves. They are best grown on mountainous blackberries, forest shrubs, alluvial soils, which are slightly acidic, so that the optimum value of the soil solution pH is from 5.2 to 6.

Results and discussion

The average number of tubers for all the tested varieties and years was 6.01 (Table 3). In the Agria variety (8.17) we determined the highest number of tubers per herb and it was statistically significantly higher compared to other investigated varieties. The smallest number of formed tubers per plant (6.19) had the varieties Kurad and compared to the variety Kenebek (6.55) was significantly lower, as well as the variety Desire (6.62), while compared to Faluk (6.99) this difference was highly significant. The variety Faluka compared to the variety Desire and the Kenebek variety had a significantly higher number of tubers. The highest number of tubers per plant in all tested varieties was achieved in the year when the highest number of PNI per plant was determined, which agrees with the results (*Momirovic et al*., 2000; *Bussan et al*., 2007; *Postic et al*., 2012a).

Table 3. Number of tubers per plant

Year / Variety	2014	2015	Average for the variety
Agria (a_1)	9.05	7.30	8.17
Faluka (a_2)	7.27	6.72	6.99
Kenebek (a_3)	6.70	6.40	6.55
Kurada (a_4)	6.67	5.72	6.19
Dezire (a_5)	6.88	6.36	6.62
Average for the year	7.31	6.50	6.91

LSD	A	B	AxB
0,05	0.304	0.216	0.423
0,01	0.517	0.368	0.728

The number of formed tubers, as well as the equalization of their size, depends on the hereditary characteristics of the variety, but also on the external influences. According to *Kopetya & Steinek* (1958), in years with normal climatic conditions, tart forming begins 4-5 weeks after sprouting. If there is enough moisture on all the columns during this period, the tubers will be formed. In the absence of moisture during this period, the plant reduces the number of tables and forms a smaller number of tubers. *Van dam et al*. (1996) and *Barkley* (2005) in their researches received a higher number of tubers per plant per lower than at higher air temperatures. These results are in line with our results. In 2014, the average temperatures were worse than temperatures in 2015. In 2014, the average number of tubers was 7.31, which is statistically significantly higher compared to 2015 (6.50). *Barcak et al.* (2003) found that genetic factors determine the possibility of the formation of columns, the number of tubers and their morphological properties. However, meteorological conditions in combination with soil properties determine the extent to which these characteristics will manifest themselves. The average mass of the tuber for all the tested varieties and the research year is 49.9 grams (Table 4). In 2014, the average mass of the tuber (61.3 grams) was statistically significantly higher, compared with the average mass of the tuber (38.4 grams) in 2015. The air temperatures that were optimal for the production of potatoes in June and July at the stage of table formation, the taper of the tuber and the beginning of the filling of tubers, followed by evenly distributed precipitation, played a key role in the formation of seed tubers in 2014, which is in line with results (*Van Dam et al*., 1996; *Jovovic*, 2011; *Postic et al*., 2012a).

Table 4. The average weight of tubers (g)

Year / Variety	2014	2015	Average for the variety
Agria (a_1)	42.2	35.4	38.8
Faluka (a_2)	73.9	26.6	50.3
Kenebek (a_3)	64.9	43.1	54.0
Kurada (a_4)	55.5	39.6	47.5
Dezire (a_5)	70.0	47.5	58.8
Average for the year	61.3	38.4	49.9

LSD	A	B	AxB
0,05	4.448	3.148	6.296
0,01	7.604	5.384	10.752

By increasing the number of pellets and calls per tuber, the number of primary trees per plant also increases, the number of planted tubers per plant is growing, but not their average mass, and vice versa, which is in agreement with the results (*Djokic*, 1988; *Bussan et al*., 2007; *Postic et al.*, 2011). The Desire variety had the highest mass of tubers in the years of research, and it did not have the largest number of PNIs formed, nor the largest number of formed tubers.

Statistical analysis of the yield (Table 5) showed very significant differences in the variety Desire (18.06 t ha^{-1}), and in comparison with the varieties Kurad (14.05 t ha^{-1}) and significant differences compared to the Agria variety and the Faluka variety, while the differences in the yield of tubers between the variety Kenebek and Kudar varieties were significant. In addition to climatic conditions and the quality of planting material, the yield of tubers is influenced by the genetic potency of varieties (*Ilin et al*., 2000; *Djukic et al*., 2000). Differences in yields of potatoes by varieties in the same year result from the genetic potential of the yield of the variety under given conditions, which is in accordance with our results. *Djordjevic* (2000) believes that the proper selection of potato varieties for the given production conditions would best exploit their genetic potential. Varieties that shrink a smaller number of tubers and form short and medium long stolons are suitable for cultivation especially in years with precipitation deficiency (*Bugarcic*, 1991), which is in line with our results.

Table 5. The yield of tubers (t ha-1)

Year / Variety	2014	2015	Average for the variety
Agria (a_1)	18.3	12.1	15.20
Faluka (a_2)	19.7	11.1	15.40
Kenebek (a_3)	20.8	13.1	16.95
Kurada (a_4)	17.3	10.8	14.05
Dezire (a_5)	22.6	14.6	18.60
Average for the year	19.74	12.34	16.04

LSD	A	B	AxB
0,05	2.26	1.15	3.19
0,01	3.86	2.24	5.47

In 2014 the average yield of tubers (19.74 t ha^{-1}) was statistically significantly higher, and compared to 2015, when the average yield of tubers was 12.34 t ha^{-1}. The layout and amount of precipitation during the vegetation period in 2014 largely met the needs of water potatoes, which largely reflected that the total yield of tubers was significantly higher compared to 2015, when a large deficit was recorded, especially in July, which influenced the watering of the tuber. Such results are in line with the research of many authors (*Tadesse et al*., 2001; *Tomasevic et al*., 2003; *Daric & Dimitrijevic*, 2009; Milic et al., 2010; *Momirovic et al*, 2010; *Jovovic et al*., 2011; 2011; *Postic et al*., 2012ab), which state that the conditions of production affect the total yield of potato tubers.

The farmers' association "Solanum produkt" currently produces seed potatoes at about 30 hectares. Table 6 shows the total production of seed potatoes in "Solanum produkt" in Rogatica. The areas on which seed potatoes have grown in recent years, and on the demand of seed potatoes on the domestic market, it was significantly influenced by the creation of optimal conditions for the storage and processing of seed potatoes.

Table 6. Total tuber yield (t ha-1)

Year / Variety	2014			2015		
	Yield (t ha^{-1})	Land area (ha)	Total yield (t)	Yield (t ha^{-1})	Land area (ha)	Total yield (t)
Agria (a_1)	18.3	9	164.7	12.1	12	145.2
Faluka (a_2)	19.7	6	118.2	11.1	7	77.7
Kenebek (a_3)	20.8	4	83.2	13.1	4	52.4
Kurada (a_4)	17.3	3	51.9	10.8	3	32.4
Dezire (a_5)	22.6	3	67.8	14.6	3	43.8
Total		25	485.8		29	351.5

Tables 7 and 8 depict the fractional structure of tubers in years 2014. and 2015. In 2014. The small tubers widespread was 1, 33%, seed tubers 88, 91% (fractions 28-35 mm and 35-55 mm) and large fraction 9, 76%. in 2015. The ratio of fractional structure was unfavorable because there was 0, 88% of large, 50, 12% seed and 49, 18 % of small tubers. in 2015. The percentage of small tubers in fractional structure was between 31.7 to 57.5% which is undesirable in seed production. High percentage of small fraction like this is affected by favorable conditions during tuber setting, and then there was dry period (month of July) which had negative effects on tuber filling. In average for all varieties and years the achieved yield of small fraction was 3,104 t ha^{-1}, seed fraction 11,925 t ha^{-1}, and large tubers 1,019 t ha^{-1}. The Agria variety had the biggest yield of small tubers, the biggest yield of the seed fraction had the Dezire variety, and of the large fraction, the Kenebek variety. In 2014. The average yield of small fraction was 0,258 t ha^{-1}, seed fraction 17,553t ha^{-1}, and large fraction 1,927 t ha^{-1}. The Kurada variety had the biggest small fraction yield, the biggest yield of the seed fraction had the Dezire variety, and the biggest yield of large fraction had the Kenebek variety. The smallest small fraction yield had the Faluka variety, varieties Agria and Kurada had the smallest yield of seed fraction, and varieties Faluka and Kurada had the smallest yield of large fraction. In 2015. The yield of small fraction was 5,954 t ha^{-1}, the yield of seed fraction 6,295 t ha^{-1}, and the yield of large fraction was 0,111 t $ha^{-1.}$ The Dezire variety had the biggest yield of large and seed fraction, and Agria the biggest yield of small fraction. The smallest yield of small fraction had the Dezire variety, and the smallest yield of seed and large tubers, the Kurada variety.

Table 7. Fractional structure (%)

Year / Variety	2014				2015				Total Variety			
	< 28 mm	28-35 mm	35-55 mm	> 55 mm	< 28 mm	28-35 mm	35-55 mm	> 55 mm	< 28 mm	28-35 mm	35-55 mm	> 55 mm
Agria	1.40	3.40	81.90	13.30	57.50	3.20	38.60	0.70	29.45	3.30	60.25	7.00
Faluka	1.10	3.30	89.00	6.60	51.40	4.40	44.40	0.80	26.25	3.85	66.70	3.70
Kenebek	1.10	5.90	80.80	12.20	48.20	5.10	45.90	0.80	24.65	5.50	63.35	6.50
Kurada	1.90	9.00	81.40	7.70	57.10	4.60	37.50	0.80	29.50	6.80	59.45	4.25
Dezire	1.15	4.85	85.00	9.00	31.70	7.00	60.00	1.30	16.43	5.93	72.50	5.15
Total Year	1.33	5.29	83.62	9.76	49.18	4.84	45.28	0.88	25.25	5.03	64.45	5.32

Table 8. Tubers yield per fraction (t ha-1)

Year / Variety	2014				2015				Total Variety			
	< 28 mm	28-35 mm	35-55 mm	> 55 mm	< 28 mm	28-35 mm	35-55 mm	> 55 mm	< 28 mm	28-35 mm	35-55 mm	> 55 mm
Agria	0.256	0.622	14.987	2,433	6.957	0.387	4.671	0.085	3.606	0.504	9.829	1.259
Faluka	0.217	0.650	17.533	1.300	5.705	0.484	4.928	0.088	2.961	0.567	11.230	0.694
Kenebek	0.229	1.227	16.806	2.537	6.314	0.668	6.013	0.105	3.271	0.947	11.409	1.321
Kurada	0.328	1.557	14.082	1.332	6.166	0.498	4.050	0.086	3.247	1.027	9.066	0.709
Dezire	0.259	1.096	19.21	2.034	4.628	1.022	8.760	0.189	2.443	1.059	13.985	1.111
Total Year	0.258	1.030	16.523	1.927	5.954	0.611	5.684	0.111	3.104	0.821	11.104	1.019

Conclusion

Providing sufficient quantities of quality seed material is one of the prerequisites for achieving high and high yields. In the Republic of Srpska there is seed production, but it is insufficient and can not meet the needs of the domestic market. Mountain-hilly areas are suitable for seed potato production. In the Rogatica municipality there are several associations of agricultural producers, as well as individual agricultural producers engaged in the production of seed potatoes. Tackling the problems of seed potato production we have come to certain conclusions:

- In the Republic of Srpska, the production of seed potatoes is organized on 80 hectares;
- In the area of Rogatica municipality, seed potato is cultivated on about 54 hectares, of which in the association of farmers "Solanum produkt" is grown on about 30 hectares;
- Agroecological conditions partially favored potato production. In both years of research, higher average temperatures were found, compared to the perennial average. In 2014, there were higher precipitation years than average, but with regular protection of crops, it was preserved from the causative agent of the disease, and significantly higher yields were achieved, compared with 2015, when a large deficit of moisture was recorded in July.
- Average number of tubers varied between 6, 50 and 7, 31. The number of formed tubers per plant was affected by the number of PNI , as well as large amount of rainfall during the forming of stolon and tuber setting;
- Average tubers mass in 2014. was 61.3 grams, and in 2015. 38.4 grams. The size of tubers was affected by their number, weather conditions and the application of agro technical measures;
- Tubers yield is affected by their number and mass, genetic potential of the variety and agro ecological conditions. The yield varied between 19.74 t ha^{-1} (in 2014.) and 12.34 t ha^{-1} (in 2015.). The biggest yield had the Dezire variety, and the smallest Kurada variety. Big fluctuation of yield was registered through the years, which was the result of lack of moisture during the tuber filling phase.
- The production of seed tubers requires a proper fractional structure of the tubers. In 2015. There was bigger widespread of small tubers, which is unfavorable in potato seed production.

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ENTOMOPATHOGENIC FUNGAL ENDOPHYTES: CAN THEY COLONIZE CUCUMBER PLANTS?

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Abstract

Entomopathogenic fungi (EPF) are considered as insect pathogens and, thus, attention is mostly focused on developing them as inundative biopesticides. Lebanese local strains of *Beauveria bassiana* and *Metarhizium anisopliae* isolated from soil have previously shown effectiveness in controlling a wide range of pests. There were no studies reporting their endophytic ability in cucumber plants. Therefore, the present study investigated the abilities of these fungal species to colonize cucumber seeds through artificial seed inoculation and to determine the endophytic activity of these fungi in resulted cucumber seedlings. The results of the germination test showed that treating seeds with EPFs and incubating at 25^{o}C for 2 days would significantly improve germination rate by more than 26% if compared to untreated seeds. Greater enhancement of germination was also observed in seeds treated with *B. bassiana* (72.5%) or *M. anisopliae* (47.5%) when grown under outdoor conditions. In addition, seedling's roots developed from treated seeds grew faster than untreated reaching 15 cm in length compared to 10 cm long control roots after 10 days of incubation. Moreover, seedlings from seeds treated with *B. bassiana* produced a significantly higher number of roots. However, seed treatments with fungal conidia had no effect on stem elongation. Ten days post-inoculation resulted seedlings were assessed for endophytic recovery from their tissues. Results show that both, *B. bassiana* and *M. anisopliae* have colonized cucumber seedlings. Significantly higher percentage recovery (100%) was observed in the cucumber plant tissues colonized by *M. anisopliae* as compared to 58.3% (cotyledon and stem) and 50 % (roots) of *B. bassiana* endophyte. No fungal growth was recorded in control. This is the first study to report a significant data on the interaction between *B. bassiana, M. anisopliae* and cucumber seedlings. However, there is a need for further research to find out if the colonized tissue with EPF can be used as an effective strategy against pests of cucumber crops.

Keywords: *Metarhizium anisopliae, Beauveria bassiana,* cucumber, colonization, plant growth promoter.

Introduction

Agriculture is the third most important sector in the Lebanon. Beside main crops including cereals, fruits, vegetables, olives, grapes, tobacco that are cultivated in the country, cucumber (*Cucumis sativus*) is represented as a very popular salad vegetable. Entomopathogenic fungi (EPF) has been documented in a variety of herbaceous and woody plants, especially *Beauveria bassiana* and *Metarhizium anisopliae* have been shown to colonize certain plant tissues endophytically. Lebanese local strains of these entomopathogenic fungi have been isolated from the soil and identified as potential candidates for future commercial products. The term "endophyte" refers to a phenomenon by which certain fungi or bacteria occur inside plant tissues without causing any apparent symptoms or harm to the host (Aly *et al*., 2011). Although, the ecological function of endophytic EPFs remains largely unknown, some studies have implicated them in plant growth as probiotics (Dora, 2013), herbivore and plant disease resistance (Vega *et al.*, 2008), increased stress tolerance of plants to abiotic factors (Rodriguez *et al*., 2009) and bioremediation of heavy metals (Bajan *et al.,* 1998). Some of the

fungi have been reported as naturally occurring endophytes while others have been introduced into plants using different techniques. The most recent findings show that EPFs are just beginning to be explored as endophytes, with interesting experimental results for the management of insect pests (Vidal and Jaber, 2015). To the best of our knowledge, there is no information in the literature of endophytic colonization of *M. anisopliae* and *B. bassiana* in cucumber plants. Therefore, the overall objective of this study was to demonstrate that EPFs could be used as probiotic (endophytes) of cucumber seeds through internal colonization of externally inoculated seeds with *M. anisopliae* and *B. bassiana* and by re-isolating the fungi from internal tissues of tested plants.

Materials and methods

This preliminary study was conducted in October 2017- April 2018 at the Lebanese University, Faculty of Agricultural Engineering and Veterinary Medicine in the laboratory of Crop Protection Department.

Fungal isolates and maintenance

Lebanese strains of *Beauveria bassiana* (L1B1) and *Metarhezium anisolpiae* (L1M1) isolated from fallow soil (Ibrahim *et al.,* 2011) were used in this study. Long term storage of the isolates was achieved by freezing conidia in 10% w/w Glycerol at -20^{o}C. When required, frozen conidia were re-hydrated by suspending in a small volume of sterile 0.03% (v/v) Tween 20 solution, placed on Potato Dextrose Agar (PDA) and incubated at 23 ±2^{o}C in the dark for 14 days. Spores from these cultures were used for subsequent experiments.

Sterilization of cucumber seeds

There are various cucumber varieties cultured in Lebanon, but Beit Alpha hybrid cucumber seeds were used in this experiment. Prior to inoculation with tested entomopathogenic fungi, seeds were soaked in sterile distilled water (dH_2O) for 15 min and then surface-sterilized by immersion in 3% sodium hypochlorite for 2 min with subsequent rinsing with sterile dH_2O and additional immersion in 70% ethanol for 2 min followed by 3 rinses with sterile dH_2O. The axenically treated seeds were stored overnight at 5 ^{0}C for synchronization of seed germination and growth. To assess the effectiveness of the sterilization method, 20 randomly selected seeds from the seeds used in the screening test were placed onto PDA media plates, incubated at 25 ^{0}C in darkness and then examined over 3, 5 and 10 days. The disinfection was considered successful when no fungal growth was observed on the PDA plate.

Preparation of fungal inoculum

Conidia from 14-days-old cultures grown on PDA were suspended in 0.03% Tween 20, shaken on a rotary shaker at 354 rpm for 30 min. Resultant suspension was filtered through sterile cheesecloth under sterile conditions to remove any hyphal fragments. Conidial concentration was determined using light microscope and hemocytometer (Fuches-Rosenthal) and later adjusted to 1 x 10^{6} conidia/ml.

Seeds inoculation

Axenic seeds were immersed into the fungal conidial suspension for 2 h and placed onto sterile moist filter paper inside Petri dishes. For the control group, seeds were immersed in sterile 0.03% Tween 20 only.

Effect of EPFs on seed germination

To determine the effects of external seed inoculation with fungal suspension on seed germination, the following experiment was conducted. Inoculated seeds were incubated at 25

^{0}C under fluorescent lighting (16h Light / 8h Dark). Germination and occurrence of cotyledons were recorded over period of 10 days (every 2 days). Sterile dH_2O was periodically added to each plate to keep the filter paper moistened. Each test was replicated 6 times with 10 seeds in each replicate and the experiment was repeated 6 times. At the same time, inoculated seeds of each treatment were cultured outside in 3 blocks with 40 replications for each treatment.

Effect of EPFs on seedling's growth

To determine the effects of external seed inoculation with fungal suspension on seedling growth, root and stem lengths and number of roots of the resulting seedlings of each treatment were measured after the completion of seed germination test.

Endophytes re-isolation

After germination test, randomly selected seedlings were surface-sterilized: seedlings were first immersed in 0.5% sodium hypochlorite for 1 min, and then in 70% ethanol for 30 sec, followed by 3 rinses in sterile dH_2O. Disinfected seedlings then were allowed to air-dry in the laminar flow hood for a few min, cut into root, stem and cotyledon parts using sterile scalpel. Obtained plant materials were placed onto PDA selective media amended with antibiotics (20 mg L^{-1} Amoxicilline), incubated at 25^{0}C for 4 weeks and inspected regularly to observe fungal outgrowth, recordings were taken one-week post-inoculation. The success of this disinfection process was assessed by plating on PDA 1 ml of residual rinse water. Percent colonization/recovery of different seedling's parts by the respective inoculated fungus was calculated following the Petrini and Fisher (1987) formula: % colonization = number of sampled plant tissue showing fungal outgrowth divided by the total number of plated plant tissue samples ×100. The test was repeated 3 times.

Statistical Analysis

The statistical analysis in this study was made using the function one way ANOVA and the means of treatments were compared using DUNCAN test, using the statistical package SPSS (2011).

Results and discussion

In the current study, seed treatment with *B. bassiana* and *M. anisopliae* did not reduce seed germination or seedling growth and did not result in the development of root disease. On the contrary, the results of the germination test showed that treating seeds with EPFs and incubating at 25 ^{0}C for 2 days would significantly improve germination rate by more than 26% if compared to untreated seeds. After this date, the rate of germination showed no significant difference between the treatments (Table 1 and Fig.1). Greater enhancement of germination was also observed in seeds treated with *B. bassiana* (72.5%) or *M. anisopliae* (47.5%) when grown under outdoor conditions (Table 1 and Fig.2). In addition, seedling's roots developed from treated seeds grew faster than untreated reaching 15 cm in length compared to 10 cm long control roots after 10 days of incubation. Moreover, seedlings from seeds treated with *B. bassiana* produced significantly higher number of roots, whereas seedlings treated with *M. anisopliae* produced the lowest number of roots per seedling. However, seed treatments with fungal conidia had no effect on stem elongation (Figs.3 and 4).

Table 1. Germination mean (%) of treated and untreated cucumber seeds (in an incubator, 2 days post treatment and under outdoor conditions seven days post treatment).

Treatment	**Germination of cucumber seeds** % ±SE˜	
	Germination (25 ^{0}C)	Germination (outdoor)
Control	^{a} 40.0 ± 0.856	^{a} 32.5 ± 0.11
M. anisopliae	^{b} 66.7 ± 0.422	^{b} 47.5 ± 0.05
B. bassiana	^{b} 68.3 ± 0.654	^{c} 72.5 ± 0.11

˜ Mean percentages (±Standard Error, SE) within a column followed by the same letter not significantly different according to Duncan test (P≤0.05).

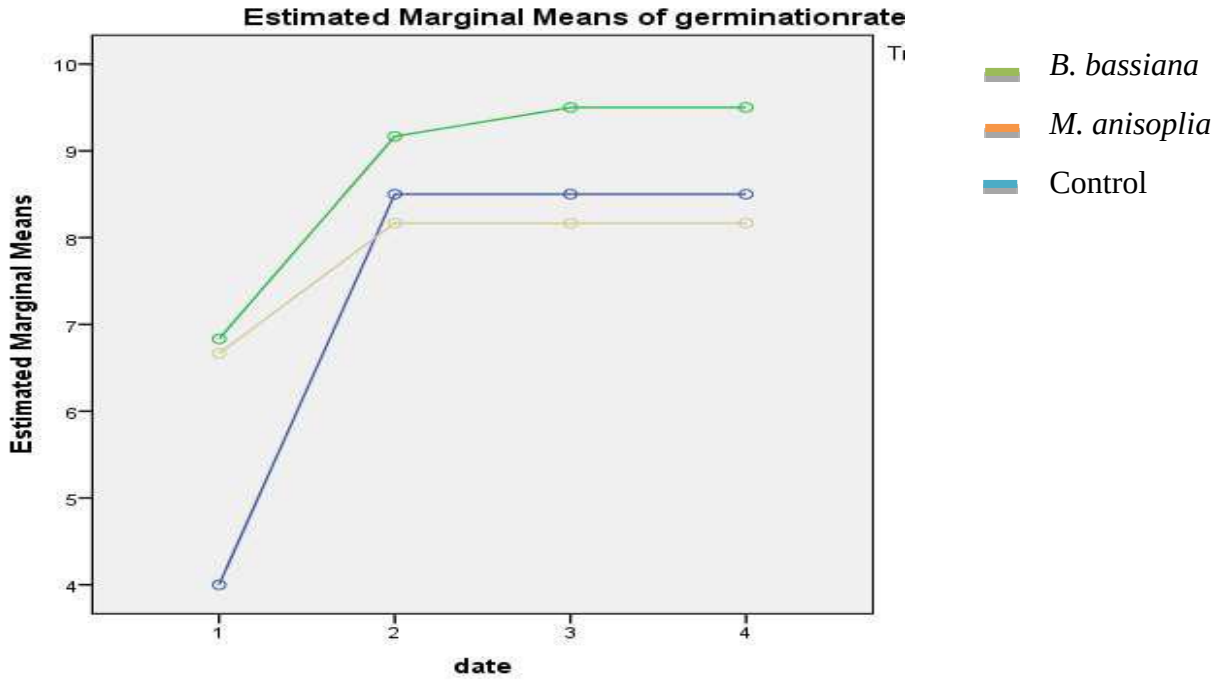

Figure 1. Germination rate of treated and untreated cucumber seeds at 25^{0}C 2 days post-treatment.

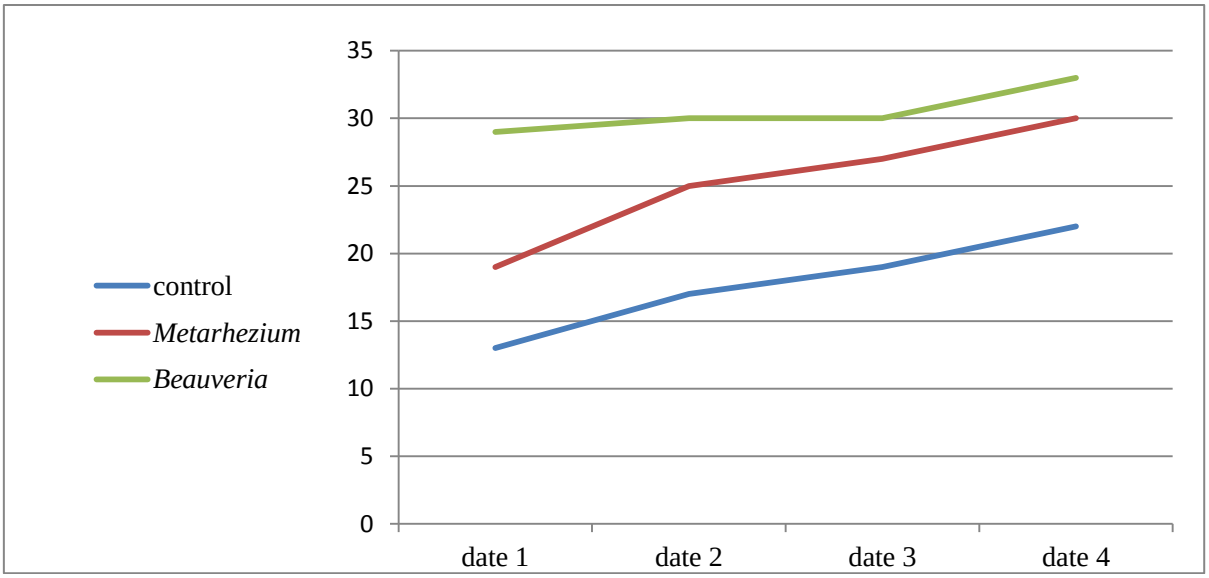

Figure 2. Germination rate of treated and untreated cucumber seeds seven days post-treatment, under outdoor conditions.

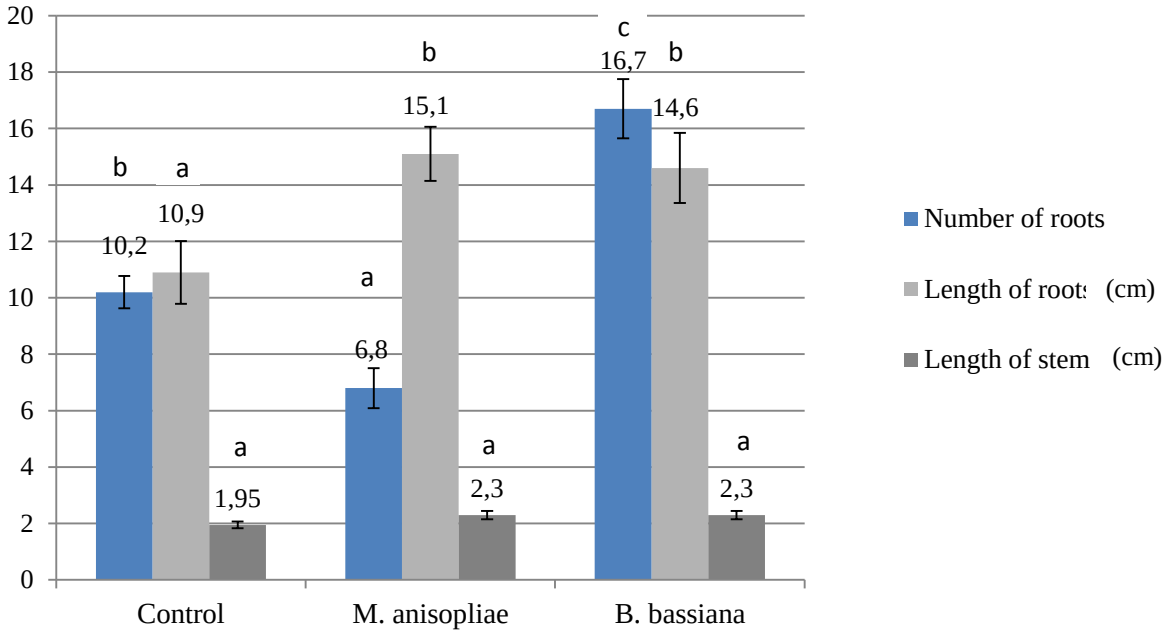

Figure 3. Physiological characteristics of roots and stems of cucumber seedlings after 10 days of incubation of treated and untreated cucumber seeds. Same letters for each parameter indicate non-significant difference according to Duncan test (P≤0.05).

Figure 4. Development and growth of cucumber seedlings untreated and treated with EPFs.

Artificial inoculation of cucumber seeds carried out by their direct submersion into conidial suspensions provided successful colonization of plant parts with tested *B. bassiana* and *M. anisopliae* isolates. Presence of the fungi in the internal tissues was confirmed by their recovery from roots, stems and cotyledon/leaf pieces on Potato Dextrose Agar (PDA) selective medium 10 days post-inoculation. Results in Table 2 and Figure 5 show that both, *B. bassiana* and *M. anisopliae*, isolates 10 days post-inoculation have colonized cucumber seedlings. Significantly higher percentage recovery (100%) was observed in the cucumber plant tissues (roots, hypocotyls and cotyledons) colonized by *M. anisopliae* as compared to 58.3% (cotyledon and stem) and 50 % (roots) of *B. bassiana* endophyte. No fungal growth was recorded in control seedlings (Table 2 and Fig.5).

Table 2. Effect of conidial seed treatments on percentage recovery of B. bassiana and M. anisopliae from different parts of cucumber seedlings.

Treatment	**Cotyledons (% ±SE)**	**Stem (% ±SE)**	**Roots (% ±SE)**
Control	0.0 ± 0.00 a˜	0.0 ± 0.00 a	0.0 ± 0.00 a
B. bassiana	58.3 ± 0.15 b	58.3 ± 0.15 b	50.0 ± 0.15 b
M. anisopliae	100.0 ± 0.00 c	100.0 ± 0.00 c	100.0 ± 0.00 c

˜ Means within a column followed by the same letter are not significantly different according to Duncan test (P≤0.05).

Figure 5. Re-isolation of EPFs from cucumber seedlings

The procedure of artificial inoculation by *Beauveria* and *Metarhizium* caused no visible defects on the germinated seeds and colonized plants did not present any physical symptoms

of damage. No fungal growth was ever observed in PDA plates with last rinse water indicating a high efficacy of surface sterilization procedure and confirming that the fungi growing from surface-sterilized plant materials onto culture media were endophytes that originated from within internal plant tissues. No *Beauveria* and *Metarhizium* were detected from control tissues, which confirms the absence of natural colonization and cross-contamination in control plants.

To our best knowledge, the artificial introduction of *M. anisopliae* and *B. bassiana* into the seed tissue followed by its successful establishment as an endophyte in other parts of resultant cucumber plant is demonstrated for the first time in present study. Interestingly, 79% of studies reported very variable results in the efficacy of artificial introduction of EPF into plants. There are works indicating that a leaf is a poor route of entry for EPF to colonize plants, as was demonstrated for *B. bassiana* on coffee seedlings (Posada *et al.*, 2007) or on tomato plants (Qayyum *et al.*, 2015). It is hypothesized that specific cuticular components might have a detrimental effect on conidium germination, but their effects have not been tested (Posada *et al.*, 2007). This study proves that seed inoculation method is a very successful approach to introduction of EPF into all parts of cucumber plants since recovery of 100% for *M. anisopliae* and 58 % for *B. bassiana* was registered in seedlings and plant parts after 10 days of cucumber seed inoculation.

Current data presented evidence that entomopathogenic fungi, not only had endophytic capacity, but could also act as plant growth promoters in cucumber. Entomopathogenic fungi could significantly increase the germination rate and seedling emergence of inoculated cucumber seeds under laboratory and outdoor conditions. Furthermore, seed-inoculated cucumber plants show significantly higher number and larger length of roots when compared to control plants. These observations prove the potential of entomopathogenic fungi in promoting plant growth and development by a symbiotic relationship that serve as extended root system possibly improving the absorption of nutrients and moisture (Sasan and Bidochka, 2012).

Conclusions

This is the first study to report a significant data on the interaction between *B. bassiana, M. anisopliae* and cucumber seedlings, and thus extends previous reports on the ability of entomopathogenic fungi to act as endophytes. However, there is a need for further research to find out if the colonized tissue with EPF can be used as an effective strategy against pests of cucumber crops.

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CWR OF GRAIN LEGUMES IN BULGARIA

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Abstract

This paper presents information from the first phase of Bulgarian and international *in situ* conservation of grain legumes CWR projects. An expedition investigation has been carried out in regions indicated in the flora of Bulgaria as well as other preliminary studies. Description of several species localities has been carried out in Strandzha Mountain and Kaliakra Natural Reserve - North Black Sea region, which during the past years of species, belonging to the group of rare species in Bulgaria: *Pisum elatius L., Cicer monbretii, Lupinus albus* and *Vicia incisa*. The number of population sand concomitant species have been defined. Seed material for *ex situ* collection has also been collected. It has been found, that the main limiting factors for disturbing condition of natural habitat sare the invasive plant species and some socio-economic reasons. We consider it is necessary to undertake a profound analysis for the situation of preserving the CWR in Bulgaria and to coordinate this activity with all scientific centers and institutions. It is imperative to prepare an action plan and monitoring.

Keywords: *Grain legumes, CWR, in situ conservation, ex situ collection, Action plan, Monitoring.*

Introduction

Crops wild relatives are of particular importance to ensure food security, human health and to preserve the biodiversity in Bulgarian flora. They are impeccable source of diverse genetic material for the breeding process. The wild relatives utilization is especially important today. Due to the introduction of modern and monoculture varieties the diversity of wild relatives decreased considerably. The *in situ* conservation has an important role for the preservation of natural resources common policy. The *in situ* method completes *ex situ* preservation system. This method is effective for all species in their natural habitats.

The lack of capacity, including the absence of effective operational framework and national plan to deal specifically with the conservation of crop wild relatives has been identified as a significant obstacle for their conservation and use.

The purpose of this paper is to develop action plan for *in situ* conservation of grain legumes- *Cicer monbretii, Pisum elatius, L. albus.*

Routes based on the flora in Bulgaria and preliminary studies carried out by Bulgarian scientists were developed *(Mihov et. al., 2001)* with the goal to confirm and establish natural habitats of several species grain legumes wild relatives *(The Bulgarian flora, The Red Book of Bulgaria*). The altitude of localities, size of the population and concomitant species is registered. This research is result from the implementation of national and international projects. A number of botanic expeditions are undertaken to the following routes:

Cicer monbretii– Strandzha Nature Park
- Kosti village
- Road to Gramatikovo village– Malko Turnovo– Ahtopol– Brodilovo village
- Malko Turnovo– Mishkova niva and Indipaskha localitie
- Along Veleka River

Lupinus albus– Strandzha Natural Park

Golyam Pazvlak locality situated in Varvara village
Pisum elatius– Northern Black sea coast
Kaliakra Archaeological Reserve
Yaylata Archaeological Reserve

Materials and methods

The research to the above mentioned areas has been conducted in 2006 -2016.
An expedition investigation has been carried out in regions indicated in the flora of Bulgaria as well as other preliminary studies. Description of several species localities has been carried out in Strandzha Mountain and Kaliakra Natural Reserve - North Black Sea region, which during the past years of species, belonging to the group of rare species in Bulgaria: *Pisum elatius L., Cicer monbretii, Lupinus albus* and *Vicia incisa*. The number of population sand concomitant species have been defined. Seed material for *ex situ* collection has also been collected.

Results and Discussion

1. *Cicer monbretii* is growing in localities in Strandzha Natural Park in an altitude up to 300 m

Important morphological features**:** A perennial, herbaceous plant with up right stem up to 80 cm high. Compound and non-pinnate leaves. The leaves are broad, elliptical, ovate, sharp toothed. The flowers are from 2 to 5, rarely single, in a loose grape-like in florescences in the axil. The banner is ovate, white. Wings are ovoid elliptic a land white. The keelis white with a purple spot. The pod is smooth, broad, oblong, brown, three-fourseminal. The seeds are round, brown or black. Depending on climatic factors the wild chickpea blossoms in the third ten days of May and ripens at the end of June / early July.
The first confirmed habitat of *Cicer monbretii* is on the road to Gramatikovo village– Malko Turnovo and has been registered by Miho Mihov in 2001.Our team discovered and marked habitats on the road to Ahtopol– Brodilovo village in 2010. The two habitats represent a group of plants or a single plant, situated not far from one another, close to the highway. Larger habitats of wild chickpea have been identified in Mishkova niva and Indipaskha localities during joint expeditions with Belgian colleagues held in 2012 and 2014. The habitats grow in proximity to oak forest on cinnamon forest soil. We observed a bigger plant groups occupying an area of 15 – 20 m^2. Although the density is not great, the plant condition is good. Predominant are the species of the families *Poacea* and *Vicia ssp.* The species growing along the streets are vulnerable to road reconstruction and expansion, while these situated in proximity to the forests by sanitation cut, timber industry, excessive tourist impact

are less vulnerable. Seeds from *Cicer monbretii* are collected and multiple growing field trials *(ex situ*) are carried out. All of them failed.

2. *Lupinus albus.* According to preliminary data of Strandzha Nature Park. Management Plan, the species is determined in Golyam Pazvlak locality at an altitude of 70 m-80 m. The locality is rocky with a slight slope.

Important morphological features: The stem is high, stright, branched, covered with white hairs. The leaves are palmated. The petals are pointed, back ovoid et the top, blunted. The stipules are long, soft, partially grown with the handle. The flowers are consistently located, with white color. The pods are oblong, fibrous, pinched with 4-6 seeds. The seeds are flattened, white or lightye llow, smooth, matt.

During the several investigations carried out by our team was discovered that the condition of *white lupin* population is good and it is not threatened by human activity. Natural disasters and erosion are considered as hazards. *Lupinus albus* is represented by single and small group of plants occupying an area of 1000 m^2 at distance varying from 10 m. to 15 m. As concomitant species is determined only *Aegilops*, because the research period coincided with the ripening phase of *white lupin*, while the other species have completed this phase. The habitat of *Lupinus albus* is markedfor *in situ* conservationand is a subject to deeper study to include it in *ex situ* collection.

3 Pisum elatius. During participation in within a project for Development of Management Plan for Kaliakra cape (situated in an altitude of 56 m.) the team has identified the species in 2000 – 2002. A joint expedition with French scientists and our registered organization for conservation of crop wild relatives and old indigenous varieties in 2016 confirmed that *Pisum elatius* grow in Kaliakra cape and is identified in Yaylata Archaeological Reserve (at altitude of 25 m).

Important morphological features: The stems are semi-upright or semi-lodged, slightly branchy, nude. The stipules are ovate and elliptical. Compound leaf with three double leaves, elliptic terminal flower with tendrils. The inflorescence has one –two petals. The small glass is nude. The corolla is purple, elliptic prolong. Wings are elliptic, round. The pod is wide, linear, yellow or brown. The seeds are round, black or dark brown.

The habitat of wild pea growing in Kaliakra cape consists of single plants spread on several areas, while the one on Yaylata Archaeological Reserve the wild pea population is bigger, situated on an area with a length of 210 m. - 215 m and grown on larger areas. The condition of the populations is good. Concomitant species are from the family of *Poacea, Vicia ssp, Lathyrus ssp.* There is nearby large habitat of red *Paeonia peregrina*. We consider as danger of species destruction the climate, erosion as well as incorrect mowing forecast against invasive plants in the Reserve. We collected seeds and conducted multiple growing field experiments (*ex situ*). All of them failed. The long term conservation of crop wild relatives should be national policy supported by the European Union institutions.

Conclusion

The conservation of grain legumes CWR has largely been neglected, due to the exclusion of the agencies responsible for the conservation of plant genetic resources for food and agriculture and those responsible for the conservation of wild plant populations in general or the habitats they grow. The annual monitoring performed at suitable vegetation periods and as well as the support from national bodies will allow long-term *in situ* conservation.

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EFFECT OF EXTRACTION CONDITIONS, HEAT TREATMENTS AND SPRAY-DRYING ON STABILITY OF ROSELLE ANTHOCYANINS AS NATURAL FOOD COLORANTS

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Abstract

The present study was designed to investigate the effect of spray-drying as microencapsulation technique on the stability of roselle calyces (R.C.) anthocyanins. Different extraction conditions were evaluated to identify the best extraction presider for extracting roselle anthocyanins. The results showed that using 2% citric acid solution by 1: 10 solids: solvent ratio with a crushed flower at 85°C for 20min. was the best condition for extraction of the red pigments from roselle calyces and recorded the highest anthocyanin yield of 1229mg/100g R.C. Total phenolic contents (TPC) and antioxidant activity was determined and the results showed that roselle calyces had TPC ranged from 12.16 to 14.45mg. gallic acid equivalent /g. depending on the extraction solvent. Methanol: water (80:20 V/V) recorded the highest TPC. Results also reflected that R.C had a strong antiradical efficiency of 0.727 and EC_{50} of 1.37μg roselle extract/μg DPPH. Thermal stability of roselle anthocyanins was investigated and the results showed that roselle extract heated at 95°C for 30 min. recorded retention value of 80.017%. The effect of three different encapsulating agents i.e. maltodextrin D.E. 18.7, gum Arabic and whey protein isolate on pigments stability was investigated. Maltodextrin DE 18.7 was found as the most effective carrier in stabilizing the pigments under the storage conditions examined with a half-life of 577.62days. The application results proved that the addition of encapsulated roselle anthocyanins as a natural color with the level of 0.3 % in a strawberry drink model system and 0.5% to jelly formulation was acceptable and can replace the synthetic color.

Key words: Roselle (Hibiscus subdariffa L), Anthocyanins, Encapsulation, whey protein, shelf-life, jelly.

Introduction

Today the food industries have an extensive range of both natural and synthetic colors. In line with the generally observed trend from synthetic dyes towards natural colorants (Pedro et al., 2016). However, because of the problems of the synthetic pigments that cause toxicity and carcinogenicity in the human body, the use of them is gradually has decreased. Therefore interest in natural pigments, that can replace synthetic ones, which caused many side effects, is increasing (Limbri et al., 2015). Recently in response to this trend, tend to use natural pigments as adding natural materials in the natural dyeing, healthy functional foods, cosmetic products for human health and safety have been gradually expanded (Kim et al., 2011). Currently, pigments of various kinds and forms have been used as additives or supplements in the food industry, cosmetics, pharmaceuticals, livestock feed and other applications. Roselle (*Hibiscus subdariffa* L.) is a tropical plant belongs to the family *Malvaceae* and is known by Egyptian consumers as Karkadah. Water extract of the Roselle calyces produces a brilliant red color and a pleasant acid test, rich in anthocyanins, and ideal for producing brilliant red colorings in many foods. Roselle anthocyanins as with most natural food colorants, suffer from inherent instability. Color stability of anthocyanins was found to be depending on a combination of various factors including: structure of anthocyanins, pH, temperature, oxygen, light, and water activity. Enzymatic degradation and interactions with food components such

as ascorbic acid, sugars, metal ions, sulfur dioxide and copigments are no less important (Duangmal et al., 2004).

Microencapsulation can be used for many different products such as encapsulation of liquid flavors, enzymes, artificial sweeteners, coloring agents, vitamins and minerals (Mahdavi et al., 2014). Various techniques have been developed for encapsulation of both food ingredients and nutraceuticals, including spray drying, spray cooling/chilling, freeze drying, extrusion, fluidized bed coating, coacervation, liposome entrapment, inclusion complexation, centrifugal suspension separation, co-crystallization and emulsions (Desai and Park, 2005).Spray-drying is the most commonly used technique, on account of it being a continuous, low cost process that produces dry particles of good quality, and for which the machinery required is readily available (Arueya et al., 2014 and Mahdavi et al., 2016).

The main targets of the present work are: First, to investigate the effect of different extraction conditions (solvents type, solvent-to-solid ratio, temperature, extraction time, and particle size) on extraction efficiency of anthocyanins from roselle calyces. Second: to produce dry red powder of roselle pigments by using spray-drying technique and three different encapsulating agents (maltodextrin 18.7, arabic gum and whey protein) and study the stability of the encapsulated pigments during storage. Finally: utilization of the encapsulated roselle extract as food colorants in a model drink and jelly.

Materials and methods

Materials

The Roselle calyces were obtained in a dried form (sun-dried) in summer 2013. The dried calyces were divided into two parts: The first part was kept as it is while the second part was crushed for 5 seconds using a blender (Braun type 4249, CombiMax (Germany). Both of the two parts were immediately packed in polyethylene bags kept away from light at low temperature (4°C) and till used. All chemicals used were purchased from Merck (Darmstadt, Germany).

Methods

Determination of total phenolic content

The Folin–Ciocalteu method was used to determine total phenolic compounds according to the methods described by Singleton, *et al.*,(1999). The total phenolic content was determined by comparing with a standard curve prepared using gallic acid (10–200 µg/ml; $Y = 0.025X + 0.2347$; $R^2 = 0.9986$). The mean of at least three readings was calculated and expressed as mg of gallic acid equivalents (mg GAE)/100 g of roselle calyces.

Determination of radical scavenging activity

The free radical scavenging activity of the anthocyanins was analyzed by using the 1,1-dipheny-2-picrylhydrazyl (DPPH) assay according to Abba Pacôme *et al.*, (2014). The scavenging or inhibition percentage was calculated according to the following equation:

$$\text{Scavenging (\%)} = \frac{(\text{abs. control} - \text{abs. sample}) \times 100}{\text{abs. control}}$$

Where: abs. is absorbance at 515nm

The measurement was performed at least in triplicate. Inhibition of coloration was expressed as a percentage, and the effective concentration 50 % (EC_{50}) was obtained from the inhibition curve.

Extraction of anthocyanins

To investigate the efficiency of extracting condition in the yield of anthocyanince from roselle calyces two different solid-to-solvent ratios (1:10 & 1:20), two temperature values (4°C &

85°C), and two extraction time 20 min & 24h.) were studied. Distilled water & citric acid 2% was used as solvents and the extraction of pigments was carried out according to the procedures described by Cissé *at al.*, (2012) with some modification as described in Table (1).

Table (1): Conditions for extracting of pigments from roselle calyces

Sample	Roselle Calyces	Solvent	Solvent: sample ratio	Temperature	Extraction time
Treatment1	Crushed flowers	Hot water	1:10	85 °C	20 min
Treatment 2	Crushed flowers	Hot water	1:20	85 °C	20 min
Treatment 3	Whole Flowers	Hot water	1:10	85 °C	20 min
Treatment 4	Whole Flowers	Hot water	1:20	85 °C	20 min
Treatment 5	Crushed flowers	Cold water	1:10	5 °C	Overnight
Treatment 6	Crushed flowers	Cold water	1:20	5 °C	Overnight
Treatment 7	Whole Flowers	Cold water	1:10	5 °C	Overnight
Treatment 8	Whole Flowers	Cold water	1:20	5 °C	Overnight
Treatment 9	Crushed flowers	2 % citric acid	1:10	85°C	20 min
Treatment 10	Crushed flowers	2 % citric acid	1:20	85°C	20 min
Treatment11	Whole Flowers	2 % citric acid	1:10	85°C	20 min
Treatment12	Whole Flowers	2 % citric acid	1:20	85°C	20 min

Total pigment content:

Total anthocyanins content of roselle extracts were determined calorimetrically according to the procedure described by Abou-Arab *et al.*, (2011).

Color diminution of roselle calyces extract (L*, a*, and b*)

The color of different samples was determined using a Chroma Meter CR-400 optical sensor (Konica Minolta Sensing, Inc., Osaka, Japan) according to the CIE Lab scale (CIE Colorimetric Committee 1974).

Microencapsulation processes

For encapsulation purposes, maltodextrin 18, whey protein concentrate and gum arabic were evaluated as wall materials the process was cured out according to Idham, et al., (2012) with some modification. Twenty grams of each carrier were dispersed in 150 ml of the pigment extract (5°Brix) and the pH was maintained at the range 2.6. Then, the mixtures were vigorously homogenized at 10,000 rpm for 15min at room temperature. The resulting mixtures were subsequently were fed into the pilot plant spray dryer (Mini Spray Dryer B-290, BÜCHI Labortechnik, Switzerland) with a nozzle atomization system with 1.5 mm diameter nozzle and main spray chamber of 500 x 215 mm. at feed flow rate of 5 cm3/min. The prepared microcapsules were collected in a cyclone and packaged to prevent light incidence and stored at room temperature for further experiments.

Degradation kinetics of the encapsulated pigments:

The degradation of roselle anthocyanins was followed periodically by measuring the coloring power of the stored samples. 0.5 gram of each encapsulated sample was dissolved in 20 mls. of distilled water on 50ml. beaker and magnetically stirred for 10 mins. The pH was adjusted to 2.6 and the volume was made up to the mark with distilled water. After filtration, absorbance was measured at 520 nm using a Spectronic 2000, Spectrophotometer, Busch and Lomb, (USA) and the coloring strength of the extract was expressed using the following formula:

$$E_{cm}^{1\%} = A_{\lambda} / CL$$

Where: $E_{cm}^{1\%}$: Extinction coefficient (55.9), A_λ : Absorbance measured at a particular wavelength, λ; C: Concentration of the anthocyanin, (g./ 100 ml of the solution) and L: Length of the cell, in cm

Degradation parameters including degradation rate constants (k) were obtained from slope of a plot of the natural log of anthocyanins retention and half-life value (T1/2) for the encapsulated roselle anthocyanins were calculated by applying a first-order reaction model.

Applications of encapsulated pigments:

Addition of encapsulated Roselle extracts to a model system of drink

A model system of a drink were prepared according to Duangmal, et al., (2004) with added roselle extract at three different concentrations (0.1, 0.2, and 0.3 % w/v, 0.1% w/v, carmine and 0.05% w/v Carmoisine) was studied.. The anthocyanins content and color were measured at zero time. The bottles were divided into two groups: the first group was stored in the refrigerator at 4-5ºC, the second was stored at room temperature (40 ±2 ºC). For each sample group, one bottle was randomly selected for analysis every week for a period of 10 weeks.

Addition of encapsulated Roselle extracts to strawberry jelly formulation

Jelly powders were prepared according to Egyptian Standard ES: 800/ 2007. Three treatments of jelly with different adding reoselle extract, were prepared with added roselle extract at concentrations of 0.167, 0.33 and 0.50%, while carmine treatments was colored with carmin at concentration of 0.167%. Control treatment was a commercial jelly powder including synthetic color (carmoisine). Jelly samples have been sensory evaluated for jelly attributes such as color, flavor, texture, Transparency, and overall acceptability.

Statistical analysis

Data were expressed as the mean± SD for five rats in each group. The data were analyzed using SPSS software package version 20.0 and values were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range tests at 5% level of significance were used to compare between means.

Results and dissection

Total phenolic and antioxidant activity of Roselle extract

The results showed that the highest phenolic compounds were found when methanol: water (80:20) was used as extracting solvent (14.245mg /g roselle calyces) followed by citric acid solution and ethanol: water (50:50) which gave 13.71mg/g and 12.83mg/g roselle calyces) respectively. On the other hand, distilled water extracted the lowest quantity of phenolic compounds. This finding could attribute to the polar character of the phenolic compounds which makes them soluble in the polar solvents such as methanol, ethanol, and water (Chinedu et al., 2011). Also, the presence of acid solvent extraction of anthocyanins is the initial step in the determination of total and individual anthocyanins prior to quantification, purification, separation, and characterization (Abba Pacôme et al., 2014 and Jafarian et al., 014).

DPPH radical-scavenging activity of the Roselle calyces extract

The results of the antioxidant activity of different concentrations of the roselle extract are shown in figure (1). The results indicated that the DPPH radical-scavenging activity of roselle calyces was occurred in a dose-dependent manner. With increasing the concentrations of roselle extract the inhibitory activity against the DPPH radical increased. The lower EC_{50} value reflects better protection action against oxidation. The concentration required to inhibit 50 % radical-scavenging effect (EC_{50}) was determined from the results of a series of concentrations tested. A lower EC_{50} value corresponds to a larger scavenging activity. The EC_{50} values of the roselle extract were EC_{50}= 1.375µg roselle extract /µg DPPH and antiradical efficiency AE (0.7272). These results reflated that when roselle extract used with a

concentration of 0.31μg roselle extract /μg of DPPH the inhibition ratio was 20.22% while the concentration of 6.94μg roselle extract /μg of DPPH recorded inhibition ratio of 90.69%.
These results indicated that there are abundant antioxidative phytochemicals present in the clyces extracts of roselle. Our results are similar to that reported by Abba Pacôme et al., 2014; Tsai et al., 2012 and Sirag et al., 2014)**.**
The strong antioxidant activity of roselle extract could be due to the presence of polyphenol compounds. Abou-Arab et al., (2011) Investigated antioxidant activity of roselle plant extracted by different solvents and indicated that the ethanol acidified with 1% citric acid extracts exhibited higher value in total antioxidant activity and recorded (EC_{50}) of 42.77 (μg/ml).

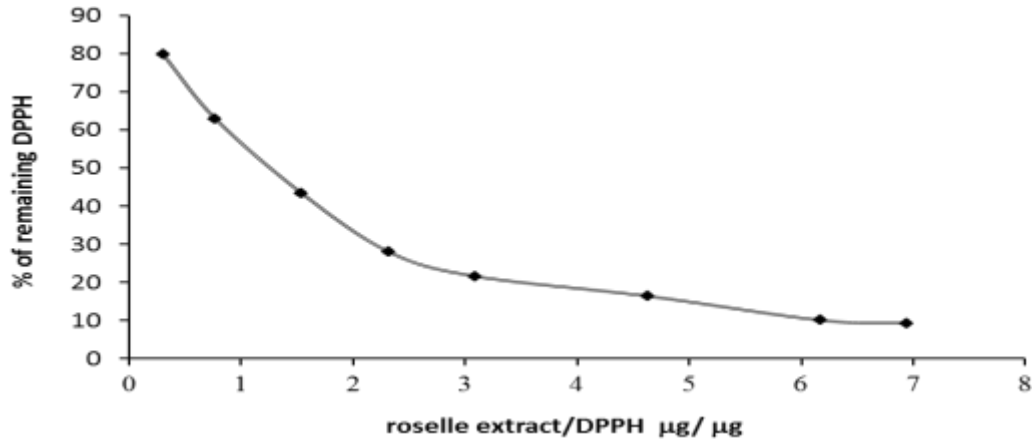

Fig. (1). Percent of remaining DPPH as function of μg of roselle extract per μg DPPH

Effect of extraction conditions on the extraction efficiency of roselle anthocyanins
The effect of extraction condition on the yield of pigments recovered, the total soluble solids and the pH of the extracts are showed in table (2). By reducing the particle size, the yield of anthocyanins was increased in all the extraction conditions examined. The crushed flower gave total anthocyanins of 0.994.18mg./100g. dry weight while the whole flower gave 912.96mg/100 dry weight under the same conditions. It could be also noted that adding citric acid to the extraction medium had a great effect in stabilizing anthocyanins, thus the extraction efficiency increased. When 2% citric acid solution was used as extraction solvent with crushed flower and 1:10 solid: solvent ratio, total anthocyanins recorded 1229.62 mg. /100g dry weight. These observations reveal that the pH value is a very important factor affecting the extraction of anthocyanins. It was reported that the extracting solution should be slightly acidic to maintain the flavylium cation form, which is red and stable in highly acidic medium Andersen and Markham, 2006 and Abou-Arab *et al.*, (2011). Likewise, Chandrasekhar et al., (2012) reported that and the mixture of 50% (v/v) ethanol and acidified water resulted in maximum anthocyanin content (390.6 mg/L). Similar results were reported by (Cisse et al., (2012) and Oancea et al., (2012) and Lambri et al., (2015). Extracting the anthocyanins at 85°C for 20min recorded higher anthocyanin content than extracting at 5°C for overnight in all condition investigated (Pedro et al., 2016).

Table (2): Extraction efficiency of different extraction conditions and color diminution of the extracts

Sample	T.S.S	pH	Total anthocyanins Mg./ 100 g/ D.W.	L*	a*	b*
Treatment 1	5	2.8	994.18	1	42.2	1.48
Treatment 2	2	2.84	760.80	11.37	75.97	19.29
Treatment 3	5	2.81	912.96	2.21	57.17	3.59
Treatment 4	2	2.83	791.64	11.51	77.3	19.53
Treatment 5	5	2.79	827.63	3	65.77	4.95
Treatment 6	2	2.92	546.95	13.6	40.33	25.25
Treatment 7	5	2.80	796.78	3.52	68.25	5.84
Treatment 8	2	2.86	567.52	13.8	39.21	23.81
Treatment 9	6.3	2.48	1,229	1.14	3.61	1.15
Treatment 10	4.1	2.58	1,079	1.68	3.46	1.16
Treatment 11	5.9	2.51	944.66	1.84	2.48	0.45
Treatment 12	4.2	2.54	902.03	1.02	2.77	0.73

Degradation kinetics and storage stability of encapsulated Roselle anthocyanins.

Kinetic studies on the degradation of roselle anthocyanins encapsulated in Maltodextrin DE 18.7, Whey protein isolate (90 %) and Gum Arabic, were carried out in dark at30ºC and control sample without carrier. changes in color strength for the different encapsulated roselle anthocyanin powders were followed by periodical measurements of absorbance to define the order of anthocyanins degradation reaction. As illustrated in Fig. (2). Plotting color strength values (Ln E_{520}) vs storage time (days) gave straight lines for the different encapsulating agents and control. Linear regression analysis showed that the degradation of roselle anthocyanins encapsulated in the three evaluated coating materials followed first-order reaction kinetics. Similar kinetic responses were reported by Martynenko, et al., (2016) and Gradinaru et al., (2003) for the degradation of pelargonidin based anthocyanins at different water activity conditions.

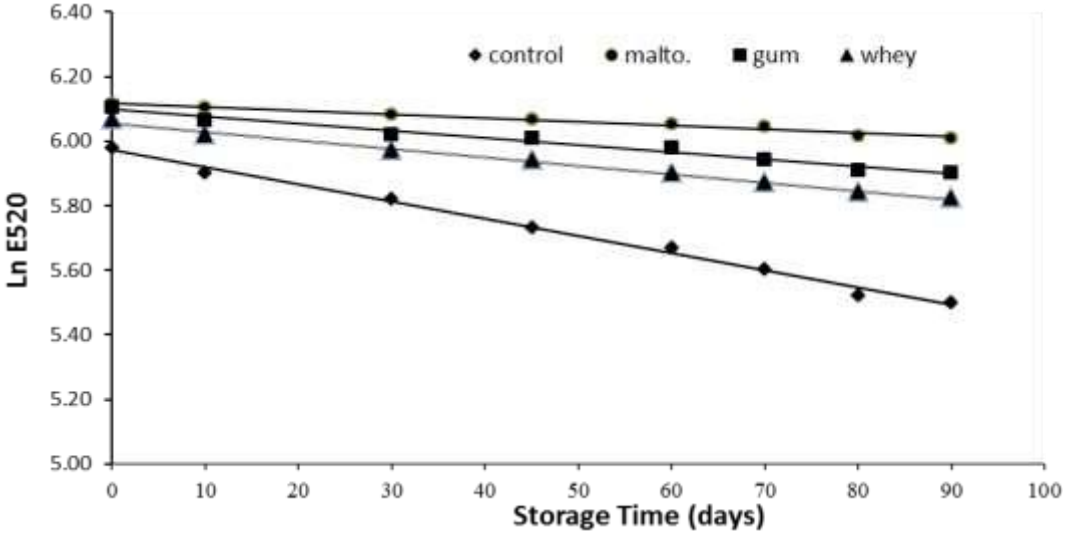

Fig. (2): First-order degradation plots for spray-dried roselle anthocyanins with different encapsulating agents during storage at 30°C in dark containers.

Degradation rate constants for anthocyanins encapsulated in the different matrices were calculated with the correlation coefficients and half-life period and the data is presented in table (3). The obtained results showed that degradation rate constant values were 5.3, 1.2; 2.2 and 2.6 X 10^3 for the control, Maltodextrin DE 18.7; Gum Arabic Whey protein isolate respectively. The highest value of the rate constants for anthocyanins degradation was

observed at for the control sample while the lowest rate constant was recorded for Maltodextrin DE 18.7. Among the polymeric matrices, which largely elongated the half-life of roselle anthocyanins, maltodextrin DE 18.7 was found as the most effective carrier in stabilizing the pigments during storage.

Table (3): Degradation rate constants for roselle anthocyanins encapsulated in different encapsulating agents during storage at 30 °C in the dark

Encapsulating agent	Rate constant ($days^{-1}$)	Correlation coefficient R^2	Half - life period (days)
Control	5.3×10^3	0.99	130.78
Whey protein	2.6×10^3	0.99	266.60
Gum arabic	2.2×10^3	0.98	315.07
Maltodextrin	1.2×10^3	0.97	577.62

The half-life period of the encapsulated roselle anthocyanins were increased feom130.78 for the control sample to; 266.60; 315.07and 577.62 days for Whey protein isolate; Gum Arabic and Maltodextrin DE 18.7 respectively. Similar kinetic responses were reported by Gradinaru et al., (2003 and Sipahii et al., 2017**)**. The could be due to the open porous structure obtained in the freeze died final product, which makes it exposed to air if the encapsulated product is not packed under vacuum or inert atmospheric condition (Gómez-Carracedo et al., 2007).

Roselle anthocyanin stability in a drink model of system.

One of the most key factors affects the applications and usage of natural colorants in the food industry is the stability during storage and handling. Roselle anthocyanin stability in a drink model system has been investigated during storage of the drink model at 40 °C & 5 °C. Three different concentration of roselle anthocyanin (0.1, 0.2 and 0.3%) were used; Carmine E 120 dosage 0.1% and Carmoisine E124 dosage 0.05% were used for comparison as the most common used red colors; Natural and synthetic respectively. The color retention was measured periodically by 10 days; the results are shown in Figure (3). When the samples were stored at 40 °C, the drink model samples were colored by the roselle anthocyanin extract lost their color faster than the drink model samples colored with Carmoisine and Carmine.

During storage at 40°C, the sample colored with roselle anthocyanin 0.1% was recorded the highest color losses and recorded a retention ratio of 26% after 70 days storage. The results reflected that the storage temperature had a very important effect on the roselle anthocyanin stability.

When the drink samples stored at 5°C, the degradation rates for the five treatments were lower than the degradation when stored at 40 °C. After 70 days storage at 5°C, the drink model samples colored with roselle anthocyanin retained 42.16, 45.37 and 68.65 % of anthocyanin content, while drink model samples with Carmine and Carmoisine retained 78.05 and 87.01 of the color content respectively.

It could be noted that with increasing roselle anthocyanin concentrations, the stability of color was increased. Therefore, colored sample with 0.1% roselle anthocyanin extract retained only 41.03 % while the sample colored with 0.3 % roselle anthocyanin extract retained 60.35 % of its color after 70 days storage at 5 °C. This is could be due to the copigmentation reaction which enhanced the stability of roselle anthocyanin. Similar findings were reported by Gris et al. (2007); González-Manzano et al., (2008).

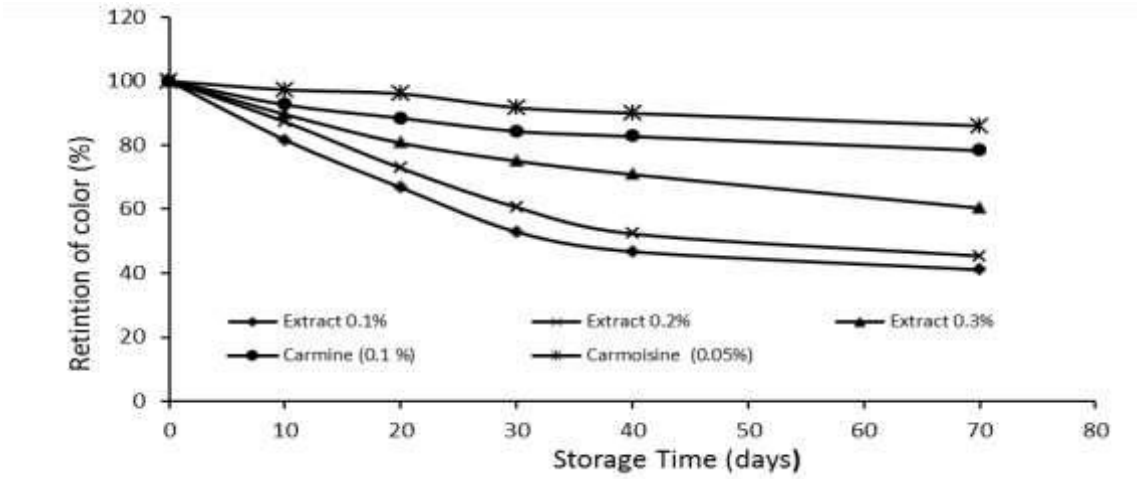

Fig. (3). Stability of roselle anthocyanin in a drink model of system stored at 5°C.compared with carmine and carmoisine.

Application of encapsulated roselle extract in jelly formulation.

The prepared jelly samples were sensory evaluated and means scores were statistically analyzed and the results are presented in fig. (4). The results indicated that no significant differences ($p > 0.05$) found between sample colored with carmoisine; carmine and sample colored with roselle anthocyanins at a level of 0.5% as coloring agent. On the other hand, there were significant differences between samples colored with roselle anthocyanins at low level 0.16 and 0.33 and the other samples. It could be notes that jelly sample colored carmoisine scored the highest value for color of (9.67) while the sample with 0.167roselle anthocyanins recorded the lowest value for color of 7.50.

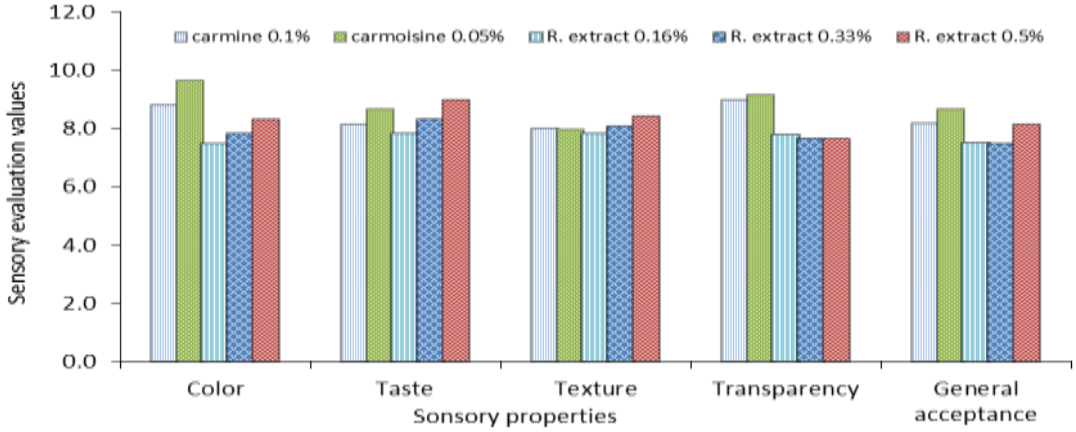

Fig. (4). Sensory evaluation of jelly samples containing encapsulated roselle extracts, carmine and carmoisine.

Regarding to the samples taste sample with roselle extract at a level of 0.5% recorded the highest taste value of 9.00. The results reflected that there were no significant differences ($p > 0.05$) between all samples except the sample colored with roselle extract at a level of 0.16% which scored the lowest value for taste (7.83). The results showed that no significant differences ($p > 0.05$) found in sample colored with carmoisine and carmine in transparency while those colored with different concentrations of roselle anthocyanins i. e. 0.16, 0.3 and 0.5% were not significantly different. Sensory data showed there were no significant differences based on texture of all samples. Concerning the overall acceptability, most of the panelists preferred the samples colored with carmoisine and carmine and samples produced with 0.5% roselle anthocyanin. Based on data collected from sensory evaluation in the

studies, adding 0.5% of roselle anthocyanins to the jelly formula gave close scores to carmoisine and carmine samples thus, indicates that the addition of the natural color with level of 0.5% to the jelly formulation was acceptable and can replace the synthetic color. Similar results were reported by Mahdavi et al., 2016.

Conclusion

Using 2% citric acid solution by 1: 10 solids: solvent ratio with a crushed flower at 85˚C for 20min. was the best condition for the red pigments from Roselle calyces. Encapsulation of anthocyanins with polysaccharides enhanced the stability of anthocyanins for efficient utilization in food systems. The storage stability result supported that maltodextrin as wall material gave the longest shelf life and the smallest change in the pigment color. Storage period significantly affect the color changes of the spray dried powder. Hence, this study signifies that the encapsulation process could stabilize and extend the shelf life of anthocyanins content. The results indicate that the addition of roselle anthocyanins as a natural color with the level of 0.3 % in the drink model system and 0.5% to the jelly formulation was acceptable and can replace the synthetic color.

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OCCURRENCE OF POTATO WART IN GEORGIA

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Abstract

Potato is one of the most important food crops in Georgia. Average yield of potato in Georgia is still low (8-11 tons/ha). Potato diseases are the main reason for yield losses. Potato wart (causal agent *Synchytrium endobioticum*) is a very harmful quarantine disease distributed in nearly 60 countries of the world including Georgia. According to official results of the observations of potato plantations and depositaries undertaken in 2009-2012 in Khulo mountainous region (western Georgia) potato wart was detected for the first time and identified in samples collected in several villages (Ghorjomi, Tkhilvana, Danisparauli, Diakonidzeebi) of Khulo municipality which was the economically important place for potato production (Gorgiladze et. al., 2014). However, the potato growers from villages Tabakhmela and Didajara observed wart symptoms earlier, in 2006 on potato cv. 'Agria', which was widely grown there for home consumption. According to the disease survey results obtained in the frame of the PhD work the incidence area of potato wart was extended. Namely, in 2016-2017 the disease was also found in 13 villages of Khulo municipality on varieties Briz, Impala, Finka, Picasso and Marfona, Saturna and in one Mestia village of the highland Zemo Svaneti region (northwest Georgia). The disease symptoms were observed on the tubers, stolons and root neck. All samples obtained were identified as *S. endobioticum* based on the morphological descriptions (OEPP/EPPO, 2004) and a specific PCR test using primers F49 and R502 (van den Boogert *et al*., 2005). The research has been continued under PhD grant of Shota Rustaveli National Science Foundation.

Keywords: *Potato wart, Georgia, Incidence area*

Introduction

Potato, which was first introduced in Georgia in 1820s, is one of Georgia's major staple and cash crops nowadays. Because of the wide range of environmental conditions in Georgia and the ability of potatoes to adapt to different conditions, potatoes grow two times a year. The area under potato cultivation varies between 30.000 and 37.000 ha per year, but the productivity still remains very low (Table 1) (GeoStat, 2017).

Table 1. Production and average yield of potato by regions

Region	Production of potato (ths. Tons)				Average yield of potato (t/ha)			
	2014	**2015**	**2016**	**2017**	**2014**	**2015**	**2016**	**2017**
Adjara AR	16.5	11.5	14.2	15.3	11.6	7.3	12.3	13.0
Kakheti	…	…	4.5	4.6	…	…	5.3	4.7
Mtskheta-Mtianeti	…	…	3.2	2.4	…	…	5.7	5.6
Samtskhe-Javakheti	99.7	101.2	160.5	103.4	13.4	10.1	14.9	9.6

Kvemo Kartli	70.6	49.8	44.1	38.0	12.3	8.2	10.5	9.3
Shida Kartli	…	…	16.0	10.5	…	…	7.9	5.8
The remaining regions	28.5	24.0	6.5	5.8	7.2	4.7	8.0	7.8
Total	**215.3**	**186.5**	**249.0**	**180.1**	**11.7**	**8.3**	**12.3**	**9.0**

Source: National Statistics Office of Georgia (2017)

According to the data of GeoStat (2017), the average yield of potato ranges from 8 to 11 t/ha. Specifically, 57% of the total volume of potato production is produced in Samtskhe-Javakheti region, 21% - in Kvemo Kartli, 9% - in Adjara, 6% -in Shida Kartli, 3 % - in Kakheti, 1% -in Mthkheta mtianeti, and 3% - in other regions (Fig. 1), which confirms the lowest productivity of potatoes.

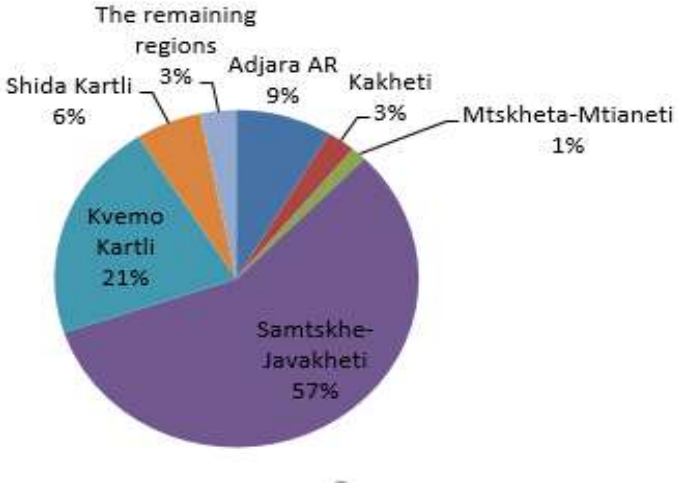

Figure 1. The percentage index of potato production per regions

In Georgia, the reduction in the potato production is due to various reasons. Namely, due to the growth of unadapted varieties, the substandard potato seeds, low level of the agrotechnical system, the lack of primary seed-growing system, and so on. Among them, pests and diseases are major constraint contributors. The most important disease affecting potato crops is the potato wart disease (PWD) which is caused by soil-borne fungus *Synchytrium endobioticum (Schilberszky)* Percival. The pathogen is an obligate parasite causing the proliferation of warts on potato tubers, stolons, the aerial buds at the stem bases, which may vary markedly in form. Deformation of leaves may also occur, with the exception of roots (OEPP/EPPO, 2017).

Potato wart disease contributes to yield losses in the range of 50-100% (Hampson, 1993; Melnik, 1998). The major agricultural problem is not only the yield losses, but also the contamination of soil with persistent resting spores of *S. endobioticum* remaining infectious for more than 20 years (Obidiegwu *et al*., 2014), that requires the long-term official control, quarantine, the prohibition of the growth of potatoes on it and legal sanctions (Hampson 1993; OEPP/EPPO, 1999, 2017). Subsequently the fungus is currently considered the most important quarantine pathogen of cultivated potato (Smith et al. 1997). It is included in the A2 list of quarantine of EPPO (OEPP/EPPO, 2004), and is also the limited distributed quarantine organism in Georgia (Order of Minister of Georgia, 2006).

S. endobioticum originates in the Andean zone of South America, from where it was introduced into Europe through the movement of infected or contaminated tubers in the

1880s. It spread widely in Europe, Asia, Africa, America and Oceania including 47 countries (EPPO/CABI, 1997). The disease has been occurring in Georgia since 2009. It was first found in two villages of Khulo Municipality. However, according to stories of growers from this region wart symptom was noted in 2006 on potato variety Agria. As the result of the survey conducted in 2012-2013 in the frame of GNSF grant, potato wart was also found in other villages of Khulo municipality (Gorgiladze et al., 2014).

Despite the studies conducted in Georgia in 2009-2013, precise details on records of the distribution of potato wart incidence for the demarcation of infested areas have not been known.

This paper presents the results of the disease survey conducted within the framework of the Ph.D. work, namely, of potato wart monitoring in producing regions of Georgia and determining the boundaries of its foci to prevent the spread of disease.

Materials and Methods

To detect the potato wart loci, determine quarantine and disease incidence area in accordance with pre-determined plan and route, the observation of the potato plantation in the fields and potato tubers in the depositories in the different potato producing regions of Georgia were conducted by expeditions depending on potato planting and harvesting time.

The infected potato tubers and other parts of plants, and soil samples were collected from fields according to standard protocols recommended by EPPO (PM 7/28 (2)) in 2016-2017. Identification of *S. endobioticum* was mainly based on microscopic observations of summer sporangia and winter (resting) sporangia (OEPP/EPPO, 2017). The specific polymerase chain reaction (PCR) was conducted for accurate detection and quantification of *Synchytrium endobioticum i*n soil samples and in plant parts (van den Boogert et al., 2005)

Results and Discussion

According to the results of the survey, the potato wart was found in 17 villages of Khulo municipality by inspection of tubers at harvest on potato varieties Dezire, Briz, Impala, Finka, Picasso, Marfona, etc, and the pathogen was also detected from the soil samples (Fig.2).

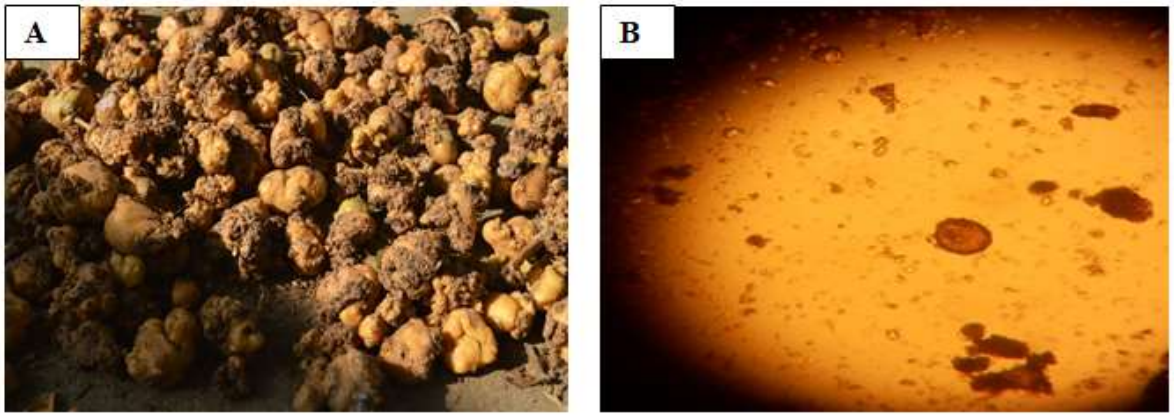

Fig. 2. A figure shows infected tubers of potato collected in the village Skvana: B figure shows the winter sporangia detected from the soil sample

As for Mestia, municipality (the highland Samegrelo-Zemo Svaneti zone of northwest Georgia), the pathogen was only detected in the soil samples in four villages of Ushguli settlement. The infested areas in Adjara AR and Zemo Svaneti are very separated (Fig.3).

Figure 3. Geographical distribution of the main places of surveys of *Synchytrium endobioticum* in Georgia

The infested areas are indicated in Table 2. Disease incidence was very high and the fields were heavily infested in the private plots of villages of Khulo municipality. Soil analyses performed in Khulo infested plots have been shown existence 5-30 sporangia per 1g of soils. Presumably, the pathogen was introduced in Georgia through infected seeding material of potato from Armenia and Turkey where the disease occurred. Areas of disease have increased because quarantine measures to prevent the spread of disease have been begun very late in Georgia.

Table 2. Areas infested by *Synchytrium endobioticum* in Georgia

Potato growing regions	**Villages**
Khulo Municipality, Adjara AR	Gorjomi
	Gorgadzeebi
	Dioknisi
	Begleti
	Tkhilvana
	Didajara
	Skvana
	Danisparauli
	Tabakhmela
	Diakonidzeebi
	Tkhilvana
	Uchkho
	Riketi
	Bodzauri
	Dzirkvadzeebi
	Iremadzeebi
	Okruashvilebi
Ushguli district, Mestia Municipality, Samegrelo-Zemo Svaneti zone,	Becho
	Etseri
	Zhibiani
	Latali

Disease surveys are continued in Georgia under PhD work and in accordance with the Order of Government of Georgia #305, 25th June, 2015 on the Rule of Potato Wart Control.

Conclusions

A survey showed that potato wart *(Synchytrium endobioticum*) is limited distributed and occurred in two regions: Khulo and Zemo Svaneti with different severity.

Acknowledgement

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THE ECONOMIC PARTNERSHIP AGREEMENT BETWEEN THE EUROPEAN UNION AND JAPAN: A COMPARATIVE ANALYSIS WITH FOCUS ON THE QUALITY STANDARDS IN THE AGRI-FOOD SECTOR

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Abstract

In 2013, the European Union (EU) initiated negotiations for an Economic Partnership Agreement (EPA) with Japan, one of the largest economies in the world and a key trading partner. After 18 rounds of negotiations, both partners agreed on the principles and implementation of this Free Trade Agreement (FTA) on 6 July 2017. The final EU-Japan agreement is expected to enter into force at the beginning of 2019 and will give both economies a major boost, lowering tariffs and trade barriers as well. Primarily, it will open new markets for agri-food export. Moreover, it is another strong example of the global supply chain matters and the sharing of values and interests. This study outlines the current situation of quality standards used in the EU and Japan. Japan, as well as the EU, have their own regulatory schemes applied to labeling, certification and trade in the agri-food sector. The purpose of this study is to investigate the differences between their standards and gain more insight into their similarities. The research has three major objectives. Firstly, the food safety regulations of the EU and Japan will be discussed more detailed. Secondly, the primary investigation of this paper is to compare those food safety standards and focus on their equivalency issues. Finally, the study insists on the necessity of understanding the importance of the emerging issues from food safety equivalency in relation to trade between the EU and Japan. Thus, the main research question, namely, till what extent are the food safety standards between the EU and Japan equivalent, will be answered and analyzed in this paper.

Keywords: *agri-food industry, food safety, quality standards, EU-Japan Economic Partnership Agreement (EPA), Free Trade Agreement (FTA).*

Introduction

The economic ties between countries are characterized by globalization. The European Union (EU) applies an open trade and investment policy with respect to the rest of the world. In that sense, the EU promotes free and fair world trade, agreeing to progressive international trade treaties as well as tearing down trade barriers at the same time as to ensure human health. Japan and the EU have been working together bilaterally for decades. They agreed on a joint Economic Partnership Agreement (EPA) in 2017, which has been signed on 17.07.2018. This Free Trade Agreement (FTA) aims to strengthen trade relations and set a strong signal in the world [EC, 2018a; EC, 2018b]. In their negotiations for EPA between 2013 and 2017, the EU and Japan aimed not only to reduce tariffs but also to establish regulatory coherence. Japan is considered a country with very high product standards in the agri-food sector and exemplary consumer protection. Nevertheless, disturbances which endangered human health occurred in the past. Cases of food poisoning, bovine spongiform encephalopathy (BSE), avian influenza and fake labeling have arisen a strong interest and awareness of food safety within the Japanese population [Morishita, 2012]. This study is motivated by the importance for both European food producers and exporters to understand Japanese food safety measures in the light of the EPA. In order to gain a comprehension of the legal framework for food safety and the quality standards of the agri-food sector of the two cooperating parties, the authors have

explained, compared and discussed these aspects in detail, focusing on Japan. The relevance of the EPA in the context of the food safety regulations and quality standards has not been appropriately analyzed yet. Moreover, this study should be useful for policymakers and exporters.

Materials and methods

An intensive desktop analysis was conducted to analyze food safety measures and to compare food safety systems in Japan and the EU under the EPA, including a literature review of available documents and reports on the outcome of the FTA negotiations and food safety regulations in Japan and the EU.

Results and Discussion

Overview of the EU-Japan Economic Partnership Agreement (EPA)

Cooperation between the EU and Japan already has a long history. In the past, several significant agreements were negotiated and adopted between the two partners. In the 1970s, the EU Commission supported European companies in their export efforts and the market entry activities in Japan by special promotional programs. In 1999, the EU-Japan Business Round Table has been established [Felbermayr et al, 2017]. In January 2002, the Mutual Recognition Agreement came into force. Later, in June 2003, the Agreement on Cooperation on Anticompetitive Activities was approved by the EU Council. In the following, the Agreement on Cooperation and Mutual Administrative Assistance came into effect on 01.02.2008. The Science and Technology Agreement between the EU and Japan was signed on 30.11.2009. Following many bilateral discussions and a constructive dialog at the EU-Japan Summit in 2011, the negotiations for an Economic Partnership Agreement, which constitutes a bilateral Free Trade Agreement (FTA), were finally officially launched on 25.03.2013. After 18 rounds of negotiations, the partners reached a basic agreement on 07.07.2017. Afterward, a few technical details were clarified and the fine adjustment has been made so that the EPA could be signed on 17.07.2018 by both partners. Ultimately, formal consent by all EU Member States and the European Parliament must be granted (ratification). In Japan, representatives from two parliaments, the Lower and Upper House, must approve the agreement. The entry into force of the EPA is expected in 2019 [EC, 2018a; Deloitte, 2018; EC, 2018b]. The aim of both partners is to engage in free and fair trade and to counteract isolationism. An important intention of the EPA is to send a strong signal to the world and counteract protectionism. The primary objective is also to strengthen international standards and reduce tariff and non-tariff barriers between the EU and Japan [EC, 2018a]. The EPA consists of 23 chapters, whereby the Agri-Food sector is directly or indirectly affected in many chapters. Chapter 2 (Trade in goods) stipulates the liberalization of Japanese agricultural imports. Nearly 99% of bilateral trade goods have been liberalized, with the exception of rice and seaweed. 91% of all import tariffs will be gradually phased out. A distinction is made between a full tariff elimination and a progressive elimination of customs duties, with product-specific annual installments beginning on the date of entry into force of the FTA. Imports of wine and other alcoholic beverages will be duty-free immediately with the entry into force of the EPA (previously 15%). Customs duties on pork meat shall be eliminated, from 4.3% to duty-free, in six equal annual installments as well as the complicated import regulations shall be reduced. For processed pork, the tariff rate will be diminished from 8.5% to 0%. Moreover, the tariff for beef will be reduced from 38.5% to 9% in 15 annual installments. Due to the FTA, a gradually reducing of import tariffs for hard cheeses from 28.9% to duty-free within the next 15 years is specified. For soft cheese and cream cheese, a duty-free quota has been implemented. Generally, the quotas for dairy products have been increased [EC, 2018a]. Other important chapters of the EPA are Chapter 3 (Rules of

origin and origin Procedures), Chapter 7 (Technical barriers to trade), Chapter 16 (Trade and sustainable development), Chapter 18 (Good regulatory practices and regulatory cooperation) and Chapter 19 (Cooperation in the field of agriculture). By virtue of chapter 6 (Sanitary and phytosanitary measures (SPS)), the two partners reaffirm their rights and obligations under the WTO. The SPS standards and regionalization in disease control have been mutually recognized. Noteworthy is Chapter 14 (Intellectual property), with which Japan recognizes 205 geographical indications (GIs) of the EU and provides trademark protection [EC, 2018b]. Significant studies which analyze the most important assumptions, objectives and scope of EPA regarding the effects on the EU economy are "On the economics of an EU-Japan Free Trade Agreement" [Felbermayr et al., 2017], "Market opportunities for EU agribusiness in the context of the EU-Japan EPA" [Fournel, 2017], "Trade Sustainability Impact Assessment of the Free Trade Agreement between the European Union and Japan" [EU, 2016] and "Assessment of Barriers to trade and investment between the EU and Japan. Copenhagen Economics" [Sunesen et al., 2009]. The EU predicts that the EPA will increase bilateral trade in goods and services by 24% [EC, 2018b].

Food Safety regulations and policy measures in the EU and Japan

This section presents the legal framework, food security policies, state and private quality standards of the EU and Japan and compares them, if possible.

Food Safety in the EU

In response to the BSE scandals, food law in the EU has been revised and restructured. As a result, the legal basis of EU food law is the General Food Law (EU-GFL) (Regulation (EC) No 178/2002) of 28 January 2002, which lays down the general principles and requirements of food law, it establishes the European Food Safety Authority (EFSA) and lays down procedures in matters of food safety. Furthermore, the Rapid Alert System for Feed and Food (RASFF) has been introduced. This law is mandatory for all EU Member States and covers the entire agri-food value chain from production until the point of consumption. The three main objectives of EU food law are settled in Article 5 of the EU-GFL. The three main objectives of EU food law are settled in Article 5 of the EU-GFL and are intended to protect public health, plant health, animal health with respect for animal welfare, prevention of fraud and deception by providing appropriate information. In April 2004, the EU adopted three further important regulations, the so-called hygiene package, which are directly linked to EU-GFL: Regulation (EC) No 852/2004 on the hygiene of foodstuffs, Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin and Regulation (EC) No 854/2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption. From all the legal bases mentioned above result the seven basic principles of EU food safety: (1) Principle of the agri-food chain with defined responsibilities, (2) Principle of corporate responsibility, (3) Principle of traceability of the origin of all products „From farm to fork", (4) Risk analysis with independent scientific risk assessment, (5) Separation of risk assessment and risk management, (6) Precautionary Principle and (7) Transparent and efficient risk communication. Furthermore, the application of the concept of Hazard Analysis and Critical Control Point (HACCP) is mandatory in the EU.

In addition to the governmental regulations valid throughout the EU, European food manufacturers also apply the international DIN EN ISO standards and private standards, for instance, IFS Food (International Featured Standard), BRC (British Retail Consortium), QS Standard (Quality and Security GmbH), GlobalGAP and GMP+. At the retail level, many supermarket chains have set their own standards that manufacturers must comply with. These standards are often stricter than EU law and a number of quality labels have emerged.

Food Safety in Japan

The Japanese administrative system is similarly structured to that in the EU and pursues the same goals. The legal framework is the Food Sanitation Law (FSL), the Food Safety Basic Law (FSBL) and the Japanese Agricultural Standard Law (JASL).

The FSL first came into force in 1947 and has been partially modified several times over the years. This law ensures the safety and hygiene of food an in May 1996, the voluntary application of HACCP has been included. In 2002, regulations on import and sale bans were added. Another milestone was the introduction of a "positive list" for agricultural chemical residues in foods in May 2003. At the same time, the Monitoring and Guidance Plan was implemented. In addition, the FSL prohibits to put foods containing harmful substances on the market and lays down guidelines for food standards, additives and packaging. Comparable with the EU basic principles is, inter alia, Article 3 of the FSL which establishes the entrepreneurial responsibility for the entire production chain of food. The law also defines the operating principles of the Ministry of Health, Labor and Welfare (MHLW), which is the food authority at the national level.

The second most important food safety basis in Japan is the FSBL, which came into force in May 2003 and builds/is based on the FSL. It sets the principles for developing a food safety regime and describes the role of the Food Safety Commission (FSC) and Justification of the Prime Minister's Office. Basically, this law is comparable to the EU-GFL.

The Law Concerning Standardization, etc. of Agricultural and Forestry Products, known as JASL, lays down the operating principles of the Ministry of Agriculture, Forestry and Fisheries (MAFF) and specifies the mandatory and voluntary labeling regulations. Also, it is linked to the Food Labeling Act, which is used in the consumer agency division. This law has been in force since 1950 and has been amended several times. It aims to improve quality, increase productivity, enhance fairness and simplicity of transactions, rationalize the consumption and utilization of agricultural and forestry products, as well as the protection of consumer interests. Due to this law, a standardized certification system (JAS system) has been established, which provides the voluntary use of the JAS label. On the one hand, an examination of the products by MAFF is required, on the other hand, the certification must be carried out by authorized certification bodies. The certification ensures a certain quality of agri-food products, which were produced by specific methods. For organic food products the use of the JAS standard is mandatory. The JAS is a consumer protection standard, so labels have been established for consumers visibility. The different JAS labels are shown in figures 1-5.

Figure 1: Logo for General JAS *Source: MAFF, 2018*	Figure 2: Logo for Specific JAS *Source: MAFF, 2018*	Figure 3: Logo for Organic JAS *Source: MAFF, 2018*	Figure 4: Logo for Production Information JAS *Source: MAFF, 2018*	Figure 5: Logo for distribution under fixed temperature control JAS *Source: MAFF, 2018*

The general JAS (figure 1) has been applied to all foods and forestry products. It confirms quality, such as grade, composition and performance. The second logo (figure 2) is awarded to certified products that conform to specific processing procedures or manufacturing methods and that have distinctive characteristics of quality, thus standing out from ordinary products. The organic label (figure 3) is not voluntary, so if an organic product is not certified according to Organic-JAS, it is not allowed to be called "organic". The JAS Production Information logo (figure 4) identifies those products that have declared their ingredients and that comply with JAS production information. For instance, in the case of beef and pork meat, the feed and veterinary medicinal products used must be disclosed. For plant products, the use of pesticides and fertilizers must be expounded. Figure 5 shows the JAS logo, which is used exclusively for Bento (lunchbox) made of rice. The essential prerequisite is, that the Bento Box be transported within the supply chain under constant fixed temperature control [MAFF, 2018].
As in the EU, in addition to governmental standards, private food safety standards also apply. Japan also distinguishes the food quality standards set by the Japanese government and the standards set by Japanese companies. For example, some supermarket chains and retailers have developed their own specific rules, which are even more stringent than government regulations. Particularly, supermarket chains are focusing on compliance with HACCP and ISO standards [Jonkert et al., 2005]. Exemplary the private standards for Good Agricultural Practices (GAP) are presented and compared below. One of the widely known private standard is GLOBALGAP, which is internationally recognized. This is a worldwide voluntary standard for Good Agricultural Practice with the aim of ensuring safe and sustainable food production around the world. The global organization defines itself as a standard provider with a certification system. Every certified company gets the possibility to use the logo (figure 6) [GlobalGAP, 2018]. Also, in Japan, a private standard was created for certification of Good Agricultural Practices, the JapanGAP (JGAP). This is the only international standard in Japan and is managed by the Japan GAP Foundation. The non-profit organization was founded in 2006 and the certification system persists since 2007. Since then, 4,113 farms have been certified (as of 03/2017). The focus is on fruits and vegetables, tea, cereals and legumes as well as animal products and livestock. Equally GlobalGAP, JGAP is a consumer protection and industry standard that aims to contribute to production safety, environmental protection and sustainable agriculture, as well as transparency along the entire value chain. The JGAP labels (figures 7 and 8) attest to the compliance with the required standards of food safety, environmental protection and occupational safety and certification schemes [JGAP, 2018].

Figure 6: GLOBALGAP-Logo *Source: GlobalGAP, 2018*	Figure 7: JGAP-Logo *Source: JGAP, 2018*	Figure 8: JGAP Certified Farm Logo *Source: JGAP, 2018*

Comparison of three important basic principles of food safety

The following section highlights and compares only the basic principles of food safety, which are often controversial issues in the negotiations of free trade agreements. It should be noted, that there are only a few fundamental differences between the EU and Japan. In the case of EPA, the different approaches have been mutually recognized by the EU and Japan.

Risk Analysis

According to the Codex Alimentarius Standard (Codex), which is recognized worldwide, risk analysis is executed using the three inter-related components of risk analysis: Risk

Assessment, Risk Management and Risk Communication. This international framework is implemented in the EU due to the Article 6 of the EU GFL, with the food law based on risk analysis. One basic principle is that Risk Assessment and Risk Management are operating separately. The risk assessment has to be carried out by an independent scientific institution. In the EU, the scientific responsibility pertains solely to the European Food Safety Authority (EFSA), based in Parma (Italy). The described separation of roles in Risk Analysis is also implemented in Japan (figure 9) and is consistent with the Codex. The Risk Assessment is ensured by the Food Safety Commission (FSC), which works separately from Risk Management. FSC was established in July 2003 after the adoption of the FSBL. The risk management is under the responsibility of the MHLW on the one hand and MAFF and the Consumer Agency on the other. For hygiene issues, MHLW is also advised by the Pharmaceutical Affairs and Food Sanitation Council. Similar to the EU, Risk Communication takes place, by all institutions and is coordinated by the Consumer Agency. This agency was founded in September 2009. The public warning of the population is ensured by the MHLW.

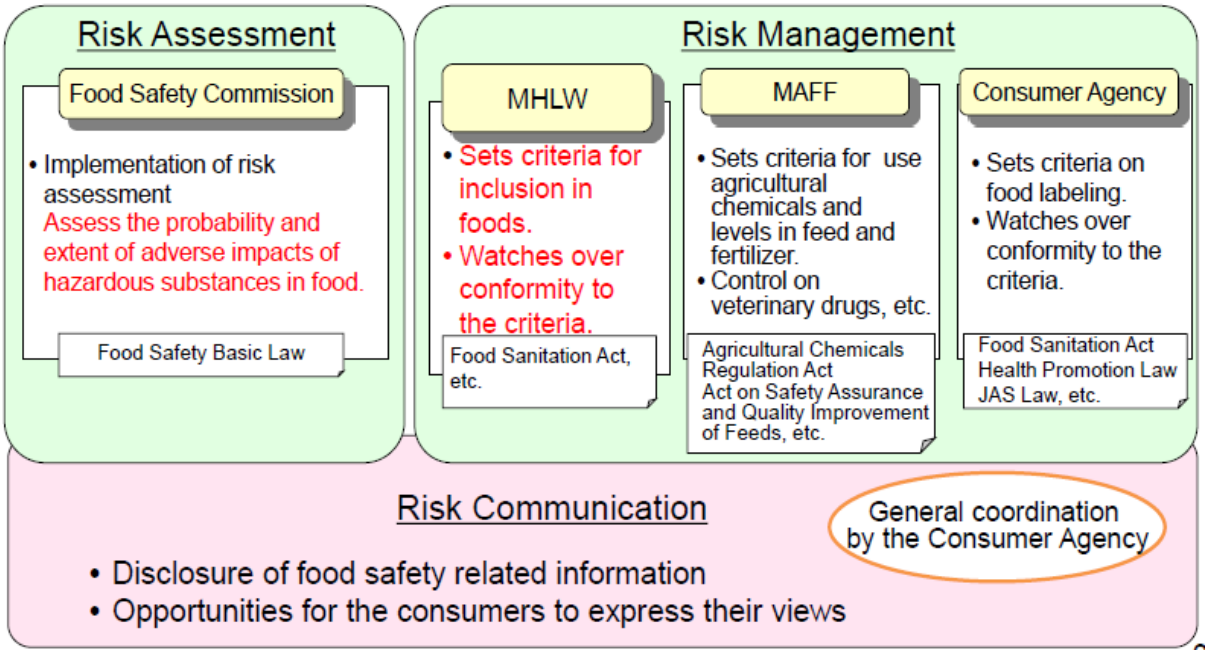

Figure 9: Risk Analysis in Japan. *Source: Sumi, 2014*

Application of the Hazard Analysis and Critical Control Point (HACCP) concept

As a quality management system, HACCP is a globally recognized system to reduce risks in the production of food. It obliges companies to analyze, control and document the entire food processing flow, from the raw material to the final product. Regulation (EC) No 852/2004 of 29 April 2004 legally required European food producers to apply the HACCP principles in conjunction with good hygiene practices. Therefore, all food manufacturers in the EU shall act according to the seven principles of the HACCP concept.

Since 1996, following the Escherichia coli O157: H7 outbreak in Sakai, the Japanese government has been recognized a need to introduce HACCP [WHO, 2006; Sumi, 2014]. So far, HACCP does not necessarily have to be applied in Japan because it is not required by national laws and regulations. As part of the FSL, only a voluntary HACCP approval system was introduced in 1996, which concerns to the production of milk and milk products, meat products, surimi-based products, low-acid canned foods and soft drinks [WHO, 2006]. In Japan, a differentiation is made between HACCP standard A and HACCP standard B. The HACCP standard A is based on the seven HACCP principles and respects the size of the companies. Imported goods, slaughterhouses and poultry processing companies belong to this category. The HACCP standard B allows more flexibility for small-sized businesses and

caterers [MHLW, 2016]. At the 7th Global Food Safety Initiative Focus Day in Tokyo on September 4, 2017, Hideshi Michino, head of department at the MHLW, announced an amendment to the FSL for 2018, which makes systematic implementation of the HACCP mandatory. The aim is to optimize the self-management of hygiene in production, raw materials and the production environment, as well as to eliminate the discrepancy between national and global standards of food safety management. This is justified by the increasing aging of the Japanese population, which raises the risk of food poisoning, the expanding globalization in food trade and the hosting of the Olympic and Paralympic Games 2020 in Tokyo [CGF, 2017].

Precautionary principle and scientific principle

Differences of risk perception pre-exist in the different doctrines. In most countries, including Japan, the *scientific principle* is the preferred approach. In the EU, the *precautionary principle* became legally binding due to Article 168 (1), Article 169 (1) and (2) and Article 191 of the Treaty on European Union and the Treaty on the Functioning of the EU. Internationally, the *precautionary principle* is recognized by the WTO, since it has been integrated into article 5 (7) of the WTO Agreement on Sanitary and Phytosanitary Measures (SPS Agreement) in 1995. In Japan, the precautionary principle is not explicitly embedded in the FSL, but there are regulations, which go along the same lines, for instance, Article 7 of the FSL. For example, the MHLW, in coordination with the FSC, may also prohibit the distribution of high-risk foods as a precautionary measure until evidence has been provided that they pose no risk to health. The precautionary principle was also determined in the EPA, namely in the following chapters: SPS-Chapter, which deals with food safety, animal health and plant health, and the chapter on technical barriers to trade, which governs technical product requirements and regulatory law [EC, 2018b].

Conclusion

This study analyzed the food safety measures and compared food safety systems in Japan and the EU under the EPA. Effective food safety regulations and policy measures play a key role in reducing potential threats to the health of humans and animals. It is well known worldwide, that both the EU and Japan have very high product standards and exemplary consumer protection. This has been verified by the results of this study. In many areas, both EPA partners have created standards that are in some cases higher than the international standards. In Japan, food is a massive political issue, reflected in the state's diverse and complex food security policy.

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STUDY ON THE ALLELOPATHIC EFFECT OF *AMARANTHUS RETROFLEXUS, DATURA STRAMONIUM* AND *PANICUM MILIACEUM* ON THE GERMINATION OF MAIZE

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Abstract

The plant extract of *Amaranthus retroflexus* showed an inhibitory effect on the development of both cotyledons and rootlets in the Petri dish trials. In the pot experiments, among the three examined weeds, dry plant residues of *A. retroflexus* hindered the germination of maize to the greatest extent. Also in the pot experiments, it showed a negative effect on the shoot length and weight, while a positive effect on the root length and weight.

A 2.5% concentration of *Datura stramonium* plant extracts showed a stimulative effect, but the 5% and 7.5% extracts inhibited the shoot and root development of germinating maize. In spite of the 60% germination rate, the incorporation of dry plant residues into the soil did not show any significant effect on the development of shoots, but a stimulative effect on root development was observed. However, the dry weight of roots exceeded the control values only at a concentration of 7.5%.

The plant extract of *Panicum miliaceum* had a stimulative effect on the shoot and root development of maize. The dry plant parts in the soil of the pots hindered the germination of maize, but significant effect on the shoot and root lengths could not be proven. At the 7.5% ratio the dry weight of shoots was higher than the control values, but all the other soil-plant part rations caused lower values.

Keywords: *dry weight, germination, weed extract, root, shoot*

Introduction

One of the solid bases of today's agricultural production is the effective control of weeds. Weeds are responsible for about 34% of yield losses globally; moreover, resistant species can be revealed increasingly (Khawar *et al.*, 2015). Improperly timed weed control measures, repetitive use of similar herbicides and spraying at an inappropriate phenological stage under unfavourable meteorological conditions can accelerate this process (Solymosi, 1990). There are about 6700 weed species having effects on the plant production worldwide, 200 of them can be considered important, which may cause serious damage on every side where agricultural production is taking place (Holm *et al.*, 1977). Modern weed control has to be performed in the spirit of integrated plant protection, which means that efforts should be made to ensure properly timed mechanical weed control, profound knowledge of the weed flora and the prediction of the expected weed coverage of the given field.

Based on these, the number of treatments with herbicides should be reduced as far as possible and combination of differently acting herbicides should be used to avoid the appearance of resistant or tolerant biotypes of weeds. The headway of herbicide resistance requires more active ingredients, but their number is finite, and in addition to it, currently used herbicides are not allowed in biological production (Soltys *et al.*, 2013). To eliminate this factor, it is important to have as many tools and technologies as possible to reduce the damage to weeds.

Understanding allelopathy helps to explain the interaction between cultivated plants and the weed flora and it may open up new opportunities for integrated and biological plant protection. The key compounds of allelopathy are the so-called allelochemicals that are synthesized by plants having allelopathic effects. These biologically active compounds

influence development of other plants in the surrounding of the emitting plant. Most of these compounds have negative effects, but they may have positive effects as well. These compounds can cause disturbance of germination or growth by upsetting photosynthesis, respiration, water balance and hormonal equilibrium, which processes are typically based on enzyme inhibitions (Rice, 1974, 1979, 1984; Putnam and Tang, 1986; Szabó, 1994; Solymosi, 1996; Szabó, 1997; Inderjit and Keating, 1999). By studying allelochemicals, exploring their mode of action, based on allelopathic processes, even new active ingredients can be produced, which can be used later in weed control. All these help to avoid the resistance of weeds and reduce the load of the environment with herbicides.

The use of allelopathic compounds can be an important component of future integrated and environmentally sound weed management, as increasing emphasis is placed on the research, development and application of environmentally friendly plant protection methods and devices.

In addition to the traditional chemical protection, integrated plant protection is preferred and the research of natural substances that can be used in organic and biological farming is also emerging.

Material and Methods

Petri dish germination trials

Green samples of the given weed plants were collected in the summer of 2015. The samples of red-root amaranth and jimsonweed derived from sunflower and maize fields of August at Bajót (Komárom-Esztergom county, Hungary), the samples of proso millet were collected in a stubble field in the vicinity of Keszthely (Zala county, Hungary).

The collected plant material, purified from soil residues, has been dried for 6 weeks under air-dry condition. At the end of the drying process, a predetermined portion of the samples was selected for the Petri dish trials, and the rest was kept for the second stage of the experiment series for pot experiments. The dried plant parts (stalk, leaf) were cut into 1-2 cm pieces with secateurs and later homogenized with a coffee grinder.

The purpose of this experiment was to prove the allelopathic effect of the donor species.

The experiment was designed with triple dilution line (2.5%, 5% and 7.5%) of plant extracts; therefore dry plant material was measured in required quantities for 200 ml of liquid, in this case distilled water, to obtain the desired solution. Plant samples had been left in the water within measuring cups for 24 hours at room temperature to allow the allelochemicals to dissolve.

At the end of the dissolution, Petri dishes were prepared. The seeds were placed on double-layer filter paper, and then were poured with 15 ml of extract (distilled water for control). For each concentration, eight replications were used, so for each of the three concentrations eight Petri dishes were ordered.

The germination experiment was performed with the seeds of 'MV Koppány' maize variety, placing 25 pieces in each cup, in four replications. As a control, only water was used.

Subsequently, the Petri dishes had been kept for 7 days at 20°C in a germinating incubator. The moisture content lost during the incubation was retrieved with water spray. As an evaluation of the experiment, the germinating seeds were individually examined on the 7^{th} day. Each concentration consisted of eight replications, each with 25 pieces of germinating seeds per repetition.

By measuring the length of the cotyledons and rootlets, 200-200 length data for each concentration were received and the average was considered as the result of the certain concentration. The same method was used in the case of control Petri dishes, where the average length of 100 cotyledons and rootlets were calculated. The data were recorded in a Microsoft® Excel spreadsheet.

This process was carried out in the same way for all the three weed species. The evaluation of seedlings treated with the extract of *Amaranthus retroflexus* was carried out on 04.11.2015, with the extract of *Datura stramonium* on 11.11.2015 and with the extract of *Panicum miliaceum* on 18.11.2015.

Pot experiment

In the second part of the experiment, the remainder part of the weed samples collected for the previous experiment were used, so their collection time and place were the same. In this case, however, 1-2 cm of dried plant pieces were break into a smaller size range of 1-6 mm by an electric coffee grinder.

Two liters of common black mould were placed in the pots, which served as a nutrient medium. To the given medium, the 2.5%, 5% and 7.5% dilution line was used, adding the plant debris required for the desired concentration. The shredded plant parts were incorporated into the soil within the upper 6 cm layer.

For each concentration, four replications were planned, so for each mixture of 2.5% and 5% and 7.5% soil-plant parts, four pots were assigned with five corn kernels per pot. The kernels were sowed in a depth of 3 cm. Four replications were designed for control; there were only black mould and corn kernels in the control pots.

The 4-4 pots, belonging to the certain concentrations, with seeds and weed residues after being injected with 200-200 ml of water were placed into a BINDER-type rearing chamber at 20°C for 30 days. The constant moisture was kept by irrigation throughout the germination period.

The evaluation took place after 30 days. The germination percentage according to certain concentrations was calculated and the dry weight and the length of roots and shoots were measured per plant. The data were recorded in Microsoft® Excel spreadsheets. The average of the repetitions of the same plant residues / soil ratio was taken to the end result of the experiment. The data were processed by single-factor analysis of variance.

All these steps were performed separately for each of the three weed species tested. The evaluation of maize treated with *Amaranthus retroflexus* samples was carried out on 19.10.2016, with *Datura stramonium* samples on 27.01.2017 and with *Panicum miliaceum* samples on 28.02.2017.

Results and Discussion

Petri dish germination trials

In the case of all the three plant extracts, the germination percentage ranged from 99% to 100% in every replication and in all concentrations, so detrimental effect of the extracts on the germination of maize could not revealed. Each seed brought a cotyledon and rootlet. The length of these cotyledons and rootlets was compared with the results of the control group to ascertain whether the extracts of weeds have an allelopathic effect on the germination.

During the evaluation, it was observed from the measured data that *Amaranthus retroflexus* extracts caused shorter lengths in the case of both the cotyledons and rootlets.

On the effect of the 2.5% dilution, the cotyledons reached 67% of the control average size, which was 25% in the case of the 5% dilution while it was 45.6% in the case of the 7.5% dilution. Thus, the strongest inhibition of shoot development was induced by the 5% solution (Doffkay, 2018).

Rootlets were also less developed in comparison to the control ones; in this case, the detrimental effect was proportional to the concentrations so the strength of inhibitory activity ranged from the smallest concentration to the highest. On the effect of the 2.5% plant extract, the rootlets reached 97% of the control size, which was 83.2% in the case of the 5% and 74.7% in the case of the 7.5% extracts.

Datura stramonium extracts also had an inhibitory effect. The average size of the cotyledons decreased proportionately with the concentration of the plant extracts, reaching 52.1% of the control size in the case of the 7.5% dilution. Rootlets showed a transient stimulatory effect at a concentration of 2.5% with more developed root components than the control ones, but in the case of 5% and 7.5% extracts growth inhibitory effect was manifested (Gerlinger, 2012).
In the case of *Panicum miliaceum*, a stimulative effect was observed, similarly to the previous results of Prutenskaya (1974) and Bellán (2017). The 5% concentration had the highest positive effect on the growth of cotyledons, in this case the average length of them was 56.9% higher than the mean values of the control. The same positive effect was observed on root growth, where the average root size was 29.8% higher than the average root size. By comparing data using single-factor analysis of variance, it can be established that there was a difference at 5% significance level between the effect of each weed extract on rootlets and cotyledons compared to controls and concentrations. These were potent inhibitory effects in the case of *Amaranthus retroflexus* and *Datura stramonium* while stimulative effect in the case of *Panicum miliaceum* (Bellán, 2017).
It can be seen that the inhibitory or stimulative effect does not necessarily follow the dilution line. The 2.5% solution of *Datura stramonium* had a stimulative effect, only the more concentrated solutions showed inhibitory effect. In the case of the *Panicum miliaceum* extracts, the 5% concentration showed the strongest stimulative effect. The reason of this may be that allelochemicals are able to quench the effect of excessive nutrients in the solution at a given concentration. In addition, consideration should be given to the inhibitory effects derived from the potential differences of solutions, which are independent from the allelopathic effects.
The more concentrated solutions are more difficult to absorb into the seeds, so they can absorb less water for germination. Additionally, plant residues contain spare nutrients besides allelochemicals, which may be released and become available for germinating seeds as germination supporting agents. These effects make it difficult to study allelochemicals.

Results of the pot experiment

Germination percentage

In the pot experiment, on the effect of the weed stalk and leaf fragments detrimental effect on germination has been appeared compared to the control group. In the control pots, all corn seeds germinated successfully, resulting in 100% germination.
During the experiment, it was revealed that the germination of maize was reduced in the case of all weeds in all concentrations compared to the control. In the greatest extent, *Amaranthus retroflexus* plant parts hindered the germination, but reversely to the proportion of plant debris, so the smallest concentration inhibited mostly the germination of corn (Doffkay, 2018). This meant a 35% germination rate.
Datura stramonium also decreased the germination, but soil-plant residue ratios did not show any significant difference in this extent (Gerlinger, 2012). In all three concentrations, the germination ratio was about 60%.
Panicum miliaceum plant parts also had a hindering effect on the germination compared to the control group. In this case, the 5% soil-residue ratio resulted in the highest plant number, similarly to the Petri dish experiment, where the 5% solution had the highest stimulative effect.

Length of roots and shoots on the effect of weed plant residues

In this experiment, stalk and leaf residues had been decomposed during germination, so besides the allelopathic effect, the nitrogen and water removal effect of the microbial bacteria involved in the decomposition had to be taken into account as well. The absence of

germination and differences in cotyledon and rootlet lengths may be attributed to this effect, apart from the effects of any allelochemicals.

During the evaluation, the length of shoots and roots were measured separately with centimetre accuracy. Their lengths were compared to the results of the control group in order to determine whether any inhibitory or stimulative allelopathic effect on germinating maize's shoot and root lengths manifested on the effect of weed stalk and leaf residues incorporated into the medium. After comparing the results, it has been found that *Amaranthus retroflexus* caused the smallest germination percentage, but the greater the amount of its plant residues in the pots, the greater the length of roots and shoots developed on the maize. Both the shoot (SD5% = 7.2%) and the root growth (SD5% = 8.8%) showed significant differences compared to the values in the control group.

In the case of *Datura stramonium*, although the average length of maize shoots decreased proportionally with concentration, it did not show significant difference. The root length averages (SD5% = 9.8) however, differed significantly. The root mass of all the concentrations exceeded its control group, so a kind of stimulative effect occurred.

In the case of *Panicum miliaceum*, there was not found any significant difference between the shoot (SD5% = 9) and the root lengths (SD5% = 9) compared to the control group.

On the effect of *Amaranthus retroflexus* plant debris, the dry weight of maize plants lagged behind the control group. The lowest dry weight value was measured at a ratio of 2.5%. The inhibitory effect was inversely affected by the increase in concentration, and at 7.5%, the dry weight almost reached the control value (Doffkay, 2018). The dry weight of roots at all three concentrations far exceeded the control group's values. Therefore, *Amaranthus retroflexus*, of which plant residues had been added to the soil, had a positive impact on the growth of the roots.

On the effect of *Datura stramonium* plant residues, with the exception of the 5% ratio, both groups showed a stimulative effect on the dry weight of the stalk. The root weight remained below the control values at 2.5% and 5%, but exceeded them in the 7.5% group.

Panicum miliaceum had a negative effect on both the root and shoot weight. Only 7.5% concentration resulted in a higher shoot weight ratio than the control group.

Conclusions

Amaranthus retroflexus plant extracts exhibit a negative allelopathic effect on maize. The *Amaranthus retroflexus* extract showed growth inhibitory effect on both the maize cotyledons and rootlets and the highest concentration caused the strongest inhibition.

In the case of *Datura stramonium* extracts, the smallest 2.5% concentration caused a stimulative effect on both cotyledons and rootlets, but the 5% to 7.5% solutions have inhibitory effects. In the case of *Panicum miliaceum*, a stimulative effect was observed for the growth of both cotyledons and rootlets in each of the three concentrations. The 5% solution exerted a stimulative effect to the greatest extent.

All weed extracts showed significantly different influence on cotyledon and rootlet development of maize.

Pot experiments

When incorporating *Amaranthus retroflexus* stalk and leaf residues into the soil, the average shoot and root lengths of germinating maize differed from the control group. The 2.5% and 5% plant-soil ratio had a negative effect on the germination of maize. In the case of root length, the 5% and 7.5% ratios showed a stimulative effect. For each of the three concentrations, the dry mass of root per plant was higher than that in the control group, however, the dry weight of shoots per plant was below the control values.

The *Datura stramonium* plant parts had a stimulative effect on the root development of maize, all three ratios showed similar effect, with the 5% group being most prominent. The effect of

residues of *Datura stramonium* on the shoot and root length could not be statistically proven, there was no significant difference among groups.
The groups treated with the residues of *Panicum miliaceum* did not show any significant difference the stimulative or inhibitory effects in this experiment were not statistically proven.
The dry plant parts of all three weeds resulted in a decrease in germination percentage in all tested concentrations compared to the control.

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THE ROLE OF SOLANUM NIGRUM IN THE SPREAD OF POTATO VIRUSES

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Abstract

Solanum nigrum is a common, high-spread weed throughout the country. It occurs commonly in arable lands and agricultural areas, and likes high nitrogenous soils. It can spread anywhere, where the right living conditions are met. The biggest problem is caused by cultures belonging to the same genus, especially in potatoes. In many ways, this relationship is unfavorable: it makes difficult to use herbicides, the weed can easily absorb many important nutrients from the crop and greatly promotes the spread of viruses from infected weeds. The aim of the experiment was to determine the virological examination of *Solanum nigrum*, and to determine the viruses appearing on the collected samples. The examined plant samples were collected in the autumn, from the potato peaks around Keszthely, Central Transdanubia, Hungary. A total of 55 *Solanum nigrum* plant samples were tested. More developed individuals with viral symptoms were collected. In order to detect viral infections, DAS ELISA serological method was used. Of 55 samples, we could diagnose viral infections in 30 cases. Epidemiological studies showed *Potato virus A* (PVA) and *Potato virus Y* (PVY) in most of the cases: PVA in 24 cases, PVY in 21 cases. In 10 samples *Tobacco mosaic virus* (TMV). *Potato virus X* (PVX) 3 samples, *Tomato mosaic virus* (ToMV) 2 samples were identified. *Tomato spotted wilt virus* (TSWV) was not detected in the collected samples. In most cases, complex viral infections were observed. Six types of virus complexes were diagnosed from 20 multiply infected plants. 12 samples showed PVY, PVA complex, PVY, PVA, TMV complex on 4 samples, PVX, PVY, PVA, TMV, ToMV complex, PVX, PVY, PVA TMV complex, PVX, PVY, PVA complex and TMV, ToMV complex appeared only at 1 plant. Our results point to the huge role played by weeds in spreading viruses. In our study, it has been shown that *Solanum nigrum* can not only carry a pathogen in most cases, but it can also carry more than one. Therefore, weed control is of particular importance.

Keywords: *Solanum nigrum, PVX, PVY, PVA, TMV, ToMV.*

Introduction

In our country, the biggest field of cultivated vegetables are from the *Solanaceae* family. This includes tomatoes, peppers, potatoes and eggplant. Plant protection is a complicated task and requires great expertise as it is advisable to apply complex protection against pathogens, animal pests and weeds. The main problem with the protection against viruses is, that it can only be prevented against them. Most importantly, it is the production and use of resistant varieties, but it can also be used to protect them against the use of virus-free propagation material, the removal of diseased plants, and the destruction of weeds and viral vectors.

Solanum nigrum is a weed belonging to the family Solanaceae. It is native to the Carpathian Basin as an Eurasian flora element. Very common, tough plant, spread throughout the country. Due to its aggressive expansion and nutritional abilities, it is also dangerous for cultivated potatoes, but also because of its viral carrier and transferring role. There are several pathogens in a single weed that can increase the chance of complex viral infection.

Our goal was the virological examination of the *Solanum nigrum* one of the most important weeds of potato. Our investigation was carried out in the spring of 2018 on samples from potato fields around Keszthely, Central Transdanubia, Hungary.

During the cultivation of potatoes, the aim is to cleanse the area from sowing to harvest. It is essential that weed has to be removed as soon as possible and this condition is maintained until the harvest. After the plant closure, potatoes can suppress the weeds until the end of the growing season. At this point a new weeding wave starts, as the foliage of the plant begins to clink and collapse for this period. The longer the ripening time, the weeding process lasts longer. (Mészáros *et al*., 2007).

PVX is almost everywhere in the world, especially where potato production is taking place. It has been shown in a variety of plant families, but mostly Solanaus species in the *Solanaceae* family are susceptible to the virus. (Horváth, 1978). PVY is the most potent and most serious damaging viral disease in potatoes. It is spread all over the world and occurs in all potato-producing countries. Its host plant circle is very extensive, especially the plants of *Solanaceae, Amaranthaceae, Chenopodiaceae* are susceptible (Edwardson, 1974, Horváth, 1983). PVA economically belongs to smaller viruses. Its significance is mainly when it comes along with PVY and PVX. It often appears in combination with PVY (Smith, 1972). It has a narrow host plant where the families of the *Solanaceae* family and two other families: *Trigonella* and *Melilotus* (Spaar *et* Hamann, 1977). TMV occurs everywhere in the world, both found in lower and higher order plants (Gibbs *et* Harrison, 1976; Horváth, 1977b). It has the most extensive host plant circle, the number is over 1000 (Horváth, 1976). Among the natural host plants it can infect economically important tobacco species (*Nicotiana* spp.), pepper (*Capsicum annuum*), tomato (*Lycopersicon esculentum*) and potato (*Solanum tuberosum*). ToMV can be found worldwide. Its main host is tomato (*Lycopersicon esculentum*), but it also infects other plants, including ornamental plants (marigolds). In addition to the plants of the family Solanaceae, it can infect some of the black currant, apple, barley, weeds, and some of the family of *Asteraceae, Chenopodiaceae, Malvaceae, Passifloraceae, Tetragoniaceae, Plantaginaceae, Cucurbitaceae, Fabaceae* (Rod *et al*., 2005; Moreira *et al*., 2003). TSWV is responsible for a number of epidemics in various regions of the world, especially in horticultural and floral plants, which often cause destructive and severe economic losses. It has a broad host plant, with the latest data currently being 1090 plant species.

Material and methods

The examined plant samples were collected in the autumn, near Keszthely, Central Transdanubia, Hungary, from potato fields. 55 samples of *Solanum nigrum* plant specimens were tested. The plants were placed individually in a polyethylene container and then frozen until the time of the laboratory test. When trying to take samples, It was tried to collect the individuals with more developed viral symptoms. The DAS ELISA (Double Antibody Sadwich-Enzyme-linked Immunosorbent Assay) method was used for the detection of viral infection, which was written by Clark and Adams in 1977. This is the most commonly used laboratory procedure for the diagnosis of plant viruses. The test does not detect the pathogen, but determines the activity of the enzyme related to the antigen or antibody, thus giving the starting point for the amount of antibodies it produces.

Results and discussion

Based on the results of the DAS ELISA serological test, the infection of the examined plant samples is shown in Table 1. by highlighting the number of samples in red.

Table 1.: Viral infection according to the DAS ELISA-method (red:infected

Solanum nigrum	1	2	3	4	5	6	7	8	9	10	11	12
A	**X***	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
B	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
C	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
D	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**
E	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**				

The first diagram shows that the number of virus-infected plants shows a changing tendency. Based on the virus epidemiological studies *Potato virus A* (PVA) and *Potato virus Y* (PVY) were detected in the highest levels. Of the 55 plant samples, 24 PVA infected platns were diagnosed, and 21 in PVY were diagnosed. In 10 samples we identified *Tobacco mosaic virus* (TMV). *Potato virus X* (PVX) 3 samples, 2 *Tomato mosaic virus* (ToMV) infected samples were observed in serological tests. The presence of *Tomato spotted wilt virus* (TSWV) was not detected in the collected samples (Figure 1).

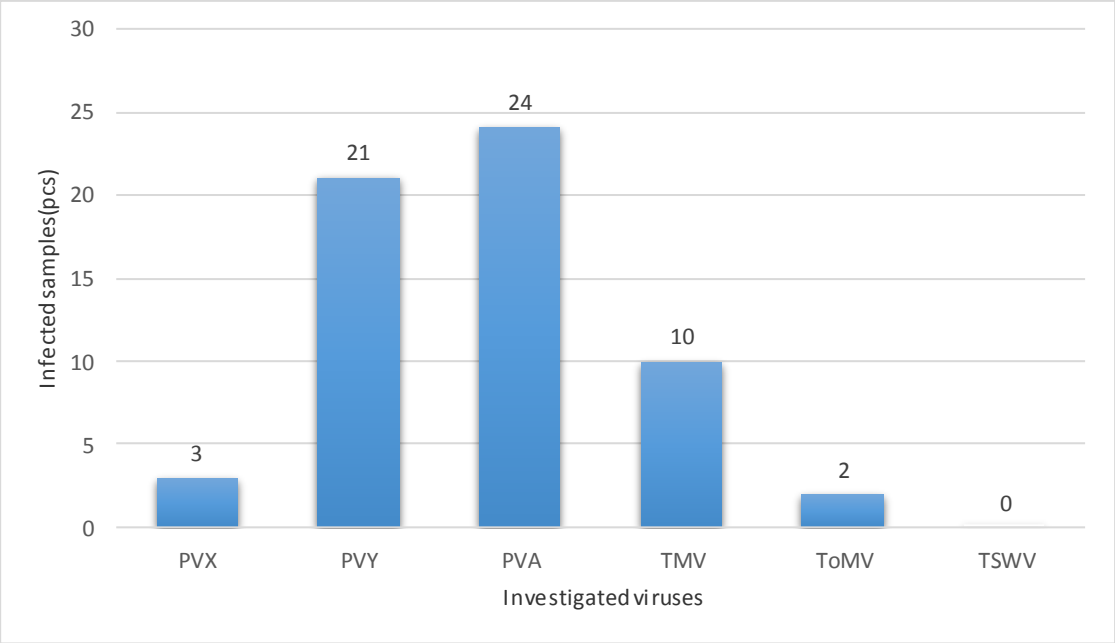

Figure 1.: Number of the infected plants

In most cases, complex viral infections can be observed. According to the number, 6 types of virus compositions were diagnosed from 20 multiply infected plants. 12 samples showed PVY, PVA complex, PVY, PVA, TMV complex on 4 samples, PVX, PVY, PVA, TMV, ToMV complex, PVX, PVY, PVA TMV complex, PVX, PVY, PVA complex and TMV, ToMV complex appeared only at 1 plant. The evolution of multi-infected plant samples is illustrated in the following diagram (Figure 2).

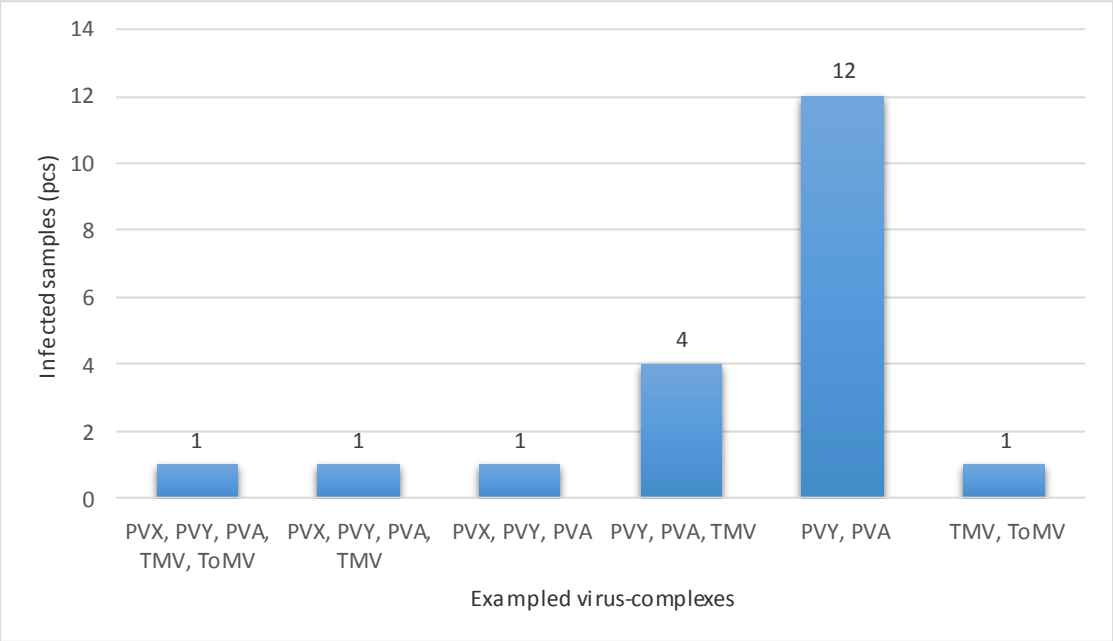

Figure 2.: The complex infections

There were simple viral infections in 9 plant samples. PVA for 5 plants, TMV for 3 and PVY for 1 (Figure 3).

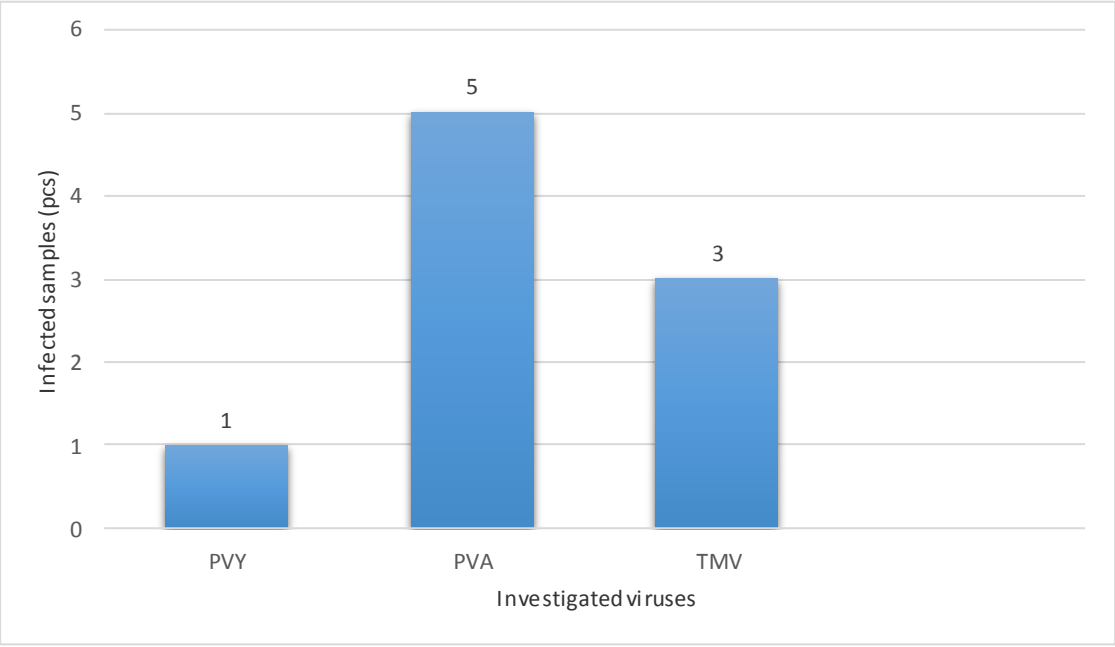

Figure 3.: The simple virus infections

Conclusions

The results of the experiment show that the weeds of cultivated potato species, especially *Solanum nigrum*, can play a significant role in the distribution of potato viruses, apart from their direct competitive effect. For this reason, it is of great importance in our cultivated crops to prevent weed control, to prevent the spread of wild *Solanum* species.

Potato virus A (PVA), *Potato virus Y* (PVY), *Tobacco mosaic virus* (TMV), *Potato virus X* (PVX) and *Tomato mosaic virus* (ToMV) occurred during the experiment. The presence of *Tomato spotted wilt virus* (TSWV) was not proven in any case.

PVA is the largest amount of pathogen detected. It is not a significant virus unless it is combined with PVY or PVX. It often appears with PVY, this is also apparent from the experiment since a total of 19 infected samples were found where these two viruses were present together. PVY is the most potent and most serious damaging viral disease in potatoes. Based on our investigations, the virus in the second most frequent samples was also present in all the viral combinations. TMV is a virus all over the world occurring in plants. During the study, it appeared on 10 infected samples alone and in combination. Its significance is mainly due to the decrease in yield, quality and dry matter content. PVX is mainly found in potato-producing areas worldwide. Of the examined plant samples, it was released in combination with only a few other viruses. The virus is mechanically easy to transfer and it is also excellent to spread with insects. ToMV is also found worldwide, most of which threatens tomatoes, but it also infects other plants. Of the collected *Solanum nigrum* samples only two showed and only in combinations where TMV was present. TSWV is responsible for many epidemics in the world, especially in horticultural and floral plants.

The results of the studies revealed that this virus was not present in any of the samples. This may also be justified by the fact that the plants under study come from arable potato culture. Protection against viruses is based on preventing and preventing infection. At present, it is not possible to treat infected plants. Most efficiently using virus-free propagating material, using biotechnological methods (resistant varieties), heat and chemotherapy, digestive cultivation, appropriate agrotechnical techniques such as stubble and other soil operations, control of vectors and virus reservoirs and, last but not least, weed control of the infectious source. In addition, due to the fact that many viruses can spread mechanically well, it is important to pay greater attention to compliance with hygiene rules in general phytosanitary regulations. Their transmission through the disinfection of tools and work clothes, frequent hand washing, and destruction of diseased plants

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INVESTIGATION OF THE IN VITRO REGENERATION OF *ASCLEPIAS SYRIACA* AND *SORGHUM HALEPENSE*

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Abstract

According to the Hungarian weed surveys, the significance of the *Sorghum halapense* and the *Asclepias syriaca* is growing significantly. Today, Johnsongrass is the most important weed of maize, and their field coverage in Hungary is over 1%. It is ranked on the 11th place on maize fields, which shows its significance. The northeastern part of the country is the most infected. The common milkweed is spreading in more and more places. In 2002 there were more than 200,000 hectares infected. Its proliferation continued both on wheat and maize fields. It is spreading predominantly in the northeastern part of the country, but its appearance can be expected anywhere. The aim of our study was to observe the regeneration of two perennial weeds, using the vegetative reproductive organs of common milkweed and johnsongrass, under controlled in vitro conditions. For this purpose, a climate chamber experiment was set up at Keszthely with different lengths of root segments (1, 3, 5 nodes and 1, 5, 10 cm), at the Plant Protection Institute, University of Pannonia. The different sized vegetative organs were regularly measured, and the renewal capabilities of the two weeds were concluded. According to our experience, the initial size of the segments had an effect on the shoots generated by them. By increasing the size of the roots the number of buds increased. The buds in the direction of the apex formed the longest shoots, because of the abolition of apical dominance. However, the larger initial size did not mean clearly higher values in the average length of the shoots. For the defense against perennials, we need to combine our capabilities, think towards an integrated approach, and if it is required, we need to use herbicides against the perennial weeds. This is the only way now, which can be used for a long period of time to suppress the foes of our crops.

Keywords: *Hungary, regeneration ability, apical dominance, Sorghum halepense, Asclepias syriaca.*

Introduction

With the advancement of cultivation technology and methods, the composition of arable weed comunities (Pinke - Pál, 2005) has also changed. A number of European researchers have been working on the follow-up of changes in weed patterns.

The significance of Hungarian perennial weeds is well illustrated by the results obtained from the National Field Weed Surveys, the principles of which were defined by Miklós Ujvárosi (1973). Records were made for plant protection purposes and found which are the most dangerous weeds and recorded their cover data and spread.

Effective regeneration of perennial weeds is served by modified underground shoots (rhizome, propagation root etc.). In the modified lifeform system of Ujvárosi (1957), the G1 group includes the *Sorghum halapense* (L.) Pers. The rhizome is a modified underground stem. Their regeneration is efficient, all rhizome segments are capable of forming a new plant after breaking up, with at least one intact axillary bud. The distance between axillary buds is 1-1.5 cm on rhizomes.

Propagation roots for reproduction are mostly run horizontally in the soil. The roots originating from them, however, penetrate deep into the ground in a vertical direction. In the

life-cycle of Ujvárosi (1957), G3 the species was named as weed with propagation roots. Propagation roots are a modified root in which adventive buds are reproduced.
Generally, a 5 cm long root segment is capable of forming a new shoot. As a result of the cultivation of the soil, the fragmented rhizomes will be regenerated according to the principle of apical dominance. Due to correlative inhibition, in most of the time, only one or two shoots will grow, while the rest will remain calm. The axillary buds that are closer to the peak groove always grow longer than the other rhizome segments, so they are polarized.
According to American researchers Johnson and Bucholtz (1962), the activity of vegetative organs has a periodicity similar to seeds. The effectiveness of herbicides is greatly influenced by the extent to which they are able to translocate in the weed plant. This process is related to the metabolism of organs (floemtransport), so we need to know the activity of regeneration functional organs (rhizomes, propagation roots).
Research by Holm (1978) and his colleagues highlighted that the johnsongrass are present in the tropical and Mediterranean countries as the world's sixth most important weed. In Romania it was declared a quarantine-weed in the 1950s. It has been grown in Hungary due to its good nutrient content and apiculture (because of its intensive nectar production).
The johnsongrass became more and more adaptive to the unfavorable, cooler climate of its initial development, so it was able to conquer new areas, the neighboring states of Slovenia, Croatia, Serbia, Bulgaria and Romania.
Thanks to the photosynthesis of C4, it has a great competitive potential against the majority of crop plants (usually C3s). They play a major role in reducing crop yields because they compete for the same resources (water, nutrients, air, sunlight, etc.). With its presence, it directly and indirectly damages our crops.
The later spread of the common milkweed, *Asclepias syriaca* L., species in Hungary has greatly contributed to the high potentials as a decorative plant in different applications. Field cultivation later has been abandoned, but the remains of the cultivated and run wild plants formed invasive centers, and the plants spread from them later caused significant damage (Botta-Dukát and Mihály, 2004). The construction of railways and roads contributed greatly to the spread of silkworm, as the competing plants were extinguished in these areas.
The potential causes of the explosive spread of common milkweed in recent years include the dry weather of the past 10 years, the increase in the number of frost-free days, the lack of stubble and plowing, soil erosion, reduced tillage, (lack of competition) or rarely targeted weed control (Kőrösmezei, 2000). It can cause natural problems as it prevents the regeneration of close-knit associations in occupied territories.
The common milkweed invasion is significant in areas degraded by anthropogenic activity. Its appearance can be combined with the planetary surface (trampling, plowing, overgrazing, erosion) or nutrient enrichment (fertilization, forest fires).

Material and methods

The aim of the study was to observe the regeneration of two perennial weeds, using the vegetative reproductive organs of johnsongrass and common milkweed, under controlled in vitro conditions.
The rhizomes of the johnsongrass and the propagation roots of the common milkweed were collected at Keszthely, Central Transdanubia, Hungary. The samples were collected on 2017.11.16 on the corner of a field plate.
The rhizomes and propagation roots were taken directly from the top 25 cm layer of the soil. Axillary buds were removed during their preparation, which allowed the control of the integrity of the buds, and thus the length of the axillary buds evolved in the early stages of regeneration. Prior to the measurements, the vegetative parts were disinfected with chlorogen and then sliced into 1, 3 and 5 nodular units for *Sorghum halapense* and 1, 5, and 10 cm

propagation root segments for *Asclepias syriaca*. From the vegetative formulas of different lengths, 10 pieces were placed in a Petri-dish lined with filter paper and 15 ml of distilled water in a 3 repetition.
To test the regeneration ability, the Petri-dishes labeled by the initial length and repetition number were placed in a thermostat. The climate chamber was kept at 20 ° C for the duration of our observations. Measurement of the shoots and roots continued until the condition of the plant parts allowed it. During the experiment, for each species we measured the total number of roots and shoots. When studying the johnsongrass, we associate the position of the buds with the length of the shoots, i.e. how many nodes develop. Knowing the situation of buds allowed us to observe the legitimacy of apical dominance.
We used Microsoft Excel and SPSS to evaluate and analyze the data. We have repeatedly applied single-factor analysis of variance considering distributions and deviations.

Results and Discussion

Sorghum halepense

In the case of 5 nodular rhizomes, the number of shoots was significantly higher than in the other two cases. The number of buds in the 3 node rhizomes was not significantly higher, but it is clear from Figure 1. that the number of nodes 3 developed more than the 1 nodus rhizome segments.
Comparing the length of shoots from various nodomous segments and evaluating our data, the following results were obtained:
On the first day of measurement, we found that the 5 nodular rhizomes had the significantly longest shoots, and in some cases reached 30 mm.
The longest shoot was 161 mm, which was placed on a 3 nodular rhizome with the other 3 shoot with over 100 mm. In the 5 nodal segments, we counted 10 shoots above 100 mm (including one 160 mm) and one over 100 mm.
Our measurements show that the starting length of the rhizomes (1, 3, 5 nodus) and thus probably the nutrient quantity did not significantly influence the length of the axillary buds developing in our present experiment.
Apart from the first measurement time, we could significantly show the dominance of the first nodes in both 3 nodus and 5 nodous segments.
From Figure 1. it can be seen that there are no significant differences between the different days of measurement in the average of the shoot lengths of the 1, 3 and 5 nodular rhizomes. The line diagram fitted to the graph illustrates that the 5 Node pieces had significantly more buds of the total time intervals of the measurements. The bar graphs show the length of the shoots on the node, while the line diagram shows the number of buds out.

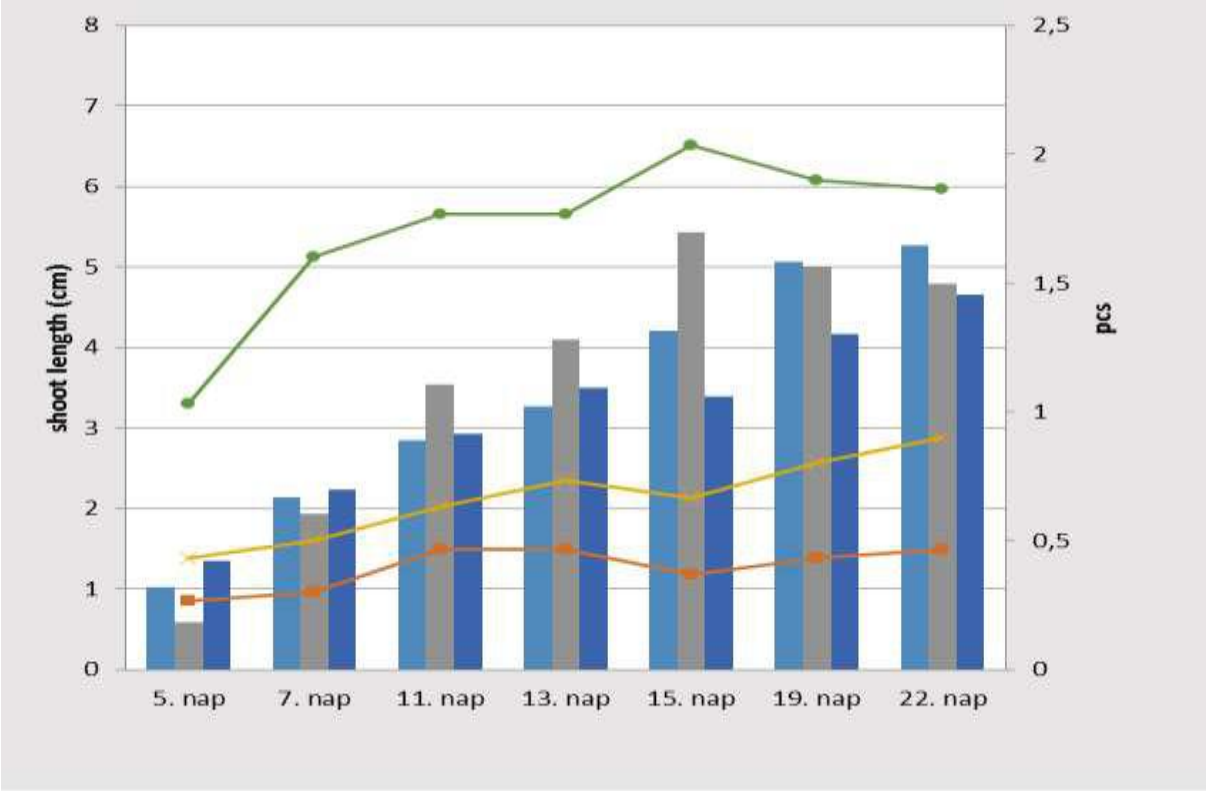

Figure 1.: Average number of sprouted buds (line diagram), average length of shoots (bar graph), depending on the days elapsed

Asclepias syriaca

The 10 cm root segments produced higher values from the start to the end of the experiment than the 5 or 1 cm counterparts. The lengths of 1 cm and 5 cm did not reach the average of 2 outbound buds on a single day of the measurements, while the 10 cm propagation roots generated an average 3-3.5 fold per segment.

There was no significant difference between the lengths of the shoots of different lengths of ripening root pieces until the 13th day. From the 13th day on, however, the difference was significant. From day 13 to day 33, the lengths of the 1 cm pieces were smaller than the other two groups, but there was no significant difference between them. From the 33rd day all three groups were significantly different. The common milkweeds propagation root, unlike the underground rhizomes of the johnsongrass, is not divided into nodes, so the position of the buds could not be fixed. But we were still curious about the relationship between the buds and the length of the shoots. For this purpose, the two data groups are displayed in a diagram, each of the measurement dates separately for the representation. At the beginning of our measurements, on the 5th day of the experiment, the number of spilled buds was 1 cm, and the 5 and 10 cm pieces had almost the same amount of bud. It can also be seen that longer propagation roots have been developed for longer shoots. However, these findings do not stop at the beginning of development. Looking at data from other days of the experiment, we see that 1cm pieces still have the smallest amount of buds and the size is the shortest. However, Figure 2. also shows that the number of buds on the 5 cm growth roots decreases by 10 cm, but the average length of shoot buds is higher, showing higher values even over the average lengths of 10 cm pieces. Thus, under the same conditions, the 5cm propagation roots sought to create longer shoots while the 10 cm segment increased the number of buds. This is probably due to the fact that 10 cm pieces had more nutrients. The bar graphs show the length of the shoots on the node, while the line diagram shows the number of buds out.

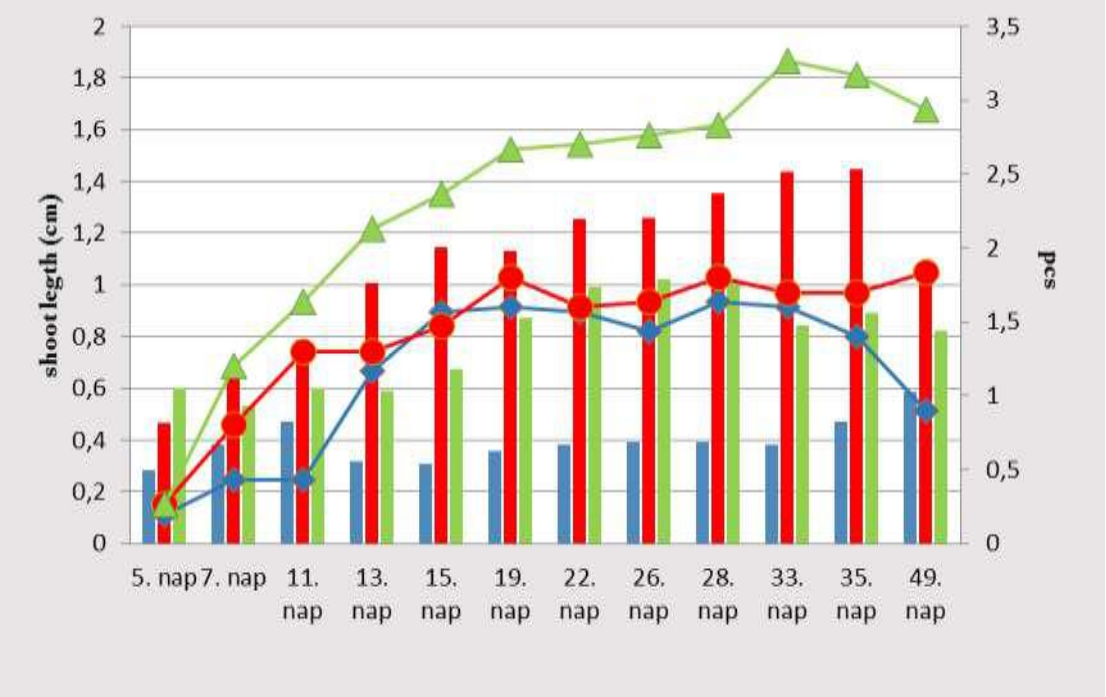

Figure 2.: The average number of sprouted buds of the common milkweed (line diagram), the average length of the shoots (bar graph), depending on the days elapsed

It was found that the number of shoots produced was also clearly influenced by the size of the fragmented vegetative reproductive organs in *Sorghum halepense* and *Asclepias syriaca*. The longer the length, the more spinning buds resulted in the experiment. We have clearly experienced the phenomenon of apical dominance, which, by regulating the tranquility of the axillary buds, plays a prominent role in the plant's species maintenance. As a result of the fragmentation, partial axillary bud activation resulted in partial dissolution of the correlative inhibition. During the experiment, the axillary buds closest to the originally apex were producing the longest shoots.

Based on our experiment, it was concluded that increasing the length of the rhizome segments does not result in significant differences in the average length of axillary buds in the case of johnsongrass. Multinodular rhizomes are presumably contain more nutrients, but due to the partial dissolution of apical dominance mentioned above, they are not concentrated in a single shoot. The case of common milkweed it eas not proved, that the longer propagation root piece would produce longer shoots, as the 5 cm root segments formed longer shoots rather than 10 cm pieces.

It is not possible to completely uproot the weeds we have tested, but we must strive to reduce their presence under integrated protection to the level of economic damage.

Conclusions

The preventive (precursor, stubble), agrotechnical, mechanical and chemical elements of our defense must be applied complexly in crop rotation. We must take care of the cleanliness of our machines, thus preventing the weeds and vegetative propagation patterns from entering our territories. The rhizomes and propagation roots are rotated by rolling into the deeper layers of the soil by deep plowing, causing anaerobic conditions into the soil. However, too deep autumn plowing can increase the chance of overlapping if vegetative formulas are already under the freezing zone. When cultivating, roots are split into smaller pieces, we can deliberately activate the formation of quiescent axillary buds and then we can defend

mechanically or with total herbicide after spraying. Good results can be achieved with soil cultivation, but in case of incorrect application, we can promote the growth of perennials. The axillary buds of the vegetative propagation staves torn together by conventional soil and sprouting tools are activated, thus increasing the perennial weeds in the area.

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EFFECTS OF SALICYLIC ACID AND PHYLAX IN THE CONTROL OF BEAN ROOT ROT DISEASE CAUSED BY *RHIZOCTONIA SOLANI* KUHN

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Abstract

This study wasconducted to detect the pathogenic fungi associated with bean stem base and root rot disease and evaluate the activity of Salicylic acid (SA) and Phylax to induce resistance in the plants against the main causal agent under field conditions. Nine species belongs to 7 genera of fungi were found associated with infected plants collected from three locations in Baghdad area at different frequency levels. *Rhizoctoniasolani* was the more dominant followed by *Fusarium solani* and *Macrophomina phaseolina*. Seven isolates belongs to *F. oxysporum, F.semitectum, F. solani, M. phaseolina* and *R.solani* have caused reduction in seed germination and increase in disease severity in contaminated soil compared with control.Amongthe tested isolates, *R.solani*isolates,RS1 and RS3, were found to bemore aggressive with totally inhibition of seed germination. It was found that seeds socking, soil application or foliage application with SA and Phylax at 2500 ppm, separately or in combination induced resistance in bean plants characterized by are striction of *R.solani*and a suppressionofdiseasesymptomsdevelopmentassociatedwithincreaseinplantfresh and dry weights. Soaking seeds in water and soil application of SA+ Phylax was found the more effective in reduction of disease incidence and severity compared with control.

Key words: *Root rot, Rhizoctonia solani, Salicylic acid.*

Introduction

The bean (*Phaseolus vulgaris* L.) is considered among the most nutritive crop in Fabaceae, where the dry seeds contain high percentage of protein,vitamins, and nutritive fibers (Buruchara, 2006; Nicolai et al, 2015). Bean crop is cultivated in the middle and southern areas in Iraq at 249 .75 Hectarewith annual yield up to 1249 ton (Statistic group, 2016).

Bean plants are subjected to infection with many soil borne pathogens including *Fusarium oxysporum,F.solani, Macrophomina phaseolina, Pythium* spp., *Rhizoctonia solani* causal agents of seed rot, seedling damping off, root and stem base rot(Matlob,2012; Binagwa et al. 2016; Marcenaro and Volkanen,2016, Maina et al. 2017). Several means were adopted to control these pathogens, mainly fungicides, but the excessive applications of fungicide have created enormous problems to ecosystem and human health (Choi and Hwang, 2011). Therefore, the research was oriented toward natural,effective and innocuous, means alternative to artificial chemicals to manage plant disease. Many compounds including Benzoic acid, Salicylic acid, Ascorbic acid and Phylax were used as foliage application, soil treatment, seed soaking, showing high inhibition activity against several fungal pathogens *in vitro* and reduced infection with root rot disease under field conditions (Katay et al. 2011; Adghough et al. 2013; Al-Juboory, 2016). The study was carried out to evaluate the efficiency of seed treatment, foliage application, or soil treatment with Salicylic acid and Phylax in controlling stem base and root rot caused by *Rhizoctoniasolani* in beans.

Materials and methods

Fungi isolation and identification

Bean plants showing stem base and root rot symptoms were collected from Al-Madaen, Al-Dora and Al-Rathwania regions in Baghdad governance. Pieces of 0.5-1 cm from rotten stem and root were surface sterilized with 2% Sodium hypochlorite for 2min, rinsed with sterilized distilled water, dried on filter papers and cultivated on potato dextrose agar(PDA) in Petri plates (ø9 cm), 4 pieces/plate. The plates were maintained at 25 ±2ºCfor 4 days. The fungi colonieswere purified and identified based on the morphological characteristics (Parameter and Whitney,1970; Barnett and Hunter,1972). Fusarium species were identified according to Leslie and Summerell (2006).The frequency percentage of fungi was calculated by the following equation:

$$\%\text{fungus frequency} = \frac{\text{No of pieces containing the fungus}}{\text{Total number of pieces}} \times 100$$

Pathogenicity test

The fungalisolates were grown on millet (*Panicummiliaceum*L.) seeds in 250 ml flasks. Millet seeds were distributed in Hu flasks, 50g/ flask, and autoclaved at 121ºCand 1.5 kg/cm^2 for 20 min. The seeds were inoculated with discs (~0.5 cm) taken from the margin of fresh fungal colonies on PDA, 5 discs/flask, and incubated at 25± 2ºCfor 15 days, with agitation every 3 days (Dewan, 1989). The inoculum was mixed with sterile soil and peat moss(2:1) at 1% in pots (10 cm dim). Sterilized millet seeds were added to other potsas control. The pots were watering and covered with polyethylene bags for 3 days and sown with surface sterilized bean seeds (5 seeds/pot). The pots were distributed in completely randomized design (CRD) with 4 replication,under greenhouse conditions. Seed germination was calculated after 10 days and the disease severity was estimated after 30 days of sowing, according to disease scale described by Matloob (2012).

0= healthy plants; 1= rolling of secondary roots; 2= discoloration of secondary roots and part of main root; 3= rotting of main root; 4= rotting and disruption of main root.

$$\%\text{ disease severity} = \frac{\text{Number of plants from 0 degree} \times 0 + .. + \text{No. of plants from 4} \times 4}{\text{Total No. of plants} \times 5} \times 100$$

Effect of Salicylic acid and Phylax application on disease development

The experiment was carried out in a field of Collage of agriculture, University of Baghdad. Bean seeds were separately soaked in water, Salicylic acid and Phylax for 24 h and sown in the field in wells (3 seeds/well) at 25 cm inter space on lines of 3 m length, 1m inter spaces, with the following treatments:

1. Seeds soaked in water and foliage application of SA+ RS3.
2. Seeds soaked in water and foliage application of Phylax + RS3.
3. Seeds soaked in water and foliage application of SA+Phylax + RS3.
4. Seeds soaked in water + RS3/ control.
5. Seeds in soil treated with SA+ RS3.
6. Seeds in soil treated with Phylax + RS3.
7. Seeds in soil treated with SA+ Phylax +RS3.
8. Seeds soaked in SA + RS3.
9. Seeds soaked in Phylax + RS3.
10. Seeds soaked in water/control.

SA and Phylax were used at 2500mg/l for foliage application and at 50ml/well for soil treatments. *R.solani* inoculum grown on millet seeds was added in groove along the lines under the wells at 50 g/m. Disease incidence, disease severity, plant fresh and dry weight were assessed after 90 days of cultivation.

Results and Discussion

Fungi isolation and identification

Results of isolation and identification showed presence nine species belong to seven genus associated to the root rot and stem bases (Table-1-) which had rot and lesion symptoms disease and the *R.solani* fungus had the priority with variance presence percentage between the regions ranged between 75 -100% followed by *F.solani*with percentage ranged between 40.5- 65.2%, while the *M.phaseolina* presented between 9.5-35.5% . These results were inagreement with Mwang-ombe*et al* ,(2007) of the spread of a soil borne fungal pathogen in the bean fields of the fungal *F.solani, M. Phaseolina*and *R. solani* by surveying the root rot of bean for ten fields in Kenya, they found that the main cause of root rot disease of beans is *R.solani* followed by the Fusarium with an appearance ranging from 5.6-65.2%. Roots rot disease spreads in many region in Babel governance and *F.solani*is the more spread fungus and it was founded in most of the tested samples with different percentage ranged between 14- 65% followed by *R.solani*and *M.phaseolina*at presence ratio 28.1 and 22.6% respectively(Matloob, 2012). Timothy *et al*, (2013) mentioned that the most important causes of root rot disease are *M.phaseolina*and *Fusariumspp* and they appeared in most samples which were collected from Latin America, Middle America and Carbine sea fields, *F.oxysporum*and*F.semitectum*appearance in Al-Maden, AL- Dora and Al- Rathwania regions samples ranged between 22.65-35.5% and 20.6-30.7 % respectively. These results agreed with many studies that explained that species of *Fusariumspp* such as *F.oxysporum, F.proliferatum ,F.semitectum*and*F.solani*were from the main causes of root rot in some field of vegetables crops(Salari*et al*,2012; Zakaria and Sasetharan, 2014). These results agreed with Al-Juboory*et al.* (2016) that isolates of *M.phaseolina*, which were isolated from watermelon plants grown with root rot disease, significantly reduced the germination rate of the seeds on the WA plant ranging from 0 to 65%.

These results agreed with finding of each of Al- Juboory(2002), Matloob (2012), Al-Mosawe (2012), that the *F.oxysporum, F.semitectum* and *F.solani* species were caused that caused root rot disease in some plants of different plant families . The results of microscopic examination revealed a number of fungi associated with the roots and bases of bean stalks such as *Alternariaalternata, Drechsleraaustraliensis* and *UlocladiumAtrum,* with ratios of less than 10.63, 5.10 and 1.7% respectively. Presence of these fungi may be attributed to their high ability in productive unit's production and to tolerance of the inconvenient condition and to their ability to stay live with host presence and their family range is wide and their large competition ability against the other soil organisms. There are some factors that contribute in rising the infection ratio of roots rot disease such as continuation on using inconvenient agricultural systems, soil fertility levels decline , use seeds collected for the same previously cultivated farms , repeating crop cultivation yearly and use sensitive species to roots rot disease infection . The incidence and severity of the disease varies according to environmental conditions and soil conditions, such as the number and type of pathogens present under certain conditions (Morris. 2017).

Table 1: Fungi accompanying to the infected roots of bean plants and ratios and regions of them.

Fungi	Frequency*%			
	Al-Maden	AL-Dora	Al-Rathwania	Average
Alternariaalternata	6.8	14.5	10.6	10.63
Drechsleraaustraliensis	6.5	3.8	5.00	5.10
Fusarium oxysporum	25.4	35.50	22.65	27.85
F. semitectum	30.7	20.6	25.5	25.60
Fusarium solani	55.0	40.5	65.2	53.56
Macrophominaphaseolina	25.7	35.5	9.5	23.56
Rhizoctoniasolani	75.0	90.8	100.0	88.6
Trichoderma harzianum	3.2	2.61	4.11	3.30
Ulocladiumatrum	2.1	3.00	0.00	1.70

$$\text{*Frequency (\%)} = \frac{\text{Number of pieces containing thefungus}}{\text{Total number. of pieces}} \times 100.$$

The result showed that *F.oxysporum* (**Fig.1** A) colony differed from white to pink to purple and it produced three kinds of Microconidia spores lifted by short tripods of kind Monophialides and Macroconidia and they were short to medium length and were divided by three barriers , Chlamidio spores is as single or pairs and it may be as groups or short chains on PDA media(Leslie and Summerel, 2006) .*F.semitectum* colony(**Fig.1** B) characterized by its brown or light brown color changes to light orange color and it forms three kinds of spores, the Macroconidia divided by 3-5 barriers , while Microconidia is rare and it is difficult to find it and Chlamidio spores is single or as chains form. *F.solani* colony characterized by its white to creamy color on the PDA media (**Fig.1** -C) and the microscopic test showed three kind of spores , they were Macroconidia, spindle to cylindrical shape , 5-7 barriers , Microconidia colony had oval to elliptical shape and 1-2 barriers resulted from monophilides kind of long tripods and Chlamidio spores was single or in pairs and sometimes as short chains (Leslie and Summerel, 2006).

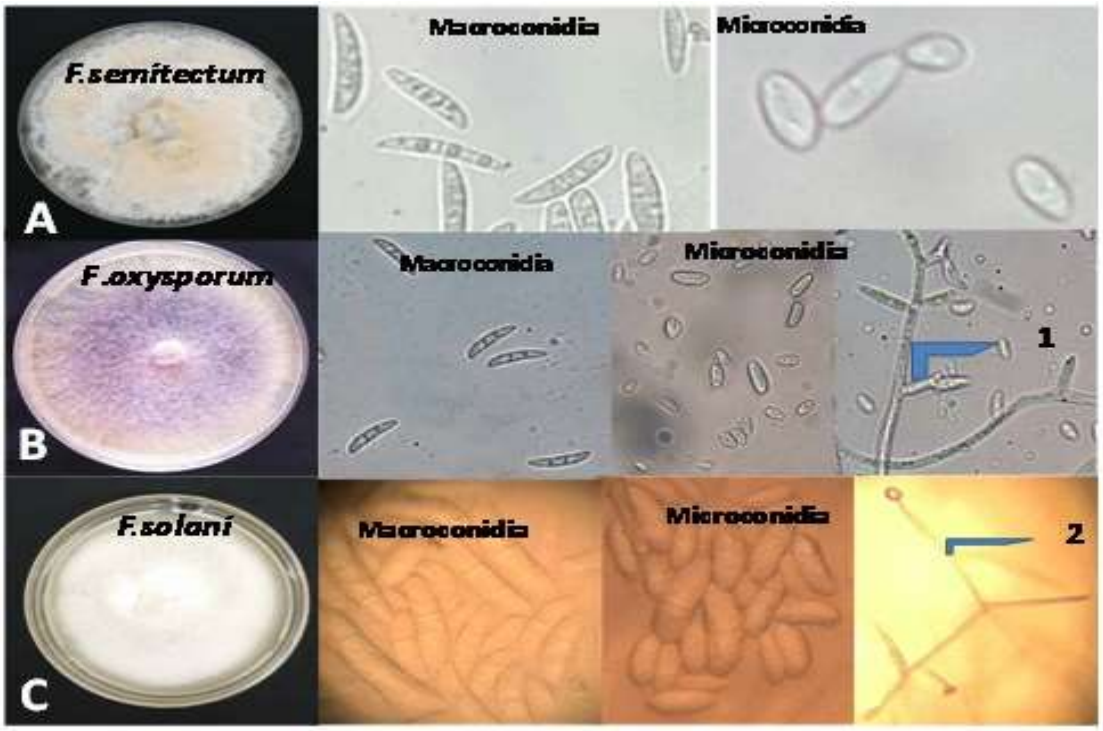


Fig.1 - Cultural and microscopic characteristics of the F species A- colony growth on PDA of *F. semitectum* , 1- short condia tripods, B- colony growth on PDA of *F.oxysporum* nature and shape of growth and Microconidia and Macroconidia , condia spores of Microconidia and Macroconidia,c- colony growth on PDA *F.solani* - long condia tripods type Mono which is classification property of fungus.

M.phaseolina isolations characterized by their fast growth on PDA compared with other fungi and by their while color that may change to dark color and then to black color(**Fig.** 2 -A) due to Microsclerotia formation and have irregular shape on surface of PDA Media(Picture 2 - B) . Disease intensity is correlated with Microsclerotia numbers in soil(Khan, 2007).

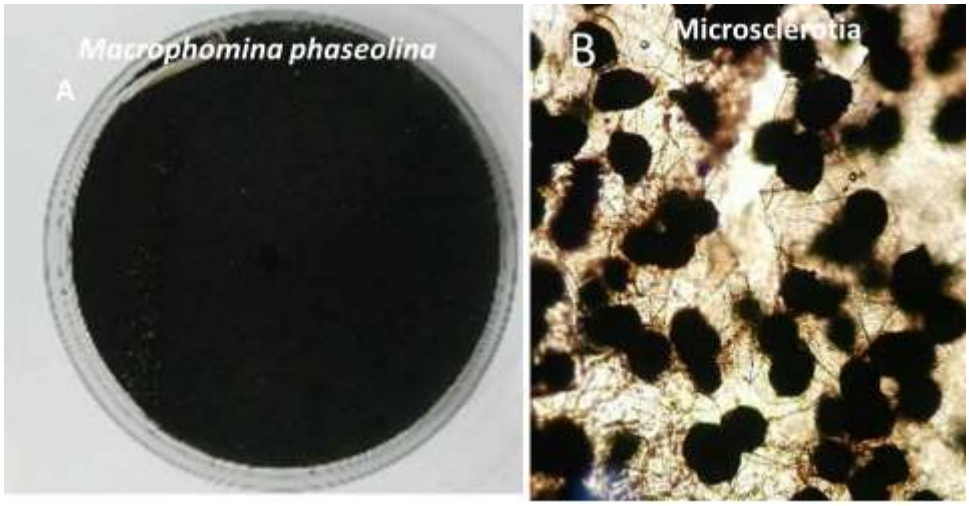
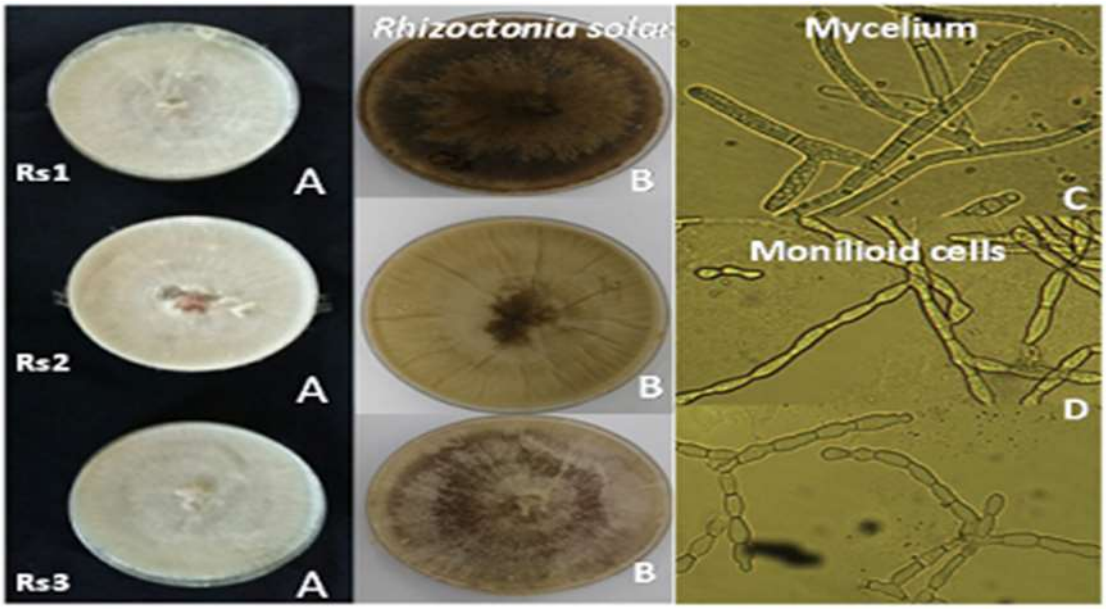


Fig 2 – Cultural and microscopic characteristics of M. phaseolina. A=M. phaseolina colony growth on PDA B= Microsclerotia x40

R.solani fungus isolation characterized showed clear variance in spinning fungus shapes and its density . Fungus colony color and size of Microsclerotia (Picture 3) colonies color characterized in the beginning of fungus threads by a white to light brown color(Picture 3-A)

and turned gradually to dark brown color with advance of colony age with variance degrees(Picture 3-B) Microsclerotia formed brown and round colony and the microscopic test showed that the fungus spinning was divided and had many standing tillers with the main fungus spinning angle and presence tears near place of branch emergence and presence of barrel cells aggregated as chains in places of chamber bodies formation(Picture 3-C) , these properties agreed with those properties which were mentioned by Parmeter and Whitney, 1970; Andersen and Stalpers, 1996).



Pathogenicity test

All the isolates obtained from plants showing rot symptom on bean stem base and root have caused significant reduction in seed germination compared with control. Variations in Virulence between the isolates were observed. The most aggressive isolates were of those belong to *R.solani*, where Rs1and Rs3 have totally inhibited seeds germination compared to 5, 10, 15, 20 and 25% germination with the isolates Rs2 , Fso2, Mp1, Fox2, Fse1,respectively. All the isolates caused significant increase in disease severity compared with control. *R.solani* isolates were found the more pathogenic with disease severity 100% for Rs1, Rs2, Rs3, compared to 79.45, 65.71, 60.31 and 54.28% withFso2, Mp1, Fox2, Fse1 respectively (Table 1).

Table 1. Effect of pathogenic fungi isolates on bean seeds germination and disease severity.

Treatments	%seeds germination	%disease severity
Fusarium oxysporum (Fox2)	20.0	60.31
F. semitectum (Fse1)	25.0	54.28
F.solani (Fso2)	10.0	79.45
Macrophominaphaseolina (Mp1)	15.0	65.71
Rhizoctoniasolani(Rs1)	0.00	100.00
R. solani (Rs2)	5.0	100.00
R.solani(Rs3)	0.00	100.00
Control	100	0.00
LSD 0.05	5.95	1.25

Each number in the table represent the mean of 4 replications

Some previous studies reported that causal agents of root rot fungi belongs to *R.solani, M.phaseolina*and *F.solani*caused reduction in seed germination up to 70% with disease severity between 40- 100% compared with control.

The pathogenicity variations between isolates may attribute to genetic and ecological variations led to differences in isolates ability to produce lytic enzymes and toxinsIt was reported that *F.solani* produce many lytic enzymes including cutinase, cellulase, polygalacturonase and protease that hydrolyze plant cell wall and penetrate into the cell. It was found that the aggressiveness of *M. phaseolina* depend on its capacity to produce toxins and lytic enzymes including, Amylase, Cellulase, Hemicellulase, Lipase and Pectinase that hydrolyze cell wall (Kaur, 2012; Kumar and Sharma, 2013). The fungus also reported to produce secondary metabolism compounds, Fusaric acid, Javanicin, polypeptides and toxins that effects cell membrane permeability and inhibits many cell enzymes(Vidhyasekaran, 1997; Azliza et al. 2014).

Effect of Salicylic acid and Phylax application on disease development

The treatment of bean seed with SA and Phylax in the presence of *R.solani* induced high reduction in disease incidence and severity compared with control. Soaking seed in water and addition SA with Phylax into the soil induced the higher reduction in disease development with disease incidence and severity 6.66% and 3.75% compared to 100% and 77.08% in control, respectively. This treatment was followed by soaking seeds in water and addition SA and Phylax separately into the soil with disease incidence and severity, 20.00% and 12.50% and 26.67% and 16.67%, respectively compared to 46.66%, 32.14%,66.67%, 41.66%, 33.33%, 12.15%in soaking seeds in water with foliage application of SA and Phylax separately and in combination respectively (Table 2).

Table 2.Activity of Salicylic acid and Phylax on bean stem base and crown rot disease development and growth parameters

Treatment	%disease incidence	% disease severity	Fresh weight kg	Dry weight g
Soaking seeds in water and SA foliage application + RS3	46.66	32.14	1.29	425.34
Soaking seeds in water and Phylax foliage application + RS3	66.67	41.06	1.26	390.65
Soaking seeds in water and SA +Phylax foliage application + RS3	33.33	12.15	1.31	430.86
Soaking seeds in water and soil application of SA + RS3	20.00	12.50	1.47	563.13
Soaking seeds in water and Phylax soil application + RS3	26.67	16.67	1.46	540.20
Soaking seeds in water and SA +Phylax soil application + RS3	6.66	3.75	1.48	574.13
Soaking seeds in SA + RS3	33.33	16.67	1.29	431.11
Soaking seeds in Phylax + RS3	46.66	25.00	1.26	382.00
Soaking seeds in water /control	0.00	0.00	1.30	428.09
Soaking seeds in water + RS3 /control	100.00	77.08	0.83	452.34
LSD 0.05	2.56	2.64	0.18	9.72

The activity of SA and Phylax in restriction of *R.solani* growth and suppression of disease symptoms development may attribute to induction of several compounds in plants mainly

phenolic compounds and phytoalexins that enhance strengthening of cell wall and prevent fungus penetration in to the cell. The restriction of disease development by SA and Phylax may be also through activation of specific set ofgenes to the plant encoding for pathogenesis related proteins(PRP) some of which possess antifungal activity. It was reported that treatment of plants with SA or one of its functional analogues, Acibenzolar-s-methyl (ASM) induce systemic resistance and activate pathogenesis – related protein (Hammer Schmidt, 1999). Meenakshi and Baldev (2013) found that soaking seed in SA induced activation of some enzymes correlated with plant defense mechanisms and accumulation of phenolic compounds that inhibit the fungal penetration into the cells (Meenakshi and Baldev, 2013). Shehata (2015) reported that soaking seeds in SA has restricted the development of root rot caused by *R.solani* associated with activation ofPRP genes. The restriction of bean stem base and root rot development by SA and Phylax was found associated with promotion of plant.

The fresh and dry weights of plants emerged from water soaked seeds in soil,1.46kgand 540.20g, 1.48kg and 574.13g respectively, 1.29 kg and 425.34g, 1.26 kg and 390.65 g, 1.31 kg and 430.86 g of plant emerged from water soaked seeds and foliage application with SA, Phylax and SA + Phylax respectively, 1.29 kg and 431.11g, 1.26 kg and 382.00g of plants emerged from seeds soaked in SA and Phylaxrespectively compared with 0.83 kg and 252.34g in control respectively (Table 2).

The promotion of plant growth may result mainly from the suppression of disease causal agent as well as considered as plant hormone can enhance plant promotion. It was reported that spraying citrus seedlings with SA at 100 mg/l induced increase in plant height, branches number, fresh and dry weights, leaves contend inchlorophyll and carbohydrate through activation many enzymes and photosynthesis(Abd-El Wahed et al. 2011).

Conclusion

Bean plants are subjected to infection with many soil borne pathogens including *Fusarium oxysporum*,*F.solani*,*Macrophomina phaseolina*, *Rhizoctonia solani* causal agents of, root and stem base rot .they found that the main cause of root rot disease of beans is *R.solani* . Many compounds including Salicylic acid, and Phylax were used as foliage application, soil treatment, seed soaking, showing high inhibition activity against several fungal pathogens *in vitro* and reduced infection with root rot disease under field conditions

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INTRODUCTION AND PHYTOCHEMICAL ANALYSIS OF MEDICINAL AND AROMATIC PLANTS IN VYTAUTAS MAGNUS UNIVERSITY 1924-2018

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Abstract

Medicinal (aromatic) plants (MAPs) are playing an important role for the solution of World Health Organization Problem *Health for everyone in 21st Century*. In Lithuania, since 1924 until, medicinal plants collections have been developed in the Sector of Medicinal and Aromatic Plants in Botanical Garden at Vytautas Magnus University (SMAPs BG VMU). In Europe unique collection of MAPs is a base for scientific researches, project-based activities, national and international studies and the object of public education. The topics and tendencies of the research on medicinal herbs have varied since that time quite a lot. Collections of medicinal plants are still an important constituent and object of the phytochemical scientific research process. The methods of chemical investigations have been changing during the distinct research periods (1st – 1924–1949, 2nd – 1949–1984, 3rd – 1984–1999, 4th – 1999–2018). The main objective of research is the introduction and phytochemical investigation of MAPs. Biodiversity of plant resources, scarce information on biologically active compounds and their properties in many species including those naturally growing in Lithuania or introduced, as well as increasing demand for natural food, food supplements and homeopathic medicine have been the main motivation aspects of the ongoing study. MAPs are sources of raw materials, required in pharmacy and phytotherapy are a part of genetic resources of Lithuania. Phenological observation, phytochemical investigation of raw material of MAPs from SMAPs BG VMU revealed that new species from foreign and local flora can be successfully introduced in Lithuania. Lithuania has good growing conditions for a great variety of MAPs.

Keywords: *Medicinal and aromatic plants, introduction, phytochemical analysis, Lithuania*

Introduction

In addressing the issue of the development of high-quality, healthy and environmentally-friendly food technologies raised by the World Health Organization in the European Health Policy document "*WHO Traditional Medicine Strategy 2013-2023*" and in the priority research, the variety of MAPs, rational, sustainable use of them is promoted.

Recently, more and more attention has been paid to the biological properties of introduced as well as local flora plants, their cultivation, medicinal plant raw materials and their phytochemical research **(**Briskin, 2000). In accordance with the requirements of the World Health Organization and Good Manufacturing Practice, medicinal plant raw materials and plant products must comply with safety, efficacy and stability parameters (GASP, 2003; Ph. Eur., 2013;).) MAPs grown in industrial plantations can control and ensure the good quality of medicinal plant material: by conducting plant biological tests, standardizing cultivation conditions, determining the optimum time of preparation of medicinal plant raw materials, drying and other conditions. Now and in the future it is necessary to study the biological properties of the introduced plants, expand the range of these plants, increase their biological diversity and accumulate national genetic resources (Radušienė and Janulis, 2004; Motiekaityte, 2006). For this purpose, since 1924 in the climate conditions of the Republic of

Lithuania, research on the introduction of medicinal plants and herbs has been carried out at SMAAPs BG VMU. Based on national and international complex, interdisciplinary research, a collection of medicinal plants has been developed, which is the subject of studies and research. In 1924-2018, the collection of medicinal plants, research issues and research trends in individual periods (1st - 1924-1949, 2nd - 1949-1984, 3rd - 1984-1999 and 4th - 1999-2018) changed, but the main tasks have remained significant, preparing scientific recommendations for medicinal plant growers and pharmaceutical raw material processing companies (Juknevičienėa and Juronis 2000; Ragažinskienė *et al.,* 2007).

The *aim of this work* is to carry out the long-term (1924-2018) scientific activities in researching the introduction of MAPs, as well as summarizing and evaluating scientific issues and developments.

The aim of the research was to carry out a retrospective analysis of the chemical composition of MAPs, in separate periods: 1st - 1924-1949, 2nd - 1949-1984, 3rd - 1984-1999 and 4th - 1999-2018 during plant introduction period, ex situ long-term outdoor collections at SMAAPs BG VMU in Central Lithuania.

Material and Methods

The object of the research is the development of studies and methods of MAPs their introduction and chemical composition in separate periods. The retrospective analysis of the explorations and methods of chemical composition of prospective MAPs and hop varieties was performed by theoretical descriptive method using scientific publications and reports as well as archival data of Botanical Garden of Vytautas Magnus University (Juknevičienė and Juronis, 2000; Maruška and Kornyšova, 2006; Ragažinskienė *et al.*,2007; Ragažinskiene and Rimkienė, 2003; Stankevičius *et al.,* 2011). MAPs research is carried out according to the scheme presented in Fig. 1.

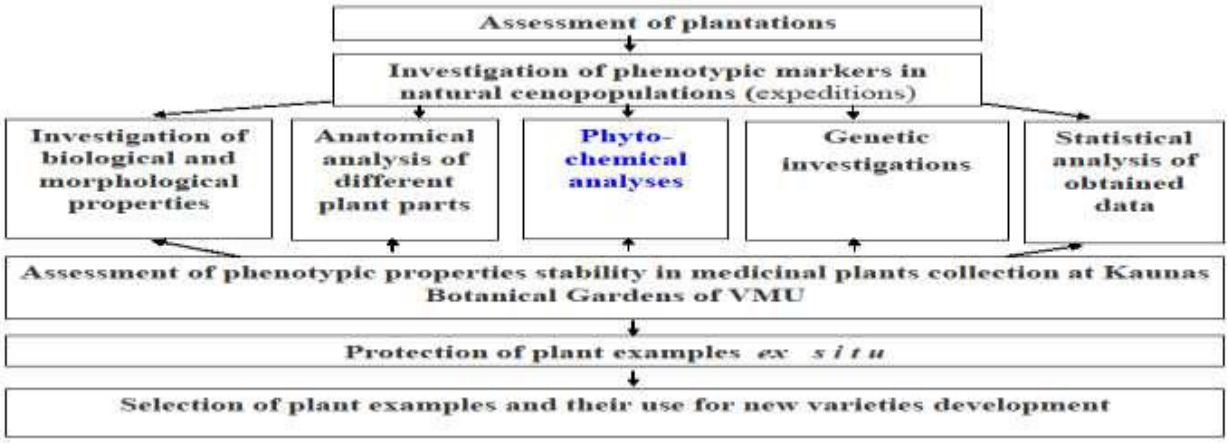

Fig. 1. Schema and Metods of the Introduction and Phytochemical Investigation of MAPs.

Results and Discussion

Retrospective analysis of the development of scientific research on the chemical composition of prospective MAPs. There were four research periods of chemical composition of medicinal (aromatic) plants: the 1st (1924-1949), the 2nd (1949-1984), the 3rd (1984-1999) and the 4th (1999-2018), which took place in the field of plants during introduction, *ex situ* field collections, at SMAPs BG VMU, Central Lithuania. This analysis highlighted the

development of research methods for plant chemical composition at separate periods and the possibility of using medicinal (aromatic) plants and their raw material.

During the 1st period chemical composition of the medicinal, technical and forest medicinal plants and medicinal herbs was carried out in the time of the introduction. 224 plant species accumulated in the collection of medicinal plants, the chemical composition of which is the quantitative analysis of essential oils, was carried out by hydro distillation. These studies supported the selection of 77 viable plant species and their cultivation in Lithuania (Grybauskas, 1954).

During the 2nd period phytochemical laboratory was founded. The chemical composition of medicinal, technical, oil, fermentation plant was investigated - the amount of essential oils by hydrodistilation method, their quality by gas chromatography, during plant introduction. The research direction was "*Sustainable use of the plant world and their protection biological bases*". During this period, the following collections of plants were created and equipped: medicinal plants, hops, rare vegetables, spice and honey plants (Penkauskienė and Rimkienė, 1991).

The 3rd period carried out research in *The consistent of functionality of variety and phytocyanosis and their sustainable use in the improvement of feed.* During this period, the chemical composition of MAPs was investigated: the amount of essential oils - by hydrodistilation method – for aerotherapy for sanatorium treatment. The quality of the medicinal plant raw material was evaluated: the qualitative analysis of flavonoids and phenolic carboxylic acids was carried out using a plane paper chromatography method. Flavanols and leaven were determined quantitatively in the terrestrial and underground parts of the plant by colorimetric methods, nitrates were titrated using ionometric methods and ascorbic acid was evaluated titrimetrically. Also, the sum of oxycinamines was calculated by recounting the chicory acid. All chemical analysis data was counted for an absolutely dry raw material (Juknevičienė and Juronis 2000; Penkauskienė and Rimkienė, 1991).

Field of research of **the 4th period** investigations of MAPs diversity and their introduction, assessment of raw material and rational, sustainable use in medicine, food industry and veterinary.

The value of medicinal plant collections. Since 1924 the pharmacognomic principle has been the development of a long-term inventory and documented collection of medicinal plants. It has already introduced 594 plant species belonging to 373 genera and 88 families. This collection contains 31 rare and endangered species of medicinal plants in Lithuania. Based on long-term introduction and chemical properties, more than 128 species of MAPs have been investigated for the status of Lithuanian national genetic resources. 54 medicinal plant species are on the list of plant genetic resources (ANGI). The 25 varieties and forms of hops are included on the list of medicinal plants and aromatic plant field collections that are assigned to Lithuanian plant genetic resources. Studies were carried out on the basis of the project "*Plant Genetic Resources Research 2000-2008*" (Grant No. 12) of the Ministry of Education and Science of the Republic of Lithuania (Ragažinskienė *et al*., 2007; 2010; Ragažinskienė and Rimkienė, 2003).

There is an exchange of medicinal plant seeds with more than 400 Botanical Gardens from different countries.

Scientific activity. Medicinal plants in collections are the subject of scientific research. Here, complex, innovative, interdisciplinary, interdisciplinary scientific work is being carried out for research of biologically active substances of medicinal (aromatic) plants together with Instrumental Analysis Open Access Center of Faculty of Natural Sciences at Vytautas Magnus University on the basis of scientific projects (Kaškonienė *et al.*, 2011; Maruška and Kornyšova, 2006; Maruška et al., 2010; Stankevičius *et al*., 2011).

The study of the methods of preparation of the medicinal plant raw material and the improvement of the drying technology in the medicinal plant raw material determined the dependence of the content of the bioactive substances – essential oils and flavonoids on the conditions of drying (natural ventilation, air velocity and flowing air flow and temperature), which are selected according to the plant's composition of the raw material components (leaves, stems, grass) and the groups of biologically active substances (Kaškonienė *et al*., 2011; Maruška *et al*., 2010).

Based on the research of these plants, 37 medicinal plant species which are promising for the pharmaceutical and food industry have been selected: *Achillea millefolium* L., *Agrimonia eupatoria* L., *Alchemilla vulgaris* L., *Althaea officinalis* L., *Angelica archangelica* L., *Arctium lappa* L., *Arnica chamisonis* Lessing., *Arnica montana* L., *Aronia melanocarpa* (Michx.) Elliott, *Artemisia absinthium* L., *Bidens tripartita* L., *Borago officinalis* L., *Calendula officinalis* L., *Chamaemelum nobile* (L.) All. = *Anthemis nobilis* L., *Convallaria majalis* L., *Melissa officinalis* L., *Mentha piperita* L., *Echinacea purpurea* (L.) Moench, *Echinacea pallida* (Nutt.) Nutt, *Glycyrrhiza glabra* L., *Geranium macrorrhizum* L., *Hyssopus officinalis* L., *Humulus lupulus* L., *Inula helenium* L., *Juglans regia* L., *Leonurus cardiaca* L., *Nigella damascena* L., *Nigella sativa* L., *Origanum vulgare* L., *Perilla frutescens* (L.) Britton, *Potentilla erecta* (L.) Raeusch., *Potentilla fruticosa* L., *Rhaponticum carthamoides* (DC.) Iljin, *Schisandra chinensis (*Turcz.) Baill., *Scutellaria baicalensis* Georgi, *Viola tricolor* L.

The research findings are presented in monographs, scientific and popular science articles, national and international scientific conferences and recommendations (Bartkienė *et al*., 2015; Ligor *et al*., 2014; Šulniūtė *et al*., 2017; Venskutonis *et al*., 2007).

Scientific knowledge for herbalist business. In the area of herbal development there is cooperation with rural communities. The geographic regions of Lithuania, under various ecological conditions, have been equipped with collections of medicinal plants and testing areas for their cultivation. In addition, scientific methods for the preparation of medicinal (aromatic) plants and medicinal plant raw materials have been introduced. A modern herbal dryer was developed, using alternative solar energy: the European Agricultural Fund for Rural Support Project: Lithuanian Rural Development Programme 2007-2013 project '"Cultivation of medicinal plants and innovative technology using of solar energy for preparation medicinal plant raw materials" (Grant No. 1 PM-PV-10-1-003015-PR001 (2011-2014) (Raila et al., 2009; Maruška et al., 2014).

Legislative justification for science and business. Participating with the Ministry of Health of the Republic of Lithuania in the legislative process, starting from 2013, *Supplemental and Alternative Health Care Act* is being developed.

Conclusion

Introduction studies of medicinal, (aromatic) plants have been carried out at Vytautas Magnus University since 1924.

In the 1st collection, 77 (out of 224) promising medicinal plant species were selected by investigating their chemical composition with hydrodistillation method.

The 2nd period founded the phytochemical laboratory, and the chemical, technical, oil, tartar chemical composition (essential oil content) was investigated by distillation method, their qualitative composition was determined by the gas chromatography method.

The 3rd (1984 – 1999) Chemical composition of medicinal (aromatic) plants: the amount of essential oils was investigated by hydrodistilation method. The quality of the medicinal plant raw material was evaluated: the quality of flavonoids and phenolic carboxylic acids was determined by plane paper chromatography, quantitatively flavonols and leaven - colorimetric, nitrates - by the ionometric method.

During the 4th period the following methods and apparatus are used for the evaluation of the chemical composition of MAPts and hop varieties: the amount of essential oil is determined by methods of hydro-distillation, super-liquid extraction and carbon black microextraction, their qualitative composition - gas chromatography and mass spectrometry, phenolic compounds – effective liquid chromatography, standard spectrophotometry and capillary electrophoresis, the activity of binding of free radicals to plant extracts was evaluated spectrophotometrically, the antioxidant and radical components of the individual extracts.

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OCURRENCE OF *TETRANYCHUS URTICAE* ON SOUR CHERRY TREES IN SOUTHEASTERN OF MACEDONIA

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Abstract

The two-spotted spider mite is probably one of the most polyphagous arthropods that feeds on tree fruits. It is distributed worldwide and is an economic pest of many crops. Its innumerable hosts include many weeds, field crops, ornamental plants, vegetables, forage crops, small fruits and tree fruits (apple, pear, peach, nectarine, apricot, cherry (sweet and sour) and plum). The research was conducted during 2017 in sour cherry orchards in southeastern of Macedonia (the region of Stip, Strumica and Gevgelija), before and after the harvest of the sour cherries (01.06. – 30.09.2017). Scouting for mites began prior the harvest. The method of monitoring two-spotted spider mite motile populations consists of sampling 10 leaves from 10 randomly selected trees (100 leaves) on an area of two ha. To estimate the number of mites per leaf, we count the number of leaves that have two or more two-spotted spider mite motile from each sample and follow the presence-absence sampling method look-up table. The results showed that after harvesting the sour cherries, during the summer and autumn, the number of two-spotted spider mite increases and exceeds the economic threshold (8-10 spiders on the leaf), so chemical treatment is required in order to control the population of the spider. The problem in the Republic of Macedonia is that sour cherries producers rarely perform postharvest treatment of the sour cherry trees, so the two-spotted spider mite population increases, which in turn contributes to a reduced yield of sour cherries.

Keywords: *two spotted spider mite, sour cherry, presence, acaricides.*

Introduction

The two-spotted spider mite is probably one of the most polyphagous arthropods and the most destructive within the family of the Tetranichidae (Vassiliou & Kitsis, 2013). It is distributed worldwide and is an economic pest of many crops, having a host range of more than 1100 species of plants (Paulo et al., 2015). Its innumerable hosts include many weeds, field crops, ornamental plants, vegetables, forage crops, small fruits and tree fruits (apple, pear, peach, nectarine, apricot, cherry (sweet and sour) and plum) (Beers H. E. and Hoyt C. S., 1993: http://jenny.tfrec.wsu.edu). *T. urticae* is particularly dominant and destructive in intensive, high-yield cropping systems, and affects crops by direct feeding. In cases of severe infestations, it reduces the area of photosynthetic activity and causes leaf abscission (Gorman et al., 2002). Two-spotted spider mite can be a problem on cherries in hot and dry years. Bronzing is the most common damage caused by high populations of two-spotted spider mites. This phenomenon can cause a reduction in photosynthesis and fruit bud initiation. Bronzing caused by two-spotted spider mite is often more gray in color than bronzing by European red mites (*Panonychus ulmi* Koch). Although bronzing presents an obvious challenge, one of the biggest threats of mites in sour cherries in a hot, dry year is "firing." Firing is an immediate result of increased temperatures and droughty conditions, most often in combination with high mite populations. Firing results in a collapse of a portion of the tree, this malformed segment can be a branch, a terminal, or a whole section of the tree. The leaves of a fired part of a tree turn brown very quickly (Fig. 1), with no prior wilting, and the overall effect is similar to fire blight in apples. Although firing may occur with low mite populations, it is found most commonly, where mite numbers are high.

Figure 1. Sour cherry trees infested with *Tetranichus urticae* in the region of Gevgelija

A major problem in the control of *T. urticae* is the response to develop resistance to many acaricides (Abd El-Moneim et al., 2012). Also, the cherry growers are often faced with the crucial decision of applying an acaricide before or after harvest. If growers intend to spray for mites before cherry harvest, the pre-harvest intervals of most acaricides are 14 days or longer, which is an important factor in the decision making process. Another confounding factor of pre-harvest mite control is that often the mite population is lower early in the season, and the numbers may not warrant an acaricide at that time. The problem in the Republic of Macedonia is that sour cherries producers rarely perform postharvest treatment, so the two spotted spider mite population increases, which in turn contributes to a reduced yield of sour cherries.

Material and Methods

The research was conducted during 2017, in six sour cherry orchards in the region of Stip, Strumica and Gevgelija (two orchards in each region), in southeastern of Macedonia, in the period of 01.06. – 30.09.2017. Scouting for mites began prior the harvest. This pre-harvest monitoring period should begin early enough to consider the long acaricide pre-harvest intervals. A fairly reliable and easy-to-use sampling method for mites is called presence-absence, or binomial, sampling (Jones, 1990). Rather than counting the number of mites on leaves, which is difficult because of their small size, presence-absence sampling requires only that the scout determine whether pest mites are present on each leaf sampled. The method of monitoring two-spotted spider mite motile populations (Morphological Identification of Spider Mites (Tetranychidae) Affecting Imported Fruits: https://www.nappo.org) consist of sampling 10 leaves from 10 randomly selected trees (100 leaves) on an area of two ha. Because spider mites are first, found in the lower center and at the top of tree canopies, spreading to the periphery over time, leaves are selected from inside the canopy, as well as from the edges. Using a 10–20x magnifying hand lens, the number of leaves from each infested tree is counted. The look-up table (Table 1) was used to estimate the number of mites per leaf. The estimated mite density for all 10 trees is collected and divided by 10 to obtain an average for the trees sampled in the block.

Table 1. Web spinning spider mite presence-absence sampling method look-up table (Jones, 1990).

Number of leaves out of 10 with at least one mite present*	Estimated number of mites per leaf
1	0.1
2	0.4
3	0.7
4	1.1
5	1.7
6	2.4
7	3.5
8	5.2
9	8.8
10	--

*Note: for each tree sampled, determine the number of leaves out of 10 with at least one mite present

On the second orchard in each site, after harvesting the sour cherries, acaricide treatment was performed. Two active substances were used: Dimetatoate, with a concentration of 0.15% (13.09.2017) and Abamectin, with a concentration of 0.1% (28.09.2017). After the treatment the leaves of the sour cherries were surveyed again with presence-absence sampling method.

Results and Discussion

During 2017, the control plantations of sour cherries in regions Stip, Strumica and Gevgelija, provided the following condition (Table 2).

Table 2. Average number of two-spotted spider mites on 100 overviewed leaves of sour cherry, in the control plantations, in Stip, Strumica and Gevgelija.

Stip									
	01.06.	15.06.	30. 06.	15.07.	30. 07.	14.08.	30.08.	15.09.	30.09.
Avg	0,65	0,89	2,78	3,26	4,68	5,03	7,92	8,08	4,52
Strumica									
	01.06.	15.06.	30. 06.	15.07.	30. 07.	14.08.	30.08.	15.09.	30.09.
Avg	1,35	2,01	2,46	2,84	4,01	5,03	7,0	8,08	4,88
Gevgelija									
	01.06.	15.06.	30. 06.	15.07.	30. 07.	14.08.	30.08.	15.09.	30.09.
Avg	2,30	3,17	3,96	4,66	6,11	5,39	8,08	8,80	4,88

According to the obtained results (Table 2), the highest occurrence of the two-spotted spider mite is on the plantations in the region of Gevgelija. This part of Macedonia is characterized by higher average temperatures, so there are more favourable conditions for the development of the two-spotted spider mite. In all examined regions, the number of mites increases in warmer months of the year, so its number is the highest in late August and early September.

Biological and cultural control for spider mite management should be the goal of every orchard pest manager. In many situations, chemical control is unnecessary and may only make the mite problem worse. The use of acaricides will eliminate the beneficial predatory mites, which are capable of keeping spider mite populations below economically damaging levels. Another reason to avoid chemical control is that populations of two-spotted spider mites have developed resistance to acaricides (Rothwell N. (2014). Managing two-spotted

spider mites in cherries: http://msue.anr.msu.edu). However, when the number of the two spotted spider mite increases and exceeds the economic threshold (8-10 spiders on the leaf), chemical treatment is required in order to control the population. The use of resistant cultivars is considered the ideal control method because they maintain the mite populations below levels of economic damage, minimize the environmental impact of pesticides at no extra costs to the farmer, and serve as an auxiliary tool in integrated pest management (Karlec et al., 2017).

The first treatment of the sour cherry was done on 13.09.2017, with the active substance Dimethoate, in a concentration of 0.15%. After treatment, it was noted that the number of two-spotted spider mites was reduced by almost 50% (Table 3). Another treatment with the active substance Abamectin was performed on 28.09.2017, in a concentration of 0.1%. When checking the number of two-spotted spider mites, it was determined that its number was reduced to 70% from the from the original population level, before first treatment (Table 3).

Table 3. Average number of two-spotted spider mites on 100 overviewed leaves of sour cherry, in the plantations treated with Dimethoate (0,15%) and Abamectin (0,1%) in regions Stip, Strumica and Gevgelija.

Active substance	Stip		Strumica		Gevgelija	
	15.09	30.09	15.09	30.09	15.09	30.09
Dimethoate	4,68	2,78	4,01	2,44	4,66	2,46
Abamectin	2,59	1,53	3,17	1,31	3,26	1,87

These results indicate that after the harvesting the sour cherries, much more favourable conditions are created for the development of the two-spotted spider mites, thus increasing their number on sour cherries. Moreover, when it exceeds the economic threshold of harmfulness, it is desirable to use chemical means for destroying the spider mites. The problem in the Republic of Macedonia is that sour cherries producers rarely perform postharvest treatment of the sour cherry trees, so the two spotted spider mite population increases, which in turn contributes to a reduced yield of sour cherries. Because of these issues, we recommend applying an acaricide after harvest, when the mite numbers are higher and when the pre-harvest intervals are no longer a concern.

Conclusions

Based on the above the following conclusions can be made:

- The number of the two-spotted spider mites is the highest in the region of Gevgelija, where there are also the most favourable conditions for its development.
- The number of two-spotted spider mites increases in the summer months, reaching the peak at the end of August and early September.
- Treatment of sour cherries with acaricides, after harvesting is necessary to reduce the number of two-spotted spider mites. In this way, the damage from the mites will be reduced and the yield of the crop will increase.

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THE EFFECTS OF ADDING LENTIL SEMOLINA ON THE NUTRITIONAL QUALITY OF FORTIFIED COUSCOUS

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Abstract

Couscous is a traditional dish for Moroccan population, based on wheat semolina and habitually consumed on Fridays and days of family reunion, its design is different according to the regions and according to the availability of the raw material (wheat, barley ...). In order to contribute to the reduction of malnutrition in Morocco, this study was carried out. Legumes including lentils have a great role in human diet, they are commonly consumed for their nutritive value specifically proteins and iron. Lentil is among the oldest and most appreciated grain legumes of the Old World. Thus, the current investigation aims to enhance the nutritive value of conventional Moroccan couscous through its preparation by incorporation of lentil semolina into its components. Four experimental variant proportions of grains legumes semolina (25, 50 and 75%) were analyzed, with couscous made of grains lentil semolina only (100%L) and of grains wheat semolina only (0%L) as control. From nutritive point of view, the higher contents of proteins, iron and potassium were found in couscous with addition of 75% of lentil semolina. In effect, the lower content was recorded for conventional Moroccan couscous, and more the percentage of incorporation increased more its content of proteins, iron and potassium increased. Contrariwise, more the percentage increased more carbohydrates and sodium contents decreased. From technological point of view, the addition of lentil semolina at 25% presented the closest results to the technological parameters of conventional Moroccan couscous. Microbiological tests revealed that couscous of wheat semolina only is a favorable environment for the growth of microorganisms. The nutritional value of lentils and their technological characteristics are useful in increasing nutritional and technological value of fortified conventional Moroccan couscous.

Key words: *Malnutrition, Couscous, Bio-fortification, Legumes, Morocco.*

Introduction

Food habits in Morocco are based on wheat products, as an energy and protein source. Bread and couscous are the most consumed. Couscous is a traditional dish specific to the African countries of the Mediterranean. In Morocco, it is consumed regularly on Fridays and days of family reunion (festivity or death). Also, the manufacture of products derived from wheat and especially durum wheat such as semolina, pasta, industrial couscous is widespread in the agri-food industry (Bar.C, 2001). Otherwise, nutrient deficiencies are a major public health problem in Morocco according to the 2016 World Nutrition Report. They affect nearly a third of the population, encouraging managers to initiate an international program to combat these deficiencies. In fact, one in three children, one in three women and one in five men suffer from nutritional deficiencies; the most widespread concerns mainly proteins and iron. The consequences of iron deficiency are detrimental to individual and community development, affecting work capacity (Basta et al., 1979), school performance (Vega-Franco et al., 1994), and stunting and mental development in children (Shultink et al., 1995, Grantham-McGregor

S). In addition, iron deficiency is manifested by anemia that can attack the immune system and thus promotion the onset of many diseases. This issue costs the Moroccan state more than 2 billion dirhams annually or 5% of gross domestic product in relation to health costs and loss of productivity. It seems that the change in food habits with a high tendency towards animal proteins is at the origin of the increase in the incidence of these deficiencies. Fortification which consists of incorporation, food resources rich in protein or micronutrients, in a staple widely accessible and consumed, figure prominently among the strategies adopted for quality improvement nutritional status of the third world (Serna-Saldivar 1999). The possibility of getting a couscous from other products such as lentils remains an interesting alternative whose purpose is to improve products quality. Legumes present an inexpensive source of proteins with a high nutritional profile and, after cereals, the next most important food source for humans (Butt & Batool, 2010). Lentils are rich in protein (20-36%) and are an excellent source of a large range of micronutrients (Wang, N et al., 2006, Thavarajah, D et al., 2007). They rank among the oldest and most appreciated grain legumes of the Old World (Smartt, 1990). Thus the purpose of this study which aims to contribute to the reduction of the incidence of malnutrition, through the incorporation of grains lentil semolina in conventional Moroccan couscous as a staple food, with studding its effects on their nutritional, technological and microbiological characteristics.

Material and Methods

This study was carried out during 2018 in the laboratories of the National Institute of Agricultural Research (INRA) in Rabat, Morocco. Samples of conventional Moroccan couscous made of durum wheat (*Triticum durum*) only (0%L), fortified couscous with lentil *(Lens culinaris)* semolina at 25, 50 and 75% rates and control couscous made of lentil semolina only (100%L) were analyzed for determining their nutritive value, technological and microbiological quality. All analytical tests were carried out in double replications. Ash content of couscous samples was determined according to AFNOR NF V03-720. Proteins were determined using the total Kjeldahl digestion procedure (McKenzie and Wallace, 1954). Crude fat was quantified using Soxhlet extraction with hexane according to AOAC. Carbohydrate (CHO) was determined by subtraction and calculated using the following equation: %CHO = 100 − (%Fat + %Protein + %Ash + %Moisture) (FAO, 2002), the results give an approximate estimate of the total amount of carbohydrates present in couscous. Minerals were determined using atomic absorption spectroscopy for iron, and using flame photometer (CORNIGEEL) for sodium and potassium (AOAC, 1984). Technological parameters were evaluated using gluten parameter measured by infrared spectroscopy (NIRS), swelling index (J. Abecassis, 1991), color index (NF ISO 11664-4, 2008), and whiteness index was calculated (Angioloni and Collar, 2009). The microbiological tests were based on the contamination level of Total Aerobic Mesophilic Flora (FMAT), total coliforms, and yeasts and molds which were researched according to NF ISO 4833 (1991). The count of each type of microorganism was done according to the law of the weighted average (J.P. Larpent, 1997).

Results and Discussion

The results about nutritive value of different sets of couscous obtained before and after cooking were presented in Tables 1 and 2 respectively. Before cooking, Ash content of conventional couscous, composite couscous and couscous made with lentil semolina only varied between 1.50 and 2.85%, high ash content was recorded for 75% (2.37). After cooking, values varied between 1.88 and 3.33%, high ash content was recorded for 75% (2.55). The ash content of food material could be used as an index of mineral constituents (Sanni et al., 2008). Before cooking, protein content was 17.20, 19.92, 21.27, 22.02 and 23.13%

respectively. After cooking, protein content was 18.98, 20.88, 21.94, 22.02, and 23.82% respectively, couscous made of lentil semolina only yielded higher contents of proteins before and after cooking. The lower content was recorded for conventional Moroccan couscous, and more the percentage of incorporation increased more its content of proteins increased. Therefore, the nutritional value increased. Protein content is an important criterion for assessing quality both for animal feed and for human food (C. Bar, 2001). Before cooking, crude fat varied between 0.20 and 1.19%, and varied between 0.20 and 0.60% after cooking. Between them, the lower percent was recorded for 50% before cooking and for 25% and 50% after cooking. Before cooking, carbohydrates content varied between 59.72 and 66.21%, and varied after cooking between 67.25 and 60.45%. Between them, the lower carbohydrates content was recorded for 50% (60.49) before cooking and for 75% (61.63) after cooking. Almost, more lentil semolina was incorporated into wheat semolina more carbohydrates percentage decreased, results concerning conventional couscous were compared to what was reported by (Stone and Morell, 2009), and generally carbohydrates account for about 65–75% of the mature wheat grain. Before cooking, iron content varied between 1.78 and 6.30mg/100g. After cooking, values varied between 2.36 and 7mg/100g. Between them, more the percentage of incorporation increased more conventional couscous content of iron increased. The results indicated that the incorporation of lentil semolina into wheat semolina affected the content of iron of semolina used for making couscous. Before cooking, potassium values varied between 300 and 852 mg/100g which are comparable to the range of 0.88 to 1.44% potassium (mean 1.16%) obtained by Bhatty (1984), and between 24 and 28 mg/100g for sodium which are also comparable to the range of 0.02 to 0.18% sodium (mean 0.04%) obtained by Bhatty (1984). After cooking, potassium values varied between 468 and 1088 mg/100g, and between 20 and 72 mg/100g for sodium. The incorporation of lentil semolina improved the mineral content. The results about technological parameters of different sets of couscous obtained before and after cooking were presented in Tables 3 and 4 respectively. Before cooking, gluten content of different samples varied between 24 and 53%. After cooking, it varied between 31 and 55 %; it seems that more lentil semolina was incorporated more this content increased, maybe it was due to a gluten contamination. Swelling index showed the following values: 2.11, 1.90, 1.70, 1.89 and 1.91 for 0, 25, 50, 75 and 100% respectively, the higher results were presented in composite couscous made with 25% (1.90) of lentil semolina. According to L.Mezroua (2011), the phenomenon of swelling results from the absorption of different amounts of water by the constitutive elements of couscous grain, he found that couscous made from wheat seems to absorb more water and swell to a level higher than couscous without gluten. The appearance, mainly the color, is an important attribute of the quality of cereal products, which affects their marketing and acceptability. The color index values of different sets of couscous were as following: before cooking, L (clarity index) varied between 55.53 and 70.19, a (brown index) varied between -2.28 and -0.56, b (yellow index) varied between 14.59 and 18.85, and IB (whiteness index) varied between 52,46 and 66,81. After cooking, L (clarity index) varied between 51.67 and 66.68, a (brown index) varied between -2.34 and 0.61, b (yellow index) varied between 15.35 and 25.10, and IB (whiteness index) varied between 45.72 and 63.31. The highest value of clarity index and whiteness index was presented by conventional couscous, and the lowest value of yellow index was presented by the same sample. More lentil semolina was incorporated in conventional couscous more clarity and whiteness index decreased, and more yellow index increased; it seems that lentil semolina color influenced wheat semolina color. The microbiological tests were based on the contamination level of FMAT, total coliforms, and yeasts and molds (Table 5). FMAT is an important hygiene indicator. The presence of fecal coliforms in food supposes a faecal pollution; they are indicators of food quality. Yeasts and molds are indicators of humidity. The results revealed that conventional couscous presented

the highest values of FMAT, fecal coliforms and yeasts and molds compared to couscous prepared of lentil semolina only which presented a high value of FMAT only and total absence of fecal coliforms, and yeasts and molds.

Table 1: Nutritive value of conventional Moroccan couscous, composite couscous and couscous made of lentil only, before cooking.

plant material		Composition							
		%					mg/100g		
Lentil	Wheat	Moisture	Ash	Proteins	Crude fat	Carbohydrates	Minerals		
							Iron	K	Na
0%	100%	13.30	2.10	17.20	1.19	66.21	1.78	300.00	24.00
25%	75%	15.10	1.50	19.92	0.60	62.88	3.48	720.00	32.00
50%	50%	15.80	2.24	21.27	0.20	60.49	5.44	680.00	28.00
75%	25%	14.10	2.37	22.02	0.40	61.11	5.70	764.00	28.00
100%	0%	13.90	2.85	23.13	0.40	59.72	6.30	852.00	24.00

Table 2: Nutritive value of conventional Moroccan couscous, composite couscous and couscous made of lentil only, after cooking.

plant material		Composition							
		%					mg/100g		
Lentil	Wheat	Moisture	Ash	Proteins	Crude fat	Carbohydrates	Minerals		
							Iron	K	Na
0%	100%	10.90	2.27	18.98	0.60	67.25	2.36	568.00	20.00
25%	75%	14.20	1.88	20.88	0.20	62.84	3.66	468.00	32.00
50%	50%	13.80	2.32	21.94	0.20	61.74	5.48	640.00	32.00
75%	25%	13.40	2.55	22.02	0.40	61.63	5.74	712.00	28.00
100%	0%	12.20	3.33	23.82	0.20	60.45	7.00	1088.00	72.00

Table 3: Technological parameters of conventional Moroccan couscous, composite couscous and couscous made of lentil only, before cooking.

Plant material		Parameters					
Lentil	Wheat	Gluten (%)	Swelling index	Color index			
				L	a	b	IB
0%	100%	24.00	2.11	70.19	-0.56	14.59	66.81
25%	75%	33.00	1.90	67.97	-2.28	18.80	62.79
50%	50%	39.00	1.70	64.50	-0.96	16.60	60.80
75%	25%	46.00	1.89	55.53	-0.80	16.80	52.46
100%	0%	53.00	1.91	60.51	-1.83	18.85	56.20

Table 4: Technological parameters of conventional Moroccan couscous, composite couscous and couscous made of lentil only, after cooking.

Plant material		Parameters				
Lentil	Wheat	Gluten (%)	Color index			
			L	a	b	IB
0%	100%	31.00	66.68	0.61	15.35	63.31
25%	75%	50.00	62.47	-2.34	19.28	57.74
50%	50%	55.00	55.20	-0.10	17.50	51.90
75%	25%	50.00	51.67	-0.42	20.37	47.55
100%	0%	53.00	51.89	-1.10	25.10	45,72

Table 5: Microbiological tests of FMAT, fecal coliforms and yeasts and molds, after cooking.

Researched germs		FMAT				Fecal coliforms				Yeasts and molds			
Dilution		10^{-1}	10^{-2}	10^{-3}	N	10^{-1}	10^{-2}	10^{-3}	N	10^{-1}	10^{-2}	10^{-3}	N
Lentil	Wheat												
0%	100%	560	292	6	$1.25*10^7$	240	36	4	$1.03*10^7$	200	80	22	$8.6*10^6$
25%	75%	430	201	10	$8.67*10^4$	3	0	0	0	14	0	0	0
50%	50%	640	246	136	$5.8*10^5$	0	0	0	0	34	1	1	$1.4*10^5$
75%	25%	320	67	43	$1.8*10^6$	5	0	0	0	65	0	0	$2.8*10^5$
100%	0%	680	265	21	$1.1*10^7$	0	0	0	0	9	2	1	0

Conclusion

It has been observed that it is possible to use grains lentil semolina to partially substitute grains wheat semolina in the elaboration of couscous without undesirable effects. Nutritionally, the higher contents of proteins, iron and potassium were found in couscous with addition of **75%** of lentil semolina, while carbohydrates and sodium contents decreased; the results revealed that as more and more lentil semolina was added to wheat semolina, there was a positive variation in composite couscous nutritional composition, which can be a good signal to achieve the main objective. From the technological point of view, lentil semolina appeared to be a suitable fortifier; its addition in a portion of **25%** showed the closest results to the technological parameters of conventional Moroccan couscous, more lentil semolina was added more its culinary quality and color were negatively influenced. Microbiological tests revealed that couscous made of wheat semolina only is a favorable environment for the growth of microorganisms and incorporation of lentil semolina may improve its hygienic quality.

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EFFECTIVENESS OF BENEFICIAL BACTERIA*PSEUDOMONAS* SPP.TO CONTROL GREY AND GREEN MOLD

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Abstract

The green mold and the grey mold, caused by *Penicillium digitatum* and *Botrytis cinerea* respectively, are the major postharvest diseases of tomatoes and citrusand other species as well. Synthetic fungicides are the primary means to control these diseases.However,thechemical residues in the food and environmental safety make it unwanted. Biological control using microbial antagonists is one of the most promising alternatives to chemical fungicides. *Pseudomonas* spp. produce many antifungal metabolites, previously shown to be effective against a wide range of fungi. In this study, the inhibitory effects of *Pseudomonas putida* Q172B, *P. fluorescens*Q110B and *P. fluorescens*Q036B, isolated from tomato roots in Agadir-Morocco, on *B.cinerea* and *P. digitatum* were examined. The effect of *Pseudomonas*strains was observed on the inhibition of mycelium elongation by production of soluble and volatile metabolites. The results showed the antagonism effect of three strains. The inhibition rate ranged from 39 to 54% for *P. digitatum*, and 66% for *B.cinerea* for all three strains. Forvolatile metabolites,100% of mycelium inhibition was recorded in *B.cinerea* for all three strains, but 44% was the maximum mycelium inhibition recorded in *P. digitatum*. Our result highlights that the *P. putida*Q172B and *P. fluorescens*(Q110B and Q036B) can be used as a non-chemical alternative treatment to control postharvest diseases of fruits.

Key words: *Biologicalcontrol, Botrytis cinerea, Penicillium digitatum, Pseudomonas fluorescens, Pseudomonas putida.*

Introduction

Pre- and postharvest diseases of fruit crops are responsible for devastating financial losses to growers and are difficult to manage with current technologies (Wisniewski *et al.*, 2003). Gray mold and green mold disease, caused by *Botrytis cinerea* and *Penicillium digitatum* respectively, are the most serious plant diseases affecting vegetables, ornamentals, and fruit crops produced in commercial greenhouses and fields all over the world. They cause large yield losses in a number of crops inpre- and postharvest (Kiss, 2003; Huang *et al.*, 2011,2012; Wang *et al.*, 2013; Di Francesco *et al.*, 2015). The treatment of those fungiis based by the conventional chemical control through repeated foliar or fruit applications of a combination of protectant and systemic fungicides.However, the growing demand of consumers worldwide for a reduction of pesticide, used on fresh fruits and vegetables, force the farmers to look for another ways. In response to this trend, physical and biological approaches have been evaluated as safer alternative to the use of chemical fungicides (Droby*et al.*, 2002; Zhang *et al.*, 2007). Biological approaches, including the use of antagonistic organisms, natural compounds, cultural practices, and biotechnology, will be used to develop new methods for

managing diseases of fruit crops (Wisniewski *et al.*, 2003). Biological control using a microorganism (or its secretions) to prevent disease, offers an attractive alternative or supplement to fungicides for the management of plant disease, without any of the negative effects of chemical control (Raupach and Kloepper, 1998; Jacobsen *et a*l., 2004).The use of bacteria as an alternative is a promising approach that can be incorporated into integrated disease management (Rosslenbroich and Stuebler, 2000). Several non-phytopathogenic bacteria, were frequently reported to protect plants against various phytopathogens (Van Wees *et al.*, 1997; Trotel-Aziz *et al.*, 2008; Verhagen*et al.*, 2010, 2011) .Various bacterialgenera such as*Bacillus* and *Pseudomonas*, were reported to be effective against a number of diseases of different fruits (Janisiewiczand Roitman, 1988; Janisiewicz. and Jeffers, 1997). Antibiotic production by these bacteria plays a major role in disease suppression (Raaijmakers*et al.*, 2002). The objective of this study is to evaluate the effect of three *Pseudomonas* strains, isolated from the rhizospheric soil of tomato plant, on*P. digitatum*and *B. cinerea*.

Material and Methods

Isolation of bacteria from tomato rhizosphere

The *Pseudomonas*strains were isolated from rhizospheric soil by adding 1 g of rhizospheric soil into 9 ml of sterile physiological water . The sample was diluted and appropriate dilutions were spread on King's B medium (King *et al.*, 1954), supplemented with 100 µg ml^{-1} of cycloheximide (Amkraz *et al.*, 2010). Three replicates were made for each sampleand incubated at 26°C for 48 h. Fluorescent colonies (UV 320nm) were purified and stored at -80°C in 40% glycerol. The three isolates were confirmed as *P. putida*Q172B and *P. fluorescens*(Q110B and Q036B) at Plant Protection laboratory - Microbiology Unit- INRA Agadir Morocco (Qessaoui *et al.*,2017). To evaluate their antagonistic activity, the supernatant were prepared for each bacterium and adjusted to 10^8 $CFUmL^{-1}$.

Isolation of *Penicillium digitatum* and *Botrytis cinerea*

The isolate of *P. digitatum* and *B. cinerea*were collected from the fruits infested of citrus and tomato respectively. The Potato Dextrose Agar(PDA) was used to isolate fungiand to obtain pure culturespurifyfungal. This isolates were confermed as *P. digitatum*and *B. cinerea*at Plant Protection laboratory - Microbiology Unit-INRAAgadir Morocco. Conidial suspensions of each fungal pathogenin Sabauroud Broth medium ($10gL^{-1}$peptone ; $20gL^{-1}$glucose) were adjusted to 10^5 spores mL^{-1}.

In vitro selection of antagonistic fluorescent pseudomonas against*P. digitatum* and *B. cinerea*

In vitro evaluation of three fluorescent *Pseudomonas* isolates were conducted using dual culture technique on PDA (Kaur, 2003). A inoculum of the individual rhizobacterial strains was applied as a stripe of 1.5 cm length equidistantly on three opposite edges of the agar medium in the petri plate using an inoculation loop. A mycelial disc of 5 mm diameter from 7 day-old culture of *B. cinerea* and *P. digitatum* was placed separately at the centre of the petri plate. Three replications were maintained for each rhizobacterium. Plates containing the pathogen alone served as control. The inoculated plates were incubated at 25°C for five days (Kaur *et al.*, 2007). After incubation period, the mycelial growth of *B. cinerea* and *P. digitatum* was recorded and the mycelial growth inhibition percentage (MGIP) was calculated using the formula:

$$\text{MGIP} = \frac{r1 - r2}{r1} \times 100$$

wherer1 is the radial growth of the fungus in the control and r2 is the radial growth of the fungus in the treated plates (Chaurasia *et al.*, 2005; Berrada *et al.*, 2012). The bacterial isolates showing maximum zone of inhibition was selected for further studies.

Production of volatile antifungal compounds (VOCs)

The effects of volatile organic compounds produced by *Pseudomonas* strains on the growth rates and activity of fungi were assessed using experimental systems that had only atmospheric connections according themethod described by Fiddaman and Rossall (1993). The antagonism of *Pseudomonas* was evaluated on King's B medium plates. After incubation for 48 h, the lid of each Petri dish was replaced by a plate containing 6mm plug of the test fungus in PDA medium. The two plates were sealed with parafilm. Controls were prepared without bacteria in the bottom plate. Petri dishes were incubated at 25°C, and observations were recorded after 5 days (Kumari and Khanna, 2014). The percentage growth inhibition (I) by VOC_S was calculated using the same formula:

$$I\% = \frac{r1 - r2}{r1} \times 100$$

wherer1 is the radial growth of the fungus in the control and r2 is the radial growth of the fungus in the treated plates.

Production of hydrogen cyanide (HCN)

To determine the production of HCN, ten *Pseudomonas*isolates were streaked onto KB agar plates supplemented with glycine(4.4 g l^{-1}). After this, the Petri plates were inverted and the filter papers impregnated with 0.5% picric acid (yellow) and 2% sodium carbonate were placed on the lids. Petri plates were sealed with parafilm and incubated at 28°Cfor 96 h. Discoloration of the filter paper from orange tobrown after incubation was considered as bacterial production of cyanide (Bakker and Schippers, 1987; Jha *et al.*, 2009).

Statistical analysis

All statistical analyses were subjected to ANOVA. Data for mycelial growth inhibition percentage (MGIP) and the percentage growth inhibition (I) by VOCs are presented as means ± standard deviation. Statistical significance was defined as$p < .05$ (Newman–Keuls test).

Results and discussion

In vitro selection of antagonistic fluorescent pseudomonas of *P. digitatum* and *B. cinerea*

Alltree fluorescent*Pseudomonas*isolates were testedagainstthe*P. digitatum* and *B.cinerea*strains inPetriplatescontainingPDA medium. All these isolates showed inhibitory activity against two fungal pathogenswithapercentage of inhibitionranging from39 %to54% for *P. digitatum* and 65 %to66% for *B.cinerea* (table 1).*P. putida* Q172B and *P. fluorescens* Q036B showed the highest inhibition of *B.cinerea and P. digitatum*.

Table 1: *In vitro* screening of bacterial isolates based on mycelial growth inhibition and HCN Production

Isolate	Code	*B.cinerea*		*P. digitatum*		HCN Production
		MGPI	I %	MGPI	I %	
P. fluorescens	Q036B	65,28±2,41[a]	100±0.00[a]	54,63±26,71[a]	14,03±2,64[a]	+
P. putida	Q172B	66,86±5,64[a]	100±0.00[a]	50,00±10.00[ab]	18,81±7,72[a]	+
P. fluorescens	Q110B	65,48±5,15[a]	100±0.00[a]	39,17±8.00[ab]	44,48±34,49[a]	+

Production of volatile antifungal compounds (VOCs)

The results of VOCs showed that those three *Pseudomonas* strains produce volatile products effective against *B. cinerea* and *P. digitatum*. An inhibition in the radial growth of the test fungiwas observed during the 5 days of incubation (table 1). These results showed that

inhibition percent ranges from 14% to 44% for *P. digitatum.* For*B.cinerea*, the radial growth is totaly inhibed (100%).

Production of hydrogen cyanide (HCN)

All three antagonistic fluorescent *Pseudomonas* isolates showed the production of HCN as indicated by the discoloration of the filter paper from orange to brown (table1). Two *Pseudomonas* strains QF72B and QF36B were found to be potential producers of HCN, causing color change from yellow to dark brown, whereas Q110B was showed an average production with a orange color (figure1).

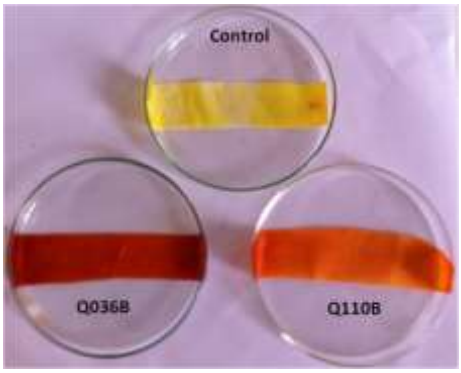

Figure 1: production of HCN on KB agar as indicated by the discoloration of the filter paper when incubated with picric acid and sodium carbonate

Three *Pseudomonas* strains belonging to the species *P. fluorescens* Q036B , *P. fluorescens* Q110B and *P. putida* Q172B have shown an efficient and significant ability to inhibit *P. digitatum* and *B. cinerea*causing green mold and grey mold, respectively. Our results concord with those that report *Pseudomonas*asamajorgenusin thebiocontrolofpathogens(Kaur *et al.*, 2007;Gao *et al.*, 2018). This inhibition canbe explained by their capacity to produce bioactive components. Some beneficial rhizobacteria can reduce pathogenic infections directly by competitionfor space or nutrients (Lugtenberg and Kamilova, 2009; Mitter *et al.*, 2013) and secretion of antibioticssuch as lipopeptides, 2,4-diacetylphloroglucinoland phenazine-1-carboxylic acid (PCA)(Jaaffar *et al.*, 2017; Paulin *et al.*, 2017). Therefore, in our experiments underlying *Pseudomonas* mechanisms against *B. cinerea*, these results are similar to that found by Chang *et al.* (2007) who showed that the supernatant of *Bacillus cereus* Q308 inhibited spore germination and germ tube elongation of *Fusarium oxysporum*, *F. solani*, and *Pythium. ultimum*. The results showed that the isolates were able to produce volatile compound such as HCN. Our results showed that VOCs producing isolates totally inhibited growth of *B.cinerea*. This results are similar with the study of Ramette *et al.*(2003) who reported that the microbial production of HCN is an important antifungal trait to control root infecting fungi.

Conclusion

Because of the potential production of volatile and diffusible antifungal metabolites, selected *Pseudomonas* strains are considered as potentialantagonists against a range of phytopathogenic fungi that infect tomatoand other crops, and thereforecould be used as potential alternative for chemical fungicides in reducing the damages caused by fungal diseases.

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RESPONSE OF SPRING BREAD WHEAT QUALITY AND YIELD PARAMETERS TO DIFFERENT DROUGHT SCENARIOS UNDER MOROCCAN CONDITIONS

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Abstract

Under climate change, the release of productive bread wheat cultivars with high quality standards is a major challenge in Moroccan wheat breeding program. The aim of the present study was to investigate drought stress influence on productivity traits and the main relevant quality parameters (grain protein content, quantity and strength of gluten) and to select new performing genotypes. Fourteen elite and national genotypes of bread wheat were evaluated in two Moroccan contrasting locations (favourable and semi-arid) during two consecutive seasons (2014-2015). Besides, electrophoretic patterns of seed storage proteins profiles using SDS-PAGE technique were performed. Results indicated that 2014 cropping season presented drier climate compared to 2015 season for both experimental sites. The analysis of variance showed significant effects of genotypes, site, year and site x year for yield traits and almost all quality parameters (P<0.001). The mean grain yield ranged from 3.92 t/ha to 2.49 t/ha under favourable and semi-arid conditions respectively. Moreover, protein content varied from 14.44% in semi-arid location to 12.52% in favourable site. The drought affected negatively all parameters at favourable and semi-arid regions. However, the genotypes behaviour at the driest year (2014) was similar for both sites based on mean comparison. Therefore, the genetic differencesin terms of quality were restricted because of drought stress.The correlation analysis showed negative correlation between yield and gluten content (r=-0.32**) at the driest environment. Also, both gluten and protein content showed negative correlation with biomass, plant height and number of grain per fertile spike.

Keywords: *bread wheat, drought, qualityparameters, yield, electrophoretic analysis.*

Introduction

Bread wheat is an important staple crop for food security worldwide, providing more than 20% of calories and protein daily requirements in addition to minerals and vitamins (Ahmed etFayyaz-ul-Hassan, 2015). In Morocco, bread wheat is the main cereal crop, covering more than 70% of cereal area, essentially cultivated under rainfed system (91%) (Jlibene, 2009).

During the last decades, the negative effects of climate change on wheat production and quality have raised, mainly because of drought stress impact, thus hindering to satisfy worldwide consumption needs and threatening worldwide food security in several regions, especially in developing countries. Forecasting models predict more frequent and intense periods of drought, especially in the Mediterranean region (IPCC, 2007; Walter et al., 2011), including Morocco. In this context, the release of productive bread wheat cultivars with high quality standards is a major challenge in current wheat breeding program,as it is associated with a number of negative correlations (Baenziger et al., 2001; Trethowan et al., 2001; Williams et al., 2008).The grain quality is judged principally on the base of its weight (usually thousand grain weight); while flour quality is mainly characterized by its protein and gluten strengthto assess good bread making quality (Békés et al., 2006; Ahmed etUl Hassan, 2015). Besides, high-molecularweight glutenin subunits (HMW-GS) play a critical role in determining dough elasticity (Shewry et al., 2002).

To gain further understanding of the effects of drought stress on grain quality attributes, this paper seeks to (1) analyse the relationship of quality and yield traits under different drought scenarios in Moroccan conditions and evaluate the relative roles of genotype, environment and their interaction in their expression; (2) determine the effects of drought stress on yield and quality parameters of cultivars; (3) determine the electrophoretic pattern of seed storage proteins profiles and (4) select performing genotypes with optimum balance between yield performance and quality parameters.

Materials and Methods

Plant materials and experimental design: Forty spring bread wheat genotypes were chosen based on their broad range of response to drought stress and yield performance and were planted in Randomized Complete Block design (RCBD) with three replications in two contrasting experimental fields (stressed and non-stressed) during 2014 and 2015 cropping seasons. Environments were defined as combinations ofseasons and sites. The germoplasme evaluated were selected from diverse origins: Gladius, Hartog, Drysdale (Australia); Nejmah-11, Nejmah-14, Shihab-12, Al-Zehraa-2, Baasha-21, Amir-2(ICARDA); Attila, Sokoll, SB003, SB165, SB069, SB062, SB109, SB169, SsrT02, SsrT09, SsrT14, SsrT16, SsrT17, SsrW35,SsrW47 (CIMMYT)and Arrehane, Achtar, Marchouch, Kanz, Amal, Massira, Aguilal, Rajae, BT05A104, BT05A106 (Morocco).

Experimental Sites*:* The study considered two contrasting experimental sites in terms of long-term average rainfall, namely "Taoujdate" and "Sidi El Aidi", belonging to the National Institute of Agricultural Research of Morocco. The Taoujdate site (TJ) represents the favorable area (non-stressed site) with an average rainfall of 470 mm. In contrast, Sidi El Aidi(SA) is located in the semi-arid area (stressed site) with an annual average of 300 mm.

Measurements*:* For yield related traits, grain yield (GY), harvest index (HI), grains number (NG), number of fertile spikes per plant (NFSP), number of grains per fertile spike (NGFS), biomass (BY) and plant height (PH) were evaluated.The superiority index (Pi) was calculated based on Lin et al. (1988) equation to determine the best performant genotypes over all environments. For quality traits, thousand grain weight (TGW), gluten content (GC), protein content (PC) and gluten strength (W) were assessed using near-infrared reflectance spectrophotometry analysis (InfraneoChoppin).

Glutenins composition analysis*:* Polyacrylimide gel electrophoresis in the presence of Sodium Dodecyl Sulfate (SDS-PAGE) following Singh et al. (1991) procedure of was carried out to analysegluteniscompostion of the studied germplasm. The HMW glutenin subunits were identified using the numbering system of Payne and Lawrence (1983) and compared with the patterns of known genotypes (standards).

Statistical analysis*:* Data were subjected to analysis of variance for each parameter to detect significance differences using F test, while genotypic means differences were judged by using Bonferroni test. The percentage of variance for each effect was calculated based on sum of squares proportion. Data were also subjected to correlation in order to elucidate the relationships among criteria and their association with grain yield performance.

Results and Discussion

Cropping season's pattern

The 2014 cropping season was drier and hotter than 2015 season for both sites, especially during reproductive stage. Besides, semi-arid site (SA) experienced hotter and drier conditions compared with the favourable site (TJ). Mean temperatures recorded at SA (13.3°C± 1.45) were hotter compared to TJ (11.54°C ± 1.15), especially during winter and autumn. Furthermore, rainfall decreased during 2014 season by 36% (-138mm) in TJ, compared to 24% (-70mm) for SA. The gap between the two sites is much larger in the best

year (-30%) than in the dry year (-13%). This result corroborates the finding of Balaghi et al. (2013)which states that drought affects all Moroccan regions during dry years.

Effects of G, E and G x E on yield and yield related traits

A previous study had already displayed grain yield variations and pattern.The combined ANOVA indicated highly significant variability over years and sites; and among genotypes ($p<0.001$)(Bennani et al., 2017). The mean grain yield ranged from 3.92 t/ha to 2.49 t/ha under favourable and semi-arid conditions respectively.

For yield related traits, the results of ANOVA showed that all factors had significant effect on the different traits studied. The environment had the largest effect exceeding 70% of the total variation (Table 1), which agrees with previous researches (Li et al., 2013).

Table 1: Analysis of variance for the yield and quality parameters

Traits	General Mean	Environment (E)	VE (%)	Genotype (G)	VE (%)	G x E	VE (%)
Yield	3.23	135.94***	94.98	1.54***	1.08	0.63**	0.44
HI	35.33	1950.7***	71.47	112.13***	4.11	50.99	1.87
BY	914.9	2336440***	83.05	79893***	2.84	56578**	2.01
TGW	35.24	5414.3***	93.45	83.02***	1.43	16.66	0.29
NGFS	30.11	1140.3***	72.03	176.1***	11.12	58.79***	3.71
NG	9256	243600000***	72.78	16180000***	4.83	7708000*	2.30
NFS/P	2.56	65.95***	95.96	0.93***	1.36	0.67***	0.97
PH	96.38	42608***	97.57	266.4*	0.61	193.2	0.44
Gluten	31.67	2692.94***	57.69	33.23***	9.26	18.64	15.58
Protein	13.47	217.921***	53.59	2.631*	8.41	1.753	16.82
W	258.5	148840***	28.93	6930***	17.51	3299	25.00

*, ** and *** represent significant level at <0.05, <0.01 and <0.001 respectively

Drought affected negatively all yield parameters as reported by earlier researches (Li et al., 2013). Based on mean comparisons, the biomass (BY) (-15%) and grain number (NG) (-14%) were the most affected at the driest year in the favorable environment (TJ14) compared to the humid one (TJ15). Whereas, at the semi-arid environment, NFSP (-49.8%) followed by BY (-22.7%) and NG (-20.6%) were the most affected (Table 2). This finding indicates that genotypes responded to the stress by reducing mainly the number of grains, biomass and also number of fertile spikes under more severe stress during anthesis period (Li et al., 2013).

Effects of G, E and G x E on quality traits

Significant differences were observed among genotypes and environments for all quality traits, which agree with Li et al. (2013) and Ahmed etFayyaz-ul-Hassan (2015) findings. In contrast, the interaction genotype x environment was not significant for any of the parameters. Yet, the environment and, to a lesser extent, the interaction were the most important factors explaining from 50 to 70% of traits variations (Table 1) as stated by Li et al. (2013) and Guzman et al. (2016). The Bonferroni test showed that genotypes behavior at the driest year (2014) was similar for both sites, recording the highest values except for TGW.

The thousand grain weight (TGW) was affected negatively by dry conditions either at TJ (-29%) or SEA (-26.4%). However, the slight drought experienced at TJ induced positive improvement in protein content and in gluten content and strength. This finding is sustained by Flagella et al. (2010) and Li et al. (2013) researches, and was associated with modification of source sink balance (Ahmed and Fayyaz-ul-Hassan, 2015), and/or even to synthetized shock proteins as protective role under stress environments (Reynolds et al., 2001). In contrast, negative impact was observed at SA under severe stress for gluten content and

strength, but not for protein content (Table 1). The combination of higher temperatures and severe drought conditions may result in alteration of the bread wheat quality in response to increased climatic variability (Fernando et al., 2014; Guzman et al., 2016).

Relationships among quality and yield traits under different drought scenarios

Important high significant correlations were found between yield and most yield related traits, except for NFSP at optimum conditions (TJ15), NGFS at slight (TJ14) and moderate (S15) stress and PH at the humid year 2015 for both sites (Table 2) depending on drought severity.

Table 2: Mean comparisons (BonferroniTest) and Pearson correlations with grain yield for traits

Traits	Meanscomparisons (Bonferroni Test)				Pearson Correlations (r)			
	TJ14	TJ15	SEA14	SEA15	TJ14	TJ15	SEA14	SEA15
Yield	3.37a	4.52b	1.93c	3.08d				
HI	36.35a	36.8ab	29.48c	38.68b	*0.64****	*0.74****	*0.62****	*0.52****
BY	907b	1070a	733c	949b	*0.68****	*0.73****	*0.81****	*0.83****
NGFS	30.7b	34.1a	26.6c	29.7b	0.32	*0.64****	*0.61****	0.25
NG	10891b	9378a	7414c	9341a	*0.79****	*0.85****	*0.94****	*0.94****
NFSP	2.81b	3.05a	1.46c	2.91b	*0.32**	0.27	*0.74****	*0.62****
PH	110.09	113.94	76.5	89.54	**-0.36*	-0.1	*0.46***	0.05
TGW	30.2b	42.55a	28.91b	39.3c	0.29	0.004	0.77	0.02
Gluten	33.25b	23.16a	34.16b	36.12c	-0.30	0.13	**-0.32*	0.09
Protein	13.96b	11.02a	14.26bc	14.62c	-0.31	0.12	-0.30	0.04
W	257.5b	206.2a	258.6b	311.9c	-0.20	0.18	-0.09	0.03

a, b and c represent the groups based on Bonferroni test comparisons

*, ** and *** represent significant level at <0.05, <0.01 and <0.001 respectively

In parallel, all quality parameters were highly correlated between them (P<0.001) (data not shown) except TGW. Gluten content and TGW showed respectively negative and positive correlations with grain yield at the driest environment (SA14), reflecting source sink balance. Other yield components and related traits showed significant correlation with quality traits. The gluten and protein content showed significant moderate negative correlation with NGFS at SA respectively at 2014 (r=-0.31; r=-0.35) and 2015 (r=-0.35; r=0.36) seasons. Besides, plant height presented moderate positive correlation with PC (r=0.31) and W (r=0.41) at slight drought conditions (TJ14) and negative one at the driest environment (S14) (r=-0.37 and r=-0.38 respectively for GC and PC), thus following an opposite pattern of height/grain yield relationships.Both lowest NGFS/ height may enable enough efficient assimilates remobilization for existing grain, even under stress (Ahmed and Fayyaz-ul-Hassan, 2015).

Seed storage proteins composition of genotypes

The frequencies of 9 alleles identified at the 3 loci Encoding HMWGs are shown in table 3. Three alleles were identified at Glu-A1, four at the Glu-B1 and two at the Glu-D1 locus. Glu-A1 (c) was the most common allele with a frequency of 62.5%. The Glu-B1 (i) showed the highest frequency (57.5%). At the Glu-D1, (d) was the preponderant allele in the bread wheat cultivars (92.25%) whereas Glu-D1(a) was present only in three genotypes (7.5%).

Table 3: Frequencies (%) of high molecular weight glutenin subunits in bread wheat at different loci Glu-A1, Glu-B1, Glu-D1

Glu-A1		Glu-B1		Glu-D1	
Subunits (Alleles)	Frequency (%)	Subunits (Alleles)	Frequency (%)	Subunits (Alleles)	Frequency (%)
1 (a)	22.5	6+8 (d)	10	5+10 (d)	92.25
2*(b)	15	7+8 (b)	20.5	2+12 (a)	7.5
Null (c)	62.5	7+9 (c)	10		
		17+18 (i)	57.5		

Results revealed the presence of favorable alleles which increase gluten strength such as 'b' and 'i' at GluB1 and 'd' at GluD1 loci in the majority of the banding patterns of the germplasm analyzed 75.5% and 92.25% respectively (Souza Costa et al., 2017).

Selection of best performing genotypes

Cultivars were found to react differently to water deficit conditions. However, their ranking over environments seems to be unchanged for quality parameters as the interaction was not significant. Based on Pi (data not shown), the best performing genotypes were AUS30355 (Pi=0.11), Gladius (Pi =0.20), Amir-2 (Pi=0.33), AUS30354 (Pi=0.27), Sokoll(Pi=0.36) and Kanz (Pi=0.32) that showed high yield across all the environments (Mean yield varies from 3.69 to 4.02t/ha) . For quality traits, we noted that all genotypes expressed good protein and gluten content for good bread making quality based on standards (CTNCLPD, 2009).

Conclusion

In general, variation was observed in genotypes and environments for each yield and quality parameter. Environment has the largest effects, affecting negatively yield and yield related traits especially biomass, grain number and thousand grain weights under drought conditions. Higher grain yields are mainly related to harvest index, biomass and grain number, while the number of fertile spikes showed great impact at the semi-arid zone. The quality parameters (gluten and protein content, strength) were positively affected by slight/moderate drought impact, while moderate/severe drought affected negatively all the traits (except protein content) due to source sink balance. Grain yield was correlated negatively with gluten content solely at the driest environment, making multi environment assessment for both yield parameters and quality very important in the breeding program. AUS30355, Gladius, Amir-2, AUS30354, Sokoll and Kanz showed high yield across all the environments, and can be recommended as best parents for drought tolerance and quality in the national breeding program.

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CHANGES IN THE ECOLOGICAL PARAMETERS OF FUNGAL COMMUNITIES COLONIZING PERENNIAL RYEGRASS AFTER THE APPLICATION OF NANOTECHNOLOGY-BASED BIOSTIMULANTS

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Abstract

Perennial ryegrass is the main forage grass used in livestock production in Poland. The quality of herbage is influenced mainly by the yield and nutritional value of grass and the presence of undesirable microbiota. Nanotechnology-based plant growth stimulants can improve plant health, germination and yield, but their impact on the microbiota colonizing plants remains unknown. Three types of silver nanoparticles, two types of titanium dioxide nanoparticles, silver nitrate, a chelated form of titanium, and an additional rate of nitrogen were applied in this study. Perennial ryegrass without stimulant treatment was the control. Grass was harvested twice during the growing season, and the biostimulants (10 ppm per 10 m^2) were applied twice after each harvest. Fungi were isolated from tillering nodes after the second harvest. Herbage yield did not differ significantly between treatments, and the highest yield was achieved in the control treatment. The applied stimulants, excluding tannic acid-reduced silver nanoparticles, improved sod density. The largest number of fungi were isolated from the control treatment and the chelated titanium treatment, and the smallest number of fungi were obtained from treatments with an additional rate of nitrogen and the silver nitrate treatment. The control treatment with a higher fertilizer rate was characterized by the highest ecological value (species richness and species diversity). The highest counts of *Penicillium* spp. (eudominance) and *Fusarium culmorum* (dominance) were observed in the titanium chelate treatment. Excluding the control, undesirable fungi were most effectively controlled in the $AgNO_3$ treatment. The remaining biostimulants exerted indirect effects and did not completely eliminate undesirable fungi (*Fusarium* spp., *Cladosporium herbarum*, *Microdochium* spp.).

Keywords: *perennial ryegrass, biostimulants, nanoparticles, fungi*.

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the main forage grass used in animal production in temperate regions. It is characterized by high yield potential and high nitrogen utilization. Perennial ryegrass has high feed value due to its high palatability and desirable chemical composition (Rath *et al.,* 2005). The surface, intercellular spaces and cells of perennial grass plants are colonized by numerous fungal species. Fungi enter into pathogenic, parasitic or mutualistic interactions with the host plant (Perez *et al.,* 2013, Sanchez-Marquez *et al.,* 2012). Pathogenic fungi can decrease the yield of perennial grass, and mycotoxins can compromise the nutritional value of forage (Thom *et al.,* 2014).

Nanotechnology-based plant growth promoters can enhance the growth and development of plants, improve germination rates and protect plants against the harmful effects of pathogenic microorganisms which decrease the quantity and quality of yield (Khodakovskaya *et al.,*. 2009, Tripathi *et al.,* 2011, Wang *et al.,* 2013, Wang *et al.,* 2014). Biostimulants containing nanoparticle adjuvants can be applied together with pesticides to reduce surface tension and

increase adhesion to plant surfaces (Bergeson 2010, Khot *et al.*, 2012). Many nanoparticles have similar properties to commercial pesticides, and they are increasingly used for plant protection (Smith *et al.*, 2008). Carbon-based nanoparticles such as fullerenes, single-wall and multi-wall carbon nanotubules, as well as inorganic nanoparticles, including metals (Au, Ag, Al and Fe) and metal oxides (TiO_2, ZnO, Al_2O_3, Fe_3O_4, Fe_2O_3, NiO, CoO, CeO_2), are most widely used (Wang *et al.*, 2014, Srivastava *et al.*, 2015, Verma *et al.*, 2018).
The aim of this study was to analyze the effects of several nanotechnology-based plant growth stimulants on fungal communities colonizing the stem bases of perennial ryegrass and producing potentially harmful secondary metabolites, and on herbage yield.

Materials and Methods

The study was carried out in the year 2017 on experimental plots in north-eastern Poland, in the didactic-experimental center of the University of Warmia and Mazury in Tomaszkowo near Olsztyn

Suspensions of silver nanoparticles

Suspensions of silver nanoparticles with the size of 10 nm, stabilized with tannic acid (TA), trisodium citrate (SBTC) and sodium hexametaphosphate (SHSH), were used in the study. The solutions reduced with the above compounds had an initial concentration of 100 mg l^{-1} (100 ppm). A solution of silver (V) nitrate ($AgNO_3$) nanoparticles with a concentration of 100 mg l^{-1} was prepared as the reference. The suspensions were reduced and prepared by Magadela Oćwieja, Ph.D. at the Jerzy Haber Institute of Catalysis and Surface Chemistry of the Polish Academy of Sciences in Cracow.

Suspensions of titanium dioxide

Two forms of titanium dioxide, TiO_2 nanoparticles with the size of 21 nm (TP) and a mineral form of TiO_2 with a stable fraction of approximately 700 nm, were purchased from Sigma Aldrich and used to prepare solutions with a concentration of 100 mg l^{-1} (100 ppm). A solution of Tytanit (Intermag, Poland), a commercial plant growth stimulant, with a concentration somewhat higher than that recommended by the manufacturer (100 ppm), was prepared as the reference.

Seed dressing formulation

The tested compounds were used for seed dressing. Grass seed samples of 10 g each were placed in capped plastic containers, and 0.2 g of industrial grade talcum powder, 0.05 g of carboxymethyl cellulose, 2 ml of every tested compound and 1 ml of demineralized water were added. The contents of each container were thoroughly combined by shaking for around 2 minutes. The mixture was dried in a laminar flow cabinet for 30 minutes and stored until sowing. The control were grass seeds combined with carrier solvents, where the active ingredient was replaced with 2 ml of demineralized water.

N1 – NPK mineral fertilizer at 60/30/60 kg/ha;
N2 – NPK mineral fertilizer at 90/30/60 kg/ha;
$AgNO_3$ – silver molecular solution in the form of silver nitrate V at a concentration of 100 mg/l.
TA – silver nanoparticles stabilized with tannic acid;
SHSH – silver nanoparticles stabilized with sodium hexametaphosphate;
SBTC – silver nanoparticles stabilized with trisodium citrate;
TiO_2 – mineral form of TiO_2 with a stable fraction of approximately 700 nm;
TP – TiO_2 nanoparticles with the size of 21 nm;
Tytanit – commercial plant growth stimulant (Intermag, Poland).

Seeding and cultivation

Grass seeds were sown manually at 35 kg ha^{-1}. Plant density was determined in each plot after seedling emergence. Weeds were removed mechanically at regular intervals.

The plot experiment involved three variants: a control treatment without an active ingredient, experimental treatments with NPK fertilization at 60/30/60, and experimental treatments with optimal NPK fertilization for perennial ryegrass at 90/30/60. Plot size was 1 m x 3 m. Every variant was performed in three replications. Grass was harvested twice during the growing season.

Foliar application of plant growth stimulants at the beginning of tillering

The tested growth stimulants were manually sprayed on leaves 4 and 8 weeks after sowing. Each biostimulant was dissolved in demineralized water to produce 2% aqueous solutions. The solutions were applied at 100 l ha^{-1}.

Forage harvest

Grass was harvested twice during the growing season. The collected herbage was weighed immediately after harvest to determine fresh weight, placed in plastic string bags and transported to the laboratory. Sod density was determined after the second harvest based on the 9-point scale proposed by Prończuk (1993).

Fungal cultures and isolation of fungi from plant material

Plant material was prepared for fungal cultures in the laboratory. Twenty-five shoot segments with a length of 1 cm each (one from each plant) were placed on potato dextrose agar (PDA). Plant tissues were previously surface disinfected with 70% ethanol for 5 s, 1% sodium hypochlorite for 20 s, and rinsed three times in sterile deionized water. The samples were incubated for 21 days at a temperature of 25°C. The colony growth index was estimated at 0.75 per leaf. Fungal colonies were transferred to fresh PDA, mycelial samples were collected after 14 days, and the fungi were identified based on the available keys.

Results and Discussion

The highest herbage yield was obtained in the treatment with the highest fertilizer rate, followed by TP and SBTC treatments. Herbage yield was lowest after the application of TiO_2. Sod density, which is determined by germination capacity, was highest in the TiO_2 treatment. Mahmoodzadeh *et al.*, (2013) demonstrated that TiO_2 improved germination capacity and early seedling growth in oilseed rape, whereas Jaberzadeh *et al.*, (2013) found that TiO_2 enhanced the early growth of wheat plants.

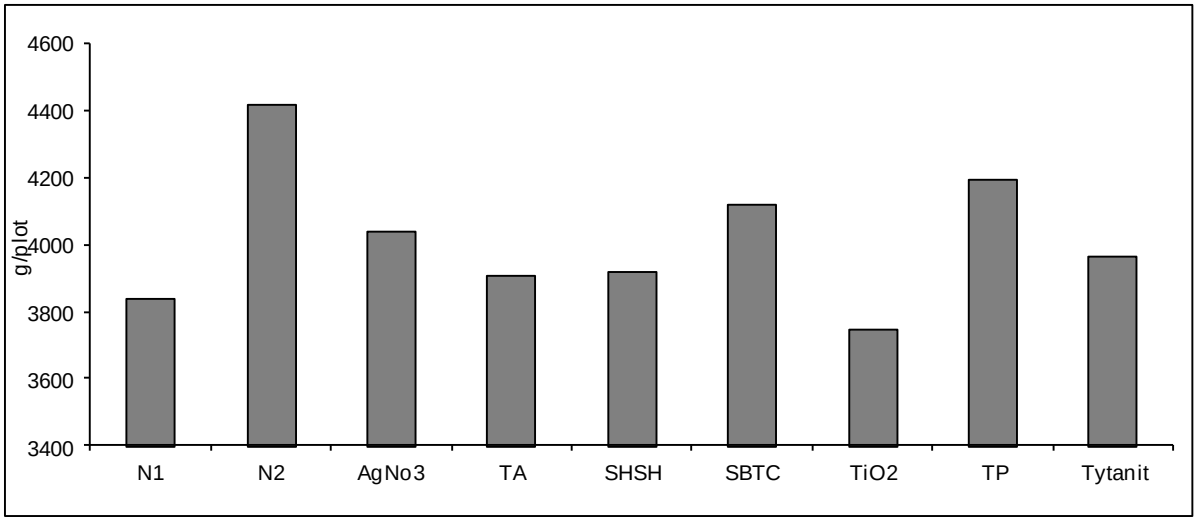

Figure 1. Weight of second-harvest perennial ryegrass herbage.

The highest number of fungal colonies were isolated from the treatment with a lower rate of nitrogen fertilizer and the treatment where the Tytanit biostimulant was applied. The treatment with a lower fertilizer rate was characterized by a high diversity of fungal species,

including four species of the genus *Fusarium*, which could influence the processing suitability of forage and herbage yield. *Microdochium nivale*, the cause of pink snow mold, was isolated from stem bases. The above pathogen was not identified only in treatments protected with silver nitrate, SHSH and TiO_2.

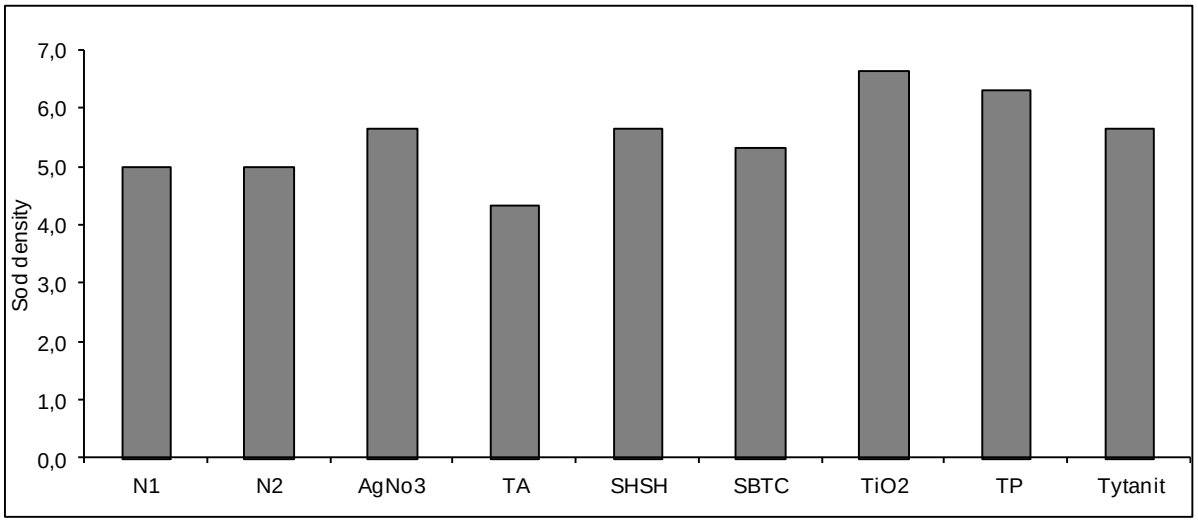

Figure 2. Sod density after the second harvest of perennial ryegrass.

Table 1. Structure of fungal communities colonizing the stem bases of perennial ryegrass

No	Fungal species	N1	N2	$AgNO_3$	TA	SHSH	SBTC	TiO2	TP	Tytanit	Total
1	*Alternaria alternata*	1	0	0	0	0	0	0	0	0	1
2	*Aureobasidium pullulans*	1	0	0	0	0	0	1	1	0	3
3	*Cladosporium herbarum*	0	0	0	3	0	0	0	0	0	3
4	*Fusarium avenaceum*	0	0	0	0	0	0	1	0	0	1
5	*Fusarium culmorum*	2	0	1	1	1	0	0	3	3	11
6	*Fusarium fujikuroi*	1	0	0	0	0	1	1	0	0	3
7	*Fusarium poae*	1	0	0	0	0	0	0	0	0	1
8	*Fusarium oxysporum*	1	0	0	0	0	0	0	0	2	3
9	*Juxtiphoma eupyrena*	0	1	2	0	2	1	2	0	0	8
10	*Microdochium bolleyi*	1	1	1	0	3	0	0	2	1	9
11	*Microdochium nivale*	1	1	0	1	0	1	0	1	1	6
12	*Mucor hiemalis*	0	0	0	1	0	0	0	0	0	1
13	*Penicillum* sp.	0	0	0	0	0	2	0	0	5	7
14	*Phoma* sp.	0	0	0	0	0	0	2	0	0	2
15	*Pyrenophora graminea*	2	1	0	0	1	2	1	2	0	9
16	*Rhizoctonia* sp.	1	0	0	0	0	0	0	0	0	1
	Total colonies	12	4	4	6	7	7	8	9	12	69
	Total species	10	4	3	4	4	5	6	5	5	
	Shannon-Wiener diversity index	0.475	0.693	0.693	0.645	0.675	0.647	0.607	0.65	0.646	
	Simpson dominance index	0.111	0.25	0.375	0.333	0.306	0.224	0.188	0.235	0.278	

Fungi of the genus *Fusarium* were not isolated from the treatment with a higher rate of nitrogen fertilizer where a total of four fungal colonies belonging to different species were isolated. Only four fungal isolates belonging to three different species were isolated in the silver nitrate treatment. The silver nitrate treatment was least colonized by fungi in the experiment. In a study by Jo *et al.*, (2009), ionic silver and silver nanoparticles effectively eliminated *Bipolaris sorokiniana* from perennial ryegrass inoculated with this fungus.

Conclusions

Titanium dioxide nanoparticles, silver nanoparticles (SBTC) and silver (V) nitrate treatments are characterized by the most desirable ratio of herbage yield to sod density. None of the tested treatments exerted a significant effect on microbial communities naturally colonizing grass surface, but all treatments decreased the abundance and species richness of fungal communities. All biostimulants improved the biometric parameters of perennial ryegrass, but none of them completely eliminated phytopathogens from grass surface.

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THE MICROBIOLOGICAL VALUE OF THE RHIZOSPHERE SOIL OF WINTER WHEAT AND WINTER OILSEED RAPE CULTIVATED IN MONOCULTURE AND IN CROP ROTATION

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Abstract

Crops are selected for cultivation in view of economic, technological and organizational considerations, which implies that some plant species are cultivated year by year. Long-term monoculture accelerates soil degradation, and it can decrease the species richness and diversity of soil microorganisms. This study analyzed the rhizosphere soil of winter wheat (*Triticum aestivum* L.) and winter oilseed rape (*Brassica napus* L.) plants grown in a 5-field crop rotation system and in long-term monoculture. The effect of the crop sequence on the counts of bacteria of the genera *Pseudomonas*, *Bacillus* and *Azotobacter*, actinobacteria and the number of TRI5 genes involved in the biosynthesis of mycotoxins in fungi of the genus *Fusarium* was determined. The crop sequence did not affect the counts of *Pseudomonas* and *Bacillus* bacteria and actinobacteria in soil from the crop rotation, but *Azotobacter* spp. were not detected. The number of TRI5 genes was relatively high in each treatment, and no significant differences were detected between treatments. In winter oilseed rape, the number of TRI5 genes was approximately three-fold lower in crop rotation than in monoculture, whereas the reverse was observed in winter wheat. It can be concluded that plants cultivated in monoculture and in crop rotation do not significantly affect the counts of bacteria stabilizing the biological value of soil and, consequently, do not lead to a significant decrease in the abundance of *Fusarium* fungi. Bacteria of the genus *Azotobacter* were eliminated in crop rotation, which indicates that they are sensitive to the crop sequence.

Keywords: *microorganisms, bacteria, monoculture, crop rotation, Fusarium.*

Introduction

Crop species are selected in view of organizational, technological and economic considerations. As a result, the proportion of cereals in the cropping system is high, exceeding 70% in many countries. Cereal production is highly profitable, and cereals are often grown year after year in short-term monocultures. Monoculture increases the risk of stem-base diseases caused by *Fusarium* fungi, *Rhizoctonia cerealis, Gaeumannomyces graminis* and *Oculimacula yallundae* (Korbas *et al*., 2001).

The area under oilseed rape has increased in recent years. Oilseed rape is sown in the same field every 2-3 years, often as a break crop in wheat monocultures (Kurowski *et al*. 2008). A short break or the absence of a break between successive oilseed rape crops in the same field increase the risk of fungal infections which negatively affect cereal stands and compromise yields. In simple crop rotation systems, oilseed rape is exposed to various pathogens, including *Plasmodiophora brassicae*, *Verticillium* spp. and *Sclerotinia sclerotiorum* (Kurowski *et al*., 2008; Korbas *et al*., 2009). Monoculture increases the risk of fungal infections, but it also contributes to the development of beneficial microorganisms. Despite the above, researchers differ in their opinions on the positive and negative effects of monoculture. Soil-dwelling microorganisms are responsible for the cycling of elements, decomposition of organic matter and degradation of xenobiotics (Hayat *et al*., 2010; Jacobsen

et Hjelmsø, 2014). Owing to their unique metabolism, soil microbes promote the growth and development of plants. Soil-dwelling microorganisms can be divided into several groups, including free-living bacteria, endophytic bacteria and symbionts (Glick, 2012; Krawczyk, 2016). Microorganisms are responsible for ecosystem functions, plant health, soil structure and soil fertility. They participate in the interactions between soil abiotic factors and higher organisms, and they are one of the most important determinants of allelopathic effects on other populations (Badura, 2006).

The aim of this study was to determine the effect of the crop sequence on the counts of desirable soil microorganisms, including bacteria of the genera *Pseudomonas*, *Bacillus* and *Azotobacter* and actinobacteria, and the number of TRI5 genes involved in the biosynthesis of mycotoxins in fungi of the genus *Fusarium*.

Materials and Methods

The experiment was performed on a bacterial community isolated from the rhizosphere soil of winter wheat and winter oilseed rape plants grown in monoculture and in crop rotation, with and without fungicide protection. Soil was obtained from a field experiment which has been conducted for 48 years in The Agricultural Experiment Station in Bałcyny by the Department of Agricultural Ecosystems of the University of Warmia and Mazury in Olsztyn (Poland). Soil samples from the winter wheat and winter oilseed rape cultivation were collected from 10 cm depth at the same time (May 2016).

The total counts of saprotrophic bacteria, bacteria of the genera *Pseudomonas* and *Azotobacter*, spore-forming bacteria and actinobacteria were determined on different media. *Pseudomonas* spp. were cultured on Pseudomonas Selective Agar (MERCK) with CFC selective agar (mixture of antibiotics), 16 g of gelatin peptone, 10 g of casein hydrolysate, 10 g of potassium sulfate, 1.4 of magnesium chloride, 11 g of agar, 10 ml of glycerin and 1 l of demineralized water. Spore-forming bacteria were cultured on TSA medium containing (per liter) 15 g of casein peptone, 5 g of sodium chloride, 5 g of soy peptone, 15 g of agar and 1 l of demineralized water. 100 µl of the sample incubated at 80°C for 15 minutes was transferred to the culture medium. Actinobacteria were cultured on a medium composed of 2 g of sodium caseinate, 0.1 g of asparagine, 4 g of sodium propionate, 0.5 g of dipotassium phosphate, 0.1 g of magnesium sulfate, 0.01 g of iron sulfate, 15 g of agar and 1 l of demineralized water. Bacteria of the genus *Azotobacter* were cultured on a medium composed of 1 g of dipotassium phosphate, 0.2 g of magnesium sulfate × water, 1 g of calcium carbonate, 0.2 of sodium chloride, 0.005 g of iron (II) sulfate × water, 10 g of glucose, 15 g of agar and 1 l of demineralized water.

Soil samples of 10 g each were collected from three sites in each treatment, and 10 g of the pooled sample was transferred to an Erlenmeyer flask containing 90 ml of sterile saline solution (0.85% NaCl) and 0.01% Tween 20. The samples were shaken in the Elpin plus 385S laboratory shaker for 30 minutes, and 1 ml of the suspension was transferred to glass test-tubes containing 9 ml of sterile saline solution. The contents of each test-tube were stirred, and 1 m of the suspension was transferred to another test-tube containing 9 ml of saline solution. The above step was repeated to perform serial dilutions with an exponential decrease in concentration from $1 \cdot 10^{-1}$ to $1 \cdot 10^{-12}$. 0.1 ml of the suspension from each solution was sampled and cultured on the appropriate solid medium. Bacterial colonies were counted after 48 h. The experiment was performed in two series (two independent soil samples) with two replications each to produce four results per treatment.

Toxin-producing fungi of the genus *Fusarium* were counted with the use of SYBR Green (5'-GCT GCT CAT CAC TTT GCT CAG-3') and Tox5-2 reverse (5'- CTG ATC TGG TCA CGC TCA TC-3') primers (Schnerr *et al*. 2001). The reaction profile involved initial denaturation at 95°C for 10 minutes, followed by 40 cycles at 95°C for 3 s, 63°C for 6 s, and

72°C for 30 s. The melt curve analysis was performed after cooling to 65°C (20°C/s) and heating to 95°C (0.5°C/s). The DNA isolated from a known number of *Bacillus subtilis* cells and *Fusarium culmorum* spores was used as the reference.
The results were processed statistically by ANOVA and Tukey's test at a significance level of a=0.05 (Statistica 12, StatSoft).

Results and Discussion

Crop rotation increases the availability of nutrients, thus contributing to the diversity of soil-dwelling microorganisms (Pereira *et al*., 2007). In the present study, the crop sequence had no significant effect on the counts of *Pseudomonas* spp., *Bacillus* spp. or actinobacteria, but the growth of *Azotobacter* spp. was not observed in soil samples from crop rotation systems (Fig. 1).

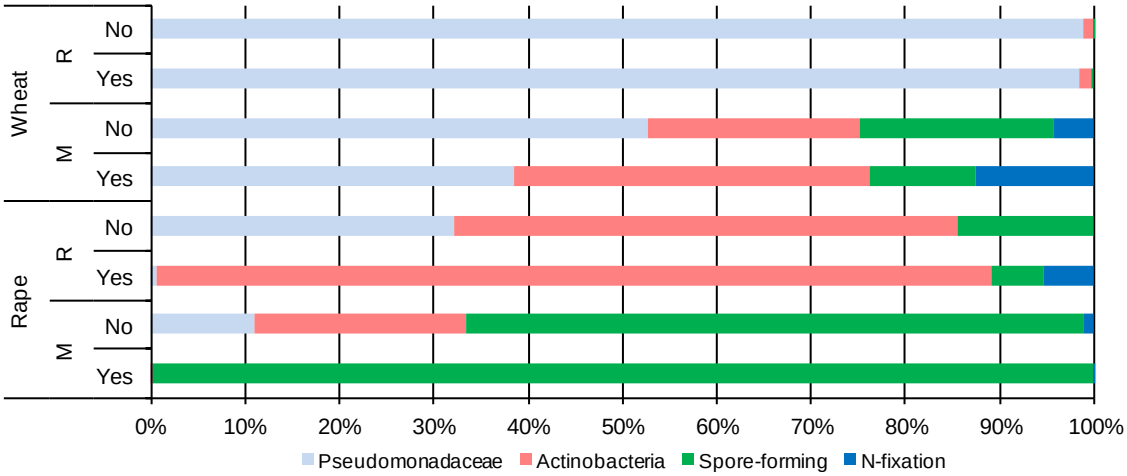

Figure 1. Frequency of occurrence of the analyzed bacterial groups in treatments (M – monoculture, R – crop rotation; Yes – chemical protection, No – no chemical protection)

According to D'Acunto *et al*. (2018), rotations with two to four crops increase soil microbial diversity by up to 4%. However, Jiang *et al*. (2011) and Jangid *et al*. (2011) found that the crop sequence had no significantly influence on bacterial communities. According to the above authors, soil-dwelling microorganisms are affected by other factors, such as soil type or locality.

Table 1. The effect of the crop sequence on bacterial counts (CFU)

Crop	**Crop sequence**	**Bacterial counts (CFU)**
Winter oilseed rape	Monoculture	3496141900 a
	Crop rotation	3688059200 a
Winter wheat	Monoculture	4976088800 a
	Crop rotation	3916700300 a

In the current study, the crop sequence had no significant effect on bacterial counts (CFU) (Table 1). According to the literature, the crop sequence (rotation or monoculture) can significantly affect bacterial communities. Alvey *et al*. (2003) and Xuan *et al*. (2012) demonstrated that the crop sequence induced significant changes in bacterial communities. Navarro-Noya *et al*. (2013) reported that bacterial counts did not differ significantly between winter wheat grown in rotation (winter wheat – corn) or monoculture. In a study by Venter *et al*. (2016), soil microbial diversity in crop rotations increased by 3.5% on average relative to

monoculture. According to the above authors, nearly two-thirds of research studies investigating the diversity of soil microbial communities in the most popular crops did not report significant differences between monoculture and crop rotation.

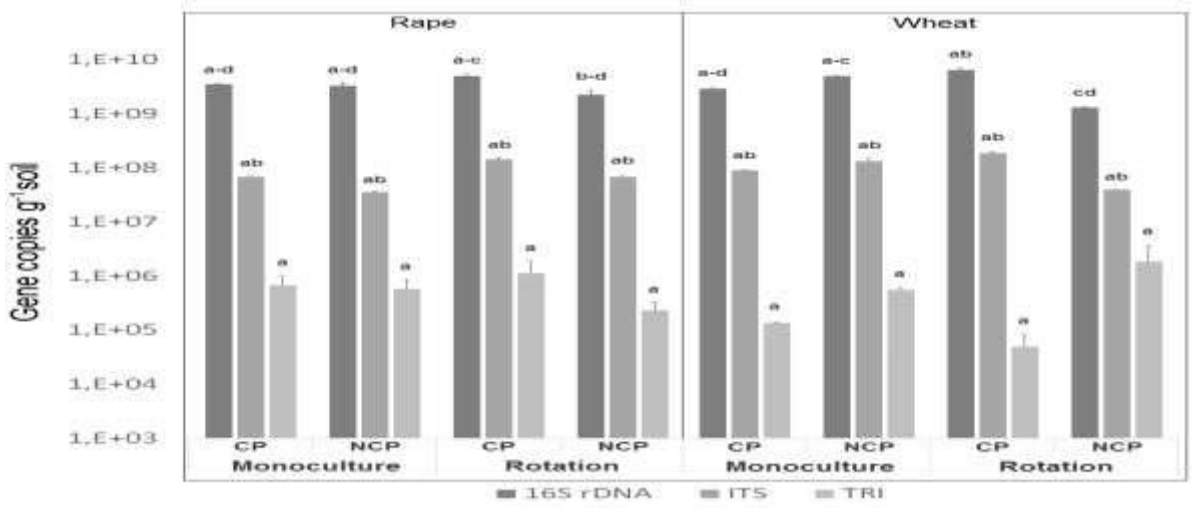

Figure 2. Size of bacterial (16S rDNA gene-dark grey column), fungal (ITS sequence-grey column) and DON-producing *Fusarium* (TRI gene-light grey column) populations quantified by qPCR (CP - chemical protection; NCP - no chemical protection).

In the present study, qPCR revealed the highest number of bacterial genes. The proportion of fungal genes was approximately 25% lower (Fig. 2). In a study by Perez-Brandan *et al*. (2014), the crop sequence significantly influenced the number of isolated fungi which was higher in the corn-soybean rotation than in the respective monocultures. In the above study, bacterial counts did not differ significantly between monocultures and crop rotations. Cereal monocultures are characterized by a higher risk of infections caused by *Fusarium* fungi and mycotoxin contamination of grain (Bateman *et al*. 2007). Crop rotations can decrease the prevalence of infections caused by pathogens of the genus *Fusarium* (Stephens *et al*. 2008). In this study, the number of TRI5 genes was approximately 20-50% lower than the number of bacterial genes in each treatment (mean: $\log_{10}$=5.9), and no significant differences were found between treatments. In winter oilseed rape, the number of TRI5 genes was approximately three-fold lower in crop rotation than in monoculture, whereas the reverse was observed in winter wheat (Fig. 2).

Conclusions

Monocultures and crop rotation systems have no significant effects on the counts of soil bacteria, and do not lead to a significant decrease in the abundance of *Fusarium* fungi. Bacteria of the genus *Azotobacter* were eliminated in crop rotation, which indicates that they are sensitive to the crop sequence.

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FUNGI ACCOMPANYING CULTIVATION OF SPRING BARLEY DEPENDING ON DIFFERENT FERTILIZATION

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Abstract

Agrotechnical treatments and plant protection are shaped by communities of soil microorganisms and colonies of crop plants. The quantitative and qualitative structure of soil mycobiota as well as the severity of disease symptoms depend on the potential of the perpetrator, variety resistance, weather conditions and fertilization. Therefore, research was undertaken to find out the quantitative and qualitative structure of the population of fungi associated with the cultivation of spring barley. The analyzed factors of the experiment were the effect of mineral fertilization in various NPK and mineral doses with the application of manure to the soil fungus community associated with the cultivation of spring barley and the identification of fungi isolated steam base. On the basis of the research, it was found that mineral fertilization supplemented with organic stimulates the number of soil microorganisms. The strongest increase in their number is associated with the application of manure together with NPK at a dose of 120/60/120 kg/ha and 40 kg/ha of Mg, while the introduction of calcium in this dose of NPK limits the soil fungi. The use of only mineral fertilizers stimulates the development of pathogens in the soil and inhibits fungi with antagonistic features, especially at doses of 120/60/120 and 120/60/120/40 kg/ha NPKMg. At this dose of NPK, after supplementation of fertilization with manure, there was an increase in the fusarium severity of foot rot, and a high dose of nitrogen (180 kg ha) along with organic fertilization stimulated the severity eyespot of cereal. In the soil fungus group, saprotrophs dominated: *Aspergillus*, *Penicillium*, *Chaetomium* and pathogenic species: *Fusarium*, *Rhizoctonia*, *Botrytis*.

Keywords: *Spring barley, fungi, fertilization*

Introduction

Soil microorganisms may co-determine the quantity and quality of crops. Knowing the interactions between microorganisms with various ecological traits, including pathogens and saprotrophs, that colonize agrocenoses may contribute to the development of an effective strategy for crop protection. Identification of the correlations between pathogens and saprotrophs affords conditions for the implementation of guidelines for integrated plant production and protection. Promoting the growing conditions wherein natural feedbacks in a microorganism community support antagonists could affect a reduction in counts of pathogens (Lenc, 2008). Soil microorganisms constitute 85% of biomass of organisms inhabiting this environment and influence many soil-forming and soil-protecting processes as well as processes which ensure the dynamics of food chain links in the agroecosystem. The rhizosphere and root hair of cereals being at early stages of development are colonized by low numbers of microorganisms, but colonization rate increases at the flowering stage. In the rhizosphere, unlike in the lower soil strata, these communities are significantly more diversified. Often, their quantitative and qualitative potential is correlated with organic remains distributed in the soil. While roots begin to decay, the re-growth of a microorganism population is observed around them, including particularly saprotrophs which are typical of the decomposing plant residues (Gregory, 2006). It is essential to keep a dynamic balance

between saprophytes and pathogens, which may be ensured by optimal fertilization and good practice of using plant protection agents. However, these measures should not disturb the qualitative and quantitative structure of microorganisms which are components of, e.g., mycorrhizas (Johansson *et al*., 2004) and affect the biometry of cultivable plants in the growing period as well as the quality and yield of crop. Dick and Tabatabai (1992) and Acosta-Martinez *et al*. (2007) have proved that organic fertilization had a more beneficial effect on the biological activity compared to mineral fertilization which improves the physicochemical parameters of soil. It needs to be remembered that nitrogen has the greatest impact on plant yield and growth; it affects both the qualitative and quantitative traits of the crop. Positive outcomes of plant fertilization with nitrogen include, primarily, an increased number of spikes and also an increased grain weight per spike, which is a very important yield-increasing factor (Liszewski 2008). When applying nitrogen as a fertilizer, consideration should be given to its form, e.g. in the ammonia form it may reduce the prevalence of *Gaeumannomyces graminis*, whereas in the nitrate form it promotes pathogen development (Kurowski *et al*., 1990). Fertilization may also influence microorganisms antagonistic to pathogens. Considering multi-faceted interactions in the natural environment compared to the in vitro conditions, including the C:N ratio in soils, contents of phosphorus and carbon in soils, their humidity and pH, temperature and UV radiation, and presence of other microorganisms, many authors have indicated no antagonistic traits in representatives of the *Trichoderma* genus against pathogens (Tanaka *et al*., 2006; Kredics *et al*., 2003) and claimed antagonist's effectiveness to be suppressed by the presence of other biological control agents and abiotic factors (Nielsen *et al*., 2014).

Therefore, it seemed advisable to identify the community of fungi appearing during cultivation of barley which is used for various purposes in many European countries even though it does not dominate in the crop structure.

Material and Methods

The experiment was conducted in the years 2015-2016 in Bałcyny. It was established in the random block design with four replications on typical grey-brown podsolic soil originating from light loam (class IIIa in the soil valuation system, very good rye complex). The experimental factors included: (1) types of fertilization: mineral (N) and mineral with manure (M), and (2) doses of mineral fertilizers NPK (1-8: 0/0/0; 60/60/60 ;120/60/60; 180/60/60; 120/60/120; 120/60/180; 120/60/120/40 (Mg);120/60/120/40/2500 (Mg/Ca).

Soil samples were collected with a sterile small shovel from each plot, from of a depth of ca. 15-20 cm close to plants of spring barley of Justina cultivar, at the 30-33 BBCH growth stage. Next, they were transferred onto sterile Petri dishes and transported to the laboratory. Afterwards, bulk samples from respective experimental treatments were prepared under sterile conditions.

The mycological analysis of soil fungi was carried out following a modified method of Mańka (1974). Quartz sand (149 g) was poured to 500-mL conical flasks. The sand was earlier sterilized in an autoclave, under the pressure of 125 atm for 25 min. Once the substrate has been cooled, 1 g of the analyzed soil form the bulk sample made of four replications of a given experimental treatment was added under sterile conditions to the flask, and flask content was mixed by shaking for 20 min. Afterwards, 25 mg of the soil mixture with quartz sand were collected and transferred onto Petri dishes. Thus prepared material was poured with cooled Martin-Johnson medium with rose bengal and streptomycin. Antibiotic addition was expected to inhibit the development of bacterial colonies on the culture medium. After 5 days of incubation in a thermostat at a temperature of 24ºC, colonies of fungi grown on the dishes were inoculated onto potato dextrose agar (PDA). The health status of stem bases was evaluated at the 87 BBCH stage of development using a 3-degreePonchet's scale modified by

Mackiewicz and Drath (1972). Severity of infections was presented in the form of the infestation index computed acc. to the method of Towsend and Heuberger (1943). Fungi were isolated from stem bases following the methodology developed by Martyniuk and Myśków (1983). Segments of stems (1 cm) with disease symptoms were excised from the collected material, rinsed and disinfected in 50% ethyl alcohol and 1% sodium hypochlorite. After 3-fold washing with sterile water, the fragments were placed onto solidified PDA medium. After a few days, the growing fungal cultures were re-inoculated on slants with PDA media and their species composition was determined based on morphotyping. Statistical calculations were based on the Tukey's test p = 0.05.

Results and Discussion

The macroscopic assessment of stem bases revealed no symptoms of take-all of cereals and showed that their infestation by *Rhizoctonia. cerealis* and *R. solani* was low and ranged from 0.5% to 3.0% in the treatment without organic fertilization. The lowest value of the infestation index was determined on the control plot and the highest one on the plot where spring barley was fertilized with the addition of calcium. In the treatment with organic fertilization, the severity of sharp eyespot (*Rhizoctonia* spp.) ranged from 0 to 2.5%, however its symptoms were not observed upon calcium application. The assessment revealed also various severity of the eyespot of cereals. In the experimental treatment with mineral fertilization, the value of the infestation index ranged from 14.0 to 41.5%. The mean severity of the eyespot of cereals reached 27.6%. The lowest infestation by *Oculimcula. yallundae* was reported on the plots with double dose of potassium and with nitrogen dose of 120 kg/ha, whereas the highest one – on plots with minimal doses of potassium and phosphorus, and with nitrogen dose of 120 kg/ha. While evaluating the health status of cereals, attention should be paid to weed control, because the presence of weeds may affect the presence of sources of infection in which the undesirable plants are vectors (Burgieł, 2002). It is also essential to diagnose various types of *O. yallundae*, which as indicated by literature data, differs in the growth rate. Type R develops slowly, whereas type W develops faster. A higher prevalence of type W is noted in Poland, whereas a higher prevalence of type R – in Europe. Today, molecular diagnostics enables even more in-depth evaluation of their pathogenicity (Nicholson *et al*., 1995; Głazek. 2002:). Organic fertilization stimulated the appearance of symptoms of the eyespot of cereals; the infestation index reached 26% on the control plot and was the lowest compared to the other experimental treatments. The strongest spring barely infestation with *O. yallundae* was observed in the treatment in which nitrogen was administered at the highest dose: 180 kg/ha. Fusarium take-all disease was the most severe among all diagnosed diseases. The mean Ip(%) values in the treatment with mineral fertilization accounted for 48.8% and in that with organic fertilization – for 58%. The highest percentage of infested stem bases (Ip(%)=57%) was demonstrated in the treatment including mineral fertilization with magnesium application. In this treatment, the lowest infestation by fungi from the genus *Fusarium* was observed on the control plot, but still it reached as much as 43.5%. Very high severity of the fusarium take-all disease was noted in the treatment involving mineral fertilization NPK 120/60/120 kg/ha coupled with the application of manure. Likewise in the combinations without manure, in this treatment the least severity of this disease was noted on the control plot. The application of magnesium and calcium did not promote health condition of plants. Experiments conducted by other research centers have proved the health status of cereals to depend on nitrogen doses. It's content in fertilizers below 100 kg/ha stimulates *Fusarium*. Pląskowska *et al*. (2002) demonstrated an increase in *F. culmorum* count at this dose of nitrogen, whereas at 200 kg N/ha she reported a high contribution of *F. avenaceum* in the fungi population. Laboratory analyses demonstrated 20 taxa of fungi, among which predominating were pathogens and fungi with weak infectious traits which pose threat to crop yield only under conditions of

plant debilitation. The most often isolated species included numerous representatives of the genus *Fusarium*, among which the highest prevalence was found for: *F. culmorum*, *F. croowellense* and *F. poae*. Also *Cochliobolus sativus* was identified among pathogens, which infested more often the stem base of barley treated only with mineral fertilizers. A study carried out by Baturo (2002) indicates that this pathogen more frequently colonizes plants from bio-cultures than these from the conventional system of cultivation. Detrimental effects of this species were emphasized by Agarwal and Sinclair (1997) who reported that seed dressing is recommended in Norway when the percentage of infested barley kernels exceeds 10% in the case of infestation with *B. sorokiniana* (*C. sativus*) and 25% in the case of infestation with fungi from the *Fusarium* spp. genus. An overview of literature data indicates that *B. sorokiniana* is a factor which deteriorates crop productivity and crop quality when high temperatures and humidity occur in May and values of weather parameters decrease in the subsequent month of the growing period. This dangerous pathogen may constitute a reservoir of infections in the subsequent season, as it survives winter in the form of mycelium on plant residues. In our study, we observed dark necrotic spots which are outcomes of the activity of genes responsible for the synthesis of helminthosporal, polygalacturanase, cellulase, and pectinesterase, which effectively penetrate the cell wall of a host and contribute to hydrogen peroxide production, which ultimately leads to the appearance of black spots of the necrotic cereal tissue. This type of fertilization tangibly promoted the population of fungi with antagonistic traits: *Trichoderma*, *Gliocladium*, and *Penicillium*. Results of our experiment demonstrate that the prevalence and qualitative diversity of taxa are determined by fertilization and doses of mineral fertilizers. A total of 262 isolates were obtained from the soil samples, among which predominating were soil fungi originating from barley culture treated with mineral fertilizers and manure (tab. 1).

Tab. 1 Taxons composition of soil microorganisms

fungi	**Treaments**																**Sum**
	1		**2**		**3**		**4**		**5**		**6**		**7**		**8**		
	N*	**M****	**N**	**M**	**N**	**M**	**N**	**M**	**N**	**M**	**N**	**M**	**N**	**M**	**N**	**M**	
Acremoniella atra (Corda)	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	2
Alternaria alternata (Fr.) Keissler	1	0	1	3	1	0	0	3	0	0	0	0	0	0	0	0	9
Aspergillus spp. (P. Micheli ex Haller)	0	0	2	1	0	2	1	0	0	0	2	20	0	37	0	0	65
Aureobasidium bolleyi (R. Sprague)	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	3
Aureobasidium pullulans (de Bary & Löwenthal)	1	0	1	0	1	0	0	0	1	0	0	0	2	0	0	0	6
Botrytis cinerea (Pers.)	0	0	1	0	0	3	0	0	1	1	0	0	1	3	0	0	10
Chaetomium spp.	4	0	1	0	1	0	0	0	0	0	2	0	0	3	0	0	11
Cladosporium cladosporioides (Fresen.)	0	0	2	0	2	0	0	0	0	1	0	0	0	0	0	0	5
Fusarium sp.(Link)	4	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	7
Fusarium avenaceum (Fr.) Sacc	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Fusarium culmorum (Wm.G. Sm.) Sacc	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2
Fusarium oxysporum Schlecht.	0	8	0	0	0	0	0	1	0	0	0	0	0	0	4	0	13
Fusarium poae (Peck) Wollenw.	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2
Fusarium solani (Mart.) Sacc.	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2
Gliocladium roseum (Bainier)	0	0	0	2	0	1	0	1	0	1	0	0	0	3	0	0	8
Cochliobolus sativus (S. Ito & Kurib.)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Humicola fuscoatra (Traaen)	3	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0	7
Humicola grisea (Traaen)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Mortierella isabelina (Oudem.)	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	2
Mucor spp. (P. Micheli ex L.)	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Oidiodendrom cerealis (Thüm.) G.L. Barron	0	0	0	0	0	0	2	0	1	0	0	0	1	0	0	0	4
Paeciliomyces spp. (Thom) *Samson*	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Penicillium sp. (Link)	2	30	2	2	1	9	2	0	0	1	1	0	0	0	3	0	53
Periconia macrospinosa (Lefebvre	1	0	1	0	2	0	0	0	1	0	1	0	0	0	0	0	6
Phoma spp.(Fr.)	0	0	0	0	0	1	0	0	0	5	0	0	0	0	0	0	6

Phomopsis spp. (Sacc. & Roum.)	0	0	0	7	0	0	2	0	0	0	0	0	0	0	0	0	9
Pythium ultimum (Ann. Bot., Lond.)	1	0	0	0	1	0	1	0	1	0	1	0	1	0	0	0	6
Rhizoctonia solani (J.G. Kühn)	0	6	0	0	0	1	1	2	0	0	1	0	2	0	0	0	13
Trichoderma harizanum (Rifai)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Verticilium sp.(Nees)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Total	19	50	14	16	10	17	10	7	9	10	12	20	13	46	8	1	262

N*- mineral fertilization, M**- mineral and manure fertilization

They represented 63.7% of all isolates and their number exceeded almost twice the number of fungal isolates obtained from the soil not treated with manure during spring barley cultivation. 95 isolates were obtained from the soil treated only with mineral fertilizers, which constituted 36.2% of the total pool of microorganisms. The lowest pool was achieved in the treatment in which soil was treated with mineral fertilizers including magnesium and calcium and with organic fertilizer. Simultaneously, as many as 46 isolates were obtained from the soil not treated with calcium, which is indicative of the inhibitory effect of this element on the development of soil microorganisms. Literature data demonstrates that an increase in nitrogen dose compared to potassium dose may contribute to cereals lodging, stimulates threat posed by pathogens, and ultimately leads to the loss of crop. In addition, experimental plots on which soil was treated with calcium and with only mineral fertilizers were characterized by decreased population numbers in the microorganism community. A similar trend of microorganism count increase upon the use of manure was demonstrated upon NPK and Mg treatment at 120/60/120/40 kg/ha during spring barley cultivation. This experimental combination also contributed to the highest count of fungi population among all plots treated only with mineral fertilizers compared to all other treatments with mineral fertilization. Small differences in counts of microorganisms under the influence of the applied fertilizers were demonstrated in treatments with the same doses of phosphorus and potassium (60 kg/ha) and with nitrogen used in doses of 60 and 120 kg/ha. The highest counts of potentially saprotrophic fungi were determined in the samples of soil treated with organic fertilizer (75 isolates). A lower contribution of saprotrophs was noted upon mineral fertilization (48 isolates) compared to the treatments with manure. The quantitative structure of pathogens proves small differences in this ecological group of microorganisms compared to the prevalence of saprotrophs and fungi with antagonistic traits. Higher counts in the fungi community were determined for these capable of decomposing dead organic matter, their higher prevalence was recorded particularly after manure application together with mineral fertilizers compared to the population of saprophytic fungi isolated from the soil treated only with mineral fertilizers. Almost three times higher prevalence was determined for the fungi potentially antagonistic to pathogens of cultivable plants in the treatment involving the use of manure. The number of isolates obtained from soil samples after mineral fertilization reached merely 14, whereas that obtained after manure application increased to 58 isolates. Among the antagonists, we identified the genus: *Gliocladium*, *Trichoderma* and *Penicillium*, but only in the treatment with manure application. In this treatment, also *Penicillium* genus was prevalent, whereas *Trichoderma harzianum* was detected in only one isolate and originated from the soil treated only with mineral fertilizers. The analysis of fungi community in particular treatments of spring barley culture with mineral fertilization at the following NPKMg doses: 120/60; 120/40; 60/120/40 60/120 kg/ha and with manure application revealed the preponderance of saprophytic fungi over the other ecological groups of fungi. An important recommendation for fertilization in the agricultural practice may be the application of manure to soil together with NPK in 120/60/180 doses, which in our study ensured no pathogenic fungi in the analyzed samples. In the control plot without organic fertilizers administered during spring barley cultivation, the prevalence of fungi antagonistic to pathogens turned out to be the highest. An overview of literature indicates that soil

microorganisms are classified into three groups: these having a positive effect on plants, these with neutral effect, and these eliciting adverse effects. A balance occurs between these three groups in the rhizosphere in natural or minimally transformed ecosystems (Nannipieri et al., 2003; Paul *et al*. 2000). The inhibiting effect of the plant growth-promoting rhizobacteria (PGPR) has been confirmed in the case of the following pathogens: *Verticillium* spp., *Sclerotium rolfsii*, *Thielaviopsis basicola*, *Pythium* spp., *Rhizoctonia solani*, *Phytophthora* spp., *G. graminis*, *Aphanomyces* spp., and *F. oxysporum* (Kloepper *et al*., 1999). In our experiment, the use of as high nitrogen dose as 180 kg per ha coupled with manure application resulted in a low prevalence of the pathogenic fungi in the soil, which increased at nitrogen doses of 60 and 120 kg per ha. This indicates that the poorly fertilized plant may be more often infected by soil pathogens, whereas at high nitrogen doses the assimilation apparatus of plants responds in making epidermis cells more delicate, thereby allowing the pathogens to easier penetrate host tissues. An equilibrium between the groups of microorganism with a tendency for an increased prevalence of fungi displaying antagonist traits was achieved in the treatments with a high dose of nitrogen coupled with calcium application. It needs to be emphasized, however, that in this group of soil samples from all plots the pathogens represented a more numerous group of microorganisms than their antagonists did, and that no microorganisms competitive to pathogenic fungi were identified in the treatment with magnesium application at 40 kg/ha and in the treatments with NPK doses of 120/60/120 kg/ha. Considering the biological activity of soil, the balance between groups of microorganisms colonizing the microrhizosphere may ensure soil fertility. The biological equilibrium is achieved in agroecosystems through optimal fertilization as well as rational, integrated use of crop protection agents (Johansson *et al*., 2004).

In summary, it needs to be emphasized that the quantitative structure of microorganisms in the soil and the activity of metabolites they synthesize, including enzymes and antibiotics, are determined many factors like e.g. water-air regime, soil pH or organic matter content. These, in turn, constitute a flexible model modifiable by the type of tillage including agrotechnical measures with fertilization treatments. The optimal mineral fertilization has a positive impact on the productivity of plants and their health status in the growing period, which ultimately determines grain quality. Knowing the pool of microorganisms accompanying the cultivation of agricultural crops is an element necessary to establish soil abundance in microorganisms posing potential threat to the quantitative and qualitative structure of crops and these which play the role of natural biostimulants of plants by improving their productivity. Analyses of the biological activity of soil are part of an integrated methodology for plant protection against pathogens.

Conclusions

Results of the study addressing soil fungi contaminating spring barley crop demonstrate that mineral fertilization coupled with organic fertilization stimulates counts of soil microorganisms. The greatest increase in their count occurred upon the use of manure together with NPK at a dose of 120/60/120 kg/ha, which additionally stimulated the severity of fusarium take-all of cereals. The application of calcium at this dose of NPK reduced the pool of soil fungi. Introducing only mineral fertilizers to spring barley cultivation stimulated pathogens development in the soil and inhibited the growth of fungi with antagonistic traits, particularly at the following doses: 120/60/120 kg/ha NPK and 120/60/120/40 kg/ha NPKMg. The soil fungal community was predominated by saprotrophs: *Aspergillus*, *Penicillium*, *Chaetomium*, and pathogenic species: *Fusarium*, *Rhizoctonia*, and *Botrytis*. Negligence of fertilizing measures and using only mineral fertilizers with calcium in the agricultural practice leads to the accumulation of pathogenic fungi in the soil and may deteriorate the health status of spring barley. The predominating symptoms of foot rot of spring barley Justina cv. were the take-all disease and eyespot of cereals. Fungi of the genus *Rhizoctonia* were of marginal

significance to the health status of spring barley. The high dose of nitrogen (180 kg/ha) coupled with organic fertilization increased eyespot severity. The predominating fungi among the isolates obtained from spring barley stem base included: *F. culmorum*, *F. croowellense*, *F. poae*, *A. alternata*, and *Gliocladium roseum*. The application of natural fertilizers stimulated the quantitative and qualitative structure of fungi colonizing the stem base of spring barley.

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THE POSSIBILITY OF APPLYING *BACILLUS* SP. SP-40 IN THE CONTROL OF *FUSARIUM* FUNGI

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Abstract

Biological control is an increasing group of plant protection methods. In comparison to fungicides, the biological control agents have a weaker impact on the natural environment. Among the various methods, the usage of antagonistic bacteria is of the outmost importance in ensuring protection against phytopathogenic fungi. The beneficial strain of bacteria can inhibit the growth of phytopathogens by using several strategies and may promote the growth of plants it colonizes. The aim of this study was to determine i) evaluation of multi-trait plant growth promoting parameters of *Bacillus* sp. SP-40; ii) the antagonistic action of used strain on *Fusarium* spp. Growth on selective medium, dual-culture test, and PCR identification of gene response to fungistatic substance production were performed to enable detection of antagonistic traits of *Bacillus* sp. SP-40. Relationships between antagonist, pathogen and plant were examined as well. Study results showed the antagonistic effect of the tested strain against *Fusarium culmorum* and *F. oxysporum* on appropriate growth media. Chitinolytic and cellulolytic activities of *Bacillus* sp. SP-40 were determined either on the selective medium. The ability of fungistatic metabolite production (surfactin, bacilysin, fengycin, bacillomycin and iturin) was affirmed by PCR. The antagonistic strain grew at 10 to 37°C, pH 5 to 9, and NaCl 5% and was resistant to $CuSO_4$ and common fungicides, insecticides and herbicides. The fluorescence microscopy analysis demonstrated that the antagonistic strain colonized *Fusarium* mycelium (hyperparasitism). Results of water-agar test showed that wheat kernels inoculated with the *Bacillus* strain were more resistant to *Fusarium* spp., and seedlings had improved health status and biometric properties. In conclusion, *Bacillus* sp. SP-40 application caused significant inhibition of *Fusarium* spp. growth on both culture medium and wheat seedlings.

Keywords*: Biocontrol, antagonistic bacteria, wheat*

Introduction

Soil is an environment inhabited by organisms being important in ensuring the proper functioning of an ecosystem. Soil microorganisms are strongly associated with the metabolism of the organic and mineral matter and with the circulation of elements in the natural environment, hence it is essential to preserve their biodiversity. An agroecosystem is an important element of nature transformed by man and its task is to provide food to the global population. However, the use of chemical agents and too dynamic plant production contribute to soil degradation and to impairment of biochemical and biological processes, which results in depletion of both biodiversity and numbers of microorganisms (Heijiden *et al*., 2008; Hinsinger *et al*., 2009).

An interesting research issue is the exploitation of microorganisms for plant growth stimulation and for plant protection against agrophages. Bacteria used to this end develop at various temperatures and remain active even at a temperature of 4°C. They produce lytic enzymes, like e.g. cellulase, lipase and protease, which are indicative of their effective organic matter degradation and nutrients redistribution, as well as of their potential to degrade undesirable microorganisms. Ecological preparations have recently become common on the

market of plant protection agents and fertilizers. Bacteria enhancing the growth of plants are referred to as plant growth-promoting bacteria (PGPB) or plant growth-promoting rhizobacteria (PGPR). These microorganisms develop in soil, rhizosphere and rhizoplane by entering into symbiotic reactions with plant root, and display a variety of properties, like e.g.: root growth stimulation, enhancement of nutrients uptake, production of phytohormones, secretion of siderophores, and synthesis of antibiotics, enzymes and substances which minimize negative outcomes of the biotic and abiotic stress. Some of the PGPRs are also commonly used in biocontrol of phytopathogens (Ahemad and Kibret, 2014; Beneduzi *et al*. 2014).

Bacteria promoting plant growth and enabling biocontrol are representatives of the following genera: *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Rhizobium*, and *Serratia*, but this group may be significantly larger (Ahemad and Kibret, 2014).

Recent works have reported on the strong effect of bacteria from the *Bacillus*, *Pseudomonas*, and also *Actinomycetes* genera on the stabilization of soil equilibrium, particularly in natural ecosystems. These bacteria produce multiple active substances which display strong fungistatic activity and, therefore, can be used as natural plant protection agents (Ayuso-Sacido and Genilloud 2004; Svercel et al., 2009; Mora *et al*., 2011) Production and application of such antibiotics is difficult, hence the most effective method is to use commercial strains of PGPB which not only promote plant growth but are also effective in minimizing the incidence of crops diseases. The aim of this study was to investigation of potential biological control of *Fusarium* sp. by antagonistic strain of bacteria that possesses many traits of plant growth promotion.

Material and Methods

Collection of soil samples and isolation of microorganisms

One kilogram of soil was collected from a stand where flaxseed was cultivated in a monoculture (48 years). The sample was transported to the laboratory and subjected to further handling procedures. First, its serial dilutions were prepared (from 10^{-1} to 10^{-10}). Then, bacteria were isolated from each dilution by inoculation onto a solid culture medium (tryptic soy agar – TSA) and incubated at 28°C for 48 h. Multiple microorganisms were isolated and their colonies displayed various morphological traits. By using methods described below, one strain which displayed the strongest antagonistic potential against *Fusarium* was isolated from this material. Its affiliation to the genus *Bacillus* was confirmed by 16S rDNA sequence analysis conducted as described in our previous work (Przemieniecki *et al*., 2018).

Determination of the antagonistic potential – dual culture

The experiment was conducted on a PDA (potato dextrose agar) medium by inoculating the culture of the tested bacteria and a phytopathogenic fungus (*Fusarium culmorum* or *F. oxysporum*) 3 cm away from each other. Thus prepared cultures were incubated at 28°C for 72 h, then mycelium growth was measured and expressed in percentage relative to the control (i.e. sterile demineralized water instead of the bacterial suspension).

Determination of selected traits promoting plant growth.

Chitinase – capability of bacteria to produce chitinase was analyzed in a culture medium composed of: monopotassium phosphate – 1.0 g, magnesium sulfate (VI) – 0.5 g, sodium chloride – 0.5 g, iron (II) sulfate (VI) – 0.01 g, manganese sulfate (VI) – 0.01 g, ammonia nitrogen — 0.3 g, agar – 15 g, and colloidal chitin – 1 g (Roberts and Selitrennikoff, 1988).

Cellulase – capability of bacteria to produce cellulase was determined on a culture medium composed of: monopotassium phosphate – 0.5 g, magnesium sulfate (VI) – 0.25 g, cellulose – 2 g, agar – 15 g, and Congo-Red – 0.2 g (Lu *et al*., 2015).

Phosphate solubilizing bacteria (PSB) – these bacteria were examined on the Pikovskay's medium composed of: glucose – 10 g, calcium phosphate – 2.5 g, ammonia sulfate (VI) – 0.5

g, sodium chloride – 0.2 g, potassium chloride – 0.2 g, yeast extract – 0.5 g, magnesium sulfate (VI) – 0.002 g, and iron (II) sulfate (VI) – 0.002 g (Nautiyal, 1999).

Assessment of activity of *Bacillus* sp. SP-40 depending on culture medium

The assessment was carried out by inoculating an overnight culture onto the TSA medium (tryptic soy agar) and its incubation at 28°C for 48h. Afterwards, colony growth potential (size, density, thickness) was evaluated compared to the control culture (TSA medium, pH~7). Colony growth was tested in the culture medium with pesticide supplementation (dose recommended by the manufacturer), NaCl concentrations of 1-7%, pH values of 5-9, and temperature ranging from 4 to 37°C (Przemieniecki *et al*., 2018).

16S rDNA sequencing

Whole DNA was isolated using a Genomic Mini AX Bacteria Spin (A&A Biotechnology), as described in our previous work (Przemieniecki *et al*., 2018). The isolated DNA was sent to an external company which performed the sequencing procedure (starters 27F-1492R).

Detection of genes responsible for the production of fungistatic substances

Genes were detected with the polymerase chain reaction (PCR) method (Mora *et al*., 2011 with modifications by Zhao *et al*., 2014) using the following starters:

Surfactin
SRFAF (TCGGGACAGGAAGACATCAT),
SRFAR (CCACTCAAACGGATAATCCTGA);
Bacilysin
BACF (CAGCTCATGGGAATGCTTTT),
BACR (CTCGGTCCTGAAGGGACAAG);
Fengycin
FENDF (GGCCCGTTCTCTAAATCCAT),
FENDR (GTCATGCTGACGAGAGCAAA);
Bacillomycin
BMYBF(GAATCCCGTTGTTCTCCAAA),
BMYMR (GCGGGTATTGAATGCTTGTT);
Iturin
ITUCF (GGCTGCTGCAGATGCTTTAT),
ITUCR (TCGCAGATAATCGCAGTGAG).

Fluorescent microscopy

Microscopic imaging was conducted with an epifluorescence microscope (Carl Zeiss). Visualization was enabled by Acridine Orange (Sigmal Aldrich). Observations were made for the mycelium grown in the dual culture assay. Images taken were subjected to processing which had no effect on the quality of results.

Seed experiment

The analyzed strain was used to dress seeds of spring wheat. Pots (120 mL) were filled with 50 mL of an aqueous agar (1%) contaminated with spores of *F. culmorum* and *F. oxysporum* with a density of 10^6, and then 20 seeds of he spring wheat cultivar Bombona were placed on the agar. Incubation was conducted in a phytotron for 14 days under the following regime: day (25°C, 12h) and night (17°C, 12h), and humidity of 80%. After incubation, analyses were conducted for: number of germinating seeds, plant height, plant colonization by the mycelium, and health status of seedlings. Non-dressed seeds cultured on the medium without fungal spores, and infected culture medium with non-dressed seeds served as the control.

Results and Discussion

The analysis of results of the antagonistic potential revealed that the growth inhibition zone in the dual culture test on PDA medium reached 30 mm in the case of *F. culmorum* and 21 mm in the case of *F. oxysporum*. Results were statistically significant at p=0.005 (very highly significant) compared to the control culture.

Table 1. Selected Plant Growth Promotion Properties of Bacillus sp. SP-40Title of the table*

Determination group	Temperature (°C)					pH			NaCl (%)			P-sol	Chit	Cel
Trait **	4	11	22	28	37	5	7	9	1	3	5	yes	yes	no
Value***	1	2	3	5	4	4	5	3	5	4	2			

* P-sol – Phosphate solubilization, Chit – chitinase activity, Cel – cellulase activity

** in addition the strain was resistant to CuSO4, mancozeb, urea derivatives - linuron and pyretroid-lambda-cyhalothrin

***value of trait: 1-very weak, 2-weak, 3, average, 4-good, 5-excelent

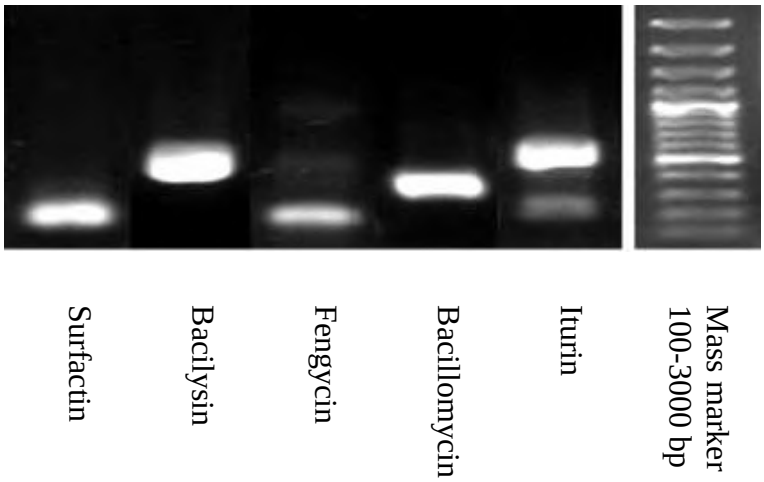

Photo 1. Detection of genes responsible for the synthesis of fungistatic substances

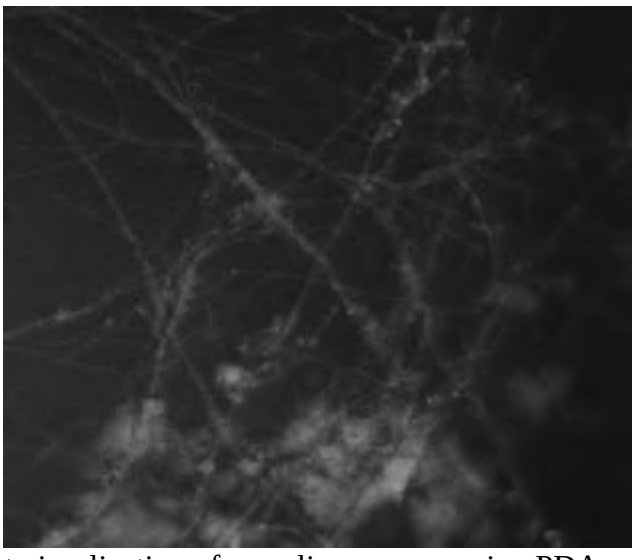

Photo 2. Fluorescent visualization of mycelium overgrowing PDA medium colonized by Bacillus sp. SP-40

Table 2. Length [cm] of seedlings and evaluation of the efficacy of spring wheat seedlings inoculation under the pressure of *Fusarium culmorum* and *Fusarium oxysporum*

Treatment	Phyllosphere	Roots	Infestation
Control for *F. culmorum*	1.0	0.8	5.0
Background	4.8	3.8	0.3
Bacillus sp. SP-40	4.5	2.8	0.5

Parameters of evaluation in the control treatment were statistically significantly different at p=0.05 from these noted for the background and the treatment with inoculated seeds

Treatment*	Phyllosphere	Roots	Infestation
Control for *F. oxysporum*	3.0	3.0	2.0
Background	4.8	3.8	0.3
Bacillus sp. SP-40	4.5	3.5	1.0

No significant differences were found at p=0.05

*0-no germination or dead plant, 1-infected seedling with 75% necrosis, 2-seedling with severe symptoms of infection and 50% necrosis, 3-seedling with severe symptoms of infection and 25% necrosis; 4-seedling with symptoms of infection and necrosis below 10%; 5-completely healthy seedling with potential sporadic necrotic lesions

The analysis of traits describing the physiological parameters of bacteria from the PGPB group (Table 1) demonstrated that the tested bacterium developed at all analyzed temperatures. The poorest development of the test strain was observed at a temperature of 4 ^{o}C, however it still maintained its activity at this temperature. Its best development was observed at 28°C. The test strain displayed good or moderate activity at temperatures of 11, 22, and 37°C. It was also able to grow in the acidic and alkaline medium, and reached optimal growth at pH 7. In addition, it turned out resistant to salinity (even 5% NaCl), but despite preserved activity at the highest tested NaCl concentration, its growth potential was found to decrease significantly. A halo zone was observed on selective media, which was indicative of strain capability for solubilization of inorganic phosphates (making phosphorus available to plants) and for degradation of chitin (production of chitinases). Colony swarming and low susceptibility to plant protection agents confirm this strain's usability in plant cultivation. The tested bacterial strain possessed all genes responsible for the production of fungistatic antibiotics (Photo 1). These assembly of active substances is typical of the bacteria from the genus *Bacillus* spp., which is their additional diagnostic trait. Microscope observations (Photo 2) revealed the ability of test strain cells to colonize *F. culmorum* mycelium along the hyphae. The clustering of microorganisms was increasing towards the mother colony (inoculation site). Culture inoculation with *Bacillus* sp. SP-40 strain significantly reduced seed infestation by *Fusarium culmorum* (improvement by 90%) and by *F. oxysporum* (improvement by 50%). An improvement was also observed in seedling growth in the treatment with the inoculated strain of the test bacteria compared to the control treatment, whereas the length of the aerial part and root of the seedlings did not differ statistically from that of the plants grown from non-inoculated seeds sown on the sterile medium (background). As demonstrated by Zhao *et al*. 2014, the antibiotic-producing *Bacillus subtilis* SG6 strongly inhibited *F. culmorum* development and caused deformations of mycelium hyphae. In addition, its application enabled significant reduction in the severity of Fusarium head blight (FHB) in field conditions as well as deoxynivalenol (DON) degradation. Microscope observations proved that the *B. subtilis* SG6 strain, likewise *Bacillus* sp. SP-40, was attached to fungus surface and colonized spores of *F. culmorum*. Our previous research (Przemieniecki *et al*., 2018) proved that the use

of *B. substilis* in wheat cultivation improved crop productivity and ensured crop protection against pathogens from the genus *Fusarium*. Seed inoculation with *B. subtilis* sp. SP-A9 strain contributed to a significant increase in grain yield.

Conclusion

With the increasing use of chemicals in agriculture, the threat to the natural environment and humans is becoming more and more real. For this reason, a search is underway for novel environment-friendly methods of plant protection and fertilization, like e.g. the application of useful microorganisms. In this study, were proved that the *Bacillus* sp. SP-40 strain isolated from arable soil displayed many traits enabling plant growth promotion and that it well adapted to various environments. Study results demonstrated also its antagonistic potential against very harmful phytopathogens from the genus *Fusarium* and its capability to colonize mycelium hyphae, which is indicative of its hyperparasitic character. The above findings coupled with results of a test indicating improvement in the health status and development of wheat seedlings confirm usability of this strain in biofertilization and biocontrol.

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CURRENT STATUS OF TOMATO LEAFMINER, *TUTA ABSOLUTA* (MEYRICK) (LEPIDOPTERA: GELECHIIDAE) IN ROMANIA

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Abstract

The tomato leafminer, *Tuta absoluta* (Meyrick) (*Lepidoptera:Gelechiidae*) is one of the most damaging invasive moths, threatening the tomato crop worldwide. It originates from South America and it was reported for the first time in Europe, in Spain, in 2006. It was reported even in Norway, in 2017. Having a spreading speed of more than 800 km in a year, *T. absoluta* reached Romanian borders most probably in 2009, when it was first found in Botoșani County. After this first interception, the pest spread in neighbouring counties as Maramures (2009), Bihor (2010), Arad (2010), Ilfov (2010) and Mures (2010). Although sustained efforts have been made to limit the spread and the Plant Protection Authority launched a national monitoring plan in 2011, while the pesticide companies have held numerous farmers' awareness sessions on the high potential of pest damage and the popularization of control measures, *T. absoluta* continues to spread in the main vegetable basins: Hunedoara (2013), Calarași (2011), Ilfov (2013), Galați (2015). According to the recent field reports, the pest started to be present outside protected areas (greenhouses and plastic film tunnels) and affect the open field tomatoes (Paradisverde, 2018). Also, the pest is now affecting and is now infesting sweet pepper, eggplants and other crops and weeds.
In this paper current status of *T. absoluta* in Romania, nine years after its first interception, is presented. Considering the pest adapts very fast to different climate conditions, it is obvious that control measures are imperatives and knowing the real spread in the territory could help Romania to take the appropriate measures.

Keywords: *Tuta absoluta; invasive pest; spreading map; Romania; citizen science.*

Introduction

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has conquered almost 60% of the total area cultivated with tomato, becoming one of the biggest problems for the vegetable producers mainly in Europe and Africa, following its escape from its native area, more than 10 years ago. It is a pest that may destroy the tomato crop completely, in a very short time, due to its extremely prolificacy and high capacity to adapt to various environmental conditions. The European Plant Protection Organisation recommended *T. absoluta* as a quarantine organism and it was included in 2004 in A1 list, as a harmful organisms not known to occur in the EPPO area (EPPO, 2004). After entering Europe (2006) and fast spreading, the pest was transferred to A2 list in 2009 (EPPO Database, 2018). Currently, the pest is spread in more than 30 European countries. In 2016, symptoms of *T. absoluta* attack were observed in Uzbekistan, which led to speculation about its presence in Central Asia (Fayad and Adiga, 2017). A recent publication confirmed the presence of *T. absoluta* in tomato greenhouse in Kyrgyzstan (Uulu *et al*., 2017) while WKN (2017) confirmed it for the first time on the southwest coast of Norway. In 2018, it was found in Lesotho (IPPC, 2018), a country where more than 80% of the territory is located at altitudes above 1800 m and in Tajikistan (Saidov *et al*., 2018).

In Canada, the United States of America, China etc., where the pest is strictly regulated as a quarantine pest, *T. absoluta* was not found until now. The United States banned the import of tomatoes from the countries where the pest is known to occur, including Romania, since 2011 (MADR, 2011).

In Romania, the pest was found for the first time in 2009. According to the National Phytosanitary Authority, *T. absoluta* was first reported in Botoşani County and then in Maramureş County (Leaotă, 2009), while Băeţan et al. (2013a) reported the first appearance in June 2009, in one market in Oradea city (Oradea County), on tomato imported from Spain. In 2010 the pest spread rapidly in Bihor, Arad, Ilfov and Mureş counties (Cean et Dobrin, 2009). In all these cases the pest was detected on tomatoes, exclusively in protected areas. At the same time, *T. absoluta* larvae have been identified on tomato fruits imported from Spain and Turkey, the respective samples coming from Cluj, Covasna and Vaslui counties (Cean and Dobrin, 2009). Immediately after the introduction to a new area, chemical treatments with synthetic insecticides were applied, as an attempt of eradication/containment of the pest. According to Zlof and Suffert (2012), in the countries with high pest pressure, growers have been spraying intensively, with up to 25 sprays per season. Because of the difficulties encountered in stopping the spread of *T. absoluta,* some countries adopted emergency control measures. According to EU notifications received from Member States, as 2008/C159/04, in Spain, until January 2009, farmers could claim financial compensation for control measures against *T. absoluta* in tomatoes, peppers, potatoes and eggplants crops (EU, 2007).

The National Phytosanitary Authority of Romania (National Phytosanitary Agency in 2011), developed and implemented in 2011 the "National Pest Control Plan for *Tuta absoluta*", which includes the monitoring plan and valuable information to prevent new outbreaks and to limit the spread. There is no economic threshold for *T. asboluta,* the treatments being applied immediately after the first adults are caught in the pheromone traps. However, as people were not too aware of the plan and measures, the expected effects were unsatisfactory.

The objective of the present paper is to highlight the level of farmers knowledge related to the *T. absoluta* in Romania, and to update the distribution map of this pest in Romania.

Material and Methods

The paper is based on an extended review of data collected from various internet data bases and social media posts. Also, a four questions online survey was carried out in the period February – May 2018, on a sample of maximum 20000 potential respondents.

The social media campaigne included a questionnaire with four questions about the year of first finding, the place of first finding (open field or protected area), the control measures taken by the farmers, what information would they look for in a control guide for *T. absoluta*.

The email campaigne included the same set of questions, that was sent by email to around 50 people.

The interest about tomato leafminer in the scientific environment, vegetable growers and pesticides supliers was also evaluated.

Results and Discussion

The interest and knowledge about *Tuta absoluta* in the scientific environment

In March 2010, in Maramureş County, at one workshop organised by the Maisadour semences seed company, the Maramureş Phytosanitary Office director, Valer Vele and the engineer Melania Oltean, introduced for the first time farmers with a new pest, tomato leafminer: insect description, spread pathways, control measures that should be taken (Gherman, 2010; TLPlus, 2017). The same phytosanitary agents responded whenever necessary in the written and audio-visual press, to warn the farmers and hobby producers about the risks posed by the pests populations' growth.

The scarce scientific publications seems not to take in consideration the economic importance of the pest. Besides the two first articles published by Cean and Dobrin (2009) and Cean (2011), about the identification and spread of this pest, only two more papers presented the spread of *T. absoluta* in the country. *T.absoluta* was indicated as a new pest for Oltenia region (Mitrea, 2013), on the sandy soils between Olt and Jiu River and in Boiu-Sicuia *et al.* (2017) found the pest in Muntenia region, Giurgiu and Ilfov counties. Băeţan (2015) dedicated a special chapter to this pest in his PhD thesis and also published four articles about tomato leafminer feeding behaviour and the spread of the pest in western Romania (Băeţan *et al*., 2013a, 2013b, 2015a, 2015b). Two teams made researches about the insecticides efficacy: Bratu *et al*. (2015) regarding spinosad, emamectin-benzoate and imidacloprid and Costache *et al*. (2014) regarding Neem saponins, *Quassia amara* and *Quassia amara*+ potassium salt. Around ten articles were published in the national Plant Health review (Sănătatea plantelor), mainly about the control strategies (Dobrin, 2013; Rinichita, 2016; Rosca, 2018).
In 2018 the National Phytosanitary Authority published a guide for the identification and control strategies of tomatoes grown in greenhouses, where a chapter is dedicated to the "Integrated Management Toolkit of the harmful organism *Tuta absoluta* Meyrick".

The interest and knowledge about *Tuta absoluta* of farmers and hobby producers
To determine the interest of farmers in *T. absoluta*, a national survey was designed. Out of ten horticultural and hobby-gardening groups where the questionaire was intended to be published, only six Facebook groups administrators agreed to publish it. From a total of around 20000 possible respondednts, only 8 answered (table 1). 7 answers were received (0,035%) on Facebook, of which three answers were enshuring that no *T. absoluta* was in their crops, in Cluj and Olt counties. On the email campaign, out of 50 emails, only 4 answers were received, which means 8% answer rate. Only one farmer agreed to allow us to monitor the population with a feromonal trap (Russel IPM lures), in March 2018, in a speenach plastic tunnel, in Bucharest (Figure 1).

Table 1. Farmers answers in *T. absoluta* survey on different social-media and email campaigns

Farmer location	**Year**	**Area of growing**	**Crop**	**Control**
Bragadiru (Ilfov)	2012	protected area	tomatoes	feromonal traps, emamectin benzoat 9,5 g/kg
	2015	open field	tomatoes	
	2018	protected area	tomatoes seedlings	
Bucharest	2015	protected area	tomatoes	clorantraniliprol
	2018	protected area	spinach	
Bragadiru (Ilfov)	2012	protected area	tomatoes	feromonal traps
Oradea (Bihor)	2012	protected area	tomatoes	formaline*, rotation
Câmpina (Prahova)	2014	protected area	tomatoes	
Şercaia (Braşov) Organic farmer	2016	protected area	tomatoes	sticky traps, repelent herbs macerate
Novaci (Giurgiu)	2013	hidroponics	tomatoes	clorpirifos metil 225 g/l on foil, DiPel®
Gheorghe Lazăr Ialomita	2013	open field	tomatoes, sweet pepper, egg-plants	clorantraniliprol

*for soil desinfection; source: author's elaboration based on the questionnaire survey results.

We could notice a certain reticence in the approach of the subject "*Tuta absoluta*" among the farmers, for different reasons. The most important could be the fear of rejection or marginalization, which could lead to the impossibility of capitalizing tomato production or tomato seedlings. Another reason might be the misleading informations which were published in a few articles, regarding the status of *T. absoluta* as a quarantine pest. According to this status, by law, it is recommended to destroy the attacked crops and to restrict the tomatoes cultivation in that area.

Regarding the guide for tomato leafminer control, the majority asked for biocontrol measures, the optimum climate condition for the pest and only one about precise treatments scheme with efficient chemical insecticides.

Figure 1. *T. absoluta*- captured adults on pheromone trap (Bucharest, March, 2018)

The interest and knowledge about *Tuta absoluta* in the commercial environment (pesticide suppliers)

The pesticide supliers were very active in disemination, organizing workshops, symposiums, conferences, open days. Few examples are given in table 2.

Table 2. Events organised by pesticides supliers

Organizer/ Place	**Date**	**No. of participants**	**Take-away message**
Marcoser, Braila	November 2014	70 farmers	The spread and losses caused by the pest in Braila are more and more severe
Syngenta, Prahova	December 2016	no data	Pest in all vegetable basins of the country, on tomatoes, potatoes, eggplants, resist up to -18°C.
Cilieni Olt	January 2017	100 farmers	Farmers were interested about *T. absoluta* control
Marcoser, Buzau	January 2017	90 farmers	The farmers were interested in *T. absoluta*, a special sessions was dedicated for the pest
Syngenta	October 2017	no data	Present in all vegetable areas, severe damage in Buzau, Galati and Ialomita counties in 2017
BASF, Galati	February 2018	no data	Presentation of a new portofolio of products against *T. absoluta*

Updated spread map of *Tuta absolut*a in Romania

By gathering all the written data we could access, an approximate distribution map of *T. absoluta* in Romania is presented (Figure 2). There were several other discussions with different consumers, researchers, pesticide supliers persons, in which the presence of the pest was mentioned in other counties, but in the lack of evidence, they could not be taken in consideration for the present map. Instead, these indications will be the basis for the future field inspections.

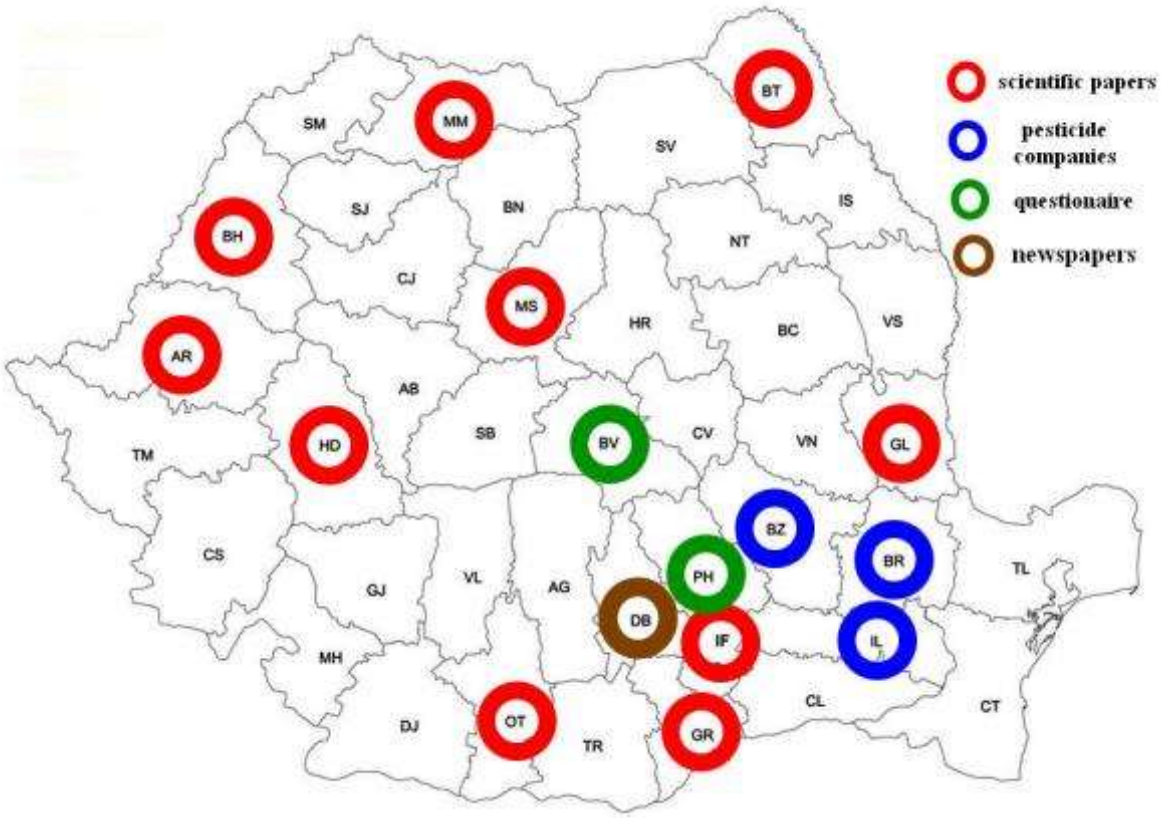

Figure 2. The current distribution of *T. absoluta* in Romania

The Ministry of Agriculture finally acknowledged in August 2018 that the threat of *Tuta absoluta* spread in all country is real and publicly asked for all farmers' involvement in control actions (Ziare, 2018, Agrointel, 2018), due to massive damages that initially started in Buzau, Galati, Ialomita (Agerpres, 2016).

Conclusions

Out of the 42 Romanian counties, the presence of *T. absoluta* was indicated in ten by scientific papers, in three by the pesticides suppliers, in two counties by our citizen science initiative and in one by a newspaper article. The three counties (Cluj, Covasna and Vaslui) where the pest where only intercepted in the import samples were not mentioned in our current distribution map. The few respondents to the e-mail and online groups survey were quite open in providing details about their work and the problems they had encountered with the tomato leafminer. There is some reticence in addressing this topic, fact which might be explained by the fear of losing the production, the future clients and even the crop. The pesticide suppliers were very active in informing farmers about the risk of infestation, pest descriptions, preventing methods, chemical control of the pest. Phytosanitary authorities have developed and implemented the national phytosanitary rules to monitor *T. absoluta* and some local staff (County Phytosanitary Offices) of the National Phytosanitary Authority supports farmers and other people involved in vegetable crops through guides, notifications and phytosanitary warning bulletins. In spite of the efforts made by the National Phytosanitary Authority in order to prevent the introduction and spread of tomato leafminer in the country, the pest continues to raise serious problems in most of our tomato production vegetable basins.

Acknowledgement

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RESEARCH OF TECHNOLOGICAL PROPERTIES OF FLOUR MIXTURES WITH AMARANTH EXTRUDATE

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Abstract

Development of technologies for specialized food products, including functional orientation, is one of the priority tasks of ensuring the quality of life of the population. Bakery products, being a product of mass consumption, can improve the structure of the population's nutrition. In this case, the dosages of the components should not only ensure the improvement of the ingredient composition, but also preserve the traditional organoleptic characteristics. For this purpose, the effect of amaranth extrudate on certain baking and functional-technological properties of flour was investigated depending on the size of the grinding and the ingredient composition of the flour mixtures. Based on the analysis of fat-emulsifying, fat and water binding abilities, the most variable in its application fineness of the grind was determined - 0.125 mm and less. 12 model mixes of flour from an amaranth extrudate and wheat flour of the highest and the first grade with various dosages were made. The optimal dosage is determined from the color flour position. A decrease in the amount of gluten was established with an increase in the mass fraction of the amaranth extrudate in the flour mix and a change in its quality. It is shown that a rational dosage of the enriching ingredient should be established taking into account the initial characteristics of batches of wheat baking flour.

Keywords: *Amaranth, extrudate, protein, functional and technological properties, enrichment.*

Introduction

One of the important tasks of recent relevance to the food industry and the agro-industrial complex of the Russian Federation is the search for and use of non-traditional raw materials in food technologies. Often it has a high content of nutrients - vitamins, minerals, proteins, etc., missing in the diet of the population. One of the representatives of this raw material is amaranth - an annual herbaceous plant with small flowers collected in thick spicate-paniculate inflorescences. More than a few dozen species of amaranth are bred in some countries as an agricultural crop. The average protein content in the amaranth is 16-18%, also it has a high content of phosphorus and calcium, vitamin E and squalene (up to 8%) - antioxidant and anticarcinogenic. Amaranth is widely used in various food technologies [Saratovskiy, 2014], in our studies it is considered as a promising enriching ingredient in the flour group of food products [Lomova, 2017]. One of the most important problems of creation enriched foods is to save its traditional view, smell and taste. This process is quite difficult because of unconventional characteristics of non-traditional raw materials and requires a few studies.

Material and methods

Several varieties of amaranth selection were screened at the Voronezh State Agricultural University and Federal State Scientific Institution and Federal Research Center for Vegetable Growing, and the light-colored grade Universal was identified as the most promising for use in bakery products [Stakhurlova, 2017]. The variety is high in protein (26%), crude fiber, copper and iron. For further processing of raw materials extrusion was chosen, because extruded products are better absorbed by the organism, since in the process of hydrothermoplastic treatment, partial degradation of the polymers of the raw material occurs [Ostrikov, 2003]. Through the laboratory mill flour was obtained with different granulometry

- 1 mm and less, 0.315 mm and less and 0.125 mm and less. Flour is a mass of light cream color with the aroma acquired after extrusion, characteristic of fried foods. To assess the properties of flour used in complex food systems, a number of technological parameters are measured, such as water and fat-binding capacity, fat-emulsifying capacity, mechanical and osmotic properties, structural anisotropy and many others. The key functional properties from the standpoint of compatibility in the formulations of food products are their water-, fat-binding capacity. This is due to the fact that the consumer properties of food systems obtained with the use of extrudates, significantly depend on the content of water and fat in them [Bogatyirev, 1994]. Solubility and water retention are important indicators which can demonstrate the ability of the extrudate to bind water and dissolve in it, characterize its carbohydrate composition, as well as its consumer properties and partial digestibility of the product [Ostrikov, 2003]. The fat-emulsifying ability of the flour was determined by suspending and emulsifying in a centrifuge. The ratio of the volume of the emulsion to the volume of the total system was estimated. To determine the water binding capacity (WBC), the sample with distilled water was centrifuged. The unadsorbed water was drained, the tubes were weighed. WBC was calculated as the ratio of the mass of water bound with flour to the original mass of the latter. One of the factors affecting the appearance of the finished bakery product, in particular, the color of the crumb, is the whiteness of flour. The whiteness is determined by the parts of the grain from which it was ground, as well as the size, the additives introduced, the storage parameters, etc. The higher the whiteness of the flour, the more starch in its composition, thanks to this the baking turns out to be lush and with a uniformly porous, "beautiful" crumb. Such flour is widely used - but contains a minimum amount of fiber, it almost lacks minerals and vitamins. The whiteness of 12 samples of mixtures of wheat flour (high and first grades) with amaranth extruded flour was investigated in order to determine the most suitable bread for baking. The determination was carried out through a laboratory whiteness meter "Blick-R3" in accordance with GOST 26361-2013. The number of automatically measured fields on the flour sample was 10. The amount of gluten was determined by a standardized method (GOST 27839-2013). The quality was examined with the help of a digital deformation meter of gluten, IDK-5M. All measurements were made in triplicate, for the final values ware taken the average ones.

Results and discussion

The results of the studies fat-emulsifying ability are shown in Figure 1.

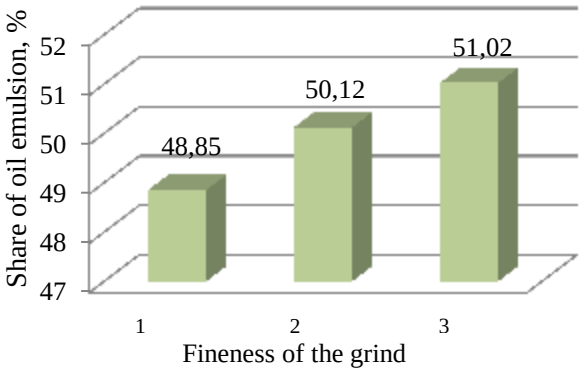

Figure 1 – Fat-emulsifying ability of the amaranth flour with the different fineness of the grind: 1 –1.0 mm and less; 2 – 0.315 mm and less; 3 – 0.125 mm and less

As shown by the results of research, the best fat-emulsifying ability was noted in amaranth flour with a grind size of 0.125 mm and less. That causes the expediency of its use in the formulations of fat-containing semi-finished products, for example, a biscuit or sugar cookie test. The introduction of amaranth flour into model mixtures increases their fat-emulsifying

ability, which can also contribute to the formation of a more porous structure of comfortable confectionery products. The results for determining the fat-binding capacity are shown in Figure 2.

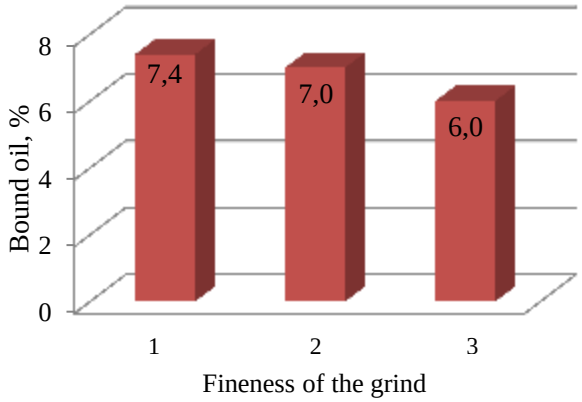

Figure 2 – Fat-binding capacity of the amaranth flour with the different fineness of the grind: 1 –1.0 mm and less; 2 – 0.315 mm and less; 3 – 0.125 mm and less

As the results of the research showed, amaranth flour with a grind size of 1.0 mm and less possesses a higher binding capacity, which makes it possible to recommend its use in bakery recipes with a high-fat content.

The results of WBC are shown in Fig. 3.

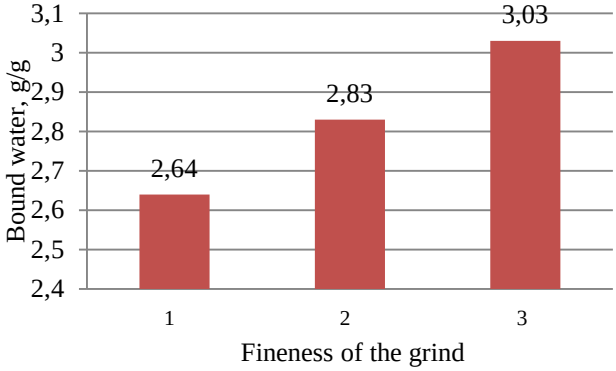

Figure 3 – Water-binding capacity of the amaranth flour with the different fineness of the grind: 1 –1.0 mm and less; 2 – 0.315 mm and less; 3 – 0.125 mm and less

As the results of the research showed that amaranth flour with a grind size of 0.125 mm or less has a better water-binding capacity in comparing with the rest samples, which allows expecting from bakery products with the introduction of amaranth flour of this size improved the shape and causes the expediency of its introduction into molded articles.

Thus, the study of the functional and technological properties of whole-hulled amaranth flour made it possible to draw the following conclusions:

- properties of flour from the extrudate, as well as the direction of its use, depending on the size of the grind;
- flour with a particle size of 0.125 mm and less is advisable to use in formulations of fat-containing flour confectionery and baked bread from high-quality wheat flour;
- flour with a size of 1.0 mm and less has a higher binding capacity, which allows us to recommend it in the technology of products with a high fat content and in shaped bakery products - due to low water binding capacity.

So, in further studies it was decided to use flour with a grind size of 0.125 mm and less. Figure 4 shows the obtained values of whiteness in mixtures of wheat flour of the highest

grade with flour of amaranth extruded with the following dosages: sample No. 1 - control (without amaranth flour); №2 - the ratio of wheat and amaranth flour 95:5; №3 - 90:10; №4 - 85:15; №5 - 80:20; №6 - 75:25; №7 - 70:30.

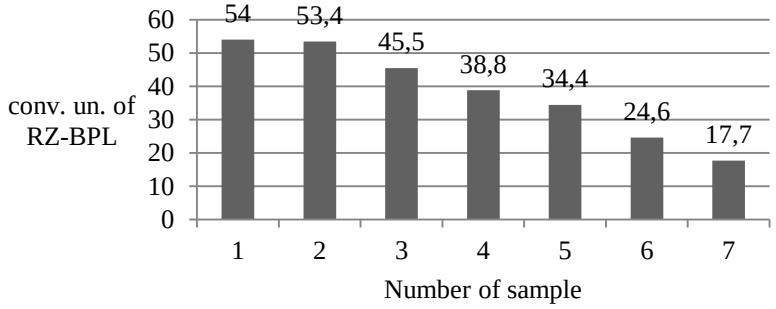

Figure 4 – Results of the study of whiteness in mixtures with wheat flour of the highest grade

Figure 5 shows the obtained values of whiteness in mixtures of wheat flour of the first grade with amaranth flour. The samples have the following relationships: No. 1 - control (without amaranth flour); №2 - the ratio of wheat and amaranth flour 95: 5; №3 - 90:10; №4 - 85:15; №5 - 80:20; №6 - 75:25; №7 - 70:30.

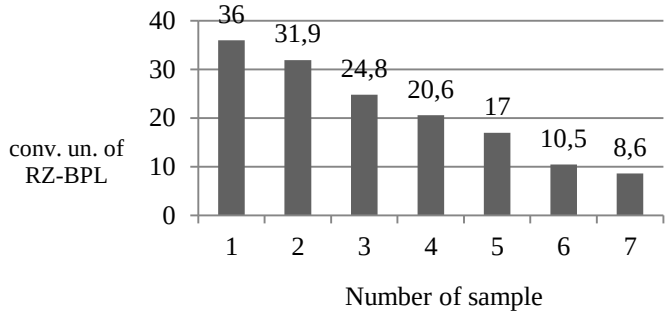

Figure 5 – Results of the study of whiteness in mixtures with wheat flour of the first grade

It was found that whiteness decreases with increasing dosage of amaranth flour in both cases. It was noted that a mixture of high-quality wheat flour with 15% amaranth flour has a whiteness of wheat flour of the first grade. Proceeding from this, it can be assumed that products baked from this mixture will not differ in color from the usual product. We assume that products made from 20% amaranth flour and 80% of the higher wheat grade will have a cream color in comparison with the traditional crumb color. The next indicator for the analysis was the content and quality of gluten, which affect the shape, condition of the crumb and other characteristics and largely determines the baking properties of the flour mixture. To assess it, the following indicators were determined: quantity and quality. Figure 6 shows the results of determining the content of gluten in mixtures of wheat flour of superior quality and flour from an amaranth extrudate. As the results of the studies show, an increase in the proportion of amaranth extrudate naturally leads to a decrease in the amount of gluten in the mixture. At the same time, a 20% dose can be considered a critical point, since further increase in the mass fraction of the amaranth extrudate determines the characteristics of the mixture, which does not allow providing traditional quality characteristics of the finished products.

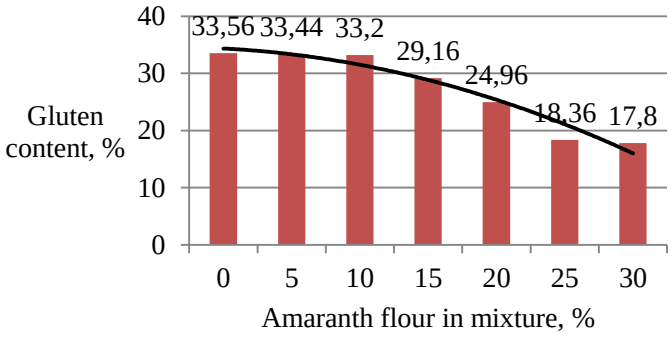

Figure 6 – The content of gluten in mixtures of wheat flour of highest grade and amaranth flour

Figure 7 shows the dynamics of changes in gluten content in mixtures of wheat flour of the first grade and amaranth.

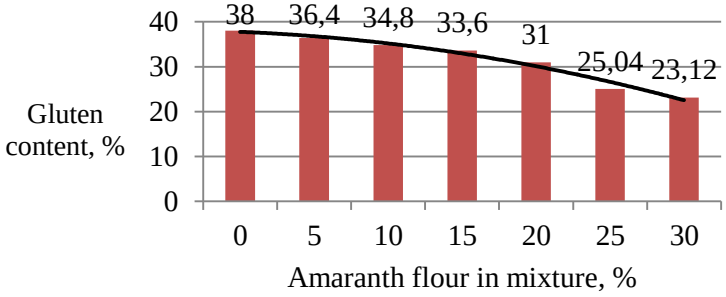

Figure 7 – The content of gluten in mixtures of wheat flour of first grade and amaranth flour

While maintaining the general dependence, the first grade of wheat flour baking can be mixed with flour from the amaranth extrudate in a ratio of 75:25. But at the same time it is necessary to pay attention to the fact that the sample of wheat flour of the first grade, accepted in the studies, initially had high baking properties. Next, the quality of gluten in the test mixtures was examined, results in Figures 8 and 9.

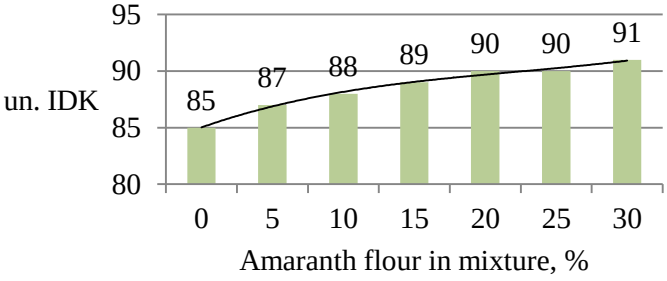

Figure 8 – Quality of gluten in mixtures of wheat bakery flour of highest grade and amaranth flour

The decrease in the quality of the gluten of flour mixture with increasing the proportion of amaranth extrudate is probably related to the water-absorbing capacity of the ingredients of the mixture. The studies conducted by us showed that the swallowing ability of the wheat flour sample of the highest grade is 1.97 g/g, while the amaranth extrudate is 3.88 g/g. Accordingly, the extrudate "pulls" water onto itself, preventing the formation of an elastic

gluten skeleton. It should be noted that the established change occurs in the limit of 6 units the IDK is unlikely to radically affect the quality of finished products.

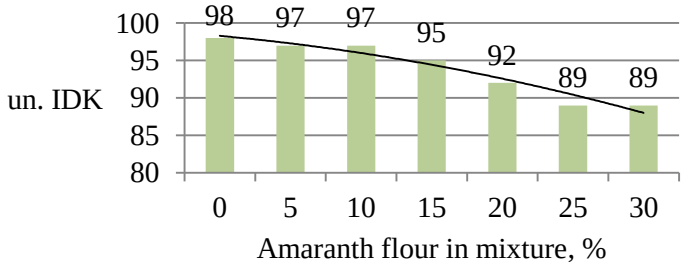

Figure 9 – Quality of gluten in mixtures of wheat bakery flour of first grade and amaranth flour

A different dependence was obtained for the flour mixture of wheat flour, baking and amaranth extrudate. But the flour sample initially had a much worse IDK characteristic. In addition to the redistribution of moisture (the water-absorbing capacity of the sample of wheat flour of the first grade was 2.11 g/g), the acidity parameters of the mixture of the activity of own proteolytic enzymes of flour could play a role in the dependence obtained. To confirm this dependence, a block of additional studies should be carried out that makes it possible to theoretically explain the regularities obtained by the influence of the amaranth extrudate on the qualitative characteristics of flour mixtures. But at the same time, it is necessary to note the individual nature of the effect of the enriching ingredients, depending on the characteristics of the batters used.

Conclusion

The conducted researches showed:

- the introduction of the amaranth extrudate naturally reduces the amount of gluten of the flour mixture. The dosage of the amaranth extrudate, which guarantees the traditional quality of baked goods, is largely determined by the characteristics of the batters used, but generally does not exceed 20-25 %, in our case it will be probably 15-20%;
- Amaranth extrudate also affects the quality of gluten, which is determined by its high water-absorbing capacity and the redistribution of moisture between the main biopolymers of the flour mix. But on the whole this influence is not so crucial as the amount of gluten.

In general, studies of functional-technological and baking properties have shown the possibility of forming high consumer properties of bakery and flour confectionery products with the introduction of extruded amaranth flour into the formulation.

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DEVELOPMENT OF CURD PRODUCT ENRICHED WITH OAT BRAN

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Abstract

The most important task of any state is the preservation and promotion of the population'shealth.The health of every person and nation is mainly determined by their rations. The malnutrition impairs metabolism and functional capacity of the digestive, heart-vascular system, nervous system and other systems. The unfavorable ecological situation, extensive use of antibiotics and chemicals, stress and other factors leading to a violation to of normal intestinal microflora, digestion and metabolism, reduce immune activity in the human body and provoke growth of the disease. The article deals with the problem of dietary food deficit. Curd product with the addition of bran was developed for the solution of this problem. The advantages of using curd product and oat bran are analyzed,andthe main properties such asproduct-quality index, vitamin and mineral composition are shown. The quality indicators for the acceptance of raw milk and oat bran are presented. When working with a curd productphysicochemical and sensorystudies were carried out. All the studies were carried out in the laboratories of the Perm State Agro-Technological University, Russia.

Keywords:*Curd products, Oat bran, Diet, Dairy products, Health.*

Introduction

With malnutrition, metabolism is disrupted, as well as disrupting the digestive, cardiovascular, nervous and other systems. Adverse ecological situation widespread use of antibiotics and chemicals, stresses and other factors lead to violations of the normal microflora of the intestine, digestion and metabolism, which reduces the immune activity of the human body and contributes to the increase in the incidence. Recently, the issue of products for dietary nutrition, their development and introduction to the consumers has gained popularity. The deficit of these products is associated with the lack of knowledge of the population about them, as well as their small assortment. Improving the structure of nutrition and public health is one of the main concepts of the state policy in the Russian Federation and the countries of the world. For protein productsthe most suitable basis with dietary properties are dairy products, in particular curd and curd products (Penzinaetal.,2013). Curd product is the most balanced in composition, nutritional and biological value for a person's diet. Supplement the diet can be food fibers, vitamins, minerals can be by combining dairy raw materials with components of plant origin. The proteins in curd are more readily digested than the protein in milk. It has been estimated that regular milk is only **32% digested** after an hour in the digestive tract, **whereas 91%** of **curd**is digested within the same period of time. It is, therefore, an ideal diet for those with sensitive digestive systems, particularly young children and elderly persons. (VahRehVah., 2007). Technologically, the proteins of milk are its most important constituents. They play important, even essential, roles in all dairy products. The roles played by milk proteins are nutritional, physiological, and physicochemical. (Hui., 2006) Vegetable raw materials are represented by grain crops and products of their processing: flour, bran, cereals. Bran is anunwanted product of grain processing. It is the outer shell of cereal, which is valued for the high content of insoluble fiber, vitamins of group B, E, PP, macro- and microelements (iron, phosphorus, sodium, magnesium, calcium and potassium) (Zhetesov.,2017). The use of bran has a positive effect on the work of the body: digestion

improves; there is a clearing of the body of heavy metals, toxins; contribute to the elimination of toxins; lowers blood sugar; contribute to rapid satiety and well satisfy hunger (Penzina.,2013).

Materials and Methods

As a material for the research, raw milk, purchased on a domestic farm, was used. In the laboratory, a curd product was obtained from this raw material. In addition to the curd product, the main component was oat bran,it was added to the finished product in a percentage of 5%, 12% and 30% of the weight of the finished curd product**.**

Milk raw materials were investigated for sensoryindicators – taste, color, odor. A research was made of the physicochemical parameters – fat, SNF, protein, density, acidity, added water. To research the indicators of milk-raw materials, «Ekomilk M» a milk quality analyzer was used. The curd product, with the addition of oat bran, has also undergone sensoryand physicochemical research - taste, color, odor, consistency, acidity, moisture, proteins, fats, carbohydrates, energy value. To research the curd product, the following equipment was used –«K 139 VELPSCIENTIFICA» Kjeldahl Apparatus, drying cabinet laboratory SNOL, centrifuge Funke-Gerber Nova Safety.

The research was carried out in the laboratories of the Perm State Ago-Technological University at the Department of Fruit and Vegetable Growing, Storage and Processing of Agricultural Products, as well as in the researchLaboratory for the development of Agro-Technologies. This experiment was conducted in one repetition, as further study of the product continues. At the time of the experiment, four batches of each variant.

Results and discussion

The main component of the curd product is raw milk were the final result of the finished product depends on its quality and indices.

Sensory indicators of the quality of raw milk, investigated in accordance with GOST 31449-2013 «Raw cow's milk. Specifications» (Table 1).This standard applies to cow raw milk (hereinafter referred to as milk), subjected to primary treatment (purification from mechanical impurities and cooling to a temperature of (4 ± 2) ° C) after milking and intended for industrial processing.

Table 1.Sensory parameters of raw milk

Indicator name	Characteristic			
	GOST 31449-2013	Sample 1	Sample2	Sample3
Consistency	Homogeneous liquid without sediment and flakes	Homogeneous liquid without sediment and flakes	Homogeneous liquid without sediment and flakes	Homogeneous liquid without sediment and flakes
Taste and smell	Pure, without foreign smells and tastes, not peculiar to fresh milk. Allowed slight forage taste and odor	Pure, without foreign smells and tastes	Pure, without foreign smells and tastes	Pure, without foreign smells and tastes
Color	From white to light cream	White	White	White

According to the sensory indicators, milk raw materials meet the requirements presented in the Russian Federation.

Important indicators for assessing the quality of milk are also physicochemical studies GOST 31449-2013 «Raw cow's milk. Specifications» (Table 2).

Table 2. Physicochemical parameters of raw milk

Indicator name	Indicator value			
	GOST 31449-2013	Sample 1	Sample2	Sample3
Mass fraction of fat,%, not less than	2.8	2.98	3.01	3.07
Mass fraction of protein,%, not less than	2.8	2.99	3.00	2.98
Th^0	From 16.0 to 21.0	18.00	18.00	17.00
Nonfat milk solids (SNF), %, not less than	8.2	8.70	8.76	8.69
Clean group, not lower than	II	II	II	II
Density, kg/m^3, not lower than	1027.0	1029.95	1030.15	1029.85

Milk raw materials purchased on domestic farming meet all quality indicators and is a good raw material for the production of a curd product with the addition of oat bran. This milk produces a curd product with a fat content of 2.5%.

At the moment, a large number of bran is produced from different plant species. Oat bran is a biologically valuable product and for the balance of proteins, fats, carbohydrates and energetic value (Table3). When oat bran is added to the curd product, it is worth considering the percentage ratio in the amount of 5%, 12% and 30%.

Table 3.Component composition of bran by species

Indicator name	Rice	Oat	Wheat	Rye
Energy value (calories)	316.00	246.00	216.00	221.00
Proteins (g)	13.35	17.30	15.55	11.20
Fats (g)	20.85	7.03	4.25	3.20
Carbohydrates (g)	28.90	66.20	64.51	32.00

Oat bran is an appropriate option, since it contains an average number of kilocalories, in contrast to others, contains the greatest amount of protein, which is ¼ of the daily intake rate.

The positive side of oat bran is the amount of carbohydrates in it. Carbohydrates are contained in the form of fiber, and it slows the absorption of sugar and fat, reduces the release of insulin in response to carbohydrate food, improves intestinal motility and helps maintain a sense of satiety (Grenchuk., 2018).

The main component of this product is a curd product, oat bran should be added to its composition. Curd product is a fermented milk product that is made from clabber by heating and removing whey. This product is traditional for Eastern and Northern Europe.

Curd product contains essential amino acids, vitamins A, B1, B2, B4, B5, B6, B9, PP, C, E, B12, H beta-carotene, rich in folic acid, calcium, iron, sodium, magnesium, copper, zinc, fluorineand phosphorus. Due to its rich composition, the curd product is well absorbed and improves the activity of the gastrointestinal tract. The recipe of the curd product with the addition of oat bran has two main components(Krus.,2006) (Table 4).

Table 4.The recipe for a curd product with the addition of oat bran

Product (g)	Control	Sample 1(5%)	Sample2 (12%)	Sample3 (30%)
Curd product	100	95	88	70
Oat bran	-	5	12	30
In total		100		

Curd product is obtained from raw milk by ripening and further heat treatment. Oat bran is steamed with hot water at t = 100˚C. The finished product is checked by independent experts, onsensory indicators (Table 5). The curd product is checked for quality indicators in accordance with GOST 31453-2013 «Tvorog. Specification».The standard applies to cottage cheese, packaged in a consumer container, made from cow's milk and / or dairy products, intended for direct use in food.

Table 5.Sensory parameters of the curd product with the addition of bran

Indicator name	GOST 31453-2013	Control	Sample 1(5%)	Sample2 (12%)	Sample3 (30%)
Consistency and appearance	Soft, smearing or crumbly with or without detectable particles of milk protein	Soft, smearing	Soft, smearing, small inclusions of bran	Soft, smearing, significant presence of bran	Soft, smearing, with a lot of bran
Taste and smell	Pure, dairy, without foreign flavors and odors	Pure, dairy, without foreign flavors and odors	Sour-milk, with a slight aftertaste of oat bran	Sour-milk, with a taste of oat bran	Sour-milk, tastes of oat bran predominate
Color	White or with a cream tint, uniform throughout the mass	White, uniform throughout the mass	White, with small patches of brown	White, with significant pieces of brown	Light cream, with brown bran particles

The control sample fully complies with GOST 31453-2013 «Tvorog. Specification», this means that the raw milk of good quality and deviations in the production of the curd product is not observed. Appearance of all samples varies considerably (Picture 1).

Sample 1 (5%). Has a slight external difference from the control sample, in appearance there are insignificant inclusions of oat bran, the consistency remained unchanged, there is a slight taste of bran, the color is white with brown impregnations.

Sample 2 (12%). This sample has a more pronounced taste of oat bran, in appearance there is a noticeable increase in the percentage of bran, compared to sample 1 and control. The color of the curd product is white, but with a high content of brown spots.

Sample 3 (30%). Appearance immediately shows that the curd product contains a significant amount of oat bran. The color became light cream with brown bran stains, a pronounced taste of the presence of oat bran. The appearance of this sample is immediately distinguished from the others in appearance.

Picture 1. Appearance of the samples under study
Control sample(A); sample 1 (B); sample 2 (C); sample 3 (D).

In addition to the sensory changes, the component composition of the curd product was also changed due to the addition of oat bran (Table 6).

Table 6. Ingredients of the curd product with the addition of bran

Indicator name	GOST 31453-2013	Control	Sample 1 (5%)	Sample2 (12%)	Sample3 (30%)
Proteins (g) not lower than	17.2	22.83	22.20	21.22	19.08
Fats (g) not lower than	1.80	2.50	2.50	2.50	2.50
Carbohydrates (g) not lower than	1.40	1.40	4.63	10.40	20.79
Humidity (%), not more than	76.00	55.20	42.10	39.87	34.50
Th^0, not more than	230	130	130	130	130
Energy value (calories)	101.00	101.00	102.25	104.40	105.50

All samples have the same fat content, this is due to the small amount of fat content in oat bran and when mixing bran with curd product, the fat remains unchanged.The calculation of the smallest significant difference (Table 7).

Table 7. Fat content in samples

Indicator name	Repetition				Sums V	Average value
	1	2	3	4		
Control	2.7	2.6	2.3	2.4	10	2.50
Sample 1 (5%)	2.2	2.6	2.5	2.5	9.8	2.50
Sample2 (12%)	2.3	2.4	2.8	2.7	10.2	2.50
Sample3 (30%)	2.6	2.4	2.1	2.8	9.9	2.50
						0
Sums P	9.8	10	9.7	10.4	39.9	1.99

With an increase in the percentage of bran, the amount of protein is slightly reduced, since the amount of protein in the bran is much lower than in the curd product (Table 8).

Table 8.Protein content in samples

Indicator name	Repetition				Sums V	Average value
	1	2	3	4		
Control	22.90	22.81	22.79	22.84	91.34	22.83
Sample 1 (5%)	22.10	21.90	22.30	22.30	88.6	22.20
Sample2 (12%)	21.22	21.52	21.06	21.11	84.91	21.22
Sample3 (30%)	19.07	19.08	19.08	19.09	76.32	19.08
						0
Sums P	85.29	85.31	85.23	85.34	341.17	17.05

Due to the significant content of carbohydrates in oat bran, their quantity increases in the curd product. The amount of carbohydrates positively affects the body, by using a curd product with the addition of bran, can make up part of the daily rate (Table 9).

Table 9.Carbohydrates content in samples

Indicator name	Repetition				Sums V	Average value
	1	2	3	4		
Control	1.3	1.4	1.4	1.5	5.6	1.4
Sample 1 (5%)	4.7	4.7	4.5	4.6	18.53	4.63
Sample2 (12%)	10.1	10.6	10.7	10.3	41.7	10.40
Sample3 (30%)	20.8	20.7	21.0	20.7	83.19	20.79
						0
Sums P	36.9	37.4	37.6	37.12	149.02	7.451

The moisture content of the finished product decreases with the addition of oat bran, as oat bran is a dry product with a small amount of humidity (Table 10).

Table 10.Humidity content in samples

Indicator name	Repetition				Sums V	Average value
	1	2	3	4		
Control	55.0	55.1	55.6	55,1	220.8	55.20
Sample 1 (5%)	42.1	43.1	42.3	40,9	168.4	42.10
Sample2 (12%)	39.8	39.0	40.2	40,4	159.46	39.87
Sample3 (30%)	34.8	34.3	34.5	34,5	138.09	34.50
						0
Sums P	171.67	171.5	172.64	170,94	686.75	34.34

Acidity remains unchanged, bran does not affect the development of microorganisms in the curd product (Table 11).

Table 11.Acidity content in samples

Indicator name	Repetition				Sums V	Average value
	1	2	3	4		
Control	129.80	131.10	131.00	129.80	521.7	130.00
Sample 1 (5%)	131.00	129.90	130.20	130.30	521.4	130.00
Sample2 (12%)	130.50	131.10	131.10	129.00	521.7	130.00
Sample3 (30%)	130.00	130.20	130.30	129.70	520.2	130.00
						0
Sums P	521.3	522.3	522.6	518.8	2085	130.00

Energy value increases due to the content of carbohydrates in the finished product. Carbohydrates slightly affect the calorie content of food than fats, but with the addition of bran in the curd product, the fat remained unchanged and the energy value increased (Table 12).

Table 12.Energy value (calories) content in samples

Indicator name	Repetition				Sums V	Average value
	1	2	3	4		
Control	101.0	101.0	101.0	101.0	404	101.0
Sample 1 (5%)	102.1	102.3	102.4	102.2	409	102.25
Sample2 (12%)	103.9	104.6	104.5	104.5	417.5	104.4
Sample3 (30%)	105.6	105.2	105.7	105.3	421.8	105.5
						0
Sums P	412.6	413.1	413.6	413	1652.3	82.615

Conclusions

Curd products with the addition of oat bran can be used in dietary nutrition. This product contains a small amount of fat, but a large amount of carbohydrates and proteins, which gives this product a good reputation. The recommended sample is sample 3 (30%). This sample has a pronounced taste of oat bran, which gives the product an unusual taste. According to the component composition, the sample is high-grade in proteins and carbohydrates, which is the most important in dietary nutrition.

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MACRONUTRIENTS CONTENT IN WHEAT STRAW AND ITS YIELD AS AFFECTED BY BACTERIAL AND MINERAL FERTILIZATION

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Abstract

Regardless of their major role in crop productivity, increased and long-term use of mineral fertilizers in agricultural production has raised concerns causing numerous environmental problems. This could be overcome by partial replacement of mineral fertilizers using bacterial inoculants, which could lead to the improvement of physical, chemical and biological soil properties, as well as help to ensure that the supply of nutrients contributing to optimized yield of crops. The study evaluated the effect of application of different rates of composite mineral fertilizers and their combination with bacterial inoculants (N-fixing *Klebsiella planticola* and *Enterobacter* spp.) on macronutrients content in straw of winter wheat and its yield. Unfertilized treatment was used as a control. The contents of nitrogen, phosphorus, potassium and crude proteins in plant samples were determined at the beginning of tillering and in full grain maturity stage, at the end of the wheat vegetation. Measuring of the straw yield was carried out at the end of the vegetation. The results of the study showed that the use of high rates of composite mineral fertilizers and their combination with bacterial inoculants resulted in increased contents of nitrogen, phosphorus, potassium and crude proteins in the wheat straw, which was noticeably observed in the stage of tillering. The highest increase in the straw yield was obtained by the same mentioned treatments, although the combination of bacterial inoculants and low rates of mineral fertilizers resulted in higher yields comparing to the use of solely low rates of mineral nutrients.

Keywords: *Wheat straw, yield, chemical composition, bacterial inoculants, composite mineral fertilizers.*

Introduction

Wheat (*Triticum aestivum* L.) is grown on every continent thanks to the large polymorphism. Along with maize, wheat represents a major crop in agricultural production in Serbia, where its cultivation in the period 2000-2009 occupied an average area of 600,171 ha with an average grain yield of 3.47 t ha^{-1} (Jocković *et al*., 2010; *Mladenov et al., 2011*). The yield and quality of wheat grain depend mainly on the balanced mineral nutrition, which is correlated with the type of soil, climate factors in the region and other agroecological factors. Nevertheless, creating an optimal conditions for growing wheat in order to achieve higher grain yields is primarily related to application of nitrogen fertilizers (Đekić *et al*., 2017). Similar could be said for the wheat straw, whose yield, besides mineral nutrition, depends on variety, soil and climate conditions. Wheat straw is the agricultural product obtained from different parts of wheat plant like stem, leaves etc., after the grain and chaff have been removed. Straw makes up about half of the yield of wheat. It has many uses, primarily as fuel, livestock bedding and fodder. An accurate content of macro and micronutrients in wheat straw can vary from cultivar to cultivar, stages of plant growth, climatic conditions and the nature of soil and fertilizers used (Safdar *et al*., 2009; Yasin *et al*., 2010; Saleem Khan and Mubeen, 2012).

Regardless of their major role in soil fertility and wheat productivity, increased use of mineral fertilizers in agricultural production can causes environmental contamination (Acosta-Martinez and Tabatabai, 2000; Alizadeh and Ghadeai, 2006). The problems concerned can be overcome by partial replacement of these fertilizers by application of microbial inoculants, in order to inhibit or stimulate certain cellular processes, including mineralization ones, thus leading to the improvement of physical, chemical and biological soil properties (Milošević *et al*., 2003; Pešaković *et al*., 2008). Regarding the preceding comments, the main purpose of this study was to evaluate the effect of different rates of composite mineral NPK fertilizers (N:P:K=15:15:15) and their combination with selected bacterial inoculants on macronutrients content in wheat straw and its yield.

Material and methods

The study was conducted on Mladenovac experimental station of Institute of Soil Science, located about 55 km south-east from Belgrade in Serbia, in 2006/2007. Average monthly air temperatures and precipitation sums for the investigated period along with multi-annual average (1990-2007) are presented in Table 1. Monthly air temperatures during the vegetation period of wheat (October 2006 - July 2007) were considerably higher in comparison with multi-annual average. The distribution of precipitation from the period January - June in 2007 was favorable, except in April, when it was measured only 3.8 mm of precipitation.

Table 1. Average monthly air temperatures and precipitation sum for the period 2006/2007 and multi-annual average.

Month	Year				Average (1990-2007)	
	2006	2007	2006	2007		
	Temperature (°C)		Precipitation (mm)		Temperature (°C)	Precipitation (mm)
January	-0.5	7.6	43.2	49.3	1.7	41.8
February	1.9	7.2	59.1	56.0	3.5	38.4
March	6.5	10.2	104.4	99.6	7.9	40.8
April	13.7	14.9	97.0	3.8	12.7	55.7
May	17.4	19.5	42.3	79.0	18.1	50.8
June	20.2	23.8	137.8	107.6	21.5	97.7
July	24.7	25.8	23.3	17.5	23.2	66.8
August	20.9	24.2	120.6	72.5	23.1	60.9
September	19.2	16.2	24.3	84.1	17.6	63.5
October	15.2	11.8	20.9	103.6	13.0	55.8
November	8.9	5.2	24.5	131.5	7.3	55.9
December	4.3	1.1	51.9	34.5	2.2	60.5
Average	12.8	14.0	-	-	12.7	-
Total	-	-	749.3	839.0	-	688.6

The studied type of soil was Eutric Cambisol (WRB, 2014). The experiment was set up in a randomized block design on 9 x 6 m^2 plot size, with three replications, based on the following variants: control (Ø, non-fertilized soil); 60 kg ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1} (N1); 120 kg ha^{-1} N, P_2O_5 and K_2O (N2); *Enterobacter* sp. strains + 60 kg ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1} (ES+N1); *Enterobacter* sp. strains + 120 kg ha^{-1} N, P_2O_5 and K_2O (ES+N2); *Klebsiella planticola* + 60 kg ha^{-1} N and P_2O_5, and 40 kg K_2O ha^{-1} (KP+N1); *Klebsiella planticola* + 120 kg ha^{-1} N, P_2O_5 and K_2O (KP+N2). Winter wheat (cv. Evropa 90) was used as a test plant in the trial and sown and harvested in optimal terms (1st decade of November in 2006, 3rd decade of June in 2007, respectively). Mineral fertilization was carried out manually for each experimental plot using composite NPK fertilizer in relation N:P:K = 15:15:15. Nitrogen (N) fertilizer was applied in the form of urea with 46% N, phosphorus (P) - in the

form of monoammonium phosphate (MAP) with 52% P_2O_5 and 11% N, and potassium (K) - as a 40% potassium salt (potassium chloride, KCl). The established amounts of potassium and 1/3 of nitrogen fertilizer were applied in the fall 2006 before sowing, and the remaining 2/3 of nitrogen fertilizer was applied during March in 2007 at the stage of tillering. The procedure for obtaining the bacterial inoculums for fertilization was previously described in study of Stanojković *et al*. (2012). Bacterial fertilization was carried out using plastic haversack sprinkler with 300.00 cm^3 m^{-2} of diluted liquid bacterial inoculums made from the pure cultures of associative N-fixing bacteria *Klebsiella planticola* (strain TSHA-91) and *Enterobacter* strains KG-75 and KG-76 and tap water. The first soil and foliar fertilization with bacterial inoculums was carried out in the stage of 2-3 formed leaves, and the second - in the tillering stage, a few days after fertilizing with mineral nitrogen. *K. planticola* was obtained from the stock culture of the Microbiology Laboratory of Faculty of Agronomy (Čačak, Serbia) and cultivated for 24 h at 28°C ± 1 on the slanting nutrient medium, while *Enterobacter* strains were obtained from the stock culture of the Microbiology Laboratory of the Center for Small Grains (Kragujevac, Serbia), where they have been isolated from the rhizosphere of wheat. These strains were cultivated for 48 h at 28°C ± 1 on the slanting nutrient medium. The preliminary analysis of the study soil, after the samples were air-dried and passed through a sieve, included the following chemical parameters: soil acidity (pH in H_2O and 1M KCl, v/v - soil:H_2O=1:5, soil:1M KCl=1:5) was analyzed potentiometrically, using glass electrode (SRPS ISO 10390, 2007); total nitrogen (N) was analyzed on elemental CNS analyzer Vario EL III (Nelson and Sommers, 1996); available phosphorus (P_2O_5) and potassium (K_2O) were analyzed by Al-method according to Egner-Riehm (Riehm, 1958), where K_2O was determined by flame emission photometry and P_2O_5 by spectrophotometer after color development with ammonium molybdate and stannous chloride; humus content was determined using Tiurin's method, modified by Simakov (Ostrowska *et al*., 1991). The yield of wheat straw without grain and chaff from each experimental plot was measured and determined directly on plots at the end of the vegetation and calculated into t ha^{-1}. The content of macronutrients in wheat samples was determined at the beginning of tillering (vegetation stage I, S1) and at the end of the vegetation period (vegetation stage II, S2). The content of phosphorus (P) and potassium (K) was determined by so called "wet" combustion, i.e. they were heated to boiling with the mixture of concentrated sulfuric (H_2SO_4) and perchloric ($HClO_4$) acids. In the obtained solution, P was determined by spectrophotometer with molybdate, and K – by flame emission photometry (Jakovljević *et al*., 1985). The content of nitrogen (N) was analyzed using elemental CNS analyzer, Vario model EL III (Nelson and Sommers, 1996), while the content of crude proteins was calculated on the basis of N content according to Licitra *et al*. (1996), using the following formula: crude proteins (%) = N (%) x 6.25 (factor for conversion of nitrogen content to crude protein). The obtained data on soil properties were presented as arithmetic means of three replicates, standard deviation values and intervals. The effects of different fertilization treatments on chemical parameters tested and yield were evaluated using ANOVA (SPSS 20.0, Chicago, USA), followed by Duncan's Multiple Range Test (DMRT). Significant differences between means were tested by the LSD test at P = 0.05 and P = 0.01.

Results and discussions

The main chemical characteristics of the study soil are presented in Table 2. According to the reference values (Šestić *et al*., 1969), the soil is characterized by very acid reaction with pH in 1M KCl 4.06, high available potassium, medium to high available phosphorus, and medium humus and total nitrogen supply.

Table 2. Main chemical characteristics of the studied Eutric Cambisol

Chemical parameter	Value (means ± standard deviation)	Intervals
pH in H_2O	4.90±0.03	4.87-4.92
pH in 1M KCl	4.06±0.05	4.00-4.10
Total N (%)	0.136±0.005	0.132-0.141
Humus (%)	2.19±0.01	2.18-2.19
Available P_2O_5 (mg $100g^{-1}$)	15.73±0.31	15.51-16.09
Available K_2O (mg $100g^{-1}$)	25.30±0.30	25.08-25.65

By analyzing the content of macronutrients in plant material at the end of the wheat vegetation period it was determined that the content of total nitrogen, phosphorus, potassium and proteins significantly depended on the fertilization treatment applied (Table 3).

Table 3. Effect of the fertilization treatments on macronutrients content in wheat straw and its yield (in t ha^{-1}).

Treatments	Total N (%)		P_2O_5 (%)		K_2O (%)		Proteins (%)		Yield
	Vegetation stage								
	S1	S2	S1	S2	S1	S2	S1	S2	S2
Ø	0.72± 0.007g	0.52± 0.005g	0.75± 0.003g	0.44± 0.003f	2.15± 0.003e	0.86± 0.009f	4.48± 0.041g	3.27± 0.028g	1.78± 0.012f
N1	0.85± 0.006f	0.63± 0.004f	0.89± 0.004f	0.58± 0.004e	2.34± 0.008d	1.19± 0.003e	5.29± 0.034f	3.90± 0.024f	3.96± 0.004e
N2	2.26± 0.005c	0.79± 0.006d	0.99± 0.004c	0.69± 0.005c	2.97± 0.057b	1.59± 0.001b	14.15± 0.031c	4.98± 0.034d	5.55± 0.285b
ES+N1	2.02± 0.005d	0.66± 0.003e	0.92± 0.004d	0.60± 0.003d	2.68± 0.009c	1.31± 0.005c	12.59± 0.029d	4.14± 0.019e	4.95± 0.003c
ES+N2	2.84± 0.004a	1.43± 0.003a	1.09± 0.001a	0.78± 0.003a	3.04± 0.005a	1.62± 0.016a	17.73± 0.025a	8.86± 0.161a	6.43± 0.004a
KP+N1	1.63± 0.006e	0.89± 0.006c	0.91± 0.004e	0.60± 0.003d	2.65± 0.006c	1.28± 0.009d	10.16± 0.038e	5.54± 0.039c	4.43± 0.007d
KP+N2	2.37± 0.007b	0.98± 0.003b	1.02± 0.002b	0.71± 0.002b	3.07± 0.010a	1.62± 0.003a	14.82± 0.041b	6.11± 0.021b	6.48± 0.005a
P value	***	***	***	***	***	***	***	***	***
LSD (0.05)	0.009	0.009	0.005	0.006	0.039	0.014	0.061	0.116	0.188
LSD (0.01)	0.013	0.013	0.007	0.008	0.054	0.020	0.080	0.160	0.262

LSD - least significant differences at P=0.05 and P=0.01; *** indicates statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; DMRT was used to compare different variants at P≤0.05, where values followed by the same letter in a column are not significantly different.

Application of high rates of mineral NPK fertilizers and their combination with bacterial inoculants has caused a significant increase in the share of nitrogen, phosphorus, potassium and crude proteins in the plant material compared to the other tested variants, which was noticeably observed in the stage of tillering, the vegetation period in which the plant growth and accumulation of nutrients are the most intensive (Čurić, 1982). This excess of microbiologicaly fixed nitrogen along with higher amounts of mineral nitrogen influenced positively on the accumulation of macronutrients in the study plant material, which is in accordance with previous researches (El-Sirafy *et al*., 2006; Behera and Rautaray, 2010).

The present study also indicated a little increase in the share of macronutrients in plant material in variants where microbial inoculation of soil and plant along with low rates of mineral NPK fertilizers was applied comparing to the application of solely low rates of mineral fertilizers. Similar results were obtained in the study of Cvijanović *et al*. (2007). The analysis of the yield of wheat straw (based on Duncan's test) showed highly significant yield differences between the applied fertilization treatments (Table 3). The highest increase in

yield was obtained by combined application of bacterial inoculants used and high rates of mineral NPK fertilizers. This was also observed in treatments with combined usage of bacterial inoculants and low rates of mineral NPK fertilizers where it was obtained higher yield comparing to the traetments with solely low rates of mineral fertilizers. Behera and Rautaray (2010) also found higher straw yields in biofertilizers + 50% NPK treatments than in treatments with solely 50% NPK. According to Josipović *et al.* (2005), the character of the applied fertilizers effects on the yield of crops also depends on the weather conditions specific to each year. It is assumed that good distribution of rainfall and temperature in the period November 2006 - June 2007 contributed largely to the achieved yields of wheat straw in the investigated agro-ecological conditions.

Conclusion

The present study demonstrated the significant positive effects of combined application of bacterial inoculants studied (*Klebsiella planticola* and *Enterobacter* spp.) and high and low rates of the composite mineral fertilizers on the yield of wheat straw (without grain and chaff). The same results were obtained regarding the effects of the mentioned applied combinations on the contents of total nitrogen, phosphorus, potassium and crude proteins in the analyzed plant material, which was noticeably observed in the stage of tillering. These data suggest that the studied bacterial inoculants can be used in further studies as the potential agents of new biofertilizers for improved wheat production and other agriculture crops.

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CHEMICAL CONTROL OF *GRAPHOLITA MOLESTA* BUSCK IN PEACH ORCHARDS

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Abstract

The oriental fruit moth *Grapholita (Laspeyresia, Cydia) molesta* Busck (Lepidoptera: Tortricidae), also known as the oriental peach moth, is one of the most destructive and economically important pests of stone and pome fruits worldwide. In Serbia, *G. molesta* represents one of the most damaging pests of peach fruits. In this work, pest susceptibility to insecticides based on indoxacarb, deltamethrin, spinetoram, and azadirachtin was assessed. Field trials were carried out in Vojvodina, the northern region of the Republic of Serbia, at localities Čerević and Mala Remeta. Trials were designed according to the EPPO method, following the pest biology and crop phenophase. Before and during the experiment, the flight of *G. molesta* adults was monitored using pheromone traps to determine the optimal timing for insecticide distribution. Products based on indoxacarb (Avaunt 15 EC), deltamethrin (Decis 2.5 EC), spinetoram (Delegat 250 WG) and azadirachtin (Ozoneem Trishul 1% EC) were 0.33, 0.5, 0.2 and 5.0 L ha^{-1} sprayed, respectively. Assessment of spray distribution efficiency was based on the number of fruit damaged by caterpillars, on a base of 100 fruits per repetition scrutiny. The results were processed using ANOVA and Fisher LSD test, while the efficiency was determined according to Abbott. Spraying application showed a efficiency in both trial localities in between 79-87% (indoxacarb), 74-91% (deltamethrin), 84-88% (spinetoram) and 74-80% (azadirachtin), compared with the untreated control sample. The highest efficiency at both sites was given by products based on deltamethrin and spinetoram, while azadirachtin had the lowest efficiency. Assessment of the number of damaged fruits was done for the all investigated insecticides, and it was at a significantly lower level in all treatments in comparison to control indicating the high sensitivity of the *G. molesta* to the tested insecticides.

Keywords: *Prunus persica, insects, plant protection, pesticides*

Introduction

Peach (*Prunus persica* L.) is one of the most widespread species of fruit in the world. Orchards are located in moderately warm areas of Europe and Asia, and in other continents also. Perspectives for growing peaches in the Republic of Serbia are very good within the areas that are known as favourable for its cultivation. Due to its rapid grow rate, peaches achieves maximum growth with a full production capacity of fruits (30, 40 t/ha and more) in six growing seasons. Despite the production is not endangered by many pests, that lead to a minimal use of chemical control actions (Mratinić, 2012), there are few pests that are often encountered during the orchard management. The most significant peach pests are Oriental fruit moth (*Grapholita molesta*), Peach twig borer moth (*Anarsia lineatella*), Aphids (*Myzus persicae* and *Brachycaudus persicae*) and the Red fruit spider (*Panonychus ulmi*). The oriental fruit moth *Grapholita (Laspeyresia, Cydia) molesta* Busck (Lepidoptera: Tortricidae), also known as the oriental peach moth, is one of the most destructive and economically important pests of stone and pome fruits worldwide. The oriental fruit moth is regularly found

in all peach plantations in central Serbia and is the economically important pest of the peach fruit production (Stamenković, 2005). In this contribution we assess the pest susceptibility to insecticides based on indoxacarb, deltamethrin, spinetoram and azadirachtin.

Material and Methods

Field trials for insecticidal control of *G. molesta* were carried out in Vojvodina, the northern region of the Republic of Serbia, at localities Čerević and Mala Remeta in 2017, in the 5 year old peach orchards, variety "Royal Gem". The trials were set before the fruit caterpillars infestations following the pest biology and crop phenophases, and designed according to EPPO method. The flight of *G. molesta* moths was monitored using the pheromone traps before and during the experiment, to determine the optimal timing for chemical protection. Products based on indoxacarb (Avaunt 15 EC), deltamethrin (Decis 2.5 EC), spinetoram (Delegat 250 WG) and azadirachtin (Ozoneem Trishul 1% EC) were foliar-applied at a rate of 0.33, 0.5, 0.2 and 5.0 L ha^{-1}, respectively, and compared with the untreated control sample. Insecticide distributions were performed on May 27, 2017. (Čerević) and on May 29, 2017. (Mala Remeta), at the beginning of the emergence of caterpillars during the second brood. Insecticide distributions were carried out with a Solo® backpack sprayer with an estimated use of 1000 litre of water/ha. Assessment of spray efficiency was based on the number of fruit damaged by caterpillars, with an overview of 100 fruits per repetition (two weeks after treatment and before harvest). The results were processed using ANOVA and Fisher LSD test for a confidence interval of 95% in statistical software R. ver 3.2.2, while the efficiency was determined according to Abbott.

Results and Discussion

The results of the efficacy test for Decis 2.5 EC, Delegat 250 WG, Avaunt 15 EC and Ozoneem Trishul 1% EC in the control of the oriental fruit moth (*G. molesta*) in peach plantations at localities Mala Remeta and Čerević are shown in table 1. The efficacy of the insecticides 14 days after treatment at locality Mala Remeta ranged from: 82.4 % (Decis 2.5 EC), 88.2% (Delegat 250 WG), 82.4% (Avaunt 15 EC) and 76.5% (Ozoneem Trishul 1% EC). After 24 days, the efficacy ranged ranged from: 73.7 % (Decis 2.5 EC), 84.2% (Delegat 250 WG), 78.9 % (Avaunt 15 EC) and 73.7% (Ozoneem Trishul 1% EC). The efficacy of the insecticides 14 days after treatment at the Čerević site ranged from: 91.3 % (Decis 2.5 EC), 87.0% (Delegate 250 WG), 82.6% (Avaunt 15 EC) and 78.3% (Ozoneem Trishul 1% EC). After 24 days of treatment, the efficacy ranged from: 90.3 % (Decis 2.5 EC), 83.9% (Delegat 250 WG), 87.1% (Avaunt 15 EC) and 80.6% (Ozoneem Trishul 1% EC).

Based on the results achieved, all the applied preparations showed satisfactory efficacy in the control of *G. molesta*. At both localities, the Ozoneem Trishul 1% EC (azadirachtin) showed the lowest efficacy and the highest was recorded in tests with Decis 2.5 EC (deltamethrin) and Delegate 250 WG (spinetoram). Although azadirachtin showed the slightly lower efficacy, its significance in pest management is reflected through the characteristics which it owns: natural (botanical) product, definitely non-toxic, 100% biodegradable, environment-friendly, entirely safe for humans and beneficial insects. The number of damaged fruits in both evaluations at the localities Mala Remeta and Čerević, was significantly lower to control which indicates a high sensitivity of the oriental fruit moth population to the studied insecticides.

The control of these tortricids mainly relies on insecticide sprays (Knight and Light, 2013), but also this control mean is threatened by the widespread development of resistance (Rodríguez et al., 2011). In 2014, six experiments were carried out in highly infested orchards in Emilia Romagna (Italy) in order to assess the efficacy of spinetoram in control of the *Cydia pomonella*. The results showed the significant activity of spinetoram with a significant reduction of the pest population (Boselli and Scannavini, 2014). Results of field experiments

(Marčić et al., 2009) have indicated a high efficacy of azadirachtin (NeemAzal-T/S) in control of *Cacopsylla pyri* and *Panonychus ulmi*. The effectiveness of insecticides (deltamethrin and indoxacarb) applied to larvae of the codling moth, *Cydia pomonella* L, was tested by Pasquier and Charmillot (2003). Both insecticides were toxic to the pest, which produced 88.6% (indoxacarb) and 99.5% (deltamethrin) mortality to codling moth larvae. The efficacy studies in chemical control of *G. molesta* should be permanent. Monitoring of the sensitivity for investigated species will take into consideration the tolerance, as well as the cross-resistance, have already been recorded worldwide (Charmilot et al., 2003).

Table 1. The efficacy of insecticides in control of *G. molesta*
(Mala Remeta and Čerević, 2017)

Mala Remeta	14 days after the treatment			24 days after the treatment		
	$\bar{x}$	Sd±	E%	$\bar{x}$	Sd±	E%
Decis (0.2 l/ha)	0,75 b	0.96	**82.4**	1.25 b	1.26	**73.7**
Delegat 250WG(0,2 l/ha)	0.50 b	0.58	**88.2**	0.75 b	0.96	**84.2**
Avaunt 15 EC (0,33l /ha)	0.75 b	0.96	**82.4**	1.00 b	0.82	**78.9**
Ozoneem Trishul 1% EC (0,5l/ha)	1.00 b	0.82	**76.5**	1.25 b	1.26	**73.7**
Control	4.25 a	1.50	/	4.75 a	0.96	/
LSD (0.05%)	1.24			1.40		
Čerević	14 days after the treatment			24 days after the treatment		
	$\bar{x}$	Sd±	E%	$\bar{x}$	Sd±	E%
Decis (0.2 l/ha)	0.50 b	1.00	**91.3**	0.75 b	0.50	**90.3**
Delegat 250WG(0,2 l/ha)	0.75 b	0.96	**87.0**	1.25 b	1.26	**83.9**
Avaunt 15 EC (0,33l /ha)	1.00 b	0.82	**82.6**	1.00 b	0.82	**87.1**
Ozoneem Trishul 1% EC (0,5l/ha)	1.25 b	1.41	**78.3**	1.50	1.29	**80.6**
Control	5.75 a	0.96	/	7.75 a	1.71	/
LSD (0.05%)	1.65			1.45		

***** $\bar{x}$ - -average number of damaged fruits; Sd± - standard deviation; E%-efficacy;

Conclusions

Based on the conducted tests to control the oriental fruit moth (*Grapholita molesta*) in two sites in Republic of Serbia (Mala Remeta and Čerević) during 2017, we propose the following conclusions:

- Results indicate high efficacy of all scrutinized insecticides: indoxacarb (Avaunt 15 EC), deltamethrin (Decis 2.5 EC), spinetoram (Delegat 250 WG) and azadirachtin (Ozoneem Trishul 1% EC), both evaluated 14 and 24 days after treatment.
- Products based on deltamethrin and spinetoram showed the highest efficacy, while azadirachtin had the lowest efficacy.
- Assessment of the number of damaged fruits for the all studied insecticides, reported a significantly lower level of infestation in comparison to the control, indicating a high sensitivity of the *G. molesta* to the tested insecticides.

Acknowledgement

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DETERMINATION OF INSECTICIDE INDOXACARB RESIDUES IN PEACH FRUITS

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Abstract

In this study, a method for the determination of indoxacarb residues in peach fruits was developed and validated. Indoxacarb belongs to oxadiazine insecticides voltage-dependent sodium channel blockers, IRAC 22A. Maximum residue level (MRL) for indoxacarb in peach fruits, set by EU legislation is 1 mg/kg. For determination of indoxacarb residues, the QuEChERS method coupled with high-performance liquid chromatographic (HPLC) analysis were carried out. The HPLC Agilent 1100 system with diode array detection and Zorbax Eclipse XDB-C18 column (50 mm × 4.6 mm, 1.8 μm) were used. The mobile phase was water and acetonitrile (25:75). The flow rate was maintained at 1.0 ml/min in isocratic mode and the injection volume was 20 μl. Chromatograms were extracted at 310 nm. Under these conditions retention time of indoxacarb was 1.501 min. Method validation was performed taking into consideration linearity, recovery, precision, matrix effect, limits of detection and quantification, completely fulfilling the SANTE/11813/2017 criteria. The limit of detection and limit of quantification were 0.006 mg/kg and 0.018 mg/kg, far below the MRLs established for indoxacarb. The average recovery ranged from 83.3 to 91.6%, for three fortification levels. The precision of the method, expressed as RSD, was 0.1% and the matrix effect was 102.39%. The results obtained in this study confirm that the proposed method can be used for the determination of indoxacarb residues in peach fruits.

Keywords: *indoxacarb, peach, residues, method validation.*

Introduction

Peach production is obstructed by a great number of pests and diseases. According to significance and frequency of occurrence, *Cydia molesta*, the Oriental fruit moth or peach moth proved to be the most important pest. First damages are visible on young shoots as wilting and drying. Symptoms on fruits, mostly made by larvae of the following generations are visible later (Gvozdenović, 1997). Lack of the adequate protection causes significant and often, even complete decay of the plantation.

With the aim of *Cydia molesta* population control in peach plantation, products based on dimethoate, chlorpyrifos, α-cypermethrin, deltamethrin, λ-cyhalothrin, pyriproxyfen and chloranthraniliprole were registered. Waiting period of these products is within the interval of 14-28 days.

As many years lasting use of products belonging mainly to pyrethroids causes the occurrence of Cydia molesta populations resistant to these compounds, the aim is to introduce new active ingredients with different action mechanism, such as insecticide indoxacarb. The mechanism of action of indoxacarb consists in blocking sodium channels, which leads to the cessation of feeding, paralysis and death of the insect. It acts primarily digestively, but it has also contact action (MacBean, 2012). According to IRAC classification, it belongs to the group 22,A (IRAC, 2017). MRL for indoxacarb in peaches in EU (Anonimus, 2018), as well in the Republic of Serbia (Official Gazette, 2018), is 1.0 mg/kg, with 14 days waiting period of products based on indoxacarb. Indoxacarb residues are expressed as a sum of indoxacarb and their R enantiomer.

Inadequate use and failure to comply waiting period can cause the occurrence of residues of used products for crop protection in the quantity above MRL. Due to this, control of their content in agricultural products by use of corresponding methods is extremely important. According to the available literature data, the analysis of indoxacarb residues in different products of plant and animal origin has been accomplished by use of GC/EC and LC-MS/MS. After extraction of the samples with acetone and n-hexane (1:2 v:v), purification was accomplished by use of SPE cartridges. Limit of detection (LOD) of this method is 0.0015 mg/kg and 0.0006 mg/kg for GC-ECD, i.e. LC-MS/MS, and the achieved yield of extraction was 72.08 and 113.74% (Xu *et al*., 2008). Determination of indoxacarb enantiomers in cucumber, tomato, apple, pear, and soil samples were performed using high performance liquid chromatography and a Chiralpak AS-H column with n-hexane-ethanol (95 + 5, v/v) as the mobile phase (Cheng *et al*., 2010). In cabbage, indoxacarb was extracted using standard QuEChERS method (Urvashi *et al*., 2012). The objective of this study was validation of the method for determination of indoxacarb residues in peach fruits.

Material and methods

Chemicals and standard solutions

Analytical standard of indoxacarb 97% purity were obtained from Dr Ehrenstorfer, Germany. Acetonitrile (MeCN; HPLC grade) was purchased from "J.T.Baker" (Darmstadt, Germany), while ultrapure water was obtained from TKA apparatus (Germany). The dispersive SP extraction (Cat. No. 5982-5650) and clean-up (Cat. No. 5982-5356) kits for QuEChERS sample preparation were purchased as ready-to-use from Agilent Technologies (USA). A stock solution of indoxacarb was prepared in acetonitrile at a concentration of 91 μg/ml. Working standard solutions for HPLC analysis were prepared by appropriately diluting the stock solution with acetonitrile, achieving concentrations from 0.036-0.91 μg/ml. Stock solutions were stored at −20 ± 2°C, and working standard solutions were stored in the dark ⩽4°C.

Sample preparation

For the analysis untreated peach fruit samples was used. Untreated samples were previously crushed and homogenized. For the recovery experiment, such prepared sample (10 g), the adequate quantity of indoxacarb standard of the end concentration of 0.1, 0.5 and 0.91 mg/kg was added and left in the fridge overnight. The extraction of indoxacarb from peach fruits was carried out with QuEChERS method (Anastassiades et al., 2003; Lehotay, 2007). Briefly, 10 ml of acetonitrile was added, vigorously shaken for 1 minute manually, then 1 minute on vortex, after which followed adding of prepared buffer mixture of salt (Agilent Cat. No 5982-5650). It was immediately intensively shaken 1 minute by hand and 1 min on the vortex. Then, the cuvettes were transferred to the ultrasonic bath for 10 minutes and centrifuged for 5 min at 3000 rpm (Figure 1).

Figure 1 Determination of indoxacarb in peach fruits – extraction and clean-up

An aliquot of 6 ml was transferred from the supernatant to a centrifuge tube and cleaned by dispersive solid-phase extraction with 900 mg of magnesium sulfate, 150 mg of PSA and graphitized carbon black. Afterward, centrifugation was carried out at 3000 rpm for 5 min. An aliquot (3 ml) from the supernatant was evaporated till volume of 1 ml, filtered through a 0.45 μm PTFE filter (Millipore, USA) and then analyzed by HPLC–DAD.

Apparatus and chromatographic analysis

Indoxacarb residue analysis was performed with Agilent technologies 1100 Series high-performance liquid chromatographic system equipped with a diode array detector, while the separation was performed on a C18 column (50 × 4.6 mm, 1.8 μm). The mobile phase was (water and acetonitrile, 25:75, v/v) with a flow rate of 1.0 ml/min and detection wavelength of 310 nm.

Results and discussion

The method for the determination of indoxacarb is based on use of reverse-phase high performace liquid chromatography. In figures 2 and 3 are presented chromatograms of indoxacarb standard in acetonitrile and in peach matrix, as well as their overlapped spectra (Picture 4), under the aforementioned conditions.

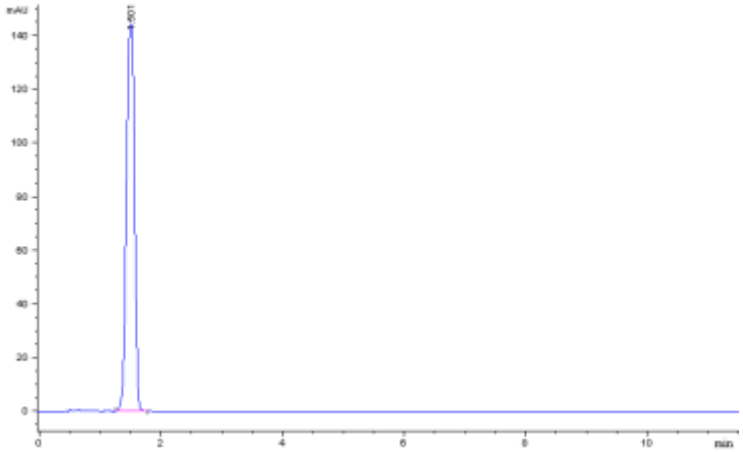

Figure 2 Chromatogram of standard indoxacarb standard solution in acetonitrile with a mass concentration of 1.0 μg / ml

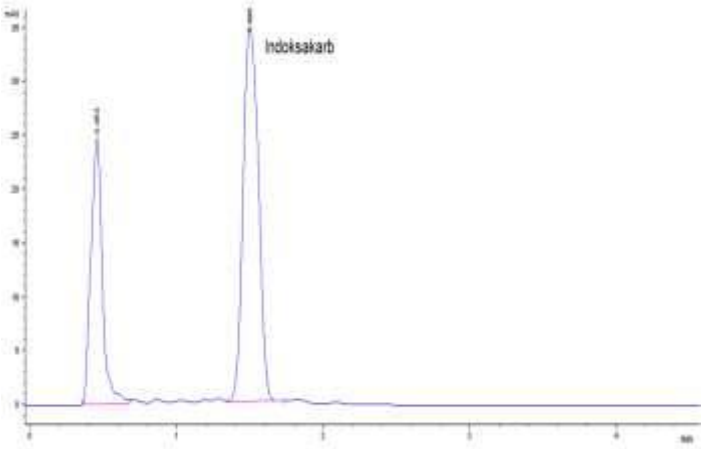

Figure 3 Chromatogram of indoxacarb standard solution in peach matrix with a mass concentration of 0,2 μg/ml

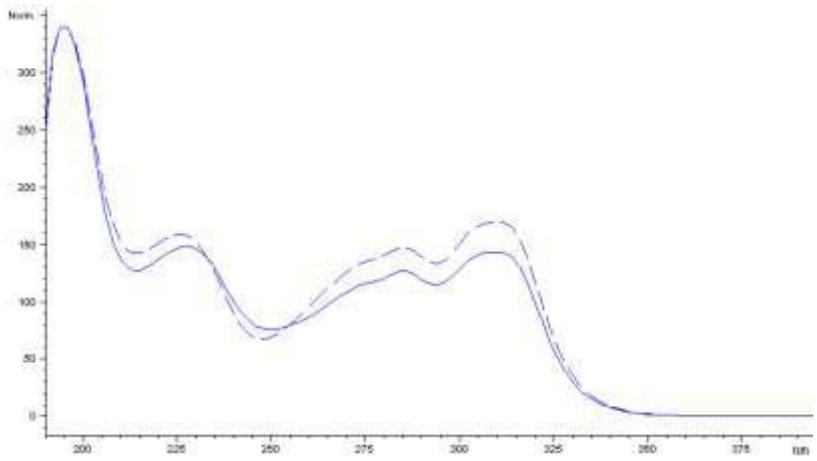

Figure 4 Indoxacarb overlapped spectrum in acetonitrile solution and in a peach matrix

The retention time of indoxacarb under the set conditions was found to be 1.501 min. Evaluation of the chromatographic conditions was carried out through the detector response linearity, the precision, and accuracy of the method, the matrix effect, as well as by determination of the detection and quantification limits.

Table 1 Validation parameters for indoxacarb determination in peach fruits

Linearity, 0.036-0.91 µg/ml	**Precision, RSD**	**Recovery**	**LOD**	**LOQ**	**ME**
r^2=0.996	0.01%	83.3 to 91.6%	0.006 mg/kg	0.018 mg/kg	102.4%

The linearity of the detector responses were evaluated at a concentration range between 0.036-0.91 µg/ml using five calibration solutions prepared in acetonitrile. In the range of the tested indoxacarab mass concentrations, a good linearity of the detectors was achieved. (r^2=0.996). The LOD (limit of detection) and LOQ (limit of quantification) were 0.006 mg/kg and 0.02 mg/kg, which are below MRL for indoxacarb set by EU (1.0 mg/kg) and Serbian legislation (0.5 mg/kg). Precision value was evaluated trough repeatability and expressed as relative standard deviation (RSD). The repeatability was checked by analysis of the indoxacarb standard prepared in peach matrix (0.6 µg/ml) on the same day, five times. Relative standard deviation (RSD) of the peak areas was 0.01%, fulfilling the criteria of RSD ≤20%. A critical aspect of pesticide residue analysis is the purification process, which is required to isolate the residues from matrix components and to reduce matrix effects (Lehotay et al., 2010). Matrix effect, i.e., signal suppression or enhancement, of the studied insecticide in peach matrix, was evaluated. For this study, the matrix effects were examined comparing the slopes of calibration curve of matrix-matched standards (MMC) and solvent-based standards (SC). Indoxacarb standards were prepared in blank matrix extract in five concentrations between 0.036-0.91 µg/ml. The influence of the peach matrix on indoxacarb signal by applicatin of this method is 102.39%, which indicates the absence of a significant impact of the matrix of the studied fruit species on the determination of indoxacarb, having in mind that good laboratory practice considers acceptable deviation of ± 15%. The accuracy of the method was carried out using blank sample spiked with a standard solution of indoxacarb insecticide at three levels. The mean recoveries for indoxacarb were 83.3 to 91.6%. According to the EU validation guideline for pesticide residues, mean recovery values should be within

the range of 70–120%, which was achieved by this method. Results of the recovery achieved in this study confirmed that the optimal recovery was obtained for indoxacarb in peach samples.

Conclusions

A simple and fast HPLC-DAD method for the determination of indoxacarb insecticide residue in peach fruits was developed. Reverse phase and isocratic elution conditions enable efficient separation of investigated insecticide. The proposed method has been validated with good recoveries and linearity, low LODs and LOQs, lower than MRLs, fulfilling the SANTE/11813/2017 criteria. The results obtained in this study confirm that proposed method is easy and reliable for the determination of the analyzed indoxacarb insecticide residues in peach fruits.

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EFFICACY OF INSECTICIDES ON *THRIPS TABACI* LINDEMAN ON ONION

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Abstract

In recent times a new technology of onion production has been introduced with getting high yields by sowing at 5cm spacing, the onion is obligationally foliarly treated with a growth hormone, maleic hydrazide in order to inhibit sprouting of stored onion bulbs in warehouses for purposes of favourable sales. In vegetation one of the most important pests is *Thrips tabaci* Lindeman, which regularly appears, it lives hidden, but the damage from *Thrips* many pathogens use as an open window for infection. The onion is used fresh for human consumption. In order for the onion production to be safe, the control of the onion demands the use of ecological and toxicological favourable insecticides. In field micro assays of 2016 and 2017 we setup assays and examined the efficacy of formetanate hydrochloride, abamectin, spirotetramat and spinosin active ingredients'. Efficiency of insecticides used in these assays depended on their persistence and ecotoxicological characteristics. On basis of the assay results, respecting the modification modes of insecticide mechanisms, formetanate hydrochloride is used at the beginning of the flight. Spirotetramat is added, which affects the new hatching of larvae, and for the time close to sowing of onion bulbs, abamectins and spinosins are used.

Key words: *onion,trips, Thrips tabaci, suppression, spirotetramate.*

Introduction

The production of onions in Serbia is taking a more important place per area and per yield. The technology of high yields requires production from seeds and the use of maleic hydrazide which secures firm bulbs and long storage in warehouses. The use of maleic hydrazide determines the production conditions:

a) Sowing of onion seeds is done by spacing of about 5.5 cm, depending on the number of rows in the garden bed,
b) Uniform germination, and
c) Healthy plants that adopt maleic hydrazide.

The most important is keeping the onion healthy i.e. avoid damages of *Thrips tabaci* (Thysanoptera, Thripetidae). This insect cutting the cuticle of the cell tissue, to suck the juices and inject eggs into the leaf tissue, creates an entrance for some pathogens (Brian, 2010; hasimran et all, 2015; Hault at all, 2016; Pouriau at all, 2019).

Photo 1. Antennae of *T. tabaci* with 7 segments (photo J. Perenčević)

Thrips regularly fly to the onion already at the beginning of vegetation and feed for two to 2 and a half months. This extended flight in the same timerepresents a problem in controlling population. Also, they live hidden and additional problem is highly efficient application of insecticides i.e. access of a.i.

The market accepts bulbs of certain standards - bulbs of 5-7 cm in diameter, 2 leaf scales, swollen base, non germinated bulbs and that are health safe. Having this in mind, the aim of this work was to test the effects of some specific insecticides on the *T. tabaci* control in quantities adapted to high technology. Choosed a.i. are at the same time ecotoxicologicaly favourable for use in onions protection.

Material and Methods

In 2016 an assay was set up on a winter onion cultivar Stutgarter Riesen within an experimental field of an Agricultural extension service with five variants, 3 treatments in 3 repetitions (Table 1).

The treatments were setup spatially by random block design. The experimental unit is one plant. One repetition is done on 15 randomly selected plants of a bed of 3 X 1.6m in size. The number of thrips was determined under the leaf petiole. We registered larvae and adults.

Threshold spraying is at the beginning of the flight of adult T.tabaci. The onion was treated the first time on May 13th, 2016, when it's flight was determined, then on May 19th and June 1st. (Table 1)

Table 1: Treatments in 2016, time application, variants and insecticide rates

Application date	Variant	Treatments	Active matter	Qty per ha
05/13/2016	**I**	Dicarzol 50 SP	formetanat hydrochloride	1 kg
05/19/2016		Dicarzol 50 SP + Movento 100 SC	formetanat hydrochloride spirotetramat	1 kg 1 L
06/01/2016		Lasser240 SC	spinosad	0.4 L
05/13/2016	**II**	Kraft 1.8 EW	abamectin	1.5 L
05/19/2106		Kraft 1.8 EW + Movento 100 SC	abamectin spirotetramat	1.5 L 1.0 L
06/01/2016		Lasser 240 SC	spinosad	0.4 L
05/13/2016	**III**	Movento 100 SC	spirotetramat	1.0 L
09/19/2016		Movento 100 SC		1.0 L
06/01/2016	**IV**	Lasser 240 SC Kontrola	spinosad	0.4 L
05/13/2016	**V**	Dicarzol 50 SP	formetanat	1.0 kg
05/19/2016		Dicarzol 50 SP	hydrochloride	1.0 kg
06/01/2016		Dicarzol 50 SP		1.0 kg

In 2017 we set up assays with onion sets of the Holland yellow cultivar, in five variants, 4 treatments and 3 repetitions. The experimental unit was one plant. One repetition was on an area of 4 rows, 5 meters, a spacing of 0.6 meters, 15 plants each were checked. The onion was checked on May 18th and 27th, June 5th, 13th and 20th, 2017. We counted larvae and adults using a 10x magnifying glass. We used the same insecticides as in the previous year in five variants of assays, but the treatment intervals were different. The assessment of the insecticide efficacy was made on the same day of the inspection. As in 2016, at the end of the assay, the bulbs were counted and measured according to variants and repetitions. The results were processed by the variant analysis.

Insecticides we used were:

1. Dicarzol 50 SP on basis of formetanat hydrochloride (carbamates). It falls into the cholinesterase inhibitores on CNS (IRAC 1A). It is considered the specific for the control of thrips, it especially has a good effect on the adults and larvae of the *T.tabaci,* and *F. occidentalis* as well. Dicarzol SP 50 was registered in Serbia in 2013, for controlling *T.tabaci* on onions at a quantity of 1 L ha^{-1}, with a waiting period of 28 days, so it is recommended for the first phase of onion development.

2. Lasser 240 SC on basis of spinosad, is an activator of nicotinic acetylcholine receptors, but on different places (IRAC 5) on neonicotines. As a product of fermentation of soil bacteria *Saccharopolyspora spinosa*, light decomposes it so it's effect is short. It is a lactone with great molecular weight (722), which limits the reception by the plant. Spinosad is adapted through the cuticle of the epidermal cell of the leaf, from where the thrips suck the juices for nutrition. It is ecotoxicologicaly favorable and it is also registered for organic production of vegetables. It is considered as the specific for the Thrips control, and especially on *Frankliniella occidentalis* and *T. tabaci*. It is recommended for the last sprinkling before harvesting of onions. It is applied a maximum of 3 times successively. It has a waiting period of 3 days on onion.

3. Movento 100 SC. Active ingridient is an spirotetramat which inhibits the new fat synthesis (IRAC 23), so the optimal time of use is at thrips larvae hatching time or those that do not have fat. The efficacy on the adult and larvae L2 and L3 is very limited, so it is recommended only in conditions of low or middle number of thrips. In the assay a quantity of 1 L ha^{-1}or with somewhat greater quantities that is registered (0.75 L ha^{-1}), because the insecticides were used in longer intervals between treatments. Movento is registered for use on tomatoes with a waiting period of 3 days.

4. Kraft 1.8 EW Active ingridient abamectin is an abamectin which stimulates the release of gama aminobutyric acids, inhibitor of the transfer of nerve signals and activates chlorine canals on CNS (IRAC 6). Produced during fermentation of soil bacteria *Streptomices avermitilis*. It falls into lactones or substances with great molecular weight (873.1) or is adopted only through cuticles in the epedermal cells of the leaf from which the thrips suck the juices. It has a greater number of metabolites which breakdown quickly in light. It is used in the afternoon when the temperature is slightly lower and the relative humidity is greater. Abamectin is also used for the last sprinkling, because it has a shorter waiting period of 14 days on tobacco, and on beans and soy beans 7 days. In order to ensure a somewhat longer efficacy between two treatments, we used a lager amount than registered or 1.5 L ha^{-1}. In the application of insecticides, wetting agent Trend 90 in a concentration of 0.1% was added.

Results and Discussion

1. Density of *T. tabaci* on check in 2016 year

On the check of May 13th, we determined that adults flew onto onions, which have a characteristic of the T.tabaci variety and among others were also seven antannae (picture 1). The population of *T.tabaci* comprises mainly of females with a lesser presence of males. The females lay a great number of eggs by rasping with ovipositors into the tissue base of the onion leaf where the larvae and adults feed (picture 3). A total of 14 *T.tabaci* adults were found (Table 1). The action threshold is the presence of *T.tabaci*, so treatments with insecticides were carried out.

Tab.1: Infestation of *T.tabaci* on the Untreated in assesments in 2016

Treatments	Preparation	Number *T.tabaci* at the time of assesment			
	Qty per ha	May 13	May 19	May 27	June 1
Untreated	-	14	21	213	197

In the assessment of May 19th, a greater number was determined (21), and on May 27th, a more massive flight of adults and hatching of larvae occured (213). In the assessment of June 1st, there were no new flights of adults, so the number of the adult *T.tabaci* was somewhat reduced (197). The number of *T.tabaci* on the 45 checked onion plants was not significant and great damage should not be expected on the onion in this year on the assayed area.

Photo number 2: Insecticide efficacy variant I on the *T.tabaci* (photo J. Perenčević)

In relation to the Untreated, Dicarzol 240 SP was used in the first treatment, then in the second with the addition of Movento 100 SC and in the third treatment Lasser 240 SC, which significantly reduced the number of *T.tabaci* larvae, and in the inspection of June 11th, only 29 larvae and adults (Table 2) or less than one thrips per plant were registered.

Photo number 3: Larve *T.tabaci* in small social group in feeding place (photo J. Perenčević)

Photo number 4. Damage of *T.tabaci* on onion stalk epidermis (photo J.Perenčević)

Tab.2: Efficacy of insecticides and variant I on *T.tabaci*

Treatments	Preparation Qty per ha	Treatment times	Number *T.tabaci* in inspections			
			May 13	May 19	May 27	June 1
Before treatment		Before setting of assay	4			
Dicarsol 50 SP	1 kg	May 13	-	8		
Dicarsol 50 SP +Movento 100 SC	1 kg 1 L	May 19	-	-	8	
Lasser 240 SC	0.4 L	May 27	-	-	-	29

3) Efficacy of insecticide variant II. on the *T.tabaci*

In this variant similar results were received as in the previous variant I. In this variant similar results were obtained as in the previous variant I. Kraft 1 EW as an adulticide in the first, Kraft 1.8 EW+Movento in the second treatment and in the third treatment only Lasser 240 SC, have shown similar efficacy in relation to *T.tabaci* (Table 3) or in relation to the Untreated number was significantly reduced.

Tab.3: Efficacy of insecticide variant II to *T.tabaci*

Treatments	Preparation Qty per ha	Treatment time	Number *T.tabaci* in inspections			
			May 13	May 19	May 27	June 1
Before treatment	-	Before treament	10			
Kraft 1.8 EW	.5 L	May 13		5		
Kraft 1.8 EW 1.5 lit/ha+Movento 100 SC 1.0 lit/ha		May 19				
Lasser 240 SC 0.4 lit/ha	0.4 L	May 27				43

4) Insecticide efficacy variant III on *T. tabaci*
The application of Movento 3 times as a larvicide, a certain increase of the number of adults on May 19 and May 27 was determined, in relation to the application of adulticides in previous variants. However, Movento affected the neonate larvae and on May 27th, a similar number of *T.tabaci* (49) was determined as in previous variants (Table 4).

Table 4: Efficacy of insecticide variant III on *T.tabaci*

Treatments	Preparation Qty per ha	Treatment time	Number *T. tabaci* in inspections May 13	May 19	May 27	Jun 1
Before treatment	-	May 13	3			
Movento 100 SC	1 L	May 13		19		
Movento 100 SC	1 L	May 19			12	
Movento 100 SC	1 L	May 27				49

Broj T.tabaci u poredima

5)Efficacy of insecticide variant V on *T. tabaci*
In implementation of Dicarzol 50 SP as an adulticide, there was no significant increase in the number of *T. tabaci* in the phase of adult flight, but Dicarzol had a weaker effect on larvae and adult *T.tabaci* were registered.

Table 5: Efficacy of insecticide variante V on *T.tabaci*

Treatments	Preparation Qty per ha	Treatment time	Number *T.tabaci* in checks May 13	May 27	May 27	June 1
Before treatment	-	-	4			
Dicarzol 50 SP	1 kg	May 13		14		
Dicarzol 50 SP	1 kg	May 19			14	
Dicarzol 50 SP	1 kg	May 29				54

The onion was not treated after July 1st, 2016. During the assessment on July 27th 2016 or 27 days after the last treatment, we recorded a difference in the number and weight of the onion bulbs per variant (Table 6).

Table 6. Number, bulb weight and % of dry matter of the Stuttgarden Riesen cultivar

Variants	Number of bulbs on an area of 40.5m^2	Bulb weight in kg on an area of 40.5m^2	% dry matter
	327	38.31	12.7
1.Kraft	311		
2.Kraft+Movento		38.35	14.78
3.Movento			
Movento 3x	326	39.10	15.18
Untreated	312	36.25	14.32
Dicarzol 3 x	319	38.59	14.16

A greater weight of the bulbs was determined in insecticide implemented variants in relation to the Untreated.
The greatest bulb weight was at the implementation of Movento. However, the statistical analysis of the bulb weight (LSD 0.05 =1.59, 0.01=2.31) showed that there is no significant influence of the application of insecticides on the mortality and feed of the thrips expressed in

the bulb yield. The increase of dry matter after the treatment of the onion with Movento is a more reliable answer of the onion, but because of the previous irrelative differences, this should be understood as a possibility (Tabela 6). Number of *T.tabaci* at the Check on onion surfaces in 2017 year

Table 7 Number of *T.tabaci* at Untreated plot

Check day	Number of ***T.tabaci*** per repetations							
	I repeat		II repeat		III repeat		Total	
	Adult	Larvae	Adult	Larvae	Adult	Larvae	Adult	Larvae
May 18.2017	8		6	6	4	1	18	9
05/26/17	1	16	3	39	4	26	8	81
05/05/17	13	49	16	225	15	120	44	394
06/13/17	9	0	6	197	31	146	46	423
06/20/17	3	92	11	252	2	258	16	602

In the Check of May 18, the flight of *T.tabaci* adults was determined (18). Nine larvae were found or hatching began the prior week. Treatment was performed on May 18.
The first time massive flight of adults was determined in period between June 5th and 13th in 2017. The onion was in good health, which increased the fertility of the females and the time and number of larvae (June 20th, 602 larvae) or 13 thrips per plant. A high number of *T. tabaci* presumes a reduction in the yield of onion bulbs. At the treatment with Dicarzol 50 SP, Dicarzol 50 SP + Movento 100 SC, 2 times and Lasser 240 SC, 2 times, the number of *T.tabaci* at the Untreated on June 20th 2017, determined 8 adults and 64 larvae. At the treatment with Kraft 1.8 EW, Movento 100 SC, 2 times and Lasser 240 SC, 2 times, the maximum number of T.tabaci was on June 13th, when 36 adults and 103 larvae were found, but on June 20th, 36 adults and 80 larvae were found. At the treatment with Movento 100 SC, 3 times, Lasser 24O SC, 2 times, the maximum number was on May 26th, when there were 10 adults and 148 larvae, but in the last Check on June 26th, there was 11 adults and 82 larvae.
At the treatment with Dicarzol 50 SP, 3 times, Lasser 240 SC, 2 times, on June 5th on the onions there were 28 adults and 123 larvae, but at the last inspection there were 17 adults and 67 larvae. As can be seen, at all treatments in relation to the Check, a very high efficacy was achieved or there was no significant damage. Number of bulbs and yield of onion on insecticide variants on the Holand yellow cultivar in 2017

Table 8: Imact of used insecticides on onion yield

Variants	Yeald per repeatation in kg			±SD	Total kg	Increase of yield in relation to Untreated (%)
	1	2	3			
1. Dicarzo 50 SP Dicarzol 50 SP+Movento100 C 2 x Lasser 240 SC	16.2	19.7	16.8	17.57 (1.87)	53.70	110.14
2. Kraft 1.8 EW Kraft 1.8 EW+Movento 100 SC Dicarzol SP 50	18.9	19.8	17.2	18.63 (1.32)	55.90	116.2
3. Movento 2 x Kraft 1.8 EW 2x	20.3	16.2	18.5	18.33 (2.06)	55.0	114.9
5. Dicarzol 4 x	18.6	14.5	17.4	16.70 (2.37)	50.10	104.7
4. Untreated	17.3	14,1	16.5	15.95 (1.66)	47.85	100.0

LSD 0.05=3.65 LSD 0.01=5.30

A determination was made of the increase of yield of onion bulbs at treatments with insecticides and especially when Movento was used 2 times. However, analysis show that this increase is not statistically significant. The strategy in controling *T. tabaci* on the onion is on maintaining a low number. Strongly recommended the first application of insecticide after adult eclosion. In the other hand, in practice spraying is often in condition of high population of *T. tabaci*. But, it is impossible to control high trips population as a consequence of hidden insect life on the plant. This was proven also in assays of both years. In the first part of vegetation during the time of intensive growth of the onion, a greater quantity of insecticides is used with spraying interval of 6 days. The economic thresholds are different and depend on the sensitivity of the onion to the of *T.tabaci*. Granshaw (2004) suggests 15 to 30 thrips per plant as a threshold level. However, at the application of spirotetramats (Movento), because of slower action, a threshold of 1 thrips per plant is suggested. In our assays, at the application of the mentioned insecticides in various combinations, the number of *T.tabaci* was continiously under the thresholds.

Conclusion

T. tabaci on the onion is successfully controlled with insecticides of different mechanisms, and have effect on the adult and the larvae such as formetanat hydrochloride, abamectin, spinosad and spirotetramat. Onions are most sensitive at the beginning of growth when protection should be carried out.Insecticides applied after adult eclosion .

Spirotetramat acts on the neonate larvae but through inhibition of synthesis of fat and the reduction of the fertility of the female. By adding an adulticide, such as Dicarzol 50 SP, Kraft 1.8 EW and Lasser 240 SC to the spirotetramat, a synergetic effect on the adult, neonate larvae and the reduction of the fertility of the female is obtained. In practical conditions, on greater areas, these preparations have satisfied protection of the onion.

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INFLUENCE OF *CURCULIO GLANDIUM* (MARSHAM, 1802) (COLEOPTERA, CURCULIONIDAE) ON TURKEY OAK (*QUERCUS CERRIS* L., 1753) (FAGALES, FAGACEAE) ACORN GERMINATION

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Abstract

Turkey oak is one of the most common oak species in Serbia. Its wood has a high calorific value, and that is why it is commonly used for firewood in Serbia. In the last few years the oak started to dieback, mainly in the northern parts of Serbia. Because of this, and many other reasons like the negligible presence of young stands in the total area, and the prevailing vegetative origin of, Turkey oak forests must be regenerated. For the natural regeneration of its forests, a significant amount of healthy acorn is needed. Unfortunately, Turkey oak acorn is frequently infested by insects. We focused on the influence of the damage caused by of one of them, *Curculio glandium* (Marsham, 1802) on the Turkey oak acorn germination. The research was conducted on three localities in the vicinity of Belgrade. Five trees from which the acorns were collected were selected at each site. Acorn rate of infestation and germination were then analyzed. The infestation rates ranged from 24 to 60% depending on the tree. There were no statistically significant differences in the infestation rates between the different localities and the individual trees. A statistically significant difference in germination was found between undamaged and damaged acorns. The average germination rate was 35.8% for the damaged, and 66.1% for the undamaged acorns. A significant difference in germination was also noted between the localities where the acorns were collected. The germination of the damaged acorns was significantly influenced by their dimensions as well. The larger acorns had a higher germination rate.

Keywords: *acorn weevil, Serbia, generative regeneration, germination rate*

Introduction

Turkey oak (*Quercus cerris* L.) is one of the most common oak species in Serbia. Its forests cover an area of about 345.200 ha (Bankovic et al. 2009) which is circa 15.3% of the total forest area in Serbia. Its wood has a high calorific value, but poor mechanical properties (Soskic 2006) and that is why it is most commonly used for firewood. In the last few years, the oak started to dieback in the northern parts of Serbia (Karadzic et al. 2017). Because of this, and many other reasons, like the negligible presence of young stands in the total area, and the prevailing vegetative origin of its forests (Bankovic et al. 2009) Turkey oak forests must be regenerated. For their natural regeneration, a significant amount of healthy acorn is needed. Unfortunately, oaks acorn is infested by a large number of pests (Hrasovec & Magdalenic 1996; Hirka 2003; Csoka & Hirka 2006; Drekic 2006; Drekic & Mihajlovic 2007, 2011; Bak-Badowska et al. 2017; Kanjevac et al. 2017). We investigated the influence of one of them - *Curculio glandium* (Marsham, 1802) (Coleoptera, Curculionidae) on Turkey oak acorn germination. Females of *C. glandium* lay their eggs in young oak acorns. Eggs hatch and the larvae start to feed on the acorns cotyledons. After the acorns drop off the trees, the larvae complete their postembryonic development, abandoning the infested fruit by a circular exit hole to bury in the ground for overwintering. The adults emerge in the spring. A portion

of the population remains in diapause in the larval stage for several years. (Forrester 1990, Mihajlovic 2008, Drekic 2007).
C. glandium is always present in oak forests in Serbia. According to Mihajlovic (2008) at normal population density 10 to 30% of acorns is infested, but during outbreaks all of the acorns (100%) can host a grub.

Material and methods

The research of the influence of *C. glandium* (Marsham. 1802) on the germination of Turkey oak acorn was carried out in 2017. The acorns were collected on three localities in the vicinity of Belgrade: Lipovica (44°38'48"N, 20°24'22"E), Zuce (44°40'59"N, 20°31'47"E), Kosutnjak (44°46'19"N, 20°25'31"E) in February. Five trees from which the acorns were collected were selected at each locality. Acorns from one tree represented one sample. One hundred acorns were randomly selected from each sample to quantify the infestation rate, based on the presence of exit holes. Then, 30 damaged and 30 undamaged acorns were taken from each sample to determine their germination rates. The acorns were placed in plastic containers filled with sand which was sterilised for 6 hours at the temperature of 200ºC. The germination rate was determined after 28 days. Acorns not used for the analyses above were used for obtaining the adults.
All the data obtained were analysed in Statistica 8.0. (StatSoft) Software. The significance of the differences in observed properties was determined by one-way ANOVA, at the level of significance $p<0.05$.

Results and discussion

The breeding got only adults of one weevil species: *C. glandium*. Its larvae damaged an average of about 39% of acorns (Table 1). No statistically significant differences in the infestation rates between the localities and individual trees on them were determined ($p>0.05$). According to Mihajlovic (2008), this infestation rate is above normal for Turkey oak in Serbia. It is considerably greater than that which Csoka & Hirka (2006) recorded on *Q. cerris* in 2001 and 2002 (3% and 18%) in Hungary.

Table 1. *C. glandium* infestation rate (%)

	Locality		
Tree number	Kosutnjak	Lipovica	Zuce
1	47.0	39.0	50.0
2	36.0	34.0	55.0
3	24.0	47.0	25.0
4	32.0	30.0	40.0
5	60.0	34.0	38.0
$\bar{x}$	39.80±14.00	36.80±6.53	41.60±11.63
$\bar{x}$	39.40 ± 10.54		

After analysing the acorns collected from each tree on each locality, it was determined that the acorns damaged by *C. glandium* were larger, i.e. they had a larger diameter ($F=10.15$, $p<0.05$). Dong et al. (2012) got similar results while examining the acorns of *Q. aliena* Blume attacked by the weevil *C. davidi* Fairmaire & L., 1878.

Table 2. Average dimensions of the analysed acorns (cm)

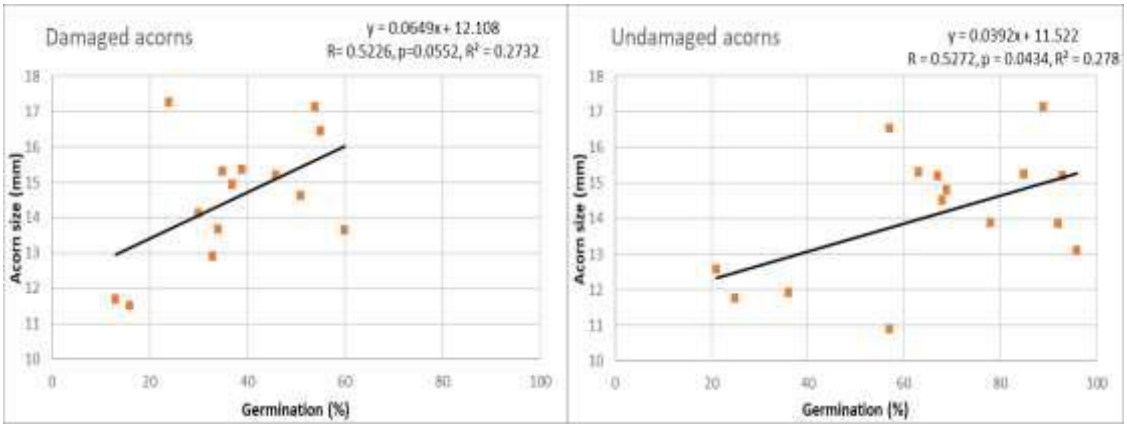
Locality	Dimension			
	Length		Diameter	
	undamaged	damaged	undamaged	damaged
Kosutnjak	27.58±4.33	27.58±3.44	13.69±2.24	13.79±1.89
Lipovica	27.58±2.80	27.85±3.27	14.90±2.01	15.62±3.27
Zuce	27.52±3.99	28.16±3.51	14.02±2.27	14.78±2.30
$\bar{x}$	27.56±3.72	27.86±3.40	14.26±2.22	14.75±2.18

Damage caused by *C. glandium* significantly affected the acorn germination rates ($F=35.51$, $p<0.05$). The average germination rate of the damaged acorns on all three localities was 35.75±15.58%, and of the undamaged 66.09±24.76%, which is 84.87% higher. Statistically significant differences in germination rates between the localities existed in cases of both damaged ($F=5.34$, $p<0.05$) and the undamaged ($F=7.73$, $p<0.05$) acorns. The highest germination rate was recorded on the acorns collected on locality Lipovica, and the lowest on those collected on locality Zuce. Individual trees did not influence germination rates ($p>0.05$). This difference in germination rates between the damaged and undamaged acorns is considerably higher than that which Hirka (2003) determined in Hungary.

Table 3. Acorn germination rates (%)

Tree Number	Locality					
	Kosutnjak		Lipovica		Zuce	
	undamaged	damaged	undamaged	damaged	undamaged	damaged
1	67.0	30.0	85.0	55.0	69.0	35.0
2	63.0	37.0	92.0	39.0	57.0	24.0
3	25.0	16.0	89.0	54.0	78.0	34.0
5	68.0	51.0	96.0	60.0	36.0	33.0
6	57.0	13.0	93.0	46.0	21.0	0.0
$\bar{x}$	56.0±17.86	29.4±15.60	91.0±4.18	50.8±8.29	52.2±23.49	25.2±14.75
$\bar{\bar{x}}$	42.7±21.13		70.9±22.07		38.7±23.33	

The existence of a positive correlation between the acorn diameters and germination rates in cases of both damaged and the undamaged acorn was determined based on the results of the regression analysis (Graph 1). Such a relation between the acorn dimensions and germination rates was also determined by Forrester (1990) and Ramos et al. (2013). According to them, that relation is even more pronounced if the acorns are damaged. Forrester (1990) and Hirka (2003) explain that by the fact that a single larva consumes a certain amount of food to finish its development. In large acorns that amount comprises a smaller part than in small ones. Because of that, the seedlings in larger acorns have a better chance of survival.



Graph 1. Influence of diameter on acorn germination rate

Results of the research conducted showed that *C. glandium* significantly reduced the acorn germination rates. They also showed us that a part of the damaged acorns still germinated (around 36%). How many of them will germinate depends on the genetic variability of the trees, severity of the damage on the acorn, the place of the damage on the acorn and environmental conditions (Forrester 1990; Yu et al. 2003; Hirka 2003; Csoka & Hirka 2006; Hou et al. 2010; Dong et al. 2012; Ramos et. al 2013; Oltean et al. 2014; Bak-Badowska et al. 2017; Bartlow et al. 2018). However, plants which grow from the damaged acorns generally grow slower and are physiologically weaker. They have a smaller height, girth, size of the leaves, amount of dry plant mass, amount of nitrogen, chlorophyll, and further (Yi & Zhang 2008).

Conclusions

Based on the above, the following conclusions can be made:

- *C. glandium* infested about 39 % of the acorns;
- Damaged acorns were larger that the undamaged ones;
- The damage caused by *C. glandium* lowered the germination rates significantly;
- The damaged acorns had a germination rate of about 36%
- The germination rates of the damaged and undamaged acorns varied significantly between the localities;
- Larger acorns had a higher germination rate.

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TERBUTHYLAZINE APPLICATION WITH HERBICIDES OF DIFFERENT MODE OF ACTION IN MAIZE CROP

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Abstract

Based on the damage caused by weeds in maize crops, the efficiency of a large number of commercial preparations with different active ingredients was examined in order to suppress dominant weed species. The experiment with terbutylazine in combination with S-metolachlor and mesotrione was placed at the following locations: Zobnatica, Ruski Krstur and Gornja Badanja (Serbia). Efficacy and phytotoxicity was evaluated according EPPO/OEPP standards. The dominant weeds were: *Abutilon theophrasti* Medic., *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Avena fatua* L., *Bilderdykia convolvulus* L., *Cannabis sativa* L., *Chenopodium album* L., *Chenopodium hybridum* L., *Cynodon dactylon* (L.) Pers., *Cirsium arvense* (L.) Scop., *Datura stramonium* L., *Galinsoga parviflora* Cav., *Helianthus annuus L., Hibiscus trionum* L., *Matricaria chamomilla* L., *Mentha arvensis* L., *Polygonum aviculare* L., *Plantago minor* L., *Potentilla reptans* L., *Rumex patientia* L., *Solanum nigrum* L., *Sonchus arvensis* L., *Taraxacum officinale* Web., *Veronica persica* Poir., *Xanthium strumarium* L. At the present weed flora in the location of Gornja Badanja, the combination of terbutylazine and S-metolachlor, applied after sowing and before the emergence of weeds and maize, had a low efficiency (59.70-66.30%). At the location of Ruski Krstur, the combination of terbuthylazine (125 g/l) and mesotrione (50 g/l), foliar applicated, had a satisfactory efficiency (88.61%) during the first and good efficacy (94.72%) during the second assessment of the present weed flora. Combination of terbuthylazine (326 g/l) and mesotrione (50 g/l), foliar applicated, had a satisfactory (81.94%) and good efficiency (92.77%) on the present weed flora at the location of Zobnatica.

Keywords: *Maize, Weeds, Terbutylazine, Mesotrione, S-metolachlor*

Introduction

Maize (*Zea mays* L.) is the third most important cereal crop in the world after wheat and rice (Mhlanga et al., 2016). Due to weeds, worldwide yield losses in maize are estimated to be around 37% (Oerke and Dehne 2004), therefore, weed control plays an important role in maize production, ensuring an acceptable yield. Weed control in maize is carried out mainly by mechanical and chemical methods, but use of herbicide is increasing, along with increases in growing areas and production costs (Kir and Dogan, 2009). Serbia quickly and efficiently adopted the achievements of modern phytomedicine, and great success has been achieved in weed control (Stefanović et al., 2011). In recent years, the focus has been shifted to foliar herbicides, with the aim of achieving their maximum efficiency, primarily due to the reduced efficiency of soil herbicides under conditions of insufficient rainfall, the limitations of using terbuthylazine, the emergence of resistant weed populations, and the introduction of foliar herbicides that are extremely effective in suppressing a large number of weed species (Radivojević et al., 2015). The application of herbicides is the most effective and economical agronomic practice for weed management in all major field crops, contributing significantly to increased crop yields (Bartucca et al., 2017). Maize crop is often characterized by a complex of weed flora, which consists of broadleaved and grass weeds (Pannacci and Onofri, 2016). This weed flora have been controlled with pre-emergence applications based on herbicide terbuthylazine, because of its broad controlled weed spectrum, superior residual

activity, great crop tolerance, good efficacy, and suitability as a partner for other active ingredients (Schulte et al., 2012). In order to minimize the application cost and optimize weed control efficacy, the use of combinations of pre- and post-emergence herbicides, as well as herbicide mixtures, has become the rule in many countries, what represents an important tool to avoid problems related to herbicide resistance (Pannacci and Onofri, 2016).

Terbuthylazine is a broad spectrum chloro-triazine herbicide that acts as a powerful inhibitor of photosynthesis (Tariba Lovaković et al., 2017). It is used for weed control in pre- and post-emergent treatment of a variety of agricultural crops as well as roads, industrial area, and railways (EFSA, 2017). Depending on the soil characteristics, terbuthylazine has a very high soil persistence, it can be present up to 17 months after the field treatments (Stipičević et al., 2015). Terbuthylazine is used as a substitute for atrazine, which has been banned in EU countries (Tariba Lovaković et al., 2017). In 2011, the European Food Safety Authority (EFSA) provided an extensive peer review of data concerning the environmental behavior and fate, ecotoxicology and mammalian toxicology, and risk assessment of terbuthylazine (EFSA, 2011). Based on these conclusions the European Commission approved the inclusion of terbuthylazine in Annex I of Council Directive 91/414/EEC and its use only as a herbicide until December 2021 (Commission Implementing Regulation (EU), 2011). S-metolachlor belongs to the acetamides chemical group and is generally applied during pre-planting, pre-emergence, or post-emergence (Milan et al., 2015). S-metolachlor is a selective herbicide, absorbed by shoots and hypocotyls, and inhibits weed germination. Terbuthylazine and S-metolachlor are widely used to control weeds in maize. Mesotrione is another important herbicide, provides pre- and post-emergence control of broadleaf weed species, primarily in maize (Mendes, 2017). It is a member of the triketone family of herbicides that is applied mainly in combination with other herbicides (Milan et al., 2015). Mode of action is to inhibit the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), which affects carotenoid biosynthesis (Mitchell et al., 2001).

The aim of this study was to determine which combination is most effective for the existing weed flora in the application of terbuthylazine with soil and foliar herbicides, depending on the weediness, agro-ecological conditions, in purpose of profitability of agricultural production.

Material and Methods

The research was conducted during 2016 and 2017 at the following locations: Zobnatica, Ruski Krstur and Gornja Badanja (Serbia). Herbicides efficiency trail was set up in maize crop by random block system, with elementary plots of 25 m^2 in 4 repetitions. Control plot, not treated with herbicides, was also included. The experiment was set up according to the EPPO/OEPP standards (2008), in order to test the efficacy and phytotoxicity of the applied herbicides in different amounts and combinations. Tests with soil and foliar herbicides were made depending on the presence and dominant weed flora. Weed control included pre-emergence treatment, where terbuthylazine was applied in combination with S-metolachlor, and post-emergence treatment in the 4-6 leaf phase of maize where terbuthylazine was applied in combination with the herbicide mesotrione. The efficacy of the treatment and the intensity of weediness was determined by counting weeds per m^2 in two assessment, 14 and 28 days after treatment in a post.em. application of herbicides, and 28 and 42 days after treatment in a pre.em. application. Based on the obtained data, the coefficient of efficiency Ce (%) of herbicides is calculated by the formula Dodel loc. cit. Janjić (1985) and represents a relative ratio between the number of destroyed weeds compared to the weeds number in the control. Visual assessment of the phytotoxicity was performed according to the European Weed Research Council (EWRC) scale (1-9). Data were analyzed statistically using analysis of variance, F, and LSD tests.

Results and Discussion

At the investigated locality of Zobnatica, Gornja Badanja and Ruski Krstur, where experiments on the efficacy of herbicides were set, the following weed species were identified: *Abutilon theophrasti* Medic., *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Avena fatua* L., *Bilderdykia convolvulus* L., *Cannabis sativa* L., *Convolvulus arvensis* L., *Chenopodium album* L., *Chenopodium hybridum* L., *Cynodon dactylon* (L.) Pers., *Cirsium arvense* (L.) Scop., *Datura stramonium* L., *Panicum crus – galli* L., *Galinsoga parviflora* Cav., *Helianthus annuus L., Hibiscus trionum* L., *Matricaria chamomilla* L., *Mentha arvensis* L., *Polygonum aviculare* L., *Plantago minor* L., *Potentilla reptans* L., *Rumex patientia* L., *Setaria glauca* L., *Solanum nigrum* L., *Sorghum halepense* (L.) Pers., *Sonchus arvensis* L., *Taraxacum officinale* Web. *Veronica persica* Poir., *Xanthium strumarium* L.

During 2016, at the locality of Gornja Badanja, application of the pre-emergence herbicides, S-metolachlor and terbuthylazine, during the first and second assessment of efficiency gave the following results presented in table 1.

Table 1. The presence of weeds in maize crop, and the efficacy of herbicides after the first and second assessment, locality of Gornja Badanja

Weed species	First assessment			Second assessment		
	Control	Basar+ Rezon (S-metolachlor 960 g/l + terbuthylazine 500 g/l)		Control	Basar+ Rezon (S-metolachlor 960 g/l + terbuthylazine 500 g/l)	
	No/m^2	No/m^2	Ce (%)	No/m^2	No/m^2	Ce (%)
Amaranthus retroflexus L.	0.60	0.30	50	0.20	0.10	0
Ambrosia artemisiifolia L.	19.90	1.60	91.90	10.10	0.20	98.00
Avena fatua L.	4.40	3.40	22.70	0.60	0.40	33.00
Bilderdykia convolvulus L.	1.10	0	100	3.80	2.90	23.60
Cynodon dactylon (L.) Pers.	1.80	0.20	88.80	4.50	0.10	97.70
Cirsium arvense (L.) Scop.	4.40	3.60	18.00	5.30	2.40	54.70
Convolvulus arvensis L.	0.80	0.20	75.00	2.70	0.90	66.60
Galinsoga parviflora Cav.	0.70	0.50	28.50	0.60	0	100
Matricaria chamomilla L.	0.20	0	100	1.20	0.10	91.60
Mentha arvensis L.	1.90	0.60	68.00	1.40	0.30	78.50
Panicum- crus-galli L.	4.90	4.10	16.30	12.40	7.50	39.50
Polygonum aviculare L.	2.70	2.20	18.50	0.20	0	100
Plantago minor Garasult.	0.90	0.10	88.80	1.10	0.20	81.80
Potentilla reptans L.	0.50	0.30	40.00	0.30	0.10	66.60
Rumex patientia L.	1.50	0.10	93.30	0.40	0	100
Sorghum halepense (L) Pers.	7.50	6.30	16.00	6.10	3.50	42.60
Sonchus arvensis L.	0.80	0	100	1.10	0.40	63.60
Taraxacum officinale Web.	3.40	1.40	58.80	2.10	0.90	57.00
Total number of weeds	**54.00**	**21.00**	**59.70**	**58.00**	**20.00**	**66.30**
Phytotoxicity (EWRC scale 1-9)	**-**	**1**	**1**	**-**	**1**	**1**

The combination of terbuthylazine and S-metolachlor, applied to the soil, had a good efficacy (> 90%) in the first assessment on: *Ambrosia artemisiifolia* L., *Matricaria chamomilla* L., *Bilderdykia convolvulus* L., *Rumex patientia* L., *Sonchus arvensis* L., and in the second

assessment on: *Ambrosia artemisifolia* L., *Cynodon dactylon* (L.) Pers., *Galinsoga parviflora* L., *Matricaria chamomilla* L., *Polygonum aviculare* L., *Rumex patientia* L. Satisfactory efficacy (75-90%) in the first and second assessment was on: *Plantago minor* Garasult, and *Mentha arvensis* L. Low efficacy (<75%) of tested herbicides in first assessment was on: *Amaranthus retroflexus* L., *Avena fatua* L., *Cirsium arvense* (L.) Scop., *Panicum-crus-galli* L., *Galinsoga parviflora* Cav., *Mentha arvensis* L., *Polygonum aviculare* L., *Potentilla reptans* L., *Sorghum halepense* (L.) Pers., *Taraxacum officinale* Web., and in the second assessment on: *Amaranthus retroflexus* L., *Avena fatua* L., *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* L., *Panicum-crus-galli* (L.), *Bilderdykia convolvulus* L., *Potentilla reptans* L., *Sorghum halepense* (L.) Pers., *Sonchus arvensis* L., *Taraxacum officinale* Web.

Application of the foliar applied herbicides based on active substance mesotrione and terbuthylazine (50 + 326 g/l), at the locality of Zobnatica, during the first and second assessment of efficiency gave the following results presented in table 2.

Table 2. The presence of weeds in maize crop, and the efficacy of herbicides after the first and second assessment, locality of Zobnatica

Weed species	First assessment			Second assessment		
	Control	Calaris Pro (mesotrione 50 g/l + terbuthylazine 326 g/l)		Control	Calaris Pro (mesotrione 50 g/l + terbuthylazine 326 g/l)	
	No/m^2	No/m^2	Ce (%)	No/m^2	No/m^2	Ce (%)
Abutilon theophrasti Medik.	2.00	0	100	2.50	0	100
Amaranthus retroflexus L.	2.00	0	100	2.75	0	100
Ambrosia artemisiifolia L.	7.25	2.50	65.52	7.25	1.50	79.31
Bilderdykia convolvulus L.	9.00	2.00	77.78	9.00	1.00	88.89
Cannabis sativa L.	9.00	3.50	61.11	9.25	1.25	86.48
Convolvulus arvensis L.	3.00	1.00	66.67	3.50	1.00	71.42
Chenopodium hybridum L.	18.50	2.50	86.49	17.50	1.00	94.28
Chenopodium album L.	15.25	1.00	93.44	13.25	0	100
Datura stramonium L.	19.50	2.00	89.74	19.25	0.75	96.10
Panicum crus – galli L.	1.00	1.00	0	1.00	1.00	0
Helianthus annuus L.	5.25	2.00	61.90	4.25	0	100
Hibiscus trionum L.	15.25	2.00	86.88	17.50	0	100
Solanum nigrum L.	3.25	0.00	100	4.00	0	100
Sorghum halepense (L.) Pers.	0.50	0.50	0	0.75	0.50	33.33
Total number of weeds	**110.75**	**20.00**	**81.94**	**108.75**	**8.00**	**92.77**
Phytotoxicity (EWRC scale 1-9)	**-**	**1**	**1**	**-**	**1**	**1**

Terbuthylazine in a higher amount (326 g/l) in combination with mesotrione (50 g/l), foliar-applied, in the first and second assessment had good efficacy (> 90%) on: *Abutilon theophrasti* Medik., *Amaranthus retroflexus* L., *Chenopodium album* L., *Datura stramonium* L., *Helianthus annuus* L., *Hibiscus trionum* L., *Solanum nigrum* L. Satisfactory efficacy (75-90%) in the first assessment was on: *Bilderdykia convolvulus* L., *Chenopodium hybridum* L., *Datura stramonium* L., *Hibiscus trionum* L., and in the second on: *Ambrosia artemisiifolia* L., *Bilderdykia convolvulus* L., *Cannabis sativa* L. Low efficacy (<75%) of tested herbicides in

first and second assessment was on: *Convolvulus arvensis* L., *Panicum- crus-galli* L., *Sorghum halepense* (L.) Pers.

At the locality of Ruski Krstur, during 2017, a combination of two active substances (mesotrione 50 g/l and terbuthylazine 125 g/l) during the first and second efficacy assessment gave the following results presented in table 3.

Table 3. The presence of weed species and the efficacy of herbicides after the first and second assessment, locality of Ruski Krstur

Weed species	First assessment			Second assessment		
	Control	Tvister (mesotrione 50 g/l + terbuthylazine 125 g/l)		Control	Tvister (mesotrione50 g/l + terbuthylazine 125 g/l)	
	No/m^2	No/m^2	Ce (%)	No/m^2	No/m^2	Ce (%)
Amaranthus retroflexus L.	2.50	0	100	3.50	0	100
Ambrosia artemisiifolia L.	12.00	1.25	89.58	14.25	0	100
Chenopodium album L.	20.00	5.00	75.00	32.50	4.50	86.15
Chenopodium hybridum L.	3.75	0.50	86.66	5.25	0	100
Convolvulus arvensis L.	2.25	0.75	66.66	4.75	1.25	73.68
Cirsium arvense (L.) Scop.	1.50	0	100	2.25	0	100
Datura stramonium L.	7.00	0.50	92.85	9.25	0	100
Solanum nigrum L.	4.25	0	100	6.00	0	100
Setaria glauca (L.) Beauv.	2.25	0	100	3.00	0	100
Sorghum halepense (s) (L) Pers.	5.50	0	100	7.50	0	100
Veronica persica Poir.	1.50	0	100	2.25	0	100
Xanthium strumarium L.	7.75	0	100	9.25	0	100
Total number of weeds	**70.25**	**8.00**	**88.61**	**99.75**	**5.75**	**94.72**
Phytotoxicity (EWRC scale 1-9)	**-**	**1**	**1**	**-**	**1**	**1**

Good efficacy (> 90%) of post emergence applied herbicides, terbuthylazine and mesotrione, in the first and second assessment was on: *Amaranthus retroflexus* L., *Datura stramonium* L., *Solanum nigrum* L., *Setaria glauca* L., *Sorghum halepense* (L.)Pers., *Veronica persica* L., *Xanthium strumarium* L. Tested herbicides in the first assessment had satisfactory efficacy (75-90%) on *Ambrosia artemisiifolia* L., *Chenopodium hybridum* L., and in the second on *Chenopodium album* L. Low efficacy (<75%) was on *Convolvulus arvensis* L., and *Chenopodium album* L.

Pannacci and Onofri (2016) showed that herbicide mixtures with terbuthylazine gave the highest control of weeds, confirming terbuthylazine as the main herbicide for weed control in maize, although thiencarbazone-methyl + isoxaflutole or s-metolachlor + mesotrione represent two valid alternatives in order to reduce the treatments based on terbuthylazine or to replace it in the future.

Based on the statistical analysis of data, there are statistically significant differences between the control, the untreated variant and the treated variants (tab. 4-6), which indicates the efficacy of the applied herbicides.

Table 4. Statistical analysis of the data after the first and second assessment, locality of Gornja Badanja

No.	Variant	I	II	III	IV	$\bar{x}$	Sd
1	Basar+ Rezon (first assessment)	32	19	24	9	21.00	9.62
2	CONTROL (first assessment)	57	51	49	51	54.00	3.46
3	Basar+ Rezon (second assessment)	22	24	18	16	20.00	3.65
4	CONTROL (second assessment)	62	58	51	61	58.00	4.96

Table 5. Statistical analysis of the data after the first and second assessment, locality of Zobnatica

No.	Variant	I	II	III	IV	$\bar{x}$	Sd
1	Calaris Pro (first assessment)	17	24	19	20	20.00	2.94
2	CONTROL (first assessment)	175	137	71	60	110.75	54.68
3	Calaris Pro (second assessment)	8	7	6	11	8.00	2.16
4	CONTROL (second assessment)	121	104	92	118	108.75	13.40

Table 6. Statistical analysis of the data after the first and second assessment, locality of Ruski Krstur

No.	Variant	I	II	III	IV	$\bar{x}$	Sd
1	Tvister (first assessment)	6	11	8	7	8.00	2.16
2	CONTROL (first assessment)	75	64	78	64	70.25	7.32
3	Tvister (second assessment)	5	6	8	4	5.75	1.70
4	CONTROL (second assessment)	103	107	89	100	99.75	7.71

I,II,III,IV - replications
$\bar{x}$ - the mean value of the number of weeds per m^2 from 4 replicates
Sd - standard deviation

Conclusions

On the basis of results of the three field experiments on maize, 29 weed species were determined, of which 25 broadleaved and 4 grass weeds. The combination of herbicide S-metolachlor and terbuthylazine on present weeds in Gornja Badanja had low efficiency (59.70–66.30%). During 2017, at Zobnatica, the combination of herbicides terbuthylazine (326 g/l) and mesotrione (50 g/l) had a satisfactory efficiency during the first assessment (81.94%), and good efficiency (92.77%) during the second assessment on the present weed flora. At the experimental field in Ruski Krstur, terbuthylazine in a lower amount (125 g/l) in combination with mesotrione (50 g/l), had a satisfactory efficiency (88.61%), and good efficiency (94.72 %) on the present weed flora. This combination of herbicide terbuthylazine and mesotrione was the most effective in this study.

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THE IMPORTANCE OF A CROP ROTATION ON MAIZE PRODUCTIVITY

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Abstract

One of important measures in agriculture is a crop rotation. It is known that a crop rotation is associated with high yields, and suppressing of pathogens and weeds. It is a cheap measure – only decision is needed. On the other hand, a crop rotation gives possibility to use different crops and different pesticides (herbicides), what could be beneficial in anti-resistant strategy. The aim of this work was to evaluate maize growing in a crop rotation with winter wheat and maize monoculture. The field trial was set up in 2009 on an experimental field of the Maize Research Institute "Zemun Polje" in Serbia. This paper presents the results from 2017– after eight years of growing maize in monoculture and after four maize-winter wheat rotations. Maize hybrids ZP 677 and ZP 606 were tested in the experiment. Herbicide combination of isoxaflutole and s-metolachlor was applied (in recommended and ½ of recommended dose) for weed control. The effects of the crop rotation on maize were evaluated by observing the leaf area and plant height (at the anthesis stage), as well as the grain yield. In the crop rotation treatments the leaf area and plant height were significantly higher when compared to maize grown in monoculture. Irrespective to unfavourable meteorological conditions, both hybrids had higher yields when grown in rotations with winter wheat, in comparison to monoculture.

Keywords: *Maize, maize-wheat rotation, monoculture*

Introduction

Integrated Weed Management System (IWMS) implies a holistic approach that can integrate a large number of methods and measures that place plants in much better possition than weeds (Harker and O Donovan, 2012). It means that, in case of weeds, do not use only herbicides for weed suppresion, but also others tools – nonchemical tools for weed suppresion. Usage of IWMS gives long-term advantages in weed control, avoiding or delaying some negative effect of herbicides application (e.g weed resistance, soil contamination etc.). Accoring to Swanton and Weise (1991) herbicides should be one of the solutions in weed management strategy, not the only one. Crop rotation is one of the method for weed suppresion and it gives a huge advantages when is applied. Advantages of crop rotation are: preserving land quality, reducing herbicide usage and provides higher grain yields achieving (Liebman et al., 2001). In case of weeds, crop rotation influence directly on weeds by reducing their number. Accriding to Bastiaans (2010) weeds life cycle and spreading are lower in crop ratation compared to monoculture. On the other hand, cultivating plants in monocultures can lead to changes in the floristic composition, contributing to the spread of resistant and therefore harmful weeds (Stefanović et al., 2011). The aim of this research was to examine potentially positive effects of crop production in relation to cultivation of corn in monoculture using herbicides, by maize measuring leaf area, plant height and grain yield.

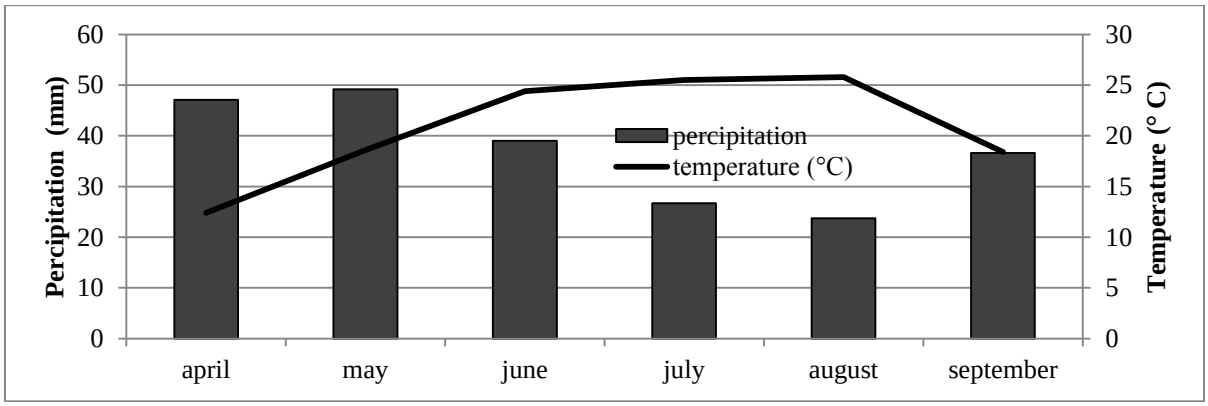
Material and Methods

The study of the effects of maize growing in monoculture and crop rotation with winter wheat began in 2009 at the field of the Maize Research Institute in Zemun Polje, on weak-carbonate chernozes. The experiment examined the influence of maize cultivation in monoculture and crop rotation with wheat. This paper presents the results obtained in 2017, that is, after eight

years of corn cultivation in monoculture and crop rotation. In each field, two hybrids of corn, hybrid of the older generation - ZP 677 and newer generations - ZP 606 were sown. The weeds in the experiment were suppressed by the herbicidal combination of isoxaflutol (Merlin flexx) and s-metolachlor (Dual Gold) in the recommended dose (RD) and ½ recommended doses. In the control variant, herbicides were not applied and it remained stunted until the first weed count. Influence of different growing systems on maize were tested by measuring leaf area, plant height in anthesis stage and grain yield after harvest.

The meteorological data are presented in Graph 1. The year 2017 was very warm, with high spring temperatures and optimal precipitation. The second part of the vegetation season was extremely warm, while the precipitation was insufficient for corn growing and developing. Obtained data were statistically processed by ANOVA and differences between means were tested by LSD test

Graph 1. Meteorological conditions in Zemun Polje during the vegetation period in 2017



Results and Discussion

According to obtained data, significant influence of applied treatments on maize parameters were recorded (Table 1). Leaf area and plant height values were significantly higher for both hybrids grown in crop rotation compared with maize monoculture. In treatment with ½ of RD maize hibrid ZP 677 had the highest values of leaf area, while the highest leaf area in ZP606 was recorded in RD treatment. Similar results were observed on maize height. Also, maize growing in crop rotation with winter wheat shows positive effects on tested parameters even in control treatments. Positive effect of maize growing in crop rotation with winther wheat was reported by Spasojević (2014). In his research, significant influence of crop rotation were observed on maize parameters, compared to maize grown in monoculture.

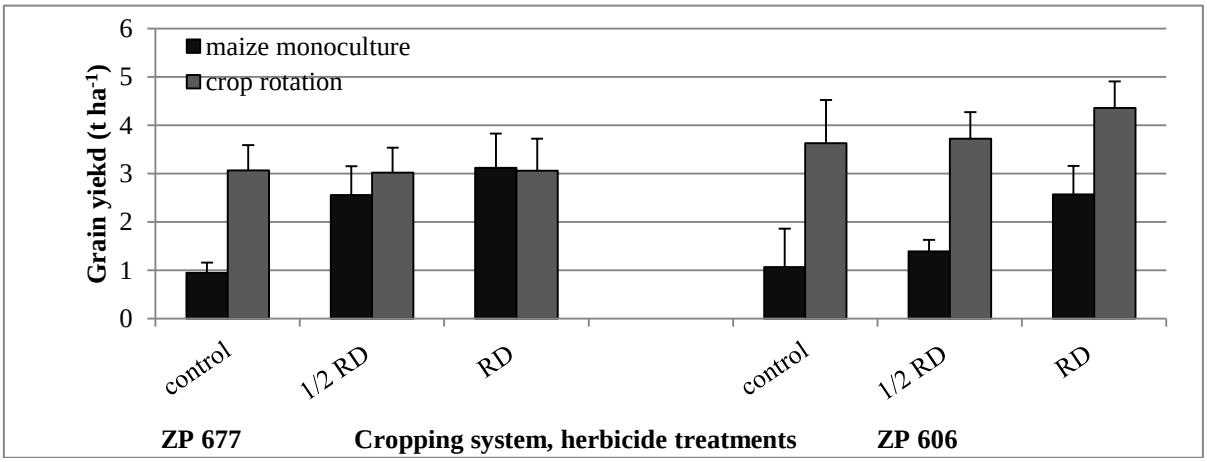
Table 1. Effects of applied treatments on maize parameters

	Leaf area (cm^2)			Maize height (cm)		
ZP 677	Herbicide treatment					
	control	½ RD	RD	control	½ RD	RD
Monoculture	1719.0	3903.6*	3870.7*	92.3	177.5*	183.4*
Crop rotation	3036.2	6496.6*	5099.6*	93.4	181.1*	178.8*
	Leaf area (cm^2)			Leaf area (cm^2)		
ZP 606	Herbicide treatment					
	control	½ RD	RD	control	½ RD	RD
Monoculture	1730.2	4282.1*	4223.6*	175.1	224.5*	213.8*
Crop rotation	4099.2	6453.1*	6942.0*	163.4	216.0*	215.6*

* - $p<0,05$ – statistical significant difference, RD – recommended dose

In the study year, meteorological conditions, especially in the second part of the vegetation, had an impact on the grain yield of both maize hybrids. The advantage of growing in crop rotation for hybrid ZP 677 was recorded in control treatments (3 t ha^{-1} in relation to 1 t ha^{-1} in monoculture). Unlike hybrid ZP 677, the hybrid of the newer generation ZP 606 had a significantly higher grain yield on all treatments in crop rotation, compared to the monoculture. Even with this hybrid, meteorological conditions influenced the reduction in yield, but to a lesser extent compared to ZP 677. The highest yield was recorded in the treatment of the recommended dose of herbicides (4.2 t ha^{-1}) (Graph 2). Among other things, the yield of maize to a great extent depends on the presence of weeds. Reduction in yield can also be over 90% if we do not apply weed control measures, and at global level, the average global loss caused by weeds is about 10% (Oerke, 2006). The decrease in the presence of weeds is directly related to the increase in yield, which is the direct effect of fertility and the application of herbicides (Simic et al., 2016). Kovačević et al. (2008) claim that the use of crop rotation in corn leads to a reduction in the presence of weeds, and therefore an increase in yield.

Graph 2. Grain yield of corn hybrids depending on the breeding and application system of herbicides



$LSD_{0.05}$ (hybrid): 1,29; $LSD_{0.05}$ (cropping system):1,24; $LSD_{0.05}$ (treatment): 1,00

Conclusions

Maize growing in crop rotation with winter wheat gives huge advantages and can be one of the solutions in IWMS. All tested parameters, leaf area maize height and grain yield, had the higher values in treatments included crop rotation. Herbicide application helped by weed suppresion, so even ½ of recommended rate of pre-em herbicides can significantly influence on weeds. This means that simple crop rotation of maize with winter wheat can be an important tool for achieving higher yields, and off course solve problem with weeds.

Aknowledements

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ECONOMIC JUSTIFICATION OF BIOLOGICAL MEASURES FOR POTATO TUBER MOTH CONTROL

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Abstract

Potato tuber moth (PTM), *Phthorimaea operculella* Zeller, represents a very dangerous potato pest in the warmer parts of the world. The harmfulness of the insect impact is reflected in the high level of tuber damage (up to the complete yield loss), as well as the complexity of its control. The damages caused by PTM to the potato production are considered to be quite significant at the locations with highest potato yields and production in Serbia. This paper will show the results of the monitoring of the occurrence and the number of imagoes (moths) of pests by using pheromones traps during 2016 and 2017 within the field experiments in the Rasina District (Serbia). The field experiment was set up at four locations. Pest control was based on the application of agro-biological and biological measures, starting from planting the potatoes, through the entire vegetation period, harvesting and storage of the tubers. The obtained results show the economic justification (13 percent lower cost of production) of agro-technical and biological measures of potato moth control in order to obtain a quality and both health and safe product, while preserving the environment at the same time.

Keywords: *Potato tuber moth, Potato, Rasina District, Biological measures, Economic justification.*

Introduction

Potato moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), represents a very dangerous pests of potatoes of warmer parts of the world, in addition to damage which is caused by the complexity of its suppression. Until recently, in Serbia, this pest was almost known only from literature, where the description was given with the poor measures of suppression and emphasized that it was not present in Serbia, but in tropical and sub-tropical areas, and in the area of the former Yugoslavia in Dalmatia and Macedonia (Tanasijević and Ilić, 1969 Tanasijević and Simova-Tošić, 1987; Igrc, 1983; Milošević,2009). The moth life cycle, under optimum conditions, lasts 30 days, so that in one year it can have up to 12 generations. An adult female, during the 10-15 days of life, lays eggs on a leaf, a tree and a tuber from which becomes larvae within 5 days (Raman, 1980). During the period of eight days, larvae eat food, creating mines into a leaf, pulling themselves up in a stalk and a tuber. The maggot stage lasts for 6-9 days (Alvarez et al., 2005). The first official data on the presence of pests in Serbia, in Leskovac area, originated in 2011 (Jovanović et al., 2013). Since 2011 pests have appeared in other areas in Serbia, as well. It will be present in Serbia and other countries in the future and can lead to major problems in the production of potatoes. In the period from 2015 to 2017, huge damage caused by this insect has been recorded in many potato production areas in Serbia, mainly in warmer locations at lower altitudes. Pests cause damage to the field and warehouse. A female lays eggs on the stalk and a leaf of potatoes, through the cracks in the ground and on the tubers where a few days later larvaes appear. The leaf with mines loses its function, and the stalks above the site of damage is decaying. In the tubes, the caterpillars make halls and such tubers can not be used due to the

high degree of damage. Damage to the tuber is the place where the injured tissue of the tuber and the pathogen causative agent of the potato tuber disease are in contact. Thus, as a secondary phenomenon, mass diseases caused by mushrooms and bacteria which causes the damp and dry decaying of the tubers, appeared (Hanafi, 1999). After extracting the tubers from the soil, in the open and in the warehouse, to their consumption, if there is an imago, the female has the ability to reproduce again.

Pest control is based on a series of measures from the planting of potatoes during the entire vegetation period, during the extraction and storage of the tuber (measurements in the field, measures taken during removal of the tuber and measure in the storage). In this paper will be presented an economic analysis of the cost-effectiveness of biological measures that reduce the number of pests and avoids its presence on above biomass and tubers, thereby reducing or completely avoiding damage in relation to chemical protection measures.

Material and Methods

In the paper, we will present the influence of Potato moth on potato plants and tuber in the Rasina District (Mačkovac, Počekovina, Radmanovo, Graševci) through gross margin (GM) cost calculation. Experiment has performed during 2016 and 2017 year by using the pheromone traps (RAG) and visual inspection of plants in the field. Data are calculated through the gross margins of the economic viability of the biological in relation to the chemical measures of the protection. The basic elements for calculation of the gross margin used in research: yield and prices, quantity and value of fertilizers, pesticides and fuels, as well as the costs of contracted services. The original data are collected in Serbian dinars (RSD), but all prices are given in EUR. Prices are calculated based on Serbian National Bank average exchange rate against major world currencies for particular year. Indicators of production value, total variable costs and gross margin are calculated according to the methodology provided by Serbian Agriculture Advisory Service. For the purpose of research we are used the Microsoft Excel for data processing and GM calculating.

Results and Discussion

In the observed period, monitoring indicates to a trend of rapid growth in the number of pest populations. The total catch on the traps at the locality of Mačkovac was 91 (2016) and 120 (2017), on the traps at Počekovina locality 28 (2016) and 53 (2017), while on the locality of Graševci and Radmanovo there were no catches of this pest. These two sites are located at an altitude of 1500 meters, which proves the previously stated claim that there is no potato moth on these altitudes. The economic threshold of harm of this type of species is 15-20 catchable imago on one trap per day. In 2016, individual specimens of potato moths were occasionally caught from the end of June to the middle of August, and then only one prominent maximum of the flight, at the end of August and the beginning of September, appeared. Unlike 2016, in 2017, the period of the year was significantly earlier and longer. The three maximum flights were recorded at the end of June, at the end of July, at the beginning of August and at the end of August.

Successful suppression and maintenance of the potato moth under the threshold of harm for potato producers is possible only by combining all available measures. Measures that are used to struggle moth damage can be divided into preventive and direct (Raman, 1980; Tsedaley 2015). Preventive measures for the control of potato moths are aimed at reducing the risk of moth attacks, and by direct measures, using insecticides, we reduce its number and the ability of an adult insect to lay eggs. Within the examination of the biological measures of the fight, in order to avoid the female laying eggs on the tubers, we prepared the soil for planting well, the plant was slightly deeper (10-15 cm) and there was good soil coverage, by forming a regular and high bank, in order to prevent "Collapse" of banks due to frequent irrigation and

heavy rains in order to protect the tubers until the moment of extraction. For planting we used undamaged tubers and planting was done earlier. By maintaining soil moisture, by more frequent irrigation with smaller inlet norms (15-20 l m-2, up to a maximum of 25 l), up to one week before taking the potato out, we avoided creating cracks in the soil through which the female is reaching the tuber on which it lay eggs. Continuous monitoring of the appearance, number and development of moths has been performed. Immediately after the formation the scarfskin of the tubers, it was accessed by their removal.
The average yield of potatoes in 2016 was 20000 $kgha^{-1}$., while in 2017 it was 28000$kgha^{-1}$. For the yield of potatoes, it can be said that it shows oscillations. The average price in 2016 was 0,1694 EUR / kg. while in 2017 it was 0,1864 EUR / kg. The average value of total variable costs in 2016 on farms that did not apply chemical measures of the fight was 2 244,53 EUR/ ha. The average value of the gross margin was 1 178, 94 EUR / ha. While in holdings, where chemical fighting measures were applied in 2016, the average price of variable costs amounted to 2 917, 66 EUR / ha. The average value of the gross margin was 506,05 EUR / ha. In 2017, the results were the following: at farms where chemical measures were not applied in order to save the total variable costs were 2 244,54 EUR / ha, while the gross margin was 3 009,45 EUR / ha. In the holdings where the protection measures were applied, the total variable costs were 2 917, 67 EUR / ha, the gross margin was 2 336, 57 EUR / ha. The results of the analysis show that the omission of the chemical treatment of the gross margin was increased by 506,42 EUR / kg in 2016, while the 2017 was increased by 672, 88 EUR / ha. From all of the above mentioned, we see that the justified omission of chemical treatment, with the adequate application of agro-technical and biological measures of fighting in order to suppress the potato moth, positively affects the gross margin of potatoes.

Conclusion

A potato moth is a pest that is present and will remain in Serbia until the conditions for its development are favorable. In order to reduce the number of pests, and to protect potatoes and prevent the occurrence of damage, it is necessary to apply a series of measures that individually applied can not produce great effects. Therefore, they need to be applied integrally by all potato producers. This requires the State, as well as the competent Ministry through the Regional Agricultural Advisory Services and Local Self-Governments, to educate the manufacturers which measures to apply in order to reduce or avoid damages from this dangerous pest. In this paper, economic cost-effectiveness and justification of the potato moth contamination by applying agro-technical and biological measures of protection using the gross margin, are shown. Gross margin is the result of the impact of yield, prices and variable costs. In the case where the price is the same, and the yield differs, the difference in treatment is reflected in the costs, therefore in the total gross margin and amounts to 25% of the more profitable production by using a new protection model. In the case where the yield is the same and the price varies, the difference is in the total gross margin and is 11% in favor of applying the new protection model. In case where both the yield and the price are the same, the difference in the total gross margin is 13 % in favor of the application of the new protection model. Of course, if both price and the yield are higher, the conventional treatment is reduced considerably. But in the case when the price and yield are lower , then it is necessary to consider how profitable the conventional treatment system is, if we look at the analysis which show how profitable the new protection model is.

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CONSEQUENCES OF EXPOSURE TO ORGANOPHOSPHATE PESTICIDES

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Abstract

The greatest challenge for the world in the upcoming period will be to feed itself without ruining its environment. An increased use of pesticides and mineral fertilizers has resulted in a large production of diverse agricultural crops and thousands of new products, which have enabled the regular supply of the market and good nutrition of the population. Due to great competition and demand, many farmers tend to use organophosphate pesticides excessively in order to increase yields. However, when spraying, farmers should, must to adhere but do not adhere to the instructions for the safe use of pesticides, i.e. they do not use appropriate personal and protective gear, which enables pesticides to enter the blood stream through inhalation and dermal exposure, negatively affecting their health. In this paper, the authors compared the relationship between the length of exposure to organophosphate pesticides and signs of disease caused by direct exposure to pesticides during spraying. In the Rasina District, the Republic of Serbia, 80 farmers were interviewed by using pre-designed questionnaires over the course of 16 months. The farmers who had been exposed to pesticide spraying reported acute signs and symptoms resulting from that exposure, such as blurred vision, burning eyes, redness and skin itching, difficulty in breathing, excessive sweating, dry throat and burning in the nose. The duration of the symptoms depended on the length of the exposure. It was concluded that there was need for raising awareness among farm sprayers on the safe use of pesticides and use of personal and protective gear while handling pesticides.

Keywords: *Organophosphate pesticides, exposure, pesticide spraying, farmers, protective gear.*

Introduction

Occupational exposures to Plant Protection Products (PPP) occur during the production, transportation, preparation and application of pesticides in the workplace (Ye et al., 2013). Exposure to pesticides is one of the most important occupational risks among farmers in developing countries (Coronado et al., 2004). Occupational exposure to pesticides is of great interest in order to identify the hazards of pesticide use and the establishment of safe methods of pesticide handling. Pesticides exposures both occupationally and environmentally cause a range of human health problems. A series of studies have documented that the easy availability of pesticides in farming households makes it a preferred means of self-harm. It has been estimated that there are 250 000 deaths annually from pesticide self-poisoning worldwide, accounting for 30% of the suicides globally (Gunnell et al., 2007).

Acute toxicity is normally the result of a single exposure and the symptoms are seen within a comparatively short time of exposure, usually within hours or days. Acute health effects may include irritation of skin or eyes or respiratory irritation. Long-term exposure to organophosphate pesticides can cause various health problems. Organophosphates are associated with well-known acute health problems such as nausea, dizziness, vomiting, headaches, abdominal pain, and skin and eye problems (MacFarlane et al., 2013; Reifenrath, 2007). A vast majority of the population in Serbia is engaged in agriculture and is therefore exposed to the pesticides used in agriculture.

In developing countries including Serbia, farmers who are engaged in the occupation of spraying pesticides in crops and plantings get the direct exposure of pesticides due to unsafe and non-preventive work practices. They do not use the Personal Protective Equipments like safety masks, gloves etc. during the aerial spraying of pesticides resulting in the entry of pesticides in the blood stream via respiratory tract through inhalation which can adversely affect respiratory system (Fareed et al., 2013). Respiratory symptoms, such as coughing, wheezing and airway inflammation, are commonly observed among people exposed to pesticides (Ye et al., 2013). Pesticides being used in agricultural tracts are released into the environment and come into human contact directly or indirectly. Humans are exposed to pesticides found in environmental media (soil, water, air and food) by different routes of exposure such as inhalation, ingestion and dermal contact. Exposure to pesticides results in acute and chronic health problems. These range from temporary acute effects like irritation of eyes, excessive salivation to chronic diseases like cancer, reproductive and developmental disorders etc (Yassi et al., 2001). The results of the study indicate the possibility that organophosphate pesticides play a role in genetic tissue damage, in the production of free radicals, in neuro-psychological disorders, and in diabetes (Jamal et al., 2016, Narayan, et al., 2013; Taghavian et al., 2016).

Organophosphates is common name for phosphorous thioesters and esters acid. In health and agriculture Organophosphates refers to a group of insecticides or nerves Agents that inhibit acetylcholinesterase, an enzyme that catalyzes the neurotransmitter hydrolysis acetylcholine in cholinergic synapses in the central and peripheral nervous system. Organophosphate pesticides are a class of chemicals defined as esters, amides, or thiol derivatives of veosfor, phosphonic, and phosphorothiocidic acid (Kavvalakis and Tsatsakis, 2012; Mangas, et al., 2016). It is necessary to emphasize that most organophosphate pesticides due to rapid degradation do not stay in the body for more than a few days (Kavvalakis and Tsatsakis, 2012). On the other hand, the highly reactive nature of organophosphate pesticides can lead to toxic effects.

Material and Methods

This paper is based on the results of the questionnaire survey on agricultural holdings in the Rasina District, the Republic of Serbia, which is famous for fruit and wine production. The study was conducted on apple-growing farms. According to the data from the Census of Agriculture (RIS, 2012), apple tree is grown in the Rasina District on an area of 1142 ha. The research was conducted in the period from May 2016 to August 2017, when Apple plantations are sprayed with organophosphate pesticides for control of pest Codling moth, *Cydia pomonella* . Usually spraying duration was from 07:00a.m. to 10:00a.m., then from 04:00p.m. to 07:00 p.m., two days per week. The farmers were examined during working days after the pesticide spraying. The organophosphate pesticide used in this study is chlorpyrifos. Respondents were exposed for six to sixteen months for this research.

Interview questionnaire

The questionnaire was designed to outline the details of land ownership, plantation where the farmers is currently working, exposure to organophosphate pesticide the use of pesticides, precautions taken, signs and symptoms related to pesticide exposure etc. Farmers who sprayed chlorpyrifos were asked if they experienced symptoms (blurred vision, itching eyes, skin infections / itching, excessive sweating, dry throat, difficulty breathing / coughing, burning nose) during or immediately after pesticide spraying. Some of these signs (blurred vision, excessive sweating and itching) were observed at the time of interview. Symptom details were collected as reports done by the farmers. The questionnaire provided information about the type of sprinklers used by the farmers for spraying, such as tractor sprayers and sprinklers. During the research, none of the respondents took precautions or used personal and

protective gear that include: protective suit, adequate protective gloves, respiratory protection masks, eye protection glasses and boots.

Data collection

The survey was conducted on a total number of 80 farmers, who were males and ranged between 20-65 years of age, belonging to same socioeconomic status. Farmers who participated in the survey were randomly selected on the basis of the initial questionnaire filled out, the fruit cultivation they grow, as well as on active engagement (full-time) for the preparation, storage and spraying of the pesticides on apples for at least six months. Analysis was done on four groups of subjects based on the duration of organophosphate exposure (i.e 15 subjects with 6 months of exposure, 20 subjects with 12 months exposure and 25 subjects with 16 months exposure). The study was compared with 20 healthy males having no previous or current occupational exposure to pesticides which were taken as control group from near by areas with same socioeconomic status. Questionnaires were asked to be filled by the all four groups of subjects. The questionnaire provided detailed information regarding the socioeconomic status, family history and personal habits which were recorded for each respondent. The purpose of the academic research was explained to all the participants and their consent is obtained.

Results and Discussion

On the basis of the questionnaire it was found that the subjects exposed to organophosphate pesticide via spraying the pesticides in their farms from 12 months and 16 months showed more prevalence in general health symptoms immediately after the spraying. The symptoms were of general nature like blurred vision, skin redness, white patches, excessive sweating, shortness of breath etc.(Table 1). An interesting aspect of this research was the prevalence of ophthalmological problems arising due to organophosphate pesticide spraying being dose and duration dependent. In the 6 months exposure period only 6.66% respondents complained of blurred vision whereas in 12 months it was 10% and in 16 months it was 36% being the highest. In the control group of same age and same social status it was only one respondents who complained of a blurred vision in 16 months of exposure, that may be because of age or environment. Burning of eyes was highly prevalent among pesticides sprayers. In 12 months of exposure duration 15% respondents complained of this symptom, and in 16 months 20% farm sprayers complained itching in their eyes which was quite significant. It was observed that there were no symptoms in the control group who were of the same age and socioeconomic status. With regard to the skin related problems in the 6 months of exposure duration only 13.33% respondents complained of skin rashes, whereas in the 12 months 20%, and in 16 months 60% of respondents complained of skin patches, redness and irritation which was also found to be highest in the longer exposure duration period. In the control group it was observed that there were no skin symptoms found in the non exposed workers. Similarly in present research respiratory related problems like excessive sweating, dry / sore throat, running / burning nose and shortness of breath / cough were found quite common in the farm pesticides sprayers exposed to organophosphate pesticides from 6 months, 12 months and 16 months. Table 1 shows that in the 6 months exposed sprayers 13.33% subjects showed excessive sweating and shortness of breath during the pesticide spraying as compared to their well matched controls. While in 12 months exposed sprayers the prevalence of symptoms were found higher, 30% sprayers complained the problem of excessive sweating, 15% sprayers reported dry throat, 25% reported burning nose, and 25% farm sprayers reported shortness of breath. Whereas in controls only one subject reported shortness breath. In the 16 months of exposure, 52% sprayers complained of excessive sweating, 32% reported dry throat, 44% reported burning nose, and 48% reported shortness of breath and cough.

While from the control group only one subject showed excessive sweating and coughing.(Table1)

Table 1. Showing the effects of organophosphate pesticides for 6, 12 and 16 months of exposure on the general parameters of eyes, skin and respiratory related symptoms in the four groups examines .

Exposure Duration	Symptoms → Groups of Subjects↓	Burning/ Stinging / Itching eyes	Blurred vision	Skin redness/white patches on skin/ skin scaling	Excessive sweating	Dry/ Sore throat	Runny/ Burning nose	Shortness of breath/ cough
6 Months	Group -1 (15)	0	1s-6.66%	2s-13.33%	2s-13.33%	0	0	2s-13.33%
	Control-1 (5)	0	0	0	0	0	0	0
12 Months	Group -2 (20)	3s -15%	2s-10%	4s-20%	6 s-30%	3s-15%	5s-25%	5s-25%
	Control-2 (7)	0	0	0	0	0	0	1s-14.28%
16 Months	Group-3 (25)	5s-20%	9s-36%	15s-60%	13s-52%	8s-32%	11s-44%	12s-48%
	Control-3 (8)	0	1s-12.5%	0	1 s-12.5%	0	0	1s-12.5%

*Source: Author's elaboration based on the questionnaire survey results.
*Note: s- subjects

From the findings of this research it can be seen that the signs and symptoms are significantly related to the duration of the exposure. Therefore, the research clearly shows that subjects who have been exposed to organophosphate pesticides for prolonged periods through spraying show a greater number of symptoms. The research showed that exposure to pesticides was mainly due to inhalation, dermal route and swallowing, since no farmer used a protective suit, adequate protective gloves, respiratory protection masks, eye protection and boots, which is mandatory prescribed in the instructions for use pesticides. It is important to note that during the interviews of the participants it was established that the farmers did not shower regularly. Most farmers in this study were not familiar with the health hazards caused by inadequate handling of organophosphates. Entrants reported two or more of the above symptoms within 24 hours of spraying and are thought to have suffered acute pesticide poisoning.
It was found that physiological changes caused by organophosphate pesticides in the above mentioned groups included ophthalmic changes, dermatological and respiratory disorders. In this regard, it is mentioned that continuous and direct exposure to pesticide eyes leads to complications such as blurred vision and eye pain (Mishra et al., 1985). Exposure to unprotected eyes by pesticides led to absorption in the eye tissues causing potential toxicity (Jaga and Dharmani, 2006). Visual problems (itching / burning eyes, blurred vision) can be related to the direct contact of pesticides with eyes due to non-compliance and non-change of eye protection measures such as protective glasses, so these findings are corroborated by this research (Singh & Kaur , 2012). The effects of organophosphate on the skin also depended on exposure time (Kishi et al., 1995). It was observed that the skin is the primary way of exposure to pesticides for farmers who handle sprays. Excluding acute poisoning, contact dermatitis is considered to be the most common health effect of pesticides, either through irritating or allergic mechanisms (Spiewak, 2000). All this confirms the previously stated assumption that the negative effects of the use of organophosphate pesticides can be minimized by the adequate application of protective equipment. The results of this study have shown similarity to some earlier toxicological tests done elsewhere. In Canada, a cross-section study of pesticide-related use and effects on the respiratory system of farmers was

carried out and the use of pesticides is associated with isolated asthma and a change in lung function (Senthilselvan et al., 1992). A worker cross – section study at the pesticide packaging also showed that, compared with the controls, pesticide-producing workers have a significantly higher risk of developing respiratory symptoms, including chronic coughing for women, nose and throat irritation for men and women (Zuskin et al 2008). These observations are in line with this research , where it has been found that most of the symptoms relate to skin and respiratory problems for farmers who have been exposed to pesticides for a long time.

Conclusion

This paper provides information related to the short-term effects of organophosphate pesticides on agricultural producers in the Rasina District, the Republic of Serbia, who worked in the most unfavorable environment without any protective gear . From the foregoing, three conclusions can be drawn. Firstly, it is necessary to continually educate farmers on the adequate use of protective gear in the handling of organophosphate pesticides, since the negative effects of organophosphate pesticides can be minimized by the adequate application of protective gear. Secondly, it is important that future research determines the correlation between the length of exposure to organophosphate pesticides, the dose of organophosphate pesticides and health problems. Thirdly, it is necessary to inform the farmers who are exposed to organophosphate pesticides about the importance of regular medical examinations.

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OCCURRENCE OF THE SEPTORIA LEAF BLOTCH CAUSAL AGENT IN SOME WINTER WHEAT CULTIVARS

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Abstract

Wheat is exposed to attack of many pathogens, so it is essential to establish their occurrence and intensity every year. Septoria leaf blotch of wheat, caused by the fungus *Septoria tritici*, is one of the most common diseases in the world and shows the significant effect on wheat production. One of the reasons for more intensive occurrence of the disease is introducing into production shorter stem cultivars, having lower leaves closer to the ground, which facilitates infection of plants during autumn. This paper aimed to establish attack intensity of the disease in some winter wheat cultivars in the conditions of natural infection. The field trials were carried out during 2014/2015 and 2015/2016 in the locality Leposavić-Lešak, at fields of the Agricultural School, with ten cultivars studied as follows: Pobeda, Evropa 90, Renesansa, Simonida, NS 40s, Zvezdana, Planeta, Kruna, Sirtaki and Euklid. Sowing was done by sowing machine. Every cultivar was sown in 46 rows, 50 m long. Soil was fertilized before sowing by 300 kg ha^{-1} of mineral fertilizer (NPK 15:15:15), and additionally, during tillering stage, by 200 kg ha^{-1} of nitrogen fertilizer LAN. The rest agrotechnical measures applied on the trial were standard. One hundred randomly selected plants were sampled from every cultivar, and grading of attack intensity in both investigation years was done during the first week of June, according to the scale of Gešele (1978), by determining infection intensity from 0-100%. The highest average infection intensity in 2015 was observed in cultivars Pobeda and NS 40s (40%), and the attack intensity in most of cultivars (Renesansa, Planeta, Kruna and Euklid) was graded as 20%. In 2016 the cultivar Pobeda also showed attack intensity of 40%. Blotches covering 30% of leaf area were observed in cultivars NS 40s, Zvezdana and Sirtaki. Infection intensity in the cultivar Kruna amounted 10%.

Keywords: *Winter wheat, Cultivars, Pathogen, Intensity, Disease.*

Introduction

Wheat (*Triticum aestivum* L.) is the most important field crop which currently occupies large area throughout the world. Denčić *et al.*, (2009) have pointed to wheat as one of the most important components of human diet according to its nutritive value. Genetic potential of Serbian cultivars for grain yield is above 12 t ha^{-1}, but the obtained average grain yield is significantly lower. Genetic potential use efficiency depends on weather conditions, applied agrotechnical measures, as well as on resistance of grown cultivars to diseases (Denčić, 2006). Having in mind parasites, i.e. causal agents of various plant diseases, as a significant factor influencing grain yield, it is essential to establish their occurrence and intensity every year. Septoria leaf blotch of wheat (*Septoria tritici*) is a disease occurring during whole vegetation period, and is one of the most common diseases in the world which shows the significant effect on wheat production. Jevtić *et* Arsenijević, (1995) point to one of the reasons for more frequent occurrence of the disease being introduction of cultivars with lower habit (dwarf and semi-dwarf cultivars), that have lower leaves closer to the ground, which enables infection during autumn. Dwarf and semi-dwarf cultivars demand an altered agrotechnique (more intensive fertilization and higher crop density), which also acts in favor

of infection. In proper conditions, primary infection occurs early and might be observed after emergence of second leaf of wheat. The attack causes necrosis of infected plant tissue, which gradually wilts and often adheres to the ground (Telečki *et* Jevtić, 2009). *Septoria tritici* is currently considered as one of the most influential parasites for global wheat production, and it is the main wheat disease in many regions throughout the world (Eyal *et al*., 1985). Greater damage can be observed in favorable weather conditions, when the disease spreads from lower leaves to upper ones. Necrosis of strongly infected leaves leads to decrease of assimilation area, which causes lowering grain yield and deteriorating grain quality (Balaž *et al*., 1995). Wiese (1987) stated that the disease was registered in about 50 countries. Leroux *et al*., (2007) and Stammler *et al*., (2008) reported Septoria leaf blotch as one of the most important diseases in Northern Europe, and Garcia et Marshall (1992) stated the same for the grain production areas of the USA. The estimated grain yield loss caused by this pathogen in Western Europe amounted between 30 and 50% (Royle *et al*., 1986).

Climate of Serbia is very favorable for occurrence and development of the disease, so it is classified in the group of very harmful diseases. Occurrence of this disease in many locations of Serbia was reported previously by Arsenijević (1965), Kostić *et* Smiljaković (1966) and Ivanović et *al*., (2001). Jevtić *et al*., (2008) stated that Septoria leaf blotch of wheat occurred regularly in Serbia, with a high infection intensity. Grujić *et al*., (1998) established by their investigation in three locations in Serbia during 1998, that the average infection intensity for 141 cultivars was 50.35%. Gudžić *et al*., (2012) reported that in 2011, at pseudogley soil type, the average infection intensity for 10 cultivars amounted 27%. Kalentić *et al*., (2006) observed significantly stronger attack of Septoria leaf blotch on winter genotypes in regard to spring ones. This paper aimed to establish attack intensity of the disease in some winter wheat cultivars in the conditions of natural infection.

Material and Methods

The investigation was carried out in the locality Leposavić-Lešak (Serbia), at fields of the Agricultural School, during 2014/2015 and 2015/2016. Coordinates of the location were the following: latitude 43º10', longitude 20°44', altitude 558 m. The following cultivars were investigated: Pobeda, Evropa 90, Renesansa, Simonida, NS 40s, Zvezdana, Planeta, Kruna, Sirtaki and Euklid. In both trial years sowing was carried out within optimal terms (October 17th, 2014 and October 24th, 2015). Sowing of the studied cultivars was done by sowing machine. Every cultivar was sown in 46 rows, 50 m long. Soil was fertilized before sowing by 300 kg ha^{-1} of mineral fertilizer (NPK 15:15:15), and additionally, during tillering stage, by 200 kg ha^{-1} of nitrogen fertilizer LAN, while weed control was done combining herbicides Tezis and Bonaca in the dose recommended by the manufacturer.

One hundred randomly selected plants were sampled from every cultivar, and grading of attack intensity in both investigation years was done during the first week of June, according to the scale of Gešele (1978), by determining infection intensity from 0-100%.

Results and Discussion

The investigation results showed Septoria leaf blotch registered at the all studied cultivars in both years (table 1). The average attack intensity in 2015 was 26%, while in 2016 it amounted 24%. During 2015 in cultivars Renesansa, Planeta, Kruna and Euklid attack intensity was 20%, which made 40% of the total studied cultivars. Leaves of cultivars Simonida, Zvezdana and Sirtaki were 30% covered by blotches caused by the parasite *Septoria tritici*. In the cultivar Evropa 90 attack intensity of 10% was observed. Pobeda and NS 40s showed the highest susceptibility, and attack intensity on them was 40%.

Table 1. Intensity of *Septoria tritici* attack to winter wheat cultivars

No	Cultivar	2015	2016
		Attack intensity	Attack intensity
1	Pobeda	40	40
2	Evropa 90	10	20
3	Renesansa	20	20
4	Simonida	30	20
5	NS 40s	40	30
6	Zvezdana	30	30
7	Planeta	20	20
8	Kruna	20	10
9	Sirtaki	30	30
10	Euklid	20	20
Average		26 %	24%

The results collected in 2016 showed several cultivars (Evropa 90, Renesansa, Simonida, Planeta and Euklid) having attack intensity of 20%. Leaves 10% covered by the blotches in 2016 were observed in the cultivar Kruna. Values of attack intensity of cultivars Zvezdana and Sirtaki were the same as in 2015, and amounted 30%, together with the cultivar NS 40s. The highest average infection by the parasite *Septoria tritici* in 2016 also was observed in the cultivar Pobeda, 40% once again. Septoria leaf blotch is the disease which represents a problem and a limiting factor in successful wheat production. Besides favorable weather conditions, development of the disease is stimulated by growing susceptible cultivars and amount of inoculum from previous vegetations (Kalentić *et al*., 2006). Creating and growing resistant cultivars has a great importance in wheat protection from the causal agent of Septoria leaf blotch. Based on their investigation, Jerković *et al*., (2005) pointed out to existing differences among cultivars in resistance to the most important causal agents of wheat diseases, while Palmer *et* Skinner (2002) stated that commercial cultivars had no adequate resistance to this parasite.

Conclusions

Septoria leaf blotch of wheat is the disease regularly occurring in Serbia, where causes economically significant damage, and shows significant effect on wheat production.

Good resistance to *Septoria tritici* in both investigated years showed cultivars Evropa 90 and Kruna. Pobeda was the most susceptible cultivar, because it had infection intensity of 40% in both 2015 and 2016. Majority of cultivars used currently for wheat production are susceptible to the causal agent of Septoria leaf blotch. Therefore, wheat breeding for resistance to this pathogen ought to be one of main goals in creating new cultivars. In addition to selection and growing resistant cultivars, adequate attention should be paid to agrotechnical and chemical measures that ought to be applied during vegetation period of wheat.

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INFLUENCE OF ARTIFICIAL INFESTATION WITH WESTERN CORN ROOTWORM EGGS ON PLANTS HEIGHT AND LEAVES NUMBER ON MAIZE

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Abstract

Western corn rootworm *Diabrotica v.* sp.*virgifera* is a pest native to America. Nowdays it presents economically one of the most important maize pests in Europe. A field experiment was carried out in Bečej (Serbia) during 2014 with Serbian maize cultivar NS-640. In experimental field, 96 plants were selected, marked, and arranged in 48 pairs. The number of leaves and plant height were recorded.. The maximum leaves number on C (control plants) was 15 during all five observations. The maximum leaves number on D (infested plants) was 14 in the first two and 15 in the last three observations. The maximum measured height on C (control) plants during five observations during vegetation was 180, 195, 300, 320 and 320cm, respectively. The maximum measured height on D (infested) plants during five observations was 178, 192, 295, 295 and 295cm, respectively. Statistical analysis shows significant differences between C and D plants based on their leaves number during the first three observations, while there were no differences between plant heights during all five observations.

Keywords: *Western Corn Rootworm, artificial infestation, maize, height, leaves*

Introduction

Western corn rootworm (WCR) *Diabrotica virgifera* sp. *virgifera* Le Conte (Coleoptera, Chrysomelidae) is maize pest native to America. WCR is first detected in Europe, in the early nineties in Serbia (Bača, 1993). Today WCR is economically very important pest of European maize fields (EPPO, 2017), with possibility to spread up to 100 km per year (Baufeld, 2003; MacLeod et al., 2004).WCR can feed on more than 20 plants from family Poaceae, but development and survival is possible only on the maize which represents reproductive WCR host plant (Clark and Hibbard, 2004). It is a univoltine, oligophagous pest, and it causes serious consequences on maize roots, on above - ground parts and on maize yields (Bača, 1993; James et al., 2005; Hummel et al., 2008; Ciobanu et al., 2009).The most important damages on maize are caused by larvae (Ciobanu et al., 2009; Wesseler and Fall, 2010). Larval attack leads to the plant lodging, and due to symptom known as "goose neck", only characteristic for WCR presence (Wesseler and Fall, 2010). Plant lodging causes mechanical losses (inability to harvest maize during mechanical harvesting) as well physiological (due to inability of the injured roots to uptake water and nutrients) (Tollefson, 2007; Dun et al., 2010). The aim of this research was to examine the impact of WCR larvae on maize morphology (i.e. leaf number and plant height) after plants were artificially infested with eggs in root zone. .

Material and method

The field experiment was carried out in Bečej municipality area, autonomous province of Vojvodina, Northern Serbia. It was performed from 2nd June until the 19th September 2014, with Serbian maize cultivar NS 640. The field chosen for experiment represents is with a low natural WCR infestation. During the experiment, 96 maize plants were selected, labelled and arranged into pairs. The plants were sawn in two rows with 1 m distance between labelled

plants. In each pair, one plant was artificially infested in root zone with 4 mL of WCR eggs 0.125% agar suspension (D plants). One mL of suspension contained 136 eggs. The other plant from the pair was the control plant, marked as C. The same amount of distilled water (4 mL) was injected in the root zone of plant C. After artificial infestation field experiment was inspected every week during four months. Inspection of plants included the measurement of heights and counting the number of leaves. The measurements of plant high were made using simple meter. Plant inspection was conducted five times on 26th June, 2nd July, 16th July, 24th July and 7th August. The differences between the heights and number of leaves among C and D plants were analysed using Two-sample t-test (Test for equality of sample variances) Gen Stat 12Th edition.

Results and discusion

During first observation the number of leaves on C plants was ranged from 8 to 15. The majority of C plants were with 12 leaves, while only one C plant had 15 leaves (Figure 1). During the second observation the number of leaves on C plants was ranges from 10 to 15. The highest number of C plants (21 plants) was with 13 leaves. During the first observation also only one C plant was with 15 leaves (Figure 1). During the third observation the number of leaves on C plants was ranges from 12 to 15. The highest number of C plants (24 C plants) was with 14 leaves, while the highest leaves number (15) was recorded on 17 C plants (Figure1). During the fourth and fifth observation the same leaves number were recorded and ranged from 12 to 15. The highest number of C plants (23 plants) was with 14 leaves, while the highest number of leaves (15) was recorded on 22 C plants (Figure1).

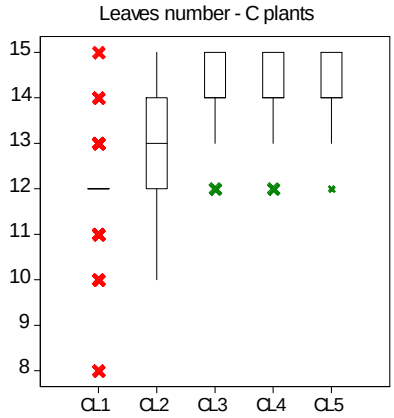

Figure 1. Number of leaves on C plants (C–control plants; L–number of leaves; 1-5 observation)

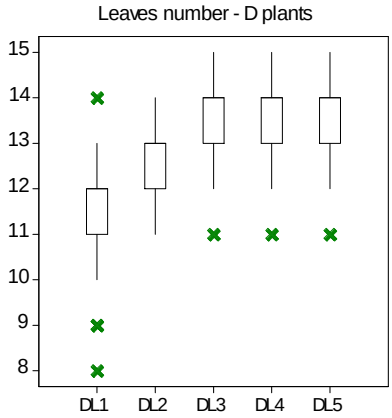

Figure 2. Number of leaves on D plants (C–control plants; L– number of leaves; 1-5 observation)

Statistical analysis shows highly significant differences in the number of leaves on C plants between 1st and 2nd as well 2nd and 3th observation, while there are no differences between 3th and 4th and 4th and 5th observation (Table 1).

Table 1. Differences between number of leaves on C plants during the field inspection

Year	Observation Leaves number	Means values ± STDTV		F	Sig.
		C plants	C plants		
2014	1st - 2nd observation	11.83 ± 1.260	12.98 ± 0.934	1.82	0.001**
	2nd -3th observation	12.98 ± 0.934	14.19 ± 0.734	1.62	0.001**
	3th -4th observation	14.19 ± 0.734	14.38 ± 0.672	1.19	0.195ns
	4th -5th observation	14.38 ± 0.672	14.38 ± 0.672	1.00	1.000ns

During the first observation number of leaves on D plants was ranged from 8 to 14. The majority number on D plants (27 plants) was with 12 leaves, with exception of one plant

with 8 and one with 14 leaves (Figure2). During the second observation number of leaves on D plants was ranged from 11 to 14. The majority number of D plants (19 plants) was with 12 leaves, while the highest leaves number (14) was recorded on 8 D plants (Figure 2). During the third observation number of leaves on D plants was ranged from 11 to 15. The highest number of D plants (22 plants) was with 14 leaves, while the highest number of leaves (15) was recorded on 7 D plants (Figure 2). During the fourth and fifth observation the same leaves number were recorded and ranged from 11 to 15. The majority number of D plants (23 plants) was with 14 leaves, while the majority number of leaves (15) was recorded on 10 D plants (Figure 2).

Statistical analysis shows highly significant differences in the number of leaves on D plants between 1st and 2nd as well 2nd and 3th observation, while there are no differences between 3th and 4th and 4th and 5th observation (Table 2).

Table 2. Differences between number of leaves on D plants during the field inspection

Year	Observation Leaves number	Means values± STDTV		F	Sig.
		D plants	D plants		
2014	1st - 2nd observation	11.48 ± 1.130	12.65 ± 0.838	1.82	0.001**
	2nd - 3th observation	12.65 ± 0.838	13.65 ± 0.887	1.12	0.001**
	3th - 4th observation	13.65 ± 0.887	13.83 ± 0.859	1.07	0.295ns
	4th - 5th observation	13.83 ± 0.859	13.83 ± 0.859	1.00	1.000ns

Statistical analysis shows highly significant differences in number of leaves between C and D plants during 3st, 4st and 5th observation, while there are no differences during the first two observations (Table 3).

Table 3. Differences in leaves number between C and D plants during the field inspection

Year	Observation	Leaves number Means values± STDTV		F	Sig.
		D plants	C plants		
2014	1st observation	11.48 ± 1.130	11.83 ± 1.260	1.24	0.150ns
	2nd observation	12.65 ± 0.838	12.98 ± 0.934	1.24	0.069ns
	3th observation	13.65 ± 0.887	14.19 ± 0.734	1.46	0.002**
	4th observation	13.83 ± 0.859	14.38 ± 0.672	1.63	0.001**
	5th observation	13.83 ± 0.859	14.38 ± 0.672	1.63	0.001**

During the first observation maximum and minimum measured heights on C plants were 180 and 110 cm, respectively. Average height of C plants in the 1st observation was 161.9 cm (Figure 4). During the second observation maximum measured heights on C plants was 195 cm, while minimum was 120 cm. Average measured heights in 2nd observation on C plants was 178.4 cm (Figure 4). In the third observation maximum heights on C plants was 300 cm and minimum 180 cm. Average heights during 3th observation on C plants was 248.9 cm (Figure 4). During the fourth and fifth observations the same maximum, minimum and average heights on C plants was recorded - 320 cm, 210 cm and 264.1 cm, respectively (Figure 4).

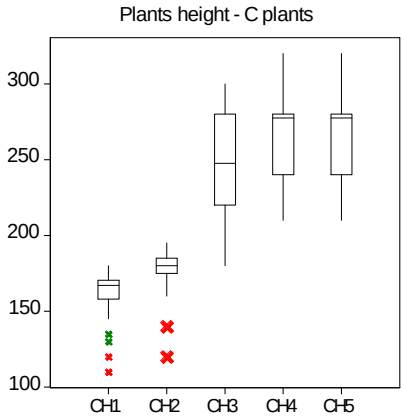

Figure 4. Plants height on C plants (C–control plants; L–leaves number; 1-5observation)

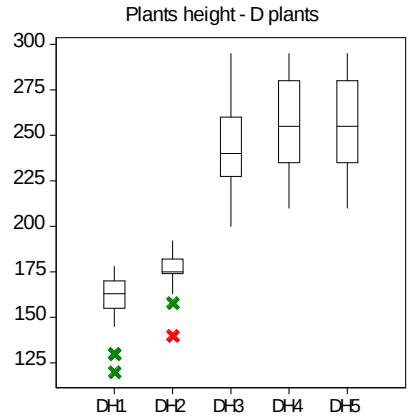

Figure 5. Plants heights on D plants (C–control plants; L–leaves number; 1-5observation)

Statistical analysis shows highly significant differences in plants height on C plants between 1st and 2nd as well between 2nd and 3th observation, while there are no differences between 3th and 4th and 4th and 5th observation (Table 4).

Table 4. Differences in plants height on C plants during the field inspection

Year	Observation Plants height	Means values ± STDTV		F	Sig.
		C plants	C plants		
2014	1st - 2nd observation	161.9 ± 14.53	178.4 ± 12.85	1.28	0.001**
	2nd - 3th observation	178.4 ± 12.85	248.9 ± 31.76	6.11	0.001**
	3th - 4th observation	248.9 ± 31.76	264.1 ± 28.60	1.23	0.015ns
	4th - 5th observation	264.1 ± 28.60	264.1 ± 28.60	1.00	1.000ns

During the first observation maximum and minimum measured heights on D plants were 178 and 120 cm, respectively. Average height of D plants in the 1st observation was 160.7 cm (Figure 5). During the second observation maximum measured heights on D plants was 192 cm, while minimum was 140 cm. Average measured heights in 2nd observation on D plants was 176.9 cm (Figure 5). In the third observation maximum heights on D plants was 295 cm and minimum was 200 cm. Average heights during 3rd observation on D plants was 243.7 cm (Figure 5). During the fourth and fifth the same maximum, minimum and average heights was recorded on D plants - 295 cm, 210 cm and 253.4 cm, respectively (Figure 5).
Statistical analysis shows highly significant differences in plants height on D plants between 1st and 2nd and 2nd and 3rd observation, while there are no differences between 3rd and 4th and 4th and5th observation (Table 5).

Table 5. Differences in plants height on D plants during the field inspection

Year	Observation Plants height	Means values± STDTV		F	Sig.
		D plants	D plants		
2014	1st - 2nd observation	160.7 ± 12.83	176.9 ± 9.243	1.93	0.001**
	2nd - 3th observation	176.9 ± 9.243	243.6 ± 23.57	6.50	0.001**
	3th - 4th observation	243.6 ± 23.57	253.4 ± 24.81	1.11	0.050ns
	4th - 5th observation	253.4 ± 24.81	253.4 ± 24.81	1.00	1.000ns

Statistical analysis shows no statistically significant differences in plants height between C and D plants during all five observations (Table 6).

Table 6. Differences in plants height between C and D plants during the field inspection

Year	Observation	Plants height Means values± STDTV		F	Sig.
		D plants	C plants		
2014	1st observation	160.7 ± 12.83	161.9 ± 14.53	1.28	0.661ns
	2nd observation	176.9 ± 9.243	178.4 ± 12.85	1.93	0.513ns
	3th observation	243.6 ± 23.57	248.9 ± 31.76	1.82	0.358ns
	4th observation	253.4 ± 24.81	264.1 ± 28.60	1.33	0.053ns
	5th observation	253.4 ± 24.81	264.1 ± 28.60	1.33	0.053ns

During the research in conditions of artificial WCR eggs infestation, Popović (2017) did not found statistically significant differences in plant height and plant diameter between artificially infested and un-infested maize plants. In the research of Tanasković et al. (2016) WCR eggs infestation caused 95.7% damages on infested plants with different rate of root damages. In conditions of artificial WCR eggs infestation Tanasković et al. (2017) reported significant differences in the level of root damages and root mass between infested and un-infested (control) maize. Artificial infestation with WCR eggs in the research of Popović et al. (2017) shows no differences in the root damages and root mass, between infested and un-infested plants. The differences between root damages on infested and un- infested plants were higher than differences in their root mass (Popović et al., 2017). These results are quite different in comparison with results recorded during this research. The available literature reports similar research of artificial WCR eggs infestation (different number of eggs), but points out consequences to the population density, size, longevity, and fecundity of emerged females. There are no available data about the influence on plant morphology parameters.
Artificially infested maize roots with WCR larvae in the research of Gavlovski et al. (1992) did not differ in fresh or dry weights or in plant heights. On the other hand, Riedell (1989) reported results of experiment with infestation with 150 second instar WCR larvae. Obtained results indicate differences in plants height, ears length and width, shoot fresh weight, ear dry weight, and husk dry weight between infested and un-infested maize plants.

Conclusion

According to this research, artificial infestation in conditions of low natural infestation caused significant statistical differences in number of formed leaves and plants height on C and D plants between 1st and 2nd and 2nd and 3th observation. Statistical analysis shows significant differences between C and D plants based on their leaves number during the first three observations, while there no differences between plant heights during all five observation.

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RESEARCH OF EXPERIMENTAL HOSTS OF ISOLATES *Colletotrichum* spp. WITH ALFALFA FROM SERBIA

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Abstract

The *Colletotrichum* spp. fungus, the anthracnose causers, are cosmopolitan and extremely aggressive. Like herbal parasites, fungus *Colletotrichum* can cause economic losses on cereals, legumes, vegetable and fruit cultures. Inoculation under controlled conditions was examined for the pathogenicity of the studied isolates of *Colletotrichum* sp. according to different plant species. In this experiment, a total of 15 plant species from 6 botanical families were inoculated. The experiment includes 10 plants of alfalfa, birdsfoot trefoil, red clover, soybeans, peas, beans, field bindweed, flax, vetch, common sainfoin, fodder kale and pepper, per isolate. The plants were inoculated by stinging and by applying parts of the colony of examined isolates at the point of the sting. Ten plants per isolate of the Timothy-grass, cat grass, wood bluegrass is inoculated by spraying a spore suspension at tested isolates. In this research, ten isolates of *C. trifolii, C. destructivum* and *C. linicola* were used. Reactions to inoculated plants that proved to be experimental hosts of the investigated isolates *Colletotrichum* spp. were mostly balanced. Around the inoculated places, the distinct necrotic strips of light brown to dark brown were distinguished. After five days, changes in the appearance of leaves necrosis and drying of plant top inoculated with isolates from the genus *Colletotrichum* were observed. The aim of this research is the ability to monitor the emergence of primary infections in the wild, for forecasting and suppressing illnesses on alfalfa.

Keywords: *Anthracnose; alfalfa; Colletotrichum destructivum*; *C. linicola*, *C. trifolii, experimental hosts*

Introduction

In countries of subtropical and tropical climates, diseases can cause significant economic losses on a large number of agricultural crops during vegetation, as well as after harvesting and during the storage. In moderate climatic zone, pathogens *Colletotrichum* spp. primarily cause the rotting and the decay of stored fruits and vegetables (Freeman *et al*., 1998).

Like all herbal parasites, *Colletotrichum* spp. fungi can cause economic losses on cereals, legumes, vegetable and fruit crops. They are significant pathogens in numerous forest and ornamental plant species, and their presence is also recorded in plants from spontaneous flora (Bailey *et* Jeger, 1992). Symptoms of infection can occur on all underground and above the ground plant organs: roots, tubers, stems, leaves, flowers and fruits. On the affected plant tissue, they cause the appearance of circular, necrotic, embedded spots, with numerous concentrically arranged fruit-bearing bodies, acervuli, from which, in the yellowish-orange matrix, conidia is released (Freeman *et al*., 1998). Species of the genus *Colletotrichum* are known as epiphytic, endophytic and saprobic organisms (Sutton, 1992; Peres *et al*., 2005).

The aim of this research is to observe the emergence of primary infections in the wild, for forecasting and suppressing illnesses on alfalfa.

Material and methods

Inoculation in controlled conditions was used to examine the pathogenicity of the studied isolates of *Colletotrichum* sp. in different plant species, i.e., an experimental set of hosts was determined. For this purpose, a total of 15 plant species from 6 botanical families were inoculated:

- Fam. Fabaceae: alfalfa (*Medicago sativa* L.), red clover (*Trifolium repens* L.), birdsfoot trefoil (*Lotus corniculatus* L.), vetch (*Vicia sativa* L.), beans (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), soy (*Glycine hispida* Max.) and sainfoin (*Onobrychis sativa* Lam.);
- Fam. Brassicaceae: oilseed rape (*Brassica napus* L.);
- Fam. Poaceae: timothy-grass (*Phleum pretense* L.), orchard grass (*Dactylis glomerata* L.) and bluegrass (*Agrostis alba* L.);
- Fam. Solanaceae, pepper (*Capsicum annuum* L.);
- Fam. Convolvulaceae, bindweed (*Convolvulus arvensis* L.) i
- Fam. Linaceae, linseed (*Linum usitatissimum* L.).

The experiment included ten plants of alfalfa, birdsfoot trefoil, red clover, soy, pea, beans, bindweed, linseed, vetch, sainfoin, oilseed rape and peppers per isolate. The plants were inoculated by pricking them and applying parts of the colony of examined isolates to the site of the prick. Prior to inoculation, the plants were sterilized by spraying the stems with 96% ethyl alcohol. After inoculation, the plants were kept in a humid chamber at a temperature of 25°C over a period of 72 hours in the constant darkness. Injured, but uninoculated plants, kept under the same conditions were used as the control.

Ten plants per the isolate: timothy-grass, orchard grass and bluegrass were inoculated by spraying the suspension of the spores of tested isolates at a concentration of $4\text{-}6x10^4$ conidia per ml (Mould *et al*., 1992). Conidia concentration was determined using hemocytometer (thoma chamber). Plants aged 6-7 weeks were cut before the inoculation, and then sprayed with suspension of the conidia of the tested isolates.

For this experiment, eight isolates were used: Coll-4 characterized as *C. trifolii* and reference isolate CBS 158.83 and isolates Coll-8, Coll-9, Coll-18, Coll-48, Coll-68, Coll-75 which are characterized as *C. destructivum* and their control isolate CC657 and isolate Coll-44 characterized as *C. linicola*.

Isolates were selected based on their morphological characteristics. All the inoculations were done by inoculum from fungi cultures aged 10 days and raised on PDA medium. The changes were monitored on a daily basis, lasting 15 days.

Results and discussion

The study of the range of experimental hosts of 10 isolates of phytopathogenic fungi from the genus *Colletotrichum* spp., classified into three morphological groups included a total of 15 plant species from six botanical families. Reactions on the inoculated plants that proved to be experimental hosts of the investigated isolates *Colletotrichum* spp. (alfalfa, red clover) were mostly uniform. Around the inoculated spot, the concave necrotic splotches of light brown to dark brown could be distinguished. After five days, changes in the shape on the leaves in the form of necrosis and drying of peak parts were observed in the plants inoculated with isolates Coll-4 and CBS 158.83 belonging to the species *C. trifolii* and with the Coll-8, Coll-9, Coll-18, Coll-48, Coll-68, Coll-75, CC576 belonging to the species *C. destructivum*. Similar reaction was found in the plants that were inoculated with the isolate Coll-44 identified as *C. linicola* (Table 1). The pea proved to be sensitive to isolates of the species *C. trifolii* (Coll-4 and CBS 158.83) and *C. destructivum* (Coll-9, Coll-18, Coll-48, Coll-68, Coll-75, CC657). Around the inoculation spot, the concave, necrotic, brown to dark brown splotches appeared (Figure 1a, 1b, 1c). Very soon after the inoculation and the occurrence of necrotic splotches

around the inoculation spot, changes were observed in the form of leaf necrosis and drying of the peak parts of the plants (Figure 2a, 2b, 2c).

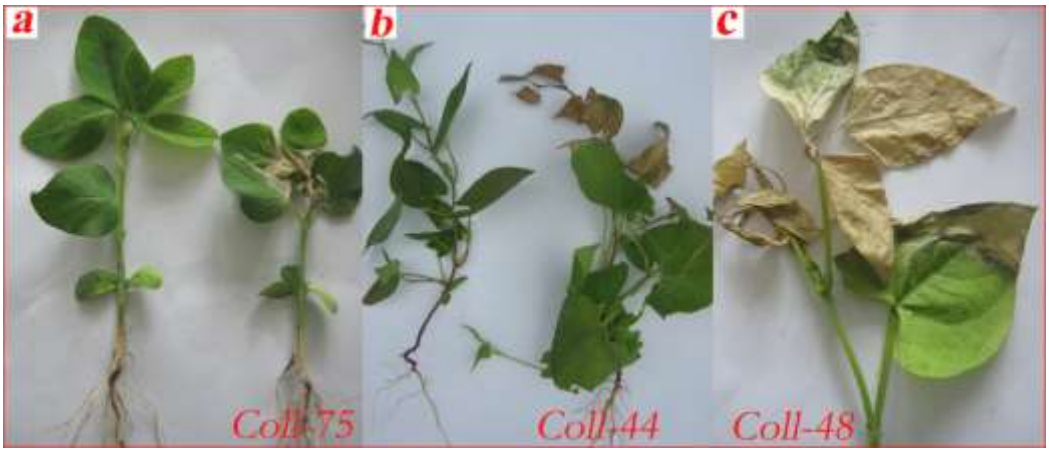

Figure 1. *Colletotrichum* spp. Soy infected with isolate Coll-75 (*C. destructivum*) (control - left, infected plant - right) (a), bindweed infected by isolate Coll-44 (*C. linicola*) (control - left, infected plant - right) (b), beans infected by isolate Coll-48 (*C. destructivum*) (c)

Isolates of *C. destructivum* (Coll-8, Coll-9, Coll-18, Coll-48, Coll-68, Coll-75 and CC657) have been shown to be pathogenic to bean plants (Figure 1c). After four weeks of inoculation, black fruit-bearing bodies (acervuli) were formed (Figure 2a). However, the isolates of *C. trifolii* (Coll-4 and CBS 158.83) did not cause any changes to the beans, and neither was the isolate *C. linicola* (Coll-44).

Figure 2. *Colletotrichum* spp. Symptoms on bean (appearance of acervuli), isolate Coll-9 (*C. destructivum*) (a), bindweed, isolate Coll-44 (*C. linicola*) (b), after four weeks and symptoms on linseed (necrosis) four days after inoculation, isolate Coll-44 (*C. linicola*) (c)

There was no change in the development of symptoms after artificial inoculation on inoculated plants of timothy-grass, orchard grass, oilseed rape, bluegrass and pepper. The three investigated species of the genus *Colletotrichum* were unable to infect any of the studied plants.

Table 1. Pathogenicity of *Colletotrichum* spp. originated from alfalfa and red clover to different plant species in artificial inoculation

Morphological groups	***C. trifolii***		***C. destructivum***							***C. linicola***	*d*
Isolates / **Plants**	Coll-4	CBS 158.83	Coll-8	Coll-9	Coll-18	Coll-48	Coll-68	Coll-75	CC 657	Coll-44	
Medicago satia	+[a] (10)[e]	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	-
Trifolium repens	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	-
Lotus corniculatus	-[b]	-	+ (7)	-	-	+ (6)	+ (8)	+ (8)	+ (7)	-	-
Vicia sativa	+ (6)	-	-	-	-	-	-	-	-	+ (10)	-
Onobrychis sativa	-	-	+/-[c](10)	+/-(10)	+/- (10)	+/- (10)	+/- (10)	+/- (10)	+/- (10)	-	-
Phleum pratense	-	-	-	-	-	-	-	-	-	-	-
Dactylis glomerata	-	-	-	-	-	-	-	-	-	-	-
Agrostis alba	-	-	-	-	-	-	-	-	-	-	-
Brassica napus	-	-	-	-	-	-	-	-	-	-	-
Phaseolus vulgaris	-	-	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	-	-
Pisum sativum	+ (8)	+ (8)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	-	-
Glycine hispida	-	-	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)	-	-
Capsicum annuum	-	-	-	-	-	-	-	-	-	-	-
Convolvulus arvensis.	-	-	-	-	-	-	-	-	-	+ (10)	-
Linum usitatissimum	-	-	-	-	-	-	-	-	-	+ (10)	-

a - positive reaction
b - negative reaction
c - hypersensitive reaction
d - control
e - number of infected plants out of 10

A total of 15 plant species from six botanical families were included in the study of the experimental hosts of 10 isolates of various species of the genus *Colletotrichum*. The plant species included in these researches have been selected as related species that are often grown in with alfalfa as mixed crops or are adjacent crops that are close to the alfalfa fields or are frequent weed species present in and around the alfalfa field. The sensitivity of these plants would indicate their possible role in the epidemiology of the genus *Colletotrichum*, the alfalfa pathogen, because they could act as a reservoir of pathogenic inoculums. These studies will continue, by expanding the number of species involved in the research, as well as attempts to detect latent or infectious diseases with lesser symptoms.

Selected isolates exhibited different ability to infect studied plants, and on plants that were infected, symptoms of necrosis and dryness developed. On the plants there were formed, more or less concave, necrotic splotches, dark brown to almost black, around an inoculated site. After 15 days of inoculation in all susceptible plants, whole plants wilted.

Isolate Coll-4 as well as the standard *C. trifolii* CBS 158.83 exhibited a uniform action on the tested plant. After inoculation, the isolates caused the occurrence of necrotic spots and the drying of all the inoculated alfalfa and red clover seedlings, exhibiting high infectivity. Both isolates exhibited high specialization for the infection of alfalfa and red clover. It should also be noted that the isolate Coll-4 has caused significant symptoms on the plants of vetch and pea, which makes it highly infectious. No isolate was able to infect sainfoin, timothy-grass, oilseed rape, orchard grass, bluegrass, soy, beans, birdsfoot trefoil, bindweed, linseed and pepper. Considering the *C. trifolii* hosts, the literature data indicate that this species exclusively parasites in the alfalfa and red clover. One group of authors stated that this species, although the primary pathogen of alfalfa, is able to infect other plants such as beans and gourds (Baxter *et al*., 1983; Liu *et al*., 2007). The results obtained in these researches are in full agreement with the statements of these authors.

The second morphological group of isolates identified as *C. destructivum* Coll-8, Coll-9, Coll-18, Coll-48, Coll-68, Coll-75, and the standard for *C. destructivum* CC657 also exhibited relative equivalence in terms of infectivity for the studied plants. They caused the occurrence of necrotic splotches and the extinction of seedlings of alfalfa, red clover, beans, pea, soy and birdsfoot trefoil. In birdsfoot trefoil only Coll-9 and Coll-18 isolates did not cause any changes. In the vetch plants, they caused an infection of smaller intensity. All seven isolates failed, under the conditions of the experiment, to infect other studied plants such as: sainfoin, ti,othy-grass, oilseed rape, orchard grass, bluegrass, bindweed, linseed and pepper. In relation to the *C. destructivum* host range, numerous authors (Baxter *et al*., 1983; Koch *et al*., 1989; Latunde-Dada *et al*., 1997; O'Connell *et al.*, 2004; Latunde-Dada and Lucas, 2007; Hyde *et al*., 2009) stated that it is broad and includes legumes such *Glycine max*, *Leucaena leucocephala*, *Lotus* spp., *Melilotus albus*, *Phaseolus lathyroides*, *Trifolium* spp., *Vigna unguiculata*, *Coronilla varia*, as well as tobacco (*Nicotiana tabacum*), dodder (*Cuscuta* spp.) and *Arabidopsis thaliana*. The results obtained during these studies are consistent with the above notes.

The third morphological group, the isolate Coll-44 identified to the level of the genus as *C. linicola*, exhibited a significant specialization in terms of the range of hosts. This isolate caused the occurrence of necrotic spots and wilting of all of the alfalfa, red clover, vetch, bindweed and linseed. This isolate was not able to infect other test plants: sainfoin, timothy-grass, oilseed rape, orchard grass, bluegrass, pepper, birdsfoot trefoil, beans, pea and soy, thus expressing similarity to the species *C. linicola*. Hyde *et al*.(2009a and 2009b) and Tunali *et al*. (2008) stated that linseed and bindweed are the hosts for the *C. linicola* species, while Latunde-Dada and Lucas (2007) noted alfalfa as an experimental host of *C. linicola* species. Considering the results of morphological identification and partial molecular characterization,

this isolate, Coll-44, shows a great similarity to *C. linicola*, which is further confirmed by a specific range of experimental hosts. Of all the examined isolates, only Coll-44 exhibited significant pathogenicity for linseed and pepper, which significantly differed from the other two determined species, *C. trifolii* and *C. destructivum*. Given the extremely complicated taxonomic status of species *C. trifolii, C. destructivum* and *C. linicola*, Johnston *et al*. (2000) stated that there are no general rules concerning their relationship between the pathogen and the host, and Freemen *et al*. (1998) emphasize that the separation of species based on the plant-host relationship does not have to be a reliable criterion, especially in the case of polyphagous, cosmopolitan and genetically variable species.

Conclusion

The study of experimental hosts confirmed the formation of morphological groups of isolates. Isolates of the first group identified as *C. trifolii*, exhibited a high level of specialization for infecting alfalfa and red clover plants. A group of isolates identified as *C. destructivum* also exhibited a high level of specialization by infections in alfalfa, red clover and bean seedlings. The third morphological group of isolates was identified as the genus *C. linicola* (Coll-44) exhibited a high level of specialization in alfalfa, red clover, linseed and bindweed plants.

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THE RESULTS OF INVESTIGATION THE IMPACT DIFFERENT COLORS NETWORKS ON YIELD AND QUALITY OF PEPPERS

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Abstract

The disadvantages of extreme high temperatures accompanied by low relative humidity, leave a whole range of consequences for most vegetable crops, first of all disruption of physiological processes, reduction in yield and quality of yields, more intense occurrence of diseases and pests. In order to reduce the negative impact of high temperatures and the direct impact of UV rays on the yield and quality of the peppers on the field of Agriculture Extension Service "Sombor" (Serbia), performed a trial experiment with the application of different color nets: blue, green, black, white and yellow. The trial experiment was performed during four years from 2014 to 2017. The results of the experiments show that the application of shading networks affect the lushness, renaissance, quality of peppers, reduction of diseases, pests, protection from the city and increase of yield. The trials were conducted with mulching with black foil in ''drop by drop'' system. In this way of production, the advantages of shading networks are simple and mobile construction, as well as the ability to use it for many years. The disadvantages of shading networks were a significant investment in production during the first year as well as the lack of effect in favorable weather conditions for the production of peppers. The application of this technology is recommended for the production of peppers on smaller surfaces as it contributes to stable and quality production.

Keywords: *High temperature, color networks, peppers.*

Introduction

In the second half of the last century, science and profession began with serious warnings that humankind on the earth's ball threatened climate change. From the middle of the last century, especially since 1970, there has been a trend of increasing the average global air temperature. High temperatures strongly affect the intensity of transpiration. Plants respond to stress by decreasing the intensity of breathing and synthesizing nutrients, as well as by increasing the reflection of radiation energy. Physiologically, injuries to plants and plant parts obtained from high temperatures depending on the intensity and length of exposure. Direct injuries lead to an imbalance of metabolism and changes that are present as membrane injuries, loss of fat, protein, and denaturation of nucleic acids (Ilić Z., et al., 2007).

In order to protect the plants and yields of the pepper from the undesirable effect of high solar radiation, it is used to shade the crops by using colored networks. Nets in color represent an additional tool in the management of light quality.

Picture number 1. The influence of high temperatures on yields of peppers (burns) on the field of PSS Sombor (photo V. Sabadoš).

Material and methods

In order to enable the production of high - quality vegetables in vegetable production, the most modern technological solutions are applied. In order to contribute to the reduction of the negative impact of extremely high temperatures we used the shading nets in the trial field of Agriculture Extension Service "Sombor" (Serbia) as a special agrotechnical measure, characteristic for the production of peppers, which significantly contributes to the reduced negative effect of abiotic factors on the growth, quality and yield of peppers. At the end of the 1960s, shading net was used in Europe. The most famous factories were a location in England and Italy. Over time, this technology has found a way into other parts of the world. Nets, in addition to basic protective use, have the function of protection against birds, insects, and wind. The benefit of the color network includes an extension of harvest time, increased yields, improved quality and agro-economic performance of the yield (Shalom Y. and Gussakovsky E.E., 2004). Filtered sunlight through a photoselective net, shows the influence and quality improvement and yield (Oren - Shamir et al., 2001). In order to solve the problem in the field of Agriculture Extension Service "Sombor" (Serbia), its implemented trial experiment with different colors of the shading net: Blue, green, black, white (rarely and dense) and yellow. An experimental trial was set in the period from 2014 to 2017. The varieties of peppers that were represented for a period of 4 years are in Madona, Slonovo uvo, Strižanka, Duga bela, Župska rana, Savo and Šorokšari. Madonna is a medium-sized variety in the type of "kapija" intended for cultivation in a protected area and an open field. The yield is fleshy, regular with two chambers, weighing 100 - 400 grams. In technological maturity, the color of the yield is yellow and in the botanical is red. Slonovo uvo is pepper "ajvaruša" with very large and fleshy fruits. The plant is very lush, forms the fruits with two or three chambers. The color of the yield from dark green turns into dark red. Slonovo uvo reaches a mass of 150-250 grams. Strižanka is in the type of "polubabura", it is yellow in technological maturity, and in the biological intense red color. It is suitable for consumption in fresh condition and industrial processing. It achieves good yields in a protected area. Duga bela is a medium early variety, strong, compact, high growth. The yield is long in the type of "kapije" that is milky white in technological maturity and in biological red color. Župska rana is a very early pepper variety. The plant is strong, compact, high growth and hanging yields. The yield is long in the type of the "kapije", which is in the technological maturity of yellow color and in the biological intense red color. The yield is light yellow, sweet and hanging. It is recommended for field production in protected areas. Šorokšari is a medium late variety. The plant is strong, compact, low growth. The fruit is in the type of "babure".

Characteristics of shading net

Manufacturers offer nets that range from 10 to 90%. Recommended vegetable density are nets that shine from 30 to 40%. They are most commonly produced from high-density polyethylene and can be used for more seasons. Shading nets are made of UV - resistant material and are suitable for use over several seasons, minimum two. Setting up nets is relatively simple. When installing the structure, it is necessary to lift the pillars over which the net is clinging and stretching. Nets are manufactured in different diameters of 1.20, 2.00, 3.60, 4.00, 6.00 and 7.50 mm depending on the purpose.

Picture number 2. Different dimensions of shading net

Nets can be found in different colors. Although it is said to be a shadow net, it at the same time reduces the amount of light, the intensity of UV rays and the temperature. Shading nets have additional functions such as making cold for domestic livestock and pets. Trials filed with the use of shading net is set in 3 repetition, the size of plots 5m², in the "drop by drop" system with the application of a mulch foil. With each variant, a control plot was set up.

Results and discussion

Results of the experiments in 2014 year

During of 2014, on-field Agriculture Extension Service "Sombor" made a trial with few different varieties of pepper: Madona, Slonovo uvo, Strižanka, Duga bela and Župska rana. Three different colors of the net were used: Blue, Green, and Yellow. In addition to the variant with the nets, a control plot was also installed.

Picture number 3. In the field of Agriculture Extension Service "Sombor" during 2014, three different colors of the network were used: Blue, Green, and Yellow (photo V.Sabadoš)

Table 1. Results in 2014 year

Variety	Control		Blue net		Green net		Yellow net	
	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage
Madona	32,5	32,0	31,8	10,0	30,2	8,0	24,7	18,0
Slonovo uvo	31,9	35,0	27,8	26,0	32,2	11,0	23,2	16,0
Strižanka	48,9	29,0	43,3	29,0	37,1	20,0	32,2	16,0
Duga bela	53,7	12,0	50,6	12,0	46,6	10,0	43,0	5,0
Župska rana	38,1	22,0	40,7	12,0	36,8	10,0	28,6	5,0

Results of the experiments in 2015 year

During of 2015, on-field Agriculture Extension Service "Sombor" made a trial with three varieties of peppers: Ljubov dlan, Slonovo uvo and Savo. Three different colors of the net were used: White (rarely), White (dense), Black and Green. In addition to the variant with the nets, a control plot was also installed.

Picture number 4. In the field of Agriculture Extension Service "Sombor" in 2015, four different colors of the nets were used: White (rarely), White (dense), Black and Green net (photo V.Sabadoš)

Table 2. Results in 2015 year

Variety	Control		White net (rarely)		Black net		Green net		White net (dense)	
	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage
Ljubov dlan	24,6	43,0	37,7	2,0	38,3	13,0	40,6	6,0	34,3	4,0
Slonovo uvo	19,2	36,0	44,4	5,0	53,0	10,0	38,8	3,0	45,3	8,0
Savo	16,2	61,0	27,6	5,0	27,6	9,0	26,4	7,0	37,8	13,0

Results of the experiments in 2016 year

During of 2016, on the field Agriculture Extension Service "Sombor", made a trial with three varieties of peppers: Ljubov dlan, SLonovo uvo i Šorokšari. Three different colors of the net were used: White (rarely), White (dense), Black and Green. In addition to the variant with the net, a control plot was also installed.

Picture number 5. In the field of Agriculture Extension Service in 2016 years, four different colors of the net were used: white (rarely), white (dense), black and green net (photo V.Sabadoš)

Table 3. Results in 2016 year

Variety	Control		White net (rarely)		Black net		Green net		White net (dense)	
	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage
Ljubov dlan	12.5	25.9	15.8	22.7	15.1	21.2	14.6	17.2	17.2	13.6
Slonovo uvo	10.5	23.5	23.2	22.7	16.5	23.3	16.3	24.0	19.7	17.3
Šorokšari	22.7	33.1	24.7	10.9	16.7	11.5	17.5	23.5	16.2	10.7

Results of the experiments in 2017 year

During of 2017, on the field of Agriculture Extension Servis "Sombor", made a trial with three varieties of pepper: Ljubov dlan, Slonovo uvo i Šorokšari. Three different colors of the net were used: White (rarely), White (dense), Black and Green. In addition to the variant with the nets, a control plot was also installed.

Picture number 6. In the field of Agriculture Extension Service "Sombor" in 2017 four different colors of the net were used: White (rarely), White (dense), Black and Green net (photo V.Sabadoš)

Table 4. Results in 2017 year

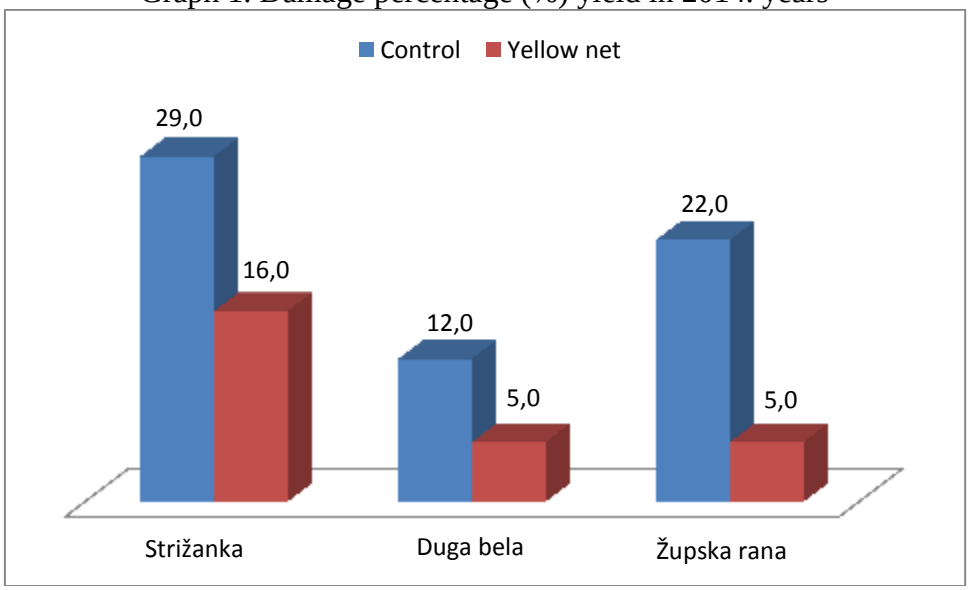
Variety	Control		White net (dense)		Black net		Green net		White net (rarly)	
	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage	Yield t/ha	% Damage
Ljubov dlan	32.1	12.6	25	6.5	42.6	14.7	54.2	8.3	47.4	12.4
Slonovo uvo	20.6	19.8	17.1	8.2	23.8	13.2	39.3	11.7	33.0	11.1
Šorokšari	27.8	19.9	19.3	12.3	17.8	18.8	20.8	14.0	14.1	16.8

Conclusion

The application of this technology is recommended for the production of table peppers on smaller surfaces, but it is also interesting for the production of blue eggplant and tomatoes. The benefit of a network of colors includes an extension of harvest time (early and later maturation) yield increase, quality improvement and total agroeconomic yield performance. (Shahak Y. and Gussakovsky E.E., 2004). In addition, the importance of network applications is also reflected in the protection of plants from the hail and insects.

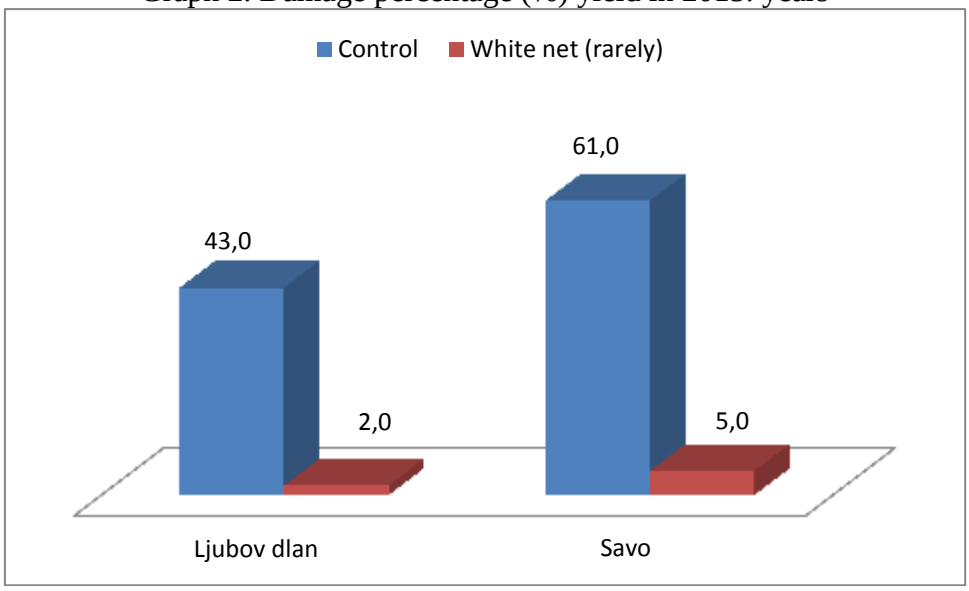
In 2014, for all the tested net (Table 1), the best results were obtained by applying a yellow net in the three examined variables, and we got lower damages than the control (Graph 1).

Graph 1. Damage percentage (%) yield in 2014. years



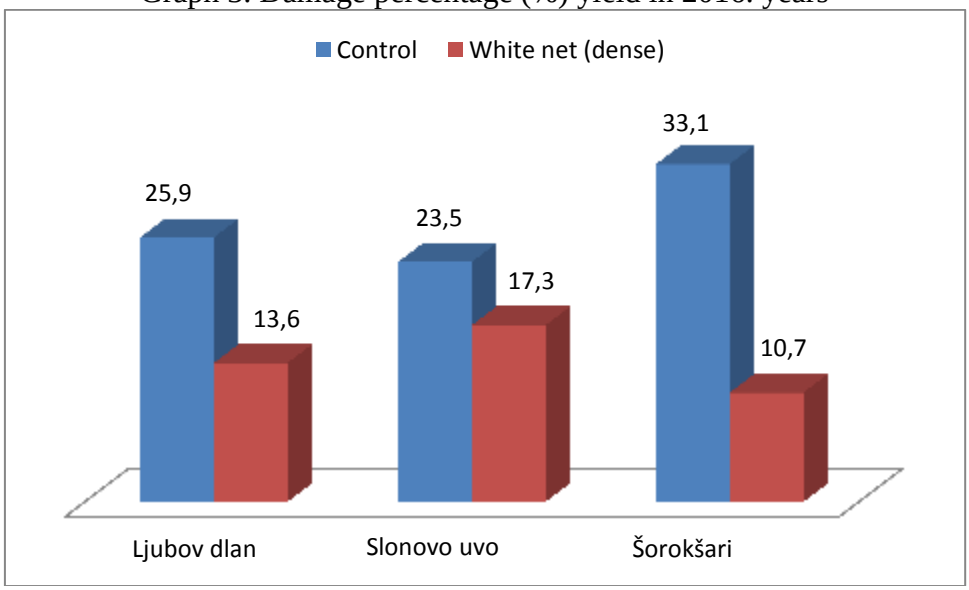
In 2015, for all the tested net (Table 2), the best results were obtained by using the white (rarely) net in the two examined variables and we got lower damages than the control (Graph 2).

Graph 2. Damage percentage (%) yield in 2015. years



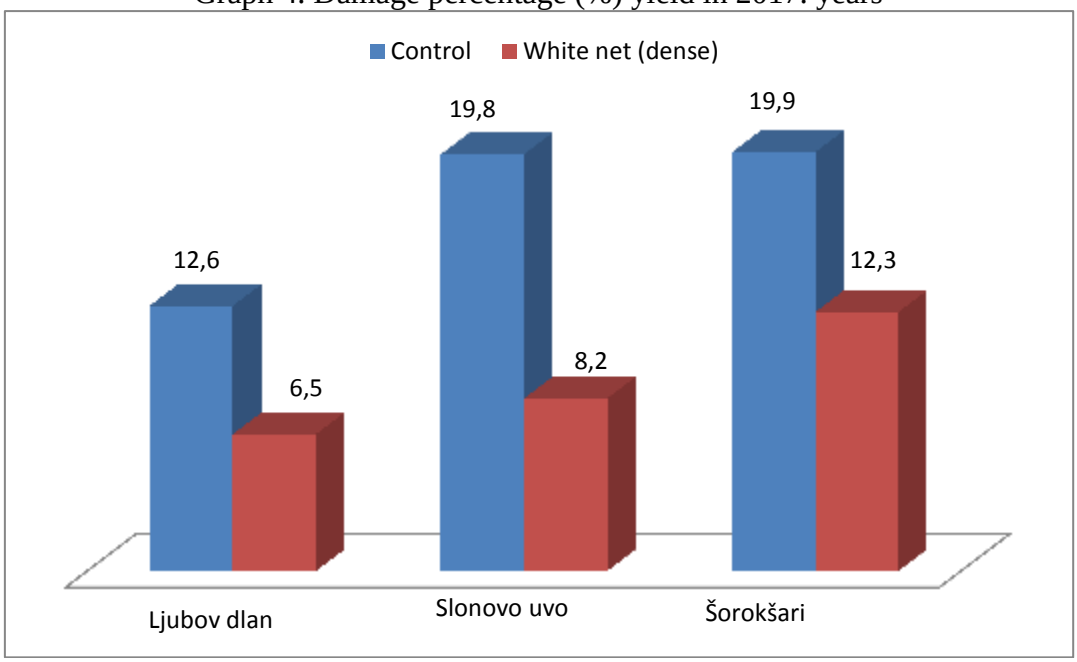
In 2016, in all the tested net (Table 3), the best results were obtained by using a white (dense) net in the three examined variables, and we got lower damages than the control (Graph 3).

Graph 3. Damage percentage (%) yield in 2016. years



In 2017, in the case of all the tested networks (Table 4), the best results were obtained by using a white (dense) net n the three examined variables, we got lower damages than the control (Graph 4)

Graph 4. Damage percentage (%) yield in 2017. years



Also, we continued testing different colors net in 2018. The disadvantages of shading nets are a significant investment in production in the first year. The benefits of the shading nets are simple and mobile construction, the use of nets for many years, the possibility of using incentive means for purchasing shading nets, stable and quality pepper production. Agronomic professions and science responded in time and tried to reduce the negative impact of climate change and abiotic stress on plants by introducing new breeding technologies, tolerant varieties, and hybrids.

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THE POTENTIAL USE OF LACTIC ACID BACTERIA AS ANTIOXIDANT AGENT IN MEAT PRODUCTS

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Abstract

Lactic acid bacteria (LAB) are commonly used and generally regarded as safe. The most significant contribution of these microorganisms to the product is to preserve the nutritive qualities of the raw material through an extended shelf life and the inhibition of spoilage and pathogenic bacteria. LAB may positively affect taste and aroma of foods. LAB can maintain the balance of gut microbial flora, enhance resistance to diseases and show immunomodulatory activities. Certain LAB strains also have other significant functions such as antioxidant activity. Antioxidant properties of some LAB strains may be derived from antioxidases such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx). SOD, CAT and GSH-Px catalyze the decomposition of superoxide anion radical scavenger, hydrogen peroxide and hydroxyl radicals/hydrogen peroxide respectively. LAB play major role during meat fermentation as they contribute to final product quality and safety. The role of LAB in fermented meat products has been investigated widely, and some LAB strains have been shown to contain antioxidant properties *in vitro*. Studies indicated that *Pedioccocus* and *Lactobacillus* species had antioxidant properties. It has been shown that lipid and protein oxidation in sausages were significantly reduced by *Pedioccocus pentosaceus*. *Lactobacillus* species were also suggested as antioxidant agents for production of fermented meat products. In this review, studies using LAB as antioxidant agent in meat and meat products are presented.

Keywords: *Meat products, Lactic acid bacteria, Antioxidant*

Introduction

The most of lactic acid bacteria (LAB) is known as "Probiotics". Probiotics have been defined as "live microorganisms, which when administered in adequate amounts, confer a beneficial health effect on the host (FAO/WHO, 2002). LAB are Gram positive bacteria, widely distributed in nature, and industrially important as they are used in a variety of industrial food fermentations. Antioxidants are substances that retard the oxidation of easily oxidizable biomolecules, such as lipids and proteins in meat products, thus antioxidants improve the shelf life of products by protecting them against deterioration caused by oxidation. The use of antioxidants in food industry is controlled by regulatory laws of a country or international standards. Although there are many compounds that have been proposed to possess antioxidant properties to inhibit oxidative deterioration, only a few can be used in food product manufacture (Coda *et. al.,* 2012). In the United States, the use of antioxidants is subject to regulations of the Federal Food, Drug and Cosmetic Act, Meat Inspection Act, Poultry Inspection Act, and other state laws (Shahidi and Zhong, 2005). Oxidative damage plays a pathological role in human body. However, ingestion of antioxidative supplements, or foods containing antioxidants, may reduce the oxidative damage in the human body (Wang *et. al.,* 2006). In response to recent demand for natural products, consumers are willing to pay significant premiums for natural foods (Sebranek and Bacus, 2007). Therefore, many researches have been focused on finding natural and safe antioxidants (Embuscado, 2015).

Recently, meat safety concerns have focused on the potential carcinogenicity of red meat and processed meat, as described by the International Agency for Research on Cancer (Xiao *et. al.*, 2018). Therefore, it is important to use natural solutions to minimize oxidative rancidity and increase products' shelf-life in the meat and poultry meat industry (Naveena *et. al.*, 2008). Of various sources, probiotics have been considered as an emerging source of effective antioxidants. Due to their long tradition of safe use and potential therapeutic benefits, the role of probiotics as an antioxidant has been started to be widely investigated (Mishra *et al*., 2015). This review intends to summarize the effects of LAB to respond to oxidative stress and oxidative-stress related problems.

Importance of Antioxidants in Meat Products

Lipid oxidation is one of the major causes of quality deterioration and is also important because it can negatively affect sensory attributes such as color, texture, odor, and flavor as well as the nutritional quality of the product (Nunez de Gonzalez et al., 2008). The meat industry uses nitrates and nitrites for the stabilization of the meat color (Honikel, 2008), prevention of the development of pathogenic microorganisms, inhibition of lipid oxidation and enhancement of product flavor (Pegg and Shahidi, 2000). Despite the antioxidant power of nitrites and nitrates, industries are searching to find healthier and greener alternatives to stop or minimize the use of these chemicals which have been surrounded with claims of negative health effects (Gassara *et al.*, 2015). These issues are focusing the meat and poultry meat industry to find economic and natural antioxidant sources without negatively affecting the quality of finished products and consumer health (Karre *et al.*, 2013).

An antioxidant can be defined as "any substance that, when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate"(Halliwell and Gutteridge, 1995). Antioxidase can transform peroxide and oxygen free radicals into hypotoxic or nontoxic compounds for antioxidant activity via oxidoreduction. These compounds mainly include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). SOD, a main antioxidant component in cells, is a specific superoxide anion radical scavenger. CAT, as an indicator of peroxidase, catalyzes the decomposition of hydrogen peroxide. And GSH-Px can scavenge hydroxyl radicals and hydrogen peroxide (Zhang *et al.*, 2017). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG) have been used as antioxidants in meat and poultry meat products (Biswas *et al.*, 2007). In response to recent claims that synthetic antioxidants have the potential to cause toxicological effects and consumers' increased interest in purchasing natural products, the meat and poultry meat industry has been seeking sources of natural antioxidants (Karre *et al.*, 2013). The role of LAB in fermented meats has been studied widely (Fadda *et. al.*, 2010), and certain LAB strains have been determined to contain antioxidant properties *in vitro*. It has been shown that numerous LAB species have SOD, GSH-Px, CAT, and NADH oxidase activities to prevent deleterious oxidative effects (Noonpakdee *et al.*, 2004).

Usage of Lab and Probiotic Lab with *Antioxidant Properties* in Meat Products

LAB strains are essential agents during meat fermentation for contributing to final product quality and safety (Liu *et. al.*, 2011). The probiotic strains which have proteolytic, lipolytic, esterolytic and other enzymatic activities, citrate utilization, and bacteriocin production may be selected for application in meat fermentations (Belgacem *et al.*, 2010). There is also considerable evidence that probiotic LAB have antioxidative properties that may be effective against oxidative stress in humans (Kullisaar *et al.*, 2003). Some studies have shown that the antioxidant activities of some LAB strains may be attributable to antioxidases (SOD, CAT, GSHPx) and their activities (Kullisaar *et. al.*, 2002). The intracellular extracts of some LAB

have metal ion chelating ability, reactive oxygen species scavenging ability, and reduction activity (Lin and Yen, 1999; Zhang *et. al.*, 2005). Several studies have shown that some LAB, including *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium breve* and *Bifidobacterium lactis*, can bind and remove heavy metals such as cadmium (Cd) and lead *in vitro* (Halttunen *et. al.*, 2008). The capacity to accumulate selenite (Se_{4+}), an essential element showing antioxidant properties (Thirunavukkarasu *et al.*, 2004) has been described for some isolates of *Enterococcus* (Pieniz *et al.*, 2011) and other LAB (Svoboda *et al.*, 2009).
Free radical scavenging activity was also described to intracellular extracts of some LAB, such as *Lactobacillus delbrueckii* (Lin and Yen, 1999), *Lactobacillus acidophilus* and *Bifidobacterium longum* (Lin and Chang, 2000). The antioxidant activity of some species of LAB has been demonstrated by *in vitro* enzymatic assays (Lin and Yen, 1999). Both intact cells and intracellular cell-free extracts of *Lactobacillus acidophilus* ATCC 4356, isolated from human intestines exhibited 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and linoleic acid peroxidation inhibition *in vitro* (Lin and Chang, 2000). Pieniz *et al.* (2014) observed the antioxidant effects of *Enterococcus durans* LAB18s by different methods, including scavenging of (2,2 azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) ($ABTS^{+}$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, and thiobarbituric acid reactive substances (TBARS) method.
Zhai *et al.* (2015) reported that *Lactobacillus plantarum* CCFM8610, showed the best binding ability and tolerance to Cd among all tested 33 strains of LAB. This strain also exhibited relatively good antioxidative properties and resistance to the simulated gastrointestinal conditions. Chen *et al.* (2015) determined the antioxidant activity of four LAB strains, isolated from Harbin dry sausage, and evaluated their antioxidant properties in both *in vitro* and a sausage model. The results of *in vitro* study showed that *P. pentosaceus* had the strongest H_2O_2 resistant ability, hydroxyl radical and DPPH radical scavenging activity, and highest reducing power and inhibition of lipid peroxidation, as well as high SOD and GSH-Px activities. Enterococci possess a competitive advantage over other microbiota in meat fermentations. The antimicrobial and antioxidant capacities of the *E. faecium* isolates from meat and dairy products revealed that indicate they could be very useful in food fermentation and feed composition. They could particularly help to inhibit pathogenic microorganisms as well as oxidative spoilage in foods and feeds (Pieniz *et al.*, 2015). *Takeda et al.* (2017) used *Lactobacillus sakei* strain no. 23 and *Lactobacillus curvatus* strain no. 28 in fermented sausage production. Researches indicated that the sausages fermented with these strains showed significantly higher antioxidant activity than those without LAB or fermented by each LAB strain. Zhang *et al.* (2017) compared the antioxidant activities of *L. curvatus* SR6 and *L. paracasei* SR10-1. The results showed that *L. curvatus* SR6 and *L. paracasei* SR10-1 significantly enhanced the antioxidant activities of sausage extracts.

Conclusions

LAB are commonly used in meat industry for fermentation and probiotic source. It has been shown that Lab are also have potential to be used as antioxidant agents in the processing of meat products. More research is needed to extend the knowledge on possible usage of LAB as antioxidant source in meat product processing.

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INVESTIGATION OF FARMERS AWARENESS ON THE USES OF CERTAIN PLANTS IN RURAL AREAS (THE CASE OF ANTALYA / TURKEY)

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Abstract

Humans have been seeking solutions for healing diseases encountered in different periods of their lives by applying different treatment methods and cures against different diseases. Natural plant products have been used throughout human history for various purposes. In fact, written records of the use of herbal medicine date back more than 5,000 years, and for much of history, herbal medicine was the only medicine. Due to complementary effect of medical treatments, alternative herbal medicine practices and cures are becoming increasingly important in developed countries. These products are complex mixtures of organic chemicals that may come from any raw or processed part of a plant. On this purpose the questionnaires were carried out in Serik, Aksu, Manavgat, Kumluca, Demre and Finike districts and villages of Antalya (Turkey) and 43 different questions were asked to 200 people living in the countryside. The alternative utilization of local plants by residents, the purpose of utilization, the consumption way, the contribution to their budget and the collection sites and collection ways were determined. As a result of this study, purslane (Portulaca oleracea L.) has been determined as most consumed plant as food material while tea and goosefoot (Chenopodium album L.) were the most commonly used plants. In disease treatments plants were mostly collected from field edges and fresh leaves were consumed. Leaf, stem and flowers were commonly used in treatments of diseases such as influenza, bronchitis and blood pressure and were commonly used as cough remover, pain reliever. These herbs are less expensive and they're a safer means of treatment than conventional medications

Keywords: *Rural areas, treatment, Antalya, weed, survey, medicinal plant.*

Introduction

Throughout their lives mankind utilize weeds for different pupropses. This purposes proved that weeds are not only harmfull also beneficial (Özer ve ark., 2003). This situation which is called ethnobotanic (human-plant relations) is short; utilization of plants by people to meet various needs and their effects on plants. Medicinal plants include plants or herbal products used in the treatment or prevention of diseases. According to the definition by World Health Organization (WHO) in 1980 medicinal plant is defined as a plant variety that can be therapeutic or preventive against certain diseases, main agent of chemical pharmaceutical synthesis (Yıldırımlı, 2004; Yapıcı ve ark., 2009). Across the world approximately 15% of 300.000 plant species are known to have pharmaceutical properties. (Palhares ve ark., 2015). Ethonobotanical studies in Anatolia dates back to beginning of Republican period and these studies are increasing day by day (Çubukçu ve Özhatay, 1987; Bulut, 2006; Doğanoğlu ve ark., 2006; Koçyiğit ve Özatay, 2006; Satıl ve ark., 2008; Yapıcı ve ark., 2009; Sarı ve ark., 2010; Kendir ve Güvenç, 2010; Faydaoğlu ve Sürücüoğlu, 2011; Tulukcu ve Sağdıç, 2011; Oğuz ve Tepe, 2017).

Alternative medicine İis the use of traditional and natural plants for treating diseases. Although some of diseases have the ability to gain resistance to synthetic medicines in a short period of time, this resistance can not be gained against herbal medicines (Özer ve

ark., 2004). The alternative medicine, called complementary medicine, is usually made with herbal products. Alternative medicine is usually applied to chronic diseases in which modern medicine is not effective. Herbal products used for alternative medicine can be used against different pathogensincluding HIV, cancer and hepatitis (Şarışen ve Çalışkan 2005; Anonim, 2009). Alternative medicine or complementary medicine is a method of treating diseases with natural herbal products (with tea or plant mixtures). These plants can either be collected from the nature or can be buy from herbalists. But the most important thing that should not be forgotten is that alternative medicine is defined as a complementary medicine and it must be remembered that the utlizisation amount creates poison (Özer ve ark., 2004; Şarışen ve Çalışkan 2005). Expert control is essential in the consumption of these plants.These plants natural plants are not only used as alternative medicine, but also in different forms such as fuel, food, shelter, spices, tea, animal food and others. In this study, a questionaire was carried out in Antalya to determine the utilization level of plants in disease treatments and other ways.

Material and method

On this purpose field studies and the questionnaires were carried out in June-September 2017 in Serik, Aksu, Manavgat, Kumluca, Demre and Finike districts and villages of Antalya (Turkey) and 43 different questions were asked to randomly selected 200 people living in the countryside.

The alternative utilization of local plants by residents, the purpose of utilization, the consumption way, the contribution to their budget and the collection sites and collection ways were determined. In addition plant observations were conducted to approve the situation of plants. The results were statistically analyzed in Plant Protection Deepartment of Faculty of Agriculture in Gaziosmanpaşa University.

Results and discussion

In this study, the socio-demogogic structure of the residentials of antalya, the plants they used, the usage forms and the diseases they treat were tried to be determined. As a result of these questionnaire, it was determined that the average age of the respondents was 37.79, the 62% of the respondents were female, 82% were married, and 34% were high school graduates. The study revealed that 29.5% of the respondents both work as worker and has an agricultural income. The participants of the questionnaire consists of livestock farmers who also consume naturally grown plants. Among natural plants kuzu kulağı was found to be collected most (53%) in winter and 81% were collected in summer. In addition 15 people who participated in the study stated that they collected more than 2 kg of semiz otu and 14 people declared that they used it in their daily consumption. Semiz otu was not only the most collected plants but the most purchased plant (13%). In general, it was determined that although fields were found suitable for collection of plants semiz out and mullet were collected from forest and katır tırnağı from seasides.

Oguz and Tepe (2017) found that plants such as sorrel, poppy were used mainly in Yüksekova. The utiliation may vary depending on local flora and wide acreage of Anatolian.

As a result of questionnaire it is determined that these plants are mainly used as food materials, the seeds, sirens, poppies and water are used for the medicine production, and also sage, lemon, thyme, mint, chamomile, rosemary, hibiscus, the sorrel were processed for tea production. The leaves were mostly used of these plants (95%) and they were collected from the fields (92%). According to Tulukçu and Sağdıç (2011), a similar results were obtained in Konya province.

Most of the collected vegetables were consumed freshly (98%), whereas these plants consumed predominantly in salads (93.5%) and meals (87.5%). Faydaoğlu and Driveroğlu 2011 mentioned the use of these plants as food and spices and raw material in medicines. Our results indicates the usage as green manure and fuel oils.

Farmers stated the possibility of pesticide residue risk (%58,5) harmfull effect on helth when directly consumed (%45,5) and they mentioned different methods such as peeling (%41), soaking in vinegar added water (%84), washing (%77,5) and utilication of alternative chemical to minimize negative effect (%3). The study by Tiryaki et al. (2010) showed that the residues of pesticides have negative effects on humans and that people are not sufficiently informed on this issue. Our work revealed that about half of the people living in the rural areas have knowledge of the effects of these pesticides on human health.

In terms of alternative medicine, 65% of the respondents stated that they prepared the medicine solution from the plants they collected and mostly prefer fresh plants (48.5%) and mainly use plant leaves (56.5%). The most commonly treated diseases are; (2%), cardiovascular diseases (11%), diabetes (29%), cancer types (6,5%), rheumatic diseases (3%), influenza (65%), hemorrhoids (16.5%), cough reliever (56.5%), pain killer (49%), blood pressure (26.5%), kidney disorders (21.5%) and others (0.05). It is known that naturally grown plants are being used to treat different diseases, which is indicated in the result of various ethnobotanical studies in different regions of our country. Yapıcı et al. (2009), reported the use of plants as wound healing, cough suppressant, sugar and kidney disorders, as a liver treatment and as a bile-reducing agent. Oguz and Tepe (2017) found that they used to treat constipation, intestinal disease, diabetes, rheumatism, headache, painkillers, heart diseases, etc. in their studies in Yüksekova. Several studies including our stud proved these results.

Conclusion

Nowadays studies regarding plant usage especially as alternative medicine have been significantly increase. Many plants have already been used in daily consumption for health for years. However, due to uncontrolled usage the necessity of control arise.

In this study it was observed that in rural parts of Antalya most of the plants were utilised as food or medicine production. The most commonly treated diseases are; prostate, flu, bronchitis, hemorrhoids, cardiovascular diseases, diabetes, cancer types, rheumatic diseases, cough suppressant, pain reliever, blood pressure, kidney disorders. In addition dried usage as tea and spice and usage as green manure soil fertilizer have been revealed.

The study is important in terms of determining the level of knowledge on the plant collection period, collection locations pesticide residues. Especially in regions with higher pesticide problems like Antalya more care should be taken in utilizing natural plants.

There is a need to work to increase the level of awareness of people to benefit more from these plants. Because many plants use limited consumption. Workshops should be organized by qualified experts to increase the level of avareness and information about the benefits and consumption of naturally grown plants should be given. Due to many plants have toxic varieties and their use can lead to serious problems the consumption must be under conrol of doctors.

In conclusion in Anatolia, rich in the point of plant species, the ways of benefiting from plants should be controlled and developed more scientifically.

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HOST RANGE AND DISTRUBUTION OF EUROPEAN MISLETOE *VISCUM ALBUM* IN NORTHWESTERN MARMARA, TURKEY

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Abstract

Weeds compete with crop plants for water, light, and nutrients leading to direct and indirect crop losses. Furthermore some weed species were a good hosts for many pathogens and insect. *Viscum album* is a semi-parasitic plant on fruit trees and schurbs which approximately provides only about half the nutrients on its own. *Viscum album* is found parasitic on more than 100 different genera and 230 different species which uses hosts for water and nutrient. This weed is reported parasitising many host plants across Turkey but host status in Northwestern Marmara Region of Turkey is not known. A field study was carried out in three provinces in Northwestern Marmara Region of Turkey to identifiy hosts frequency and density of *V. album*. The parasitic weed was mostly found in wild pear, pear, almond, plum, apricot, oak, spruce tree, willow, cherry and poplar trees in Edirne, Kırklareli and Tekirdağ provinces (Turkey). The incidence of weed was higher in Tekirdağ especially on pear trees. Tree death due to higher weed infection was observed on cherry and pear trees. According to our observations a correlation between the number of mistletoe shrubs on the host and tree health status was observed in infected fruit trees. Significant increase in damage on trees was determined paralel to increase of number of misletoe schrubs. Dried branches were observed in upper parts of infected trees. The infections in upper branches were found more destructive on examined trees. The geographic distribution map, occurance rate, host status and damage level were comprehensively detailed.

Keywords: *Misletoe, Hosts, Northwestern Marmara Region.*

Introduction

Viscum album, commonly known as European mistletoe, is a species of mistletoe in the family Viscaceae. This plant is a semi-parasitic perannial evergreen plant that grows on the stems and branches of deciduous species of trees. European misletoes have yellow-green color, egg-shape to elongated form leaves, yellow flowers, nearly in 1 m diameter green brown branches.

Misletoe cause significant reduction on growth and fruit production of host plants. These plants has higher transpiration rate and lower leaf water potential. Thus plant has lower water use efficiency for photosynthesis and consumes host plants water. This may create drough stress in host plants and may significantly affetc plant growth under low rainfall conditions (Mathiasen et al. 2008; Rigling et al. 2010; Mutlu et al., 2016). In addition many fungi and bacteria may invade into host trees from misletoe infeted canes (Varga et al. 2012).

Mistletoe is mostly polinated by insects such as sap-sucking bugs (*Cacopsylla visci, Pinalitus viscicola* and *Hypseloecus visci*,), the Mistletoe Marble Moth (*Celypha woodiana*), the Mistletoe Weevil (*Ixapion variegatum*) but wind polination is also reported (Hawksworth & Wiens 1996; Barney et al., 1998; Brigges, 2010).

On the other hand birds are responsible for dispersal of weed seeds. More than 11 bird vectors of European misletoes were reported including European bee-eater (*Merops apiaster*), Blackcap (*Silvia atricapilla*), the great tit (*Parus major*), the Eurasian jay (*Garrulus*

glandarius), Eurasian magpie (*Pica pica*), Eurasian blue tit (*Cyanistes caeruleus*), the Eurasian nuthatch (*Sitta europea*), the common wood pigeon (*Columba palumbus*). This evergreen plant has small white berries which contain 1-4 seeds and birds comes to feed even in winter. When feeding sticky misletoe seeds stick to their beaks. After birds ingest seeds by feeding they excrete seeds onto tree branches where the seeds germinate and begins to uptake water and minerals from its host plant with its newly emerged haustoria. Mistle thrush (*Turdus viscivorus*), fieldfare (*Turdus pilaris*), waxwing (*Bombycilla garrula*) can disperse seeds by this way in 20 km distance. Blackcap (*Sylvia atricapilla*) is another vector which only eats berry and leaves seed on bark (Zuber, 2004).

European mistletoe is divided into several subspecies. These species are morphologically very similar so that host plant is the easy way to differentiate. In Europe, four subspecies including *Viscum album subsp. album, V. album subsp. abietis*; *V. album subsp. austriacum; Viscum album subsp. creticum* were recorded (Zuber, 2004; Varga et al., 2014).

At present misletoe is widely distributed throughout Europe, Asia and America (Watson 2001). Several studies revealed presence and damage of European misletoe in wide range of host plants. The number of host plants and distribution rate arise due to higher incidence rate of vectors. Approximately 452 species in 96 genera of 44 families were identified as a host of misletoe (Barney et al., 1998). More than 118 hosts mainly *Tilia cordata, Betula pendula, Acer platanoides, Sorbus aucuparia, P. canadensis, P. nigra,* and *Salix alba* were determined in Poland (Stypinski 1997; Kolodziejek et al., 2013). Three trees including *Populus nigra, P. canadensis* and *Malus domestica* were found as preliminary hosts in Belgium (Stanton et. al., 2010). In Croatia, 52 hosts (33 autochthonous and 15 allochthonous species, two cultivars and two hybrids) recorded whereas host number decrease to 25 hosts (21 autochthonous and four allochthonous species) in Slovakia (Idzojtic et al., 2006). The majority of hosts belonged to the Rosaceae, Salicaceae, Aceraceae, Betulaceae, Fagaceae, Juglandaceae, Tiliaceae, Hippocastanaceae, Ulmaceae, Oleaceae, Fabaceae, Moraceae and Viscaceae families.

On the other hand, *Viscum album* was found in the trees of whitethorn, wild pear, pear, almond, apple, plum, apricot, sour cherry, locust, Canada poplar and willow in Turkey (Üstüner et al., 2015). According to Iversen (1944), both summer and winter temperatures restrict the geographic distribution of mistletoe. *V. album* can grow in an average temperature above 15°C lowest temperature and can tolerate without frost damage till –8°C in winter (Skre, 1979). Most parts of Turkey has quiet temperate weather which creates appropriate condition for misletoe ditribution, seed germination and growth. The climate cconditions are quiet well for polinator insects and vector birds as well.

This study was carried out in Northwesteern Marmara Region of Turkey. A survey was carried out in Edirne, Kırklareli and Tekirdağ Provinces. The current host status of misletoe is not well known. The geographic distribution map of misletoe in three provinces, occurance rate, host status and damage level were comprehensively detailed.

Material and method

A four year field survey was carried out in the summer of 2013-2017 to assess the distribution of the European mistletoe. Field observations were carried out in 6 different localities, 16 locations in Edirne, Kırklareli and Tekirdağ Provinces. The survey area covered approximately 9.000 km^2 area. GPS coordinates of survey area are 41°16'19" N - 26°41'05" E; 40°39'52" N - 26°42'00" E; 40°45'44" N - 26°40'05" E; 40° 57' 51.7500" N - 27° 6' 35.4312" E; 40° 38' 58.2612" N - 27° 8' 43.8360" E; 40° 42' 8.5320" N - 27° 15' 51.9228" E; 40° 42' 40.6944" N - 27° 10' 39.7128" E; 40° 57' 21.6864" N - 27° 26' 27.6684" E; 40° 41' 17.5128" N - 26° 10' 13.1268" E. The Region has humid and semi-humid weather with annual average temperature of 8-15°C. Several plant obersvations were performed on walnut, oak, peach,

pear, wild pear, wild cherry, cherry, pomegranate, apple, willow, poplar, oak, spruce, acacia, appricot, plum, lime, pine, plane, ash.
Leaf and flower samples were collected from predominant host trees (Figure 1). Each samples were put into pocket, labeled seperately and brought to laboratory in cold bag.

Figure 1. Flower and leaf of European misletoe *Viscum album*

The herbarium of misletoes were prepared by slow drying of plants in air flow incubator at 30°C. Herbariums of weed were deposited in Tekirdag Viticulture Research Institute. Identification of mistletoes were carried out by examining plant morphology, flower colour, host plant. The identifications were performed in Herbology Laboratory at Gaziosmanpaşa University, Turkey.
Presence or absence of misletoe was recorded in location. The infection degree of host plants was assessed using skala of Spálavský, 2001: 0 – No infection: no mistletoe was found on the tree (0 %), 1 – Rare occurrence: the tree is partially infected (1–10 %), 2 – Scattered occurrence: - 11–40 % of crowns on tree are infected 3 – Abundant occurrence: the tree is infected with higher misletoe volume approximately 41–70 %, 4 – Mass occurrence: The tree is infected strongly; 71–100 % of tree is infected.

Results and discussion

The european misletoe *Viscum album* was identified on wild pear (*Pyrus elaeagrifolia*), pear (*Pyrus communis*), almond *(Amygdalus communis*), plum (*Prunus domestica*) , apricot (*Pyrus armeniaca*), oak (*Quercus ilex*), spruce tree (*Picea* spp.), willow (*Salix* spp.), cherry (*Prunus avium*), acacia (*Robinia pseudoacacia*), whitehorn (*Crataegus monogyna*) and poplar (*Populus nigra*) 11 trees in Edirne Kırklareli and Tekirdağ. The parasitic weed was mostly found in wild pear, pear, almond, plum, apricot, oak, spruce tree, willow, cherry and poplar trees in Edirne, Kırklareli and Tekirdağ provinces (Turkey). No mistletoe shrub was found on coniferous trees, apple, pomegranate, walnut, lime, plane and ash trees during our field experiments. Tepe (1997) and Üstüner et al. (2015) obtained similar results in Central and Southern Anatolia. Tepe (1997) stated higher incidence of weed infestation in *P. communis*, *P. armenica*, *Amygdaus* spp., *P.elaegnifolia*. The infection degree of host plants was given in Table 1.

Table 1. Host plants and infection degree of host plants in Northwestern Marmara Region of Turkey

Host plant	Infection degree	Host plant	Infection degree
Pyrus elaeagrifolia	1	*Quercus ilex*	0
Pyrus communis	2-3-4	*Crataegus monogyna*	2
Amygdalus communis	2	*Populus nigra*	2
Picea spp.	1	*Prunus domestica*	1-2
Salix spp.	1	*Prunus armeniaca*	1-2
Malus domestica	0	*Tillia cordata*	0
Robinia pseudoacacia	1	*Juglans regia*	0
Prunus avium	3-4-5	*Fraxinus*	0
Quercus ilex	0	*Pinus* spp.	0
Crataegus monogyna	2	*Platanus* spp.	0

The incidence of weed was higher in trees in Tekirdağ especially on pear trees. Poplar was another forest tree highly attached with parasitic weed along roads in some locations in Tekirdağ. About 2/4 of poplar trees in İbribey location of Malkara was infected and the infection degree on trees was inside the scale of 11-40 %. There are many reports on frequent distribution of poplar (Pochzai et al., 2011). In addition higher incidence was observed on wild pear, almond and plum trees in Edirne and Kırklareli

According to our observations a correlation between the number of mistletoe shrubs on the host and tree health status was observed in infected fruit trees. There is large difference between the bush number and the host trees. The bush number on trees ranged between 2 to 21 in host plants. The higher bush number and schrub number was counted on pear, wild pear and poplar trees while this number was lowest in spruce tree and willow

Becides dried branches were significant in upper parts of heavy infected trees. The infections in upper branches were found more destructive on examined trees. Significant increase in damage on trees was determined paralel to increase of number of misletoe schrubs. Thus tree death due to higher weed infection was observed on cherry and pear trees. Two cherry trees in Malkara and one pear tree in Şarköy was almost dead due to heavy weed damage (Figure 2).

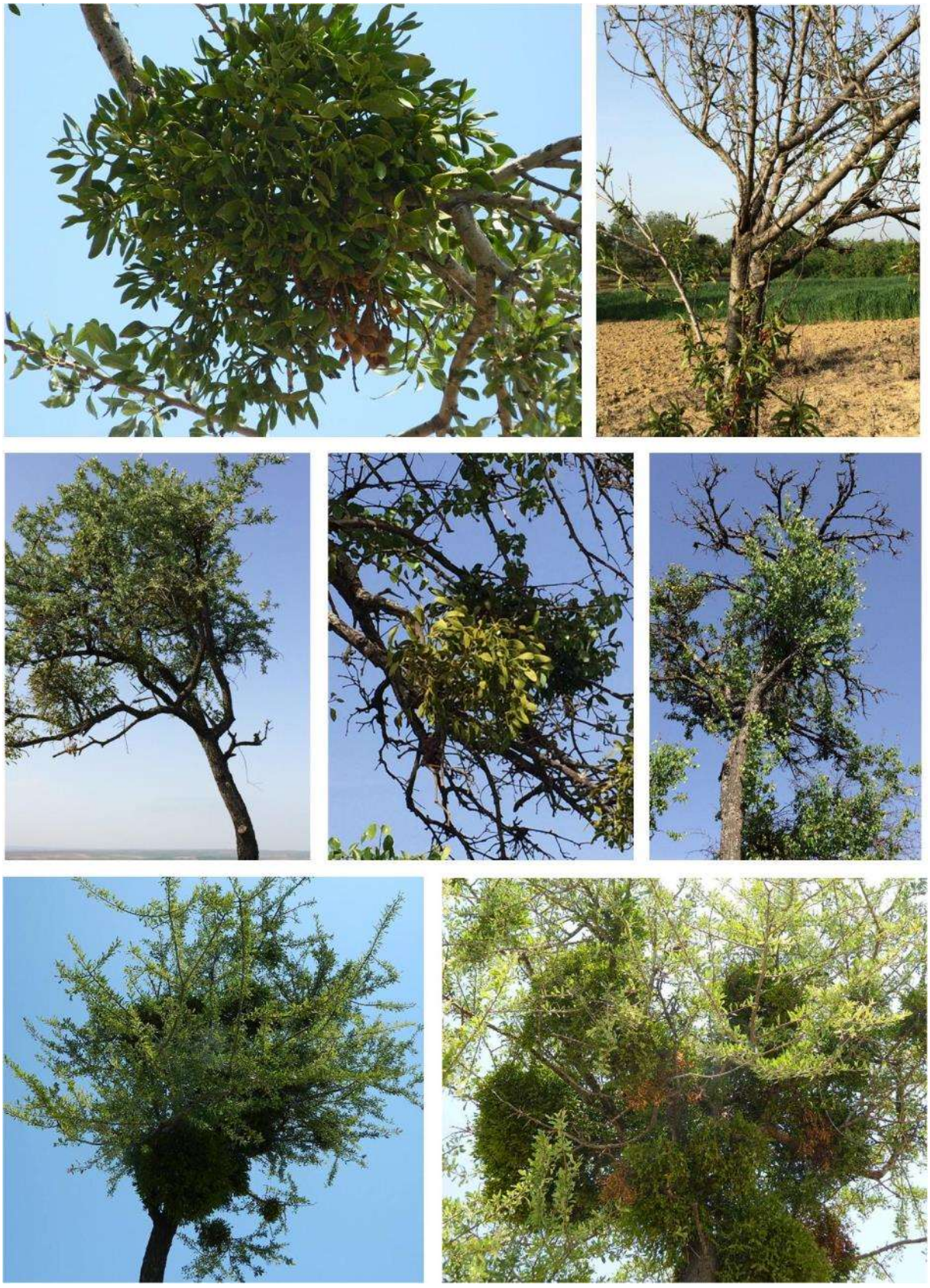

Figure 2. Mistletoe infected plant and weed shrubs on host trees

Conclusion

Viscum album semiparasitic weed was identified in 12 different host plants. Most frequent plants were cherry, pear, wild pear and poplar. The damage level and shrub number varied depending on host plant. The control of mistletoe dispersal is not so easy due to several vector birds in the environment. On the other hand climate conditions are very suitable for seed germination and weed growth. Several precautions like cutting and throwing away shrubs in infected trees can be done to minimize weed distribution in the region

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EFFECTS OF ANTAGONIST BACTERIA AGANIST WALNUTS BACTERIAL BLIGHT DISEASE IN *IN VITRO* CONDITIONS

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Abstract

Walnut (*Juglans* spp.) crops are among the most economically important tree crops worldwide and cultivated to a different extent in all conditions. Bacterial blight (*Xanthomonas arboricola* pv. *juglandis*) is one of the main diseases of walnut that reduce the yield. Bacterial symptoms have been reported on all succulent tissues such as shots, leaves, buds, female flowers, catkins and nuts. The walnut leaves are more susceptible than other plant parts. Points of infection begin as water-soaked areas which develop into brown necrotic areas. Walnut bacterial blight is difficult to control because the bacteria overwinter and survive in dormant buds. So biological control is very important against walnut blight. The aim of this study was to determine whether bacterial antagonist could be used to control *Xanthomonas arboricola* pv. *juglandis.* In total, 69 potentially bacterial antagonists were isolated of healthy walnuts leaves. These samples were screened for their biological control activity against *Xanthomonas arboricola* pv. *juglandis* with dual culture methods in *in vitro* conditions. As a result of these study 48 antagonist bacterial strains obtained effective against *Xanthomonas arboricola* pv. *juglandis* with inhibition zone diameter ranging from 4.6 to 16.6 mm. Interestingly, among 21 effective antagonist bacteria, all of them totally inhibited the growing ability of pathogen *Xanthomonas arboricola* pv. *juglandis*. We clearly observed that these antagonist bacteria have a higher efficacy for *Xanthomonas arboricola* pv. *juglandis* biocontrol.

Keywords: *Xanthomonas arboricola* pv. *juglandis, juglan, biological control, dual culture method*

Intoruction

Walnut blight disease, caused by *Xanthomonas arboricola* pv. *juglandis* (Xaj), is one of the most important and common bacterial disease which effects production of walnut (*Juglans regia* L.) plants. Typical symptoms occur on all succulent tissues including shoots, leaves, rachis, petioles, buds, female flowers, catkins and nuts. Infections begin as translucent water-soaked areas which develop into brown to blackish greasy necrotic areas (Miller and Bollen, 1946). Lesions, which are often surrounded by a yellow-green halo, are initially circular but often expand into angular spots. Bacterial walnut blight caused by *Xanthomonas arboricola* pv. *juglandis* (Xaj) is one of the most serious and economically important diseases of walnut, occurring in all major walnuts-growing areas of the world (CABI-EPPO, 2001). There are no effective control methods, thus bacterial walnut blight may cause considerable yield losses (up to 70%) under favorable conditions for the disease (Gironde et al., 2009; Lang and Evans, 2010; Mulrean and Schroth, 1981). Copper-based compounds have been the only means of control for more than 40 years, but their efficacy is often limited (Gardan et al., 1993). Copper-resistant strains of Xaj have been isolated from walnut orchards where intensive copper spraying was used for disease control (Gardan et al., 1993). The way to reduce the damages caused by this disease has been to use periodical copper treatments although they are not always effective (Brault et al., 1990, Gardan et al., 1993, Radix et al., 1998) Biological control of walnut blight pathogen may be an alternative to chemical control. The aim of this

study was evaluated the effectiveness of potential antagonist bacteria against *Xanthomnas arboricola* pv. *juglandis* in *in vitro.*

Material and method

Survey

In the years 2016 and 2017 between May and July, leaf and fruit spots were observed on walnut cultivars grown in different orchard in Kütahya in Turkey. Leaves showing potential symptoms of walnut bacterial blight were collected. The samples were cut from trees and placed into paper bags and transported in the laboratory. A total of thirty five diseased plant samples were collected.

Isolations from diseased samples

Small pieces of tissue from leading edges from spots were macerated in 3 mL of nutrient broth and allowed to release 10 or 15 min. The suspensions were streak into nutrient agar (NA). Yellows colonies were grown on NA and considered as Xanthomonas.

Pathogenicity test on walnut fruits

Inoculums of each isolate were prepared from 48 hour bacterial cultures grown on NA. The bacteria were inoculated on immature walnut with sterile needle and incubated at 25 ^{0}C for three or five days.

Biochemical tests

Isolates were tested for Gram reaction, levan production, arginine dihyrolase, pectolitic activity on potato, hypersensitive reaction on tobacco and starch hydrolysis (Lelliott et al. 1996, Scaad et al. 2001).

Isolation from healthy samples

Healthy walnut leaves samples were collected and bacteria were isolated on King's medium B. Colonies were observed. After that the isolates were suspended and injected in tobacco leaf tissue between veins after 24 h.

Results and discussion

Pathogenicity and biochemical tests from diseased samples

All strains from walnut produced water suckedgi spot and necrotic area on immature walnut. According to pathogenicity test result, obtained re-isolate on immature walnut. All starins were negative for levan, arginine dihyrolase, pectolitic activity and positive for starch hydrolysis and hypersensitive reaction on tobacco. According to test result twenty strains identified as *Xanthomnas arboricola* pv. *juglandis.*

Isolations from healthy samples

All isolates weren't caused water sucked spots of tobacco leaf tissue between veins after 24 h.
Effects of antagonist bacteria were used against *Xanthomnas arboricola* pv. *juglandis* in petri dishes. Sixty-nine candidate antagonist bacteria were selected according to result of hypersensitive reaction and investigated for their ability to suppress bacterial growth *in vitro* conditions by dual culture test. Totally of forty-eight antagonist bacterial strains obtained effective against *Xanthomnas arboricola* pv. *juglandis* with inhibition zone diameter ranging from 4.6 to 16.6 mm.

All the isolates studied, originating from diseased and healthy walnut leaves. They were identified with biochemical and pathogenicity tests.
Candidate antagonist bacteria isolates were selected with hypersensitive reaction on tobacco leaves. According to results of dual culture test among totally of twenty one the most effective antagonist bacteria, all of them totally inhibited the growing ability of pathogen *Xanthomnas arboricola* pv. *juglandis*. Bacterial isolates could be use a possible bio-control agent against walnut bacterial blight disease.

Conclusion

Further studies should be conducted on identification and determination of mode of actions and *in vivo* activities of the most efficient bacterial isolates against disease agent.

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EFFICACY OF ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA*ISOLATE GOPT-228 AGAINST *GONIOCTENA FORNICATA* (BRUGGEMAN)

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Abstract

The lucerne beetle, *Gonioctena fornicata* (Brüggeman) (Coleopteran: Chrysomelidae)causes significantdamages on alfalfa. Both adults and larvae eat the leaves, flowers, leaf buds, young shoots and leaf stalks of alfalfa. Biological control methods are environmentaly safe and friendly ways of controling this pest. Entomopathogenic fungi are promising biocontrol agents for this pest under field conditions. At the present study, control potential of the entomopathogenic *Beauveria bassiana* isolate (GOPT-228) were tested against adults of *G. fornicata* at five different concentrations ($1x10^3$, $1x10^5$, $1x10^7$, $1x10^8$, and $1x10^9$ spores/ml). The experiment was laid out in completely randomized block design with 5 replications and replicated 2 times. Mortalities were recorded on the 1st, 3rd, 5th, and 7th days of incubation. Dead insects were transferred in humid sterile 90 mm glass petri dishes for 14 days to determine the mycosis rates. Mortality has been observed from the 3rd day and increased with increase in incubation period and inoculum concentration.Mortality rate of 100% was obtained at the end of the 7thin $1x10^9$spores/ml.The results showed that *B. bassiana* with LC50 and LC90 equal to 2×10^5 and 4.8×10^7 spores/ml The results of the study showed that B. bassiana isolate GOPT-228 can be used as a bioinsecticides against *G. fornicata* adults.Further study will be conducted to see performance of the isolate under field conditions.

Key words: *Entomopathogenic, Beauveria bassiana,Gonioctena fornicate, lucerne beetle*

Introduction

Alfalfa, *Medicago sativa* L., is a perennial flowering plant of the pea family (Fabaceae) and cultivated as significant forage crop lasting longer period than any other crop. Alfalfa has a very great yield potential, and it is also one of the most palatable and nutritious forage crops. Due to the high protein and vitamin content, alfalfa is the main component of the diet of dairy cattle (Mustafa et al. 2014). Lucerne beetle *Gonioctena fornicata* (Chrysomelidae) occurs in central and southern Europe, Turkey and most parts of the world, where it causes serious damage to *M. sativa* (Heyden et al. 1906, Blunck 1954). The beetle overwinters in the adult stage in its breeding-places, at a depth of 5-20 cm or more in the soil. Adults become active in spring, when the days become sunny and warm, with the air temperature above 20 ^oC and the soil temperature at least 11 ^oC. Emergence from the soil takes place over 15 days or more, and the beetles move to alfalfa plants and feed on the young leaves (Kovancı, 1982, Yıldırım et al., 1996). In Turkey, both adults and larvae feed on *M. sativa,Vicia sativa* and *Trifolium sp*.. Both the larvae and adults damage alfaalfa, eating the leaves, buds, leaf buds, young shoots and tips of stems (Kasap, 1988, Aslan et al., 2003; Coşkuncu and Gençer, 2006). In May, losses can exceed 60% of green mass and reach 100% of seeds (Grigorov, 1976). This pest gives one generation a year. The natural enemies of this species in the Turkey are insufficiently known.Burgio et al. (1992) examined the efficacy of the biological insecticide Novodor FC, which is a *Bacillus thuringiensis* subsp. t*enebrionis*-based formulation, against young (first- to third-instar) larvae and adults of *G. fornicata*. The young larvae and adults of this pest were fed on lucerne leaves (Medicago sativa) treated with 5 ml Novodor FC per litre

of water. All the larvae were killed within 2 days of feeding on the treated leaves. Adult mortality was significantly increased (by 44%) after 5 days of feeding on treated leaves, and after 7 and 10 days it had reached 52 and 72%, respectively. Atay et al. (2015) suggested entomopathogenic *Beauveria bassiana* isolates can be used for the control of *G. fornicata*. The local *B. bassiana* isolates usedagainst the adults, caused more than 90% mortality after 7 days.Biological control methods are environmentaly safe and friendly ways of controling this pest.

Aim of the present study was to evaluate the susceptibility of the adult stage of *G. fornicata* to infection by the isolate of GOPT-228 *B. bassiana* isolate.

Material and Methods

Fungal Isolate Entomopathogen *Beauveria bassiana* isolate GOPT-228 were selected for the experiment based on its tendency to sporulate at 27 ^{o}C and high virulance. *Beauveria bassiana* culture was obtained from culture collection in Phytopathology laboratory, Department of Plant Protection, Faculty of Agriculture at Gaziosmanpasa University Tokat, Turkey.

Invitro Bioassay. The isolate GOPT-228 was grown on Potato Dexrose Agar (PDA) medium at 27 ^{o}C for 4 weeks to get sporulation. The conidia were harvested under sterile conditions by flooding the plate with 10 ml sterile distilled water containing 0,02% tween 80 then scraping the colony with sterile glass hokey stick. Spore suspension was filtered through four layer steril cheeseclotes to remove mycelia. The concentrations of conidial suspensions were determined, using a Neubauer hemocytometer.The conidial suspensions were stored at 4 ^{o}C for up to 1 week until used in the assays. The viability of conidia was determined by spread-plating 0.1 ml of the suspension on the PDA plates. A sterile microscope cover slip was placed on each plate. Plates were incubated at 27 ^{o}C and examined after 24 h. The percentage of germination was determined by counting 100 spores for each plate, and over 95% of the spores germinated.For the dose-mortality test, *Gonioctena fornicata*adults were collected from infested alfalfa fields in Tokat province, Turkey. Healthy adults were randomly selected and used for bioassays. Conidial suspensions of fungal isolates were prepared as described above, and a series of dilutions was preparedas 1×10^3 , 1×10^5, 1×10^7 , 1×10^8 , and 1×10^9 conidia/ml.Ten adults were dipped inconidia suspension for 5 seconds than transferred into the glass petri dish (90 mm in diameter) containing fresh alfalfa as a food.The experiment was laid out in completely randomized block design with 5 replications and replicated 2 times. The control insects treated withsterile distilled water containing 0.02% tween 80. Mortalities were recorded on the 1st, 3rd, 5th, and 7th days of incubation. Dead insects were transferred in humid sterile 90 mm glass petri dishes for 14 days to determine the mycosis rates.

Statistical analysis

The data was analyzed by analysis of variance (ANOVA) and the means compared by Tukey's multiple comparison test. All statistical analyzes were carried out using the SPSS Release 16 packet program. Lethal concentrations (LC_{50} and LC_{90}) values were calculated by probit analysis (Finney 1978).

Results and Discussion

Limited number of researchers performed studies on effectivness of entomopathogenic *Beauveria bassiana* against *Gonioctena fornicata*(Atay, et al., 2015).However use of entomopathogenic fungi to control the other alfalfa weevils have been studied by various people worldwide (Roberts et al., 1994, Radcliffe and Flanders, 1998, Mustafa et al., 2014, Yücel et al., 2015, Reddy 2016). *B. bassiana* was found to be an effective controlling agent for *G.fornicata.* However, there existed a range in mortality according to the concentration of

conidia incorporated in the tereatment. Previous studies showed an increase in mortality at the higher conidia concentration produced the highest percentage of mortality (Atay, et al., 2015).The results of present study agrees with the results where higer dosage produced the highest percentage of mortality (Atay, et al., 2015).Theisolates tested and type of formulation used may also essential to an understanding of the conciderable differences in the activity of isolates tested in different studies. *B. bassiana* isolate GOPT-228 found to be pathogenic to the*G.fornicata* tested. Mortality produced by the fungus was confirmed on the basis of the steriomycroscop observarion of mycelial growth on the cadavers. In the dose-response tests of *B. bassiana*GOPT-228 adults'mortality has been observed from the 3rd day and increased with increase in incubation period and inoculum concentration. Themortality reached 100% within 7 days after inoculation of the conidial concentration of 1×10^9conidia/ml, and there was a significant difference among concentrations (df= 5, $P < 0.05$) (Table 1). Adults' mortality rate also reached 84% within 7 days after application of the 1×10^8conidia/ml concentration, and there was a significant difference among concentrations (df= 5, $P < 0.05$) (Table 1).The LC_{50} and LC_{90} values of isolate *B. bassiana*GOPT-228 were calculated as 2×10^5 and 4.8×10^7conidia/ ml against adults based on probit analysis, respectively (Table 2).Mycosis was observed on all the treatments accept the control.

Table 1. Mortality of *Gonioctena fornicata* exposed to different doses (1×10^3, 1×10^5, 1×10^7, 1×10^8, and 1×10^9 conidia/ml) of *Beauveria bassiana* isolates GOPT-228 and controls over 7 days after treatment

	Mortality±SEM* (%)			
Doses(conidia/ml)	**1st day****	**3rd day**	**5th day**	**7th day**
1x10^3	0.00±0.00a***	8.00±3.74ab	24.00±4.00ab	32.00±2.00b
1x10^5	4.00±2.44ab	18.00±2.00ab	28.00±4.89b	52.00±4.89c
1x10^7	4.00±2.44ab	20.00±0.00b	30.00±4.47bc	66.00±2.44d
1x10^8	8.00±2.00b	24.00±4.00b	48.00±4.89cd	84.00±4.00e
1x10^9	20.00±0.00c	40.00±0.00c	64.00±4.00d	100.00±0.00f
Control	0.00±0.00a	2.00±2.00a	6.00±2.44a	6.00±2.44a

* SEM: Standard error of the mean;
** DAT: Days after treatment;
*** Means in a column followed by the same letter are not significantly different ($P < 0.05$).

Table 2. Lethal dose (LD50 and LD90) values of adult of Gonioctena fornicata treated Beauveria bassiana isolate GOPT-228.

Isolates	**Slope±SE***	**LD_{50} (95% fiducial limit)**	**LD_{90} (95% fiducial limit)**	**χ^{2**}**
GOPT-228	0.377±0.02	$2x10^5$ ($6x10^4 – 5x10^5$)	$4.8x10^7$($1.4x10^6$-$2.9x10^8$)	12.2

*Slope value(±standart deviation) of dose-mortality respose of *Gonioctena fornicata* to *Beauveria basiana* isolate GOPT-228
**Pearson χ2 value (α=0.05)

Conclusion

In conclusion, the results of the study showed that *B. bassiana* isolate GOPT-228 can be used as a bioinsecticides against *G. fornicata* adults. Further studies are ongoing for determination of the efficacy of this isolate under field conditions.

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INVESTIGATION OF TOMATO YELLOW LEAF CURL VIRUS (TYLCV) STRAINS IN CUKUROVA REGION

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Abstract

Tomato yellow leaf curl virus (TYLCV) is a viral agent causing significant economic losses worldwide on tomato (*Lycopersicum esculentum*) cultivation in greenhouses. The symptoms caused by TYLCV on infected tomato plants are stunting, small chlorotic leaves with upward curling. Many different strains of TYLCV have been reported from various parts of the World. There are many difficulties in the symptom based differentiation of this virus. This study was carried in 2017 to identify and characterize TYLCV strains in Cukurova region, south-eastern Mediterranean coast of Turkey. With this aim, a total of 83 tomato leaf samples were collected and tested on TYLCV presence by PCR. Two different strains of TYLCV (TYLCV-IL, TYLCV-Mild) were identified in Cukurova region with strain specific primers. Samples that gave no PCR products with TYLCV-IL and TYLCV-Mild specific primers were tested with set of primers amplifying the coat protein (CP) coding gene. Phylogenetic analysis of selected TYLCV-IL and TYLCV-Mild isolates proved that they were related to other isolates from several countries. Although, phylogenetic analysis of CP of the two isolates from Mersin province had similarities with most of the TYLCV strains and isolates from other countries. These results showed that TYLCV had many viral variants being a threat for tomato cultivation in greenhouses in the Mediterranean region of Turkey and should be further investigated in detail.

Keywords: *TYLCV, PCR, Characterization, Tomato, Turkey*

Introduction

Tomato is one of the most consumed vegetable which can be cultivated in greenhouses or field all around the world and also in Turkey. It's the seventh most cultivated plant after maize, rice, wheat, potato, soybean and cassava (Bergougnoux, 2014). There are many known viruses effecting the quantity and quality of tomato cultivation. *Tomato yellow leaf curl virus* (TYLCV) belongs to *Begomovirus* genus in *Geminiviridae* family with single stranded DNA (ssDNA) genome structure. TYLCV infected tomato plants are declined with small chlorotic and upward curled leaves from margins (Hull, 2009). This DNA virus can cause almost 100% yield losses with early infection, especially in autumn sessions' greenhouse tomato cultivation (Czosnek, 2007). The first report of TYLCV presence in Cukurova region was published in 1978 (Yilmaz, 1978). TYLCV-Israel and TYLCV-Mld strains are detected in the most of the countries as major viral agents for tomato cultivation (Lefeuvre *et al.*, 2010). The aim of our study was to investigate the presence and diversity of TYLCV's common strains (TYLCV-IL and TYLCV-Mild) by using PCR based approach and phylogenetic analysis.

Material and Methods

Material

The survey was conducted in the autumn 2017 in tomato cultivation areas at Adana and Mersin provinces of Cukurova region of Turkey. The young leaf samples were collected from 83 tomato plants with the suspicion of TYLCV infection.

Methods

Infected plant leaves with TYLCV symptoms (upward curled and chlorotic leaves, stunted plants) were sampled, put in plastic bags and brought to laboratory under optimum conditions. The leaf samples were stored +4°C prior to DNA extraction. Four primer sets for TYLCV-IL TYv2337-TYc138 (Anfoka *et al.,* 2009) with TYLCV1840F-IL2642 (Lefeuvre *et al*., 2007); for TYLCV-Mild: TYLCV1840F-Mild2354R (Lefeuvre *et al*., 2007); for TYLCV coat protein : V1 (Kim *et al*., 2011) were used for PCR based detection PCR products were analysed in agarose gel electrophoresis. PCR products of selected isolates were sequenced by Erdem Lab, Turkey.The obtained sequences of selected isolates were analysed with MEGA7 programme (Kumar, 2016). The aligned sequences were compared after BLAST analysis and phylogenetic trees were reconstructed (Neighbour-joining method– Bootstrap: 1000 replicates).

Results and Discussion

The characteristic symptoms of TYLCV which cause declined tomato plants with upward curled chlorotic leaves were observed during our survey and described symptoms were also reported by numerous authors (Antignus and Cohen, 1994; Rochester *et al.,* 1994; Crespi *et al.,* 1995; Bananej *et al.,* 2004; Köklü *et al.,* 2006; Boukhatem *et al.,* 2008; Pakniat *et al.,* 2010; Van Brunschot *et al.,* 2010; Al-Ali *et al.,* 2016) as typical symptoms for TYLCV infected tomato plants..

DNA isolation of 83 tomato leave samples with suspicion of TYLCV was done and all the samples was tested to investigate the presence of TYLCV strains by molecular methods with using primers sets given in Table 1.

Table 1. Primer sets to detect TYLCV

Primer Sets	**Primer Sequence (5'-3')**	**PCR products**	**Ref.**	**Diagnosis**
TYv2337 TYc138	ACGTAGGTCTTGACATCTGTTGAGCTC AAGTGGGTCCCACATATTGCAAGAC	634 bp	Anfoka *et al.,* 2009	TYLCV-IL
V1 (CP)	ATGTCGAAGCGWCCA TTAATTTKRTAYTGAATCATAGAA	777 bp	Kim *et al.,* 2011	Coat Protein
TYLCV-1840F Mild-2354R	GGTCTACGTCATCAATGAC AGGGAGCTAAATCCAGTT	514 bp	Lefeuvre *et al.,* 2007	TYLCV-Mild
TYLCV-1840F IL-2642R	GGTCTACGTCATCAATGAC ACACCGATTCATTTCAAC	802 bp	Lefeuvre *et al.,* 2007	TYLCV-IL

One isolate from Adana (Sarıçam) were positive for TYLCV-Mild. In addition to this, two isolates from Mersin were mixed infected with both strains. To be able to confirm the presence of TYLCV-IL, a different primer set (TYv2337/TYc138) was used and 15 positive isolates were detected from Mersin province. Primer set for V1 (coat protein coding region) was used for isolates which were negative for both strains, despite the fact that they were showing similar symptoms. PCR products of a fragment from a TYLCV's coat protein genome region were observed in five isolates from Mersin.

Seven TYLCV-IL (634 bp), one TYLCV-Mild (514 bp), and two of TYLCV's V1 coat protein region (777 bp) isolates were sequenced. Obtained sequences were aligned with the sequences of isolates from other countries. Reconstructed phylogenetic trees are given in figures 1-3.

Table 1 Example of Coding TYLCV isolates in Cukurova region

Name	Viral agent	License Plate	County	Isolate No.
TYLCV-33:E:1	TYLCV	33(Mersin)	E(Erdemli)	1
TYLCV-01:S:1	TYLCV	01(Adana)	S(Sarıcam)	1

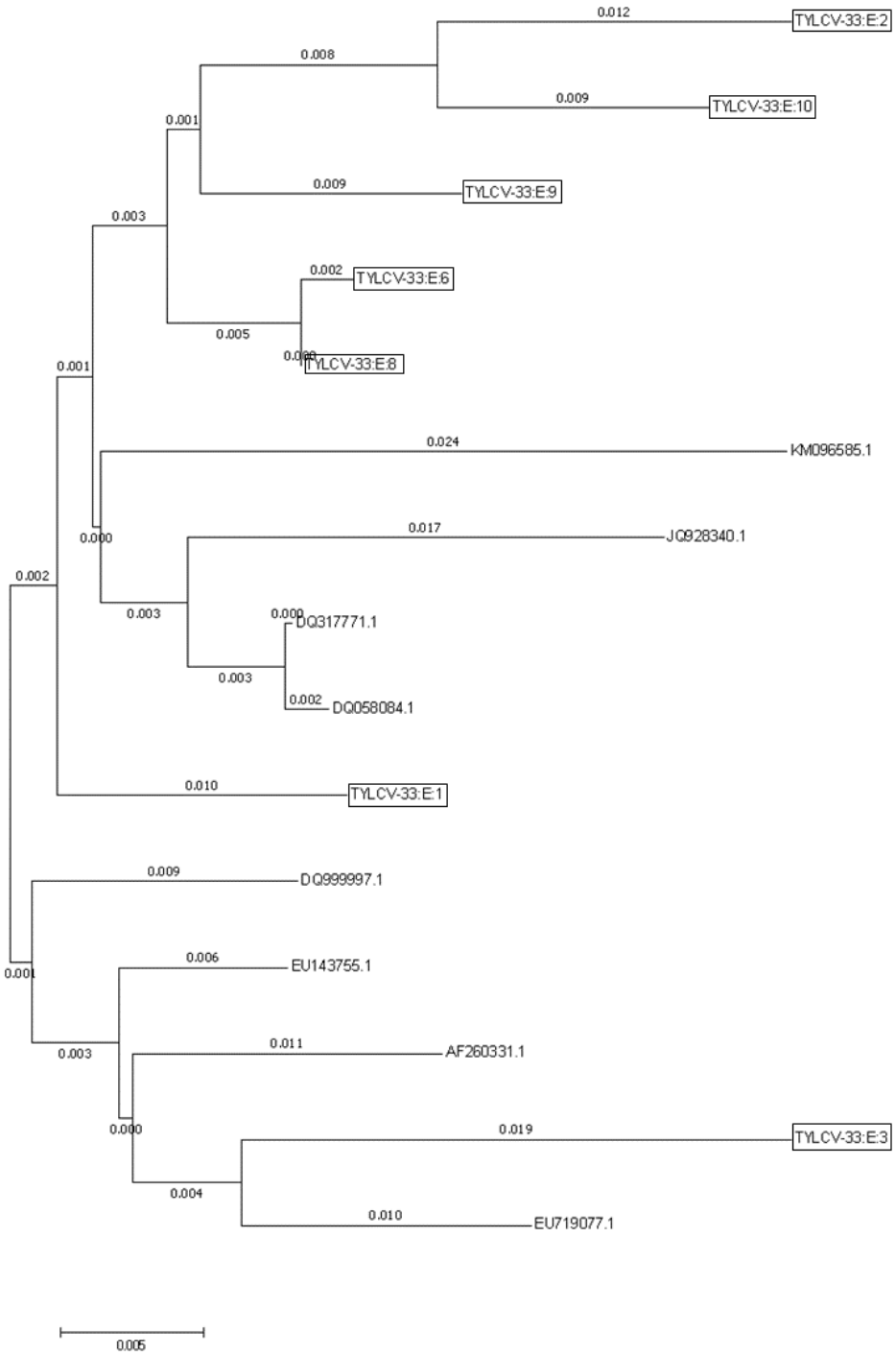

Figure 3. Phylogenetic tree reconstructed from the nucleotide sequences of the TYLCV isolates amplified with TYv2337/TYc138 primers

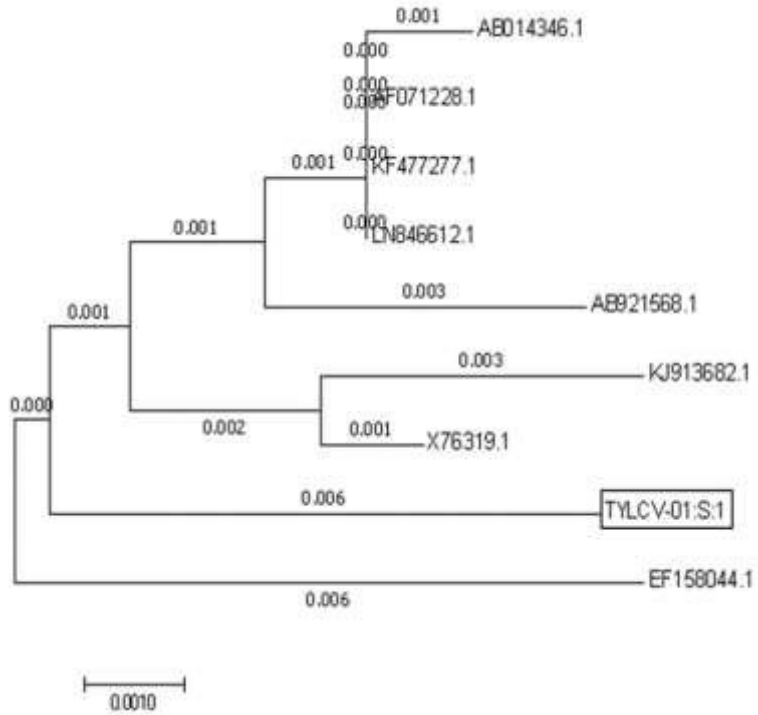

Figure 4. Phylogenetic tree reconstructed from the nucleotide sequences of the TYLCV isolates amplified with TYLCV1840F-Mild2354R primers

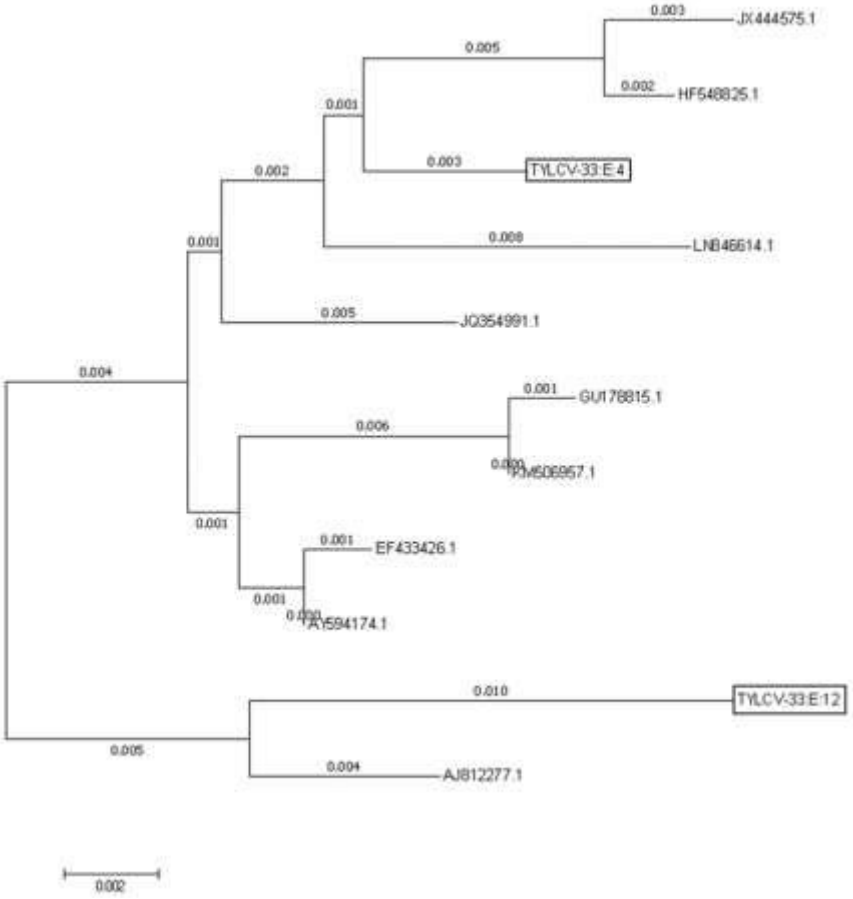

Figure 5. Phylogenetic tree reconstructed from the nucleotide sequences of the TYLCV isolates amplified with V1 primers

Our study is the first report regarding the molecular detection of the TYLCV strains in Cukurova region, south east region of Mediterranean. The presence of TYLCV was first reported in 1978 (Yılmaz., 1978). Köklü *et al.* (2006) also reported the presence of TYLCV in this region in one sample using molecular techniques. In this study, we analyzed 83 samples from different parts of the Cukurova region. According to the results, TYLCV-Mild was detected in 3,6% of the samples, and TYLCV-IL in 18% of the samples. in 6% of the samples we were unable to identify the TYLCV strain. In the rest of the 60 samples (72,4%) TYLCV was not detected by PCR method.

Conclusion

Our results showed that TYLCV is significantly present in analysed samples from the Cukurova region Further studies should be conducted including a greater number of samples throughout the Cukurova region. A combination of biological and molecular tests may give appropriate and precise results on the TYLCV presence. The design of a new strain-specific primers for TYLCV detection may also be a proper way to improve diagnostics.

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THE IMPACT OF COMMERCIAL PREPARATION *BACILLUS THRUGIENSIS VAR. KURSTAKI* ON THE *THAUMETOPOEA PITYOCAMPA* (DEN. & SCHIFF.) (LEP.: THAUMETOPOEIDAE)

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Abstract

The pine processionary moth (*Thaumetopoea pityocampa* (Den. & Schiff) (Lepidoptera: Thaumetopoeidae), which is a common pest spread all over the world, causing economic loss, as well as health problems in humans and animals. Since mechanical and chemical control methods against the pine processionary moth have not achieved permanent or prolonged success and due to the negative effects of chemical control on resistance and environment, the importance of biological control measures has been emphasized in recent years. *Bacillus* spp is an important entomopathogen, especially in the biological control against Lepidoptera. In this study, 3 different doses (0.75, 1.5 and 3 g/1 lt water) of a commercial preparation containing *B .thrugiensis var κurstaki* obtained from Kazakhstan Research Institute were applied in 4 replications to the larvae of pine processionary moth obtained from natural populations. 1 kg plastic containers were used in the study and 10 larvae were placed in each container. The study was conducted at 22 ± 2 o C and 70% humidity. The study was observed for 5 days and deaths were noted. At the end of the 5th day of the study, the mortality rate was 60% for dose 0.75 g/1 lt water, 75% for dose 1.5g/1lt water and 90% for dose 3g/1lt water. As a result, it has been determined that the *B. thrugiensis var. κurstaki* preparation can be used at a dose of 3g/1lt water in the control of the pine processionary moth.

Keywords: *Bacillus thrugiensis var. κurstaki*, commercial preparation, pine processionary moth, *Thaumetopoea pityocampa.*

Introduction

One of the factors that threatens sustainability of forests in Turkey is pests. Among these pests, pine processionary is important due to the harm it does. Pine processionary (*Thaumetopoeo pityocampa* Schiff) is a moth that gives damage in pine forests particularly in Mediterranean, Egean, and Marmara regions. *Thaumetopoea pityocampa* Schiff is a pest that lives by feeding on acicular leaves of Calabrian pine (*Pinus brutia*), black pine (*Pinus nigra*), aleppo pine (*Pinus halepensis*), and scotch pine (*Pinus sylvestris*) in Turkey (Özkazanç, 2002). This pest feeds on pine leaves and spins a web on these trees. Pine processionary is reported to cause a loss around 60% in our forests (Anonymous 1995; Kanat *et al* 2002, 2004). Also, trees that are weakened due to this pest feeding on them become vulnerable for secondary pests such as scoltyds. In addition to these damages, the hair on larvae cause respiratory distress, conjunctivitis and allergic reactions such as asthma in humans and animals (Ekerbiçer, 2002; Kanat,2002). As fighting against pine processionaries is difficult, chemical and mechanical treatments are frequently used. Due to negative effects of chemical treatments on the environment and human health, biological treatments are becoming more common. In biological fighting against pine processionaries, there are studies on the use of parasites and predators (Bescelli 1969, Torun, 1975, Eroğlu & Oğurlu 1993, Avcı, 2000, Kanat & Mol 2008) and the use of microbial preparates (Bescelli 1969, Özkazanç 1986, Orhankaya & Can 2004). Entomopathogens play important roles in natural regulation of various pests and harmful populations. Many types of entomopathogens (fungus, bacteria,

nematod) are produced commercially and are being used as a biological treatment in fighting againsts pests around the world.

Bacillus thuringiensis (Bt) is a gram positive soil bacteria that has an economical value, and has a toxic effect on various larvaes of pests. Bt has been used as a bioinsecticide successfully in organic farming. Bt products maintain control by causing damage in the midgut of larvaes during larva state of various species of pests or by caousing septisemia (Raymond, 2010). Commercial formulations of parasporal crystal proteins produced during sporulation are done and used for biological control (Tamez-Guerra,2004). Bt strains are the most commonly studies entomopathogen due to being environmentally safe and species-specific (Han,2004). Insecticidal crystal proteins (ICP) show toxicity against several agricultural and forest pests as well as disease vectors. Different strains of Bt have limited or comprehensive toxic effect on pests from the orders of Lepidoptera, Diptera and Coleoptera. Studies on the effect mechanism of Cry toxins are mostly focused on Lepidoptera pests (Bravo, 2007). While these products are commonly used as biological treatment around the world, its use in our country is limited. However, recently several researchers in our country have been conducting comprehensive research on Bt products (Kurt, A., 2005; Bozlağan, İ.,2007;Tatar D.2008; Sezen K.2008; Azizoğlu U 2009;Öztürk F2008; Bozloğan İ 2010;Yılmaz S2011, Yılmaz S.2011, Azizoğlu U2011, Tekin S 2012).

This study aims to examine the effects of a commercial preparate (Ak kolebek) with B. thrugiensis var κurstaki obtained from Kazakhstan Research Institute on larvae of pine processionary obtained from pine trees on Ondokuz Mayis University campus.

Material and Methods

The larvae of *Thaumetpoae pitycompae* used in this study were obtained from the cultured larvae sacs that were obtained from pine trees on Ondokuz Mayis University campus. 3 different doses of Ak kobelek (*B. thrugiensis var κurstaki*) preparate (It was obtain from Kazakhstan Research Institute) were used (0,75 g/l; 1,5 g/l ve 3 g/l). Blotting paper were placed in plastic containers with the dimensions of 10x10x10cm and 10 larvae were placed in each container and pine needles were placed as food. The study was conducted in a labratory with 4 repetitions under the conditions of 22±2 oC and 70% humidity. Larvae used in this study were obtained from one colony and they all were the same age. 10 larvae were placed in each container and 5 ml of different doses of the biological preparate was sprayed to the container. This treatment was monitored for 5 days and the dead larvae were noted every day. According to the initial pest number, the daily dead pest number was ratioed to determine the percentage of death. Obtained data were analysed using SPSS 21.0. LD50 and LD90 values were calculated by completing a probit analysis. Also, a variance analysis was completed on the death ratio for each different doses in the same day and the means were compared by completing a Duncan multiple range test ($p<0.05$).

Results and Discussion

The preparate with *Bacillus thrugiensis* was effective on pine processionaries, however there were differences between the doses. As the dose increased after the treatment, the ratio of death due to the dose increased. Particularly after the 3. day, deaths became more significant. At the end of the 5th day on Thaumetpoae pitycompae larvae, the death ratio was 60% with the dose of 0.75 g/l , 75% with the dose of 1.5 g/l, and 90% with the dose of 3 g/l (Figure 1). The performance of a single application of *B. thrugiensis var κurstaki* against T. pityocampa that has been observed in the field (20–85% mortality) is related to a great variability in both the application dose and some population parameters of *T. pityocampa* (Battisti et al., 1998). Isolate *Bacillus thuringiensis* subsp. morrisoni had a promising insecticidal effect on third instar larvae of *T. pityocampa* (up to 70% mortality within 10 days) (Ince et al., 2008). The

bioinsecticides included in *Bacillus thuringiensis subsp. kurstaki* (Btk). The vulnerable performance of a single application has been observed in the field trial of these bioinsecticides with mortality rates ranging from 97 to 99% in Turkey's pine forests (Cebeci et al., 2010). *Thaumetopoea solitaria* of larval high mortality (78%-100%) was attained in the first week after the treatment especially for the first 3 larval stages with the application of the highest 3 concentrations of *Bacillus thuringiensis var. kurstaki* (104, 105, and 106 μg l–1) (Er et al., 2007).

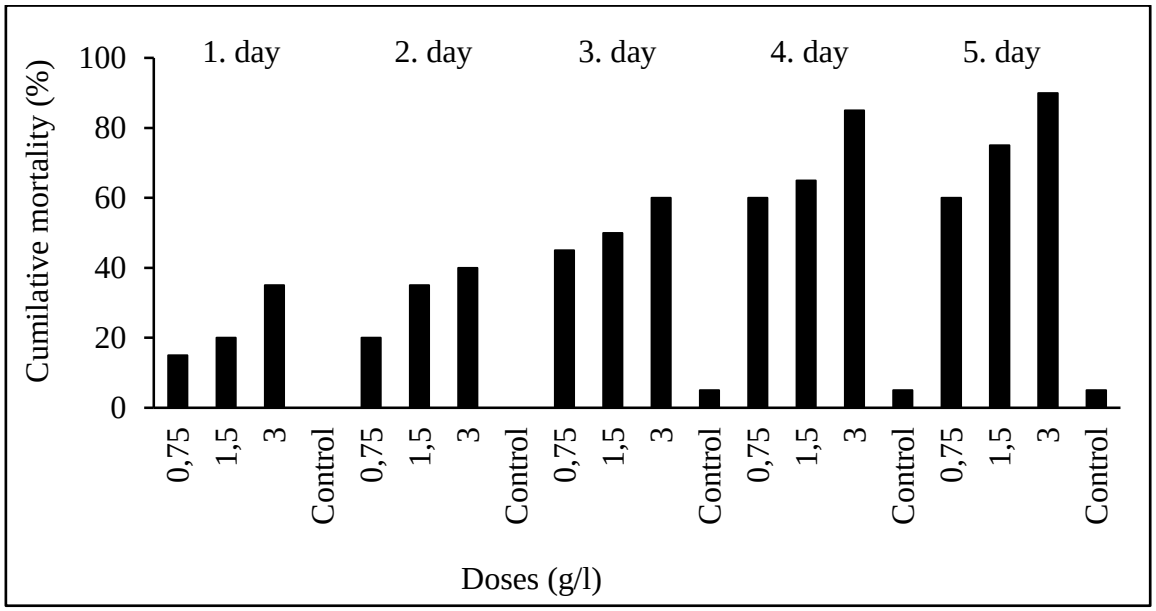

Figure 1. Distribution of death rates of commercial bacteria preparate in different doses by day.

In the study conducted with biopreparate with *Bacillus thrugiensis*, only the LD50 and LD90 values for the 5th day were determined. LD50 values was 0.65 while LD90 was 2.47 (Table 1).

Bacillus thrugiensis is used as an entomopathogen in fighting against several bugs that are harmful for plants. It is used particularly for Lepidoptera larvae (Nikolaos,1998). There are commercial preparates such as Bio-T plus, Foray 76B that includes B. Thrugiensis bacteria that are licensed for pine processionary larvae in Turkey . The Ak Kobelek preparate used in this study is licensed for certain lepidoptera larvae in Kazakhstan (Cotton bollworm, Cabbage butterfly, American white butterfly, Codling moth, Gypsy moth, Grapevine moth).

Table 1. The after treatment of pine processionary with the commercial bacterial preparate.

Days	**Doses (g/l)**	**Death rates (%)**	**LD_{50}**	**LD_{90}**
1.day	0,75	15,00±5,00ab*	4,98	9,80
	1,5	20,00±12,58a		
	3	35,00±8,16ab		
	Control	0,00±0,00b		
2.day	0,75	20,00±8,16a	6,14	14,68
	1,50	35,00±17,08a		
	3	40,00±16,33 a		
	Control	0,00±0,00a		

3.day	0,75	45,00±22,17ab		
	1,50	50,00±17,32ab		
	3	60,00±14,14 a	1,96	4,86
	Control	5,00±5,00 b		
4.day	0,75	60,00±16,33a		
	1,5	65,00±9,57 a		
	3	85,00±9,57 a	1,12	2,92
	Control	5,00± 5,00 b		
5.day	0,75	60,00±15,00a		
	1,5	75,00±5,00 a		
	3	90,00±5,77 a	**0,65**	**2,47**
	Control	5,00± 5,00 b		

*The doses within same day are compared statistically. Same letters in the same line representative of each day show that there is no a statistically significant difference between the doses.

Conclusion

With this study, it was found that the preparate used was also effective on pine processionary larvae. As this study was conducted under labratory conditions, it would be beneficial to conduct the same study in the field. Also, it is important that a comparative study on the effectiveness of commercial preparates used in control against pine processionary in Turkey.

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THE EFFECT OF SYNERESIS ON PHYSICAL PROPERTIES AND MINERAL CONTENTS OF YOGHURTS

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Abstract

Syneresis values of yoghurt samples ranged from 50.00% (with carrageenan 0.25%) to 67.44% (with carrageenan 0.10%). Syneresis level of yoghurt samples with orange were ranged from 60.16% to 64.64%. Viscosity values of samples changed depending on additives. The mineral contents of yoghurt samples were determined by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). Ca contents of yoghurt samples changed from 4155.0 mg/kg (with orange 0.15%) to 6464.64 mg/kg (with carrageenan 0.15 %). K contents of yoghurt samples ranged from 6383.81 mg/kg (with orange with 0.15%) to 10626.32 mg/kg (with carrageenan 0.15%). Generally, mineral contents of liquid phase decreased according to the solid phase of yoghurt samples. While Ca contents of liquid phase change between 274.5 mg/kg (with lemon 0.10%) and 1510.95 mg/kg (with lemon 0.25%), K contents ranged from 406.56 mg/kg (with lemon 0.10%) to 2025.40 mg/kg (with carrageenan 0.25%).

Key words: *pectin, pectin sources, yoghurt production, syneresis, minerals, ICP-AES*

Introduction

Milk has great importance in human nutrition. It is processed to various products with the aim of facilitating its consumption and improving its storability (Çopur *et al.*, 1993). Different stabilizers are used in order to prevent the separation, which is a quality criterion and to give the yoghurt the desired consistency (Hall, 1975; Basset, 1983). Hydrocolloids defined as stabilizers are used to increase consistency, to reduce serum separation and to impartstability tothe lactic acid gel in fermented dairy products like yogurt (Gönç and Gahun, 1980). Agar is a stabilizer other substances used in the production of yogurt (Saldamlı, 1985). In addition, pectin used as stabilizer reduces the amount of cholesterolin the blood at the same time. Pectin that in the form of natural colloidsand methoxylated galacturonic acid in its structure is found in the form of protopectin in plant roots, leaves and fruits (Gönç and Gahun,1980). Pectin is used in yoghurt to preserve the acidity of the products and to increase their shelf life. The primary use of pectin in the food production industry is as a gelling agent (Basset, 1983; Tamime and Robinson, 1985; Tayar *et al.*, 1995). The aim of this study was to inhibit syneresis and to keep minerals in solid phase of yoghurt in yoghurt manufacture by using pectic, carrageenan,and pectin sources like orange, lemon and grapefruit.

Material and methods

Material

In this experiment, cow milk with 9% dry matter was used. Milk was pasteurized in a binary at 85 ^{o}C for 30 min. After heating, it was cooled to the 45^{o}C. Yoghurt culture at the 2% level and stabilizers (orange, lemon, grapefruit, carrageenan, pectin) at 0, 05, 0.10, 0.15, 0.20, and 0.25% concentrations were added in to cooled milk, and then incubated till 4.6 pH. After incubation, yoghurt samples were kept for 1 h in a refrigerator (+4^{o}C) (Tekinşen, 1976).

Determination of Syneresis Level

25 gr of yogurt samples were put into centrifuge tubes (50 mL volume). The samples were centrifuged at 4000 rpm for 15 minutes. Liquid phase of each yogurt sample was transfered into different flask, and weight of liquid phase was measured. Syneresis of yogurts (%) were determined.

Determination of mineral contents

About 0.5 g solid and liquid phase yoghurt samples were digested by using 5ml of 65% HNO_3 and 2 ml of 35% H_2O_2 in a closed microwave system (Cem-MARS Xpress). The volumes of the digested samples were completed to 20 ml with ultra-deionized water and mineral concentrations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-AES; (Varian-Vista, Australia) (Skujins,1998). Working conditions of ICP-AES were given in Table 1.

Statistical analyses

All analyses were carried out three times and the results are mean±standard deviation (MSTAT C) of yoghurt samples (Püskülcü and İkiz, 1989).

Results and discussion

Syneresis level of yogurt samples with orange were ranged from 60.16% to 64.64%. The syneresis level of yoghurt samples with0.1% and 0.15% orange decreased. The yoghurts with added 0.05% orange and 0.05% grapefruit also showed nearly same tendency to syneresis than plain yoghurt whilst increased amount of grapefruit increased syneresis level. The addition of different amounts of pectin caused more syneresis when compared with plain yoghurt. However, change of amount didn't affect syneresis level. Minimum syneresis level was obtained from 0.05% lemon added yogurt whereas syneresis level increased due to raising of amount of lemon. In 0.05% and 0.1% carrageenan added yoghurts more syneresis was observed than plain yoghurt. Scarcely, the increase of carrageenan produced a decrease in syneresis. Maximum syneresis was found in yogurt which contain 0.1% carrageenan. The most appropriate additives and their amounts were found as orange (0.05%, 0.1%, 0.15%), grape fruit (0.05%), lemon (0.05%, 0.2%, 0.25%) (Table 2).

Syneresis and titratable acidity values of yoghurt samples are presented in Table 2. Syneresis values of yoghurt samples ranged from 50.00% (with carrageenan 0.25%) to 67.44% (with carrageenan 0.10%). Generally, syneresis level of yoghurt contained orange skin at the different contentrations were found partly low compared with other samples. In addition, acidity values of most of yoghurt samples were found the same (1%). But acidity values of a few yoghurt samples were found between 1.64% (with carrageenan 0.10%) to 1.76% (with lemon 0.15%). Generallay, syneresis, had not affected to the acidity levels of yoghurt samples. Acidity did not more change depending on syneresis and concentrations.

Viscosity results of yogurt samples are given in Table 3. Viscosity values of samples changed depending on additives. In addition, viscosity values decreaset at increasing rpm levels. Generally, viscosity values of yoghurt with pectin and carrageenan were found higher than those of other samples. These differences can be probably due to using of different fruit materials. It was established the lowest viscosity values in 100 rpm in yoghurt samples. But, viscosity values of yoghurt contained pectin and carrageenan were found partly higher than those of yoghurt contained orange, grapefruit and lemon.

Mineral contents of solid phase of yogurt samples are given in Table 4. Ca, K, Mg, Na, P and S of solid phase of yoghurt samples were found as major elements. Ca contents of yoghurt samples changed from 4155.0 mg/kg (with orange 0.15%) to 6464.64 mg/kg (with carrageenan 0.15 %). K contents of samples ranged from 6383.81 mg/kg (with orange with 0.15%) to 10626.32 mg/kg (with carrageenan 0.15%). Mg contents were found between 603.20 mg/kg (with orange 0.15%) and 911.96 mg/kg (with carageenan 0.15%). While Na

contents of yoghurt samples change between 1377.99 mg/kg (with orange 0.15%) and 2171.10 mg/kg (with carrageenan 0.15%), P contents ranged from 4808.46 mg/kg (with orange 0.15%) to 6844.14 mg/kg (with carrageenan 0.15 %). In addition S content of samples changed between 2953.57 mg/kg (with orange 0.05%) and 5088.97 mg/kg (with carrageen 0.15%). B, Cu, Fe and Zn content of yoghurt samples ranged from 4.02 mg/kg (with pectın 0.05%) to 6.79 mg/kg (with pectin 0.15%), 1.48 mg/kg (with lemon with 0.20%) to 3.51 mg/kg (with orange 0.10%), 15.26 mg/kg (with lemon 0.20%) to 24.76 mg/kg (with carrageenan 0.05%) and 14.61 mg/kg (with orange 0.15%) to 23.72 mg/kg (with petin 0.25%)respectively. Mineral contents of yoghurt samples contained several ingredients were found partly high than that of control group. Generally, mineral contents of yoghurts contained pectin and carrageenan were found higher compared with other ingredients.

Mineral contents of liquid phase of yoghurt samples are summarized in Table 5. Generally, mineral contents of liquid phase decreased according to solid phase of yoghurt samples. While Ca contents of liquid phase change between 274.5 mg/kg (with lemon 0.10%) and 1510.95 mg/kg (with lemon 0.25%), K contents ranged from 406.56 mg/kg (with lemon 0.10%) to 2025.40 mg/kg (with carrageenan 0.25%). Na contents were found between 136.68 mg/kg (with lemon 0.10%) to 593.82 mg/kg (with pectin 0.25%).P contents of liquid phase ranged from 178.08 mg/kg (with lemon 0.10%) to 670.15 mg/kg (with orange 0.10%). S contents of samples were found between 43.05 mg/kg (with lemon 0.10%) and 450.27 mg/kg (with pectin 0.25%). Mg contents of liquid phase ranged from 15.12 mg/kg (with lemon 0.10%) to 115.64 mg/kg (with lemon 0.20%). B, Cu, Fe and Zn contents of liquid phase of yoghurt samples were found between 1.53 mg/kg (with lemon 0.10% and pectin 0.25%) and 3.10 mg/kg (with orange 0.05%), 0.25 mg/kg (with grapefruit 0.15%) and 0.41 mg/kg (with pectin 0.10%), 2.37 mg/kg (with grapefruit 0.25%) and 4.09 mg/kg (with lemon 0.20%) and 0.96 mg/kg (with lemon 0.10%) and 3.89 mg/kg (with orange 0.10%), respectively. Heavy metal contents of liquid phase of yoghurt samples considerably decreased compared with solid phase of yoghurt. Different stabilizers are used in order to prevent the separation which is a quality criterion and to give yoghurt the desired consistency (Çopur *et al.*, 1993). Çopur *et al.*, (1993) reported that titratable acidity values of yoghurt contained pectin at the different concentration (0.01 to 0.50%) were determined between 1.05% and 1.18%. According to the sensorial evaluations, the samples which contain gelatin and gelatin-pectin more preferred. As a result, it has been thought that gelatin and gelatin-pectin can be used as the level of 0.6% and 0.6 +0.05% respectively in yoghurt manufacture (Atasever, 2004). Christensen (1991) reported that the using of pectin with other additive had affected on the structure of yoghurt manufacture.As a result, the use of available stabilizersmay bemore appropriate to prevent to the separation of serum phase in yoghurt production. Thus, minerals penetrated into the serum phase will have been trapped in the solid phase.

Table 1 Working conditions of ICP-AES

Working conditions of ICP-AES:	
Instrument	:ICP-AES (Varian-Vista)
RF Power	: 0.7-1.5 kw (1.2-1.3 kw for Axial)
Plasma gas flow rate (Ar)	: 10.5-15 L/min. (radial) 15 " (Axial)
Auxilary gas flow rate (Ar)	:1.5 "
Viewing height	: 5-12 mm
Copy and reading time	:1-5 s (max.60 s)
Copy time	: 3 s (max. 100 s)

Table 2. Syneresis and titratable acidity results of yogurt samples

Additive	**Concentration (%)**	**Syneresis (%)**	**Titratable Acidity (% Lactic acid)**
Orange	0.05	62.00±1.26*	1.00±0.04
	0.10	60.00±2.35	1.00±0.01
	0.15	60.00±1.87	1.00±0.05
	0.20	64.28±1.67	1.00±0.09
	0.25	64.64±1.29	1.00±0.07
Grapefruit	0.05	62.32±2.31	1.72±0.11
	0.10	63.00±2.09	1.00±0.09
	0.15	66.64±1.79	1.69±0.13
	0.20	66.92±1.63	1.00±0.03
	0.25	66.00±1.71	1.00±0.01
Lemon	0.05	57.52±1.49	1.00±0.07
	0.10	63.00±2.53	1.00±0.03
	0.15	63.00±2.81	1.76±0.21
	0.20	62.00±2.74	1.00±0.09
	0.25	61.84±1.68	1.00±0.07
Pectin	0.05	66.88±1.39	1.72±0.11
	0.10	67.00±1.72	1.00±0.03
	0.15	65.00±2.49	1.00±0.01
	0.20	66.00±2.85	1.67±0.17
	0.25	67.00±2.93	1.00±0.09
Carrageenan	0.05	65.20±2.57	1.00±0.03
	0.10	67.44±1.86	1.64±0.09
	0.15	61.00±1.37	1.66±0.21
	0.25	50.00±1.29	1.00±0.13
Control	-	61.00±2.42	1.00±0.07

*mean ±standard deviation

Table 3. Viscosity results of yogurt samples

Additive	Viscosity (cP)							
	0.5 rpm	**1 rpm**	**2.5 rpm**	**5 rpm**	**10 rpm**	**20 rpm**	**50 rpm**	**100 rpm**
Orange	-	81700	25400	12100	7300	4100	1600	500
	-	143800	48000	25100	11300	4700	1500	600
	-	112100	37700	18800	11600	5500	2300	1100
	-	719100	87500	33900	16600	7900	2400	1000
	-	288000	118400	54000	28000	15500	5300	1600
Grapefruit	-	191900	69800	30500	13300	6600	1800	200
	-	310700	104400	44400	16500	7000	2000	300
	-	141800	53000	30300	15300	7900	3100	400
	-	478400	165500	71800	32100	13700	4000	900
	-	269900	74300	39400	17000	7800	2700	1300
Lemon	-	125600	52800	29200	16100	9400	3400	2100
	-	313700	98100	42100	18900	8300	2900	1200
	-	556000	84100	34100	15900	8000	2200	400
	-	123600	48800	25300	12200	5600	1500	700
	-	659500	128100	53500	25900	12400	3900	1200
Pectin	-	180200	64900	28400	14600	6400	2000	1300
	-	111700	35700	21500	12600	6700	2500	1500
	-	624400	74300	32700	15000	6600	2400	1300
	-	185900	67600	32700	13800	7300	2500	1400
	-	-	81800	35600	16400	7700	3300	1900
Carrageenan	-	115500	43400	19000	9400	3600	1400	1000
	-	365700	102900	41600	18200	8200	2900	1100
	-	260500	77000	31000	12400	5400	2000	1100
	-	168700	38900	19700	12100	4900	1700	1100
Control	588400	258400	92600	41200	18700	7700	2000	600

Table 4. Mineral content of solid phase of yogurt samples (mg/Kg)

	Additive	Concent-rations (%)	Al	Co	Mo	Ca	B	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	S	Se	Zn
SOLID PHASE OF YOGURT	Orange	0.05	0.00	0.12	0.31	4397.89	4.95	0.09	0.00	1.80	16.76	6825.41	633.71	1.10	1492.67	0.23	5259.38	0.00	2953.57	0.00	15.11
		0.10	0.00	0.33	0.39	4166.38	4.95	0.09	0.00	3.51	24.26	6531.71	623.61	1.23	1410.87	0.22	5294.10	0.00	3151.35	0.00	15.60
		0.15	0.00	0.19	0.27	4155.20	5.99	0.02	0.00	2.96	18.66	6383.81	603.20	1.03	1377.99	0.02	4808.46	0.00	3098.25	0.00	14.61
		0.20	0.00	0.31	0.22	4352.28	4.83	0.05	0.00	1.72	17.19	6960.07	695.49	1.09	1446.29	0.51	5805.16	0.00	3566.41	0.01	15.48
		0.25	0.00	0.22	0.28	4726.35	5.51	0.09	0.00	2.25	23.65	7499.84	731.10	1.14	1615.10	0.94	6043.93	0.00	3567.00	0.00	16.63
	Grapefruit	0.05	0.00	0.15	0.30	4688.58	4.82	0.06	0.00	2.88	25.39	7181.78	690.37	1.14	1552.79	0.27	5674.53	0.00	3270.75	0.00	16.97
		0.10	0.00	0.16	0.27	4663.89	5.35	0.10	0.00	2.11	18.88	7418.57	708.76	1.11	1575.46	0.24	5890.04	0.00	3510.64	0.00	16.33
		0.15	0.00	0.31	0.49	4514.50	4.69	0.08	0.00	2.29	21.34	7641.70	724.51	1.10	1591.87	0.32	6276.46	0.00	4197.82	0.00	16.45
		0.20	0.00	0.13	0.42	4389.08	4.65	0.07	0.00	1.86	21.56	7311.59	711.31	1.11	1535.20	0.11	6027.99	0.00	4161.99	0.00	15.89
		0.25	0.00	0.17	0.20	5187.07	5.65	0.11	0.00	2.03	20.50	8274.84	817.46	1.16	1734.01	0.12	6624.77	0.00	4104.07	0.32	17.48
	Lemon	0.05	0.00	0.15	0.31	4249.10	4.44	0.07	0.00	2.14	20.37	6725.13	609.21	1.10	1473.85	0.67	5136.25	0.00	3012.38	0.00	14.87
		0.10	0.00	0.08	0.29	4674.67	4.54	0.06	0.00	1.77	18.76	7401.79	702.00	1.05	1580.72	0.45	5741.25	0.00	3249.35	0.00	16.13
		0.15	0.00	0.21	0.35	5486.94	5.07	0.08	0.00	2.81	21.19	8242.03	798.84	1.11	1757.51	0.38	6264.12	0.00	3512.25	0.00	18.06
		0.20	0.00	0.22	0.31	4463.00	4.18	0.05	0.00	1.48	15.26	7326.65	692.76	1.05	1530.15	0.62	5873.67	0.00	3455.58	0.77	15.53
		0.25	0.00	0.36	0.30	4741.70	5.52	0.05	0.00	1.75	18.12	7732.18	730.71	1.10	1600.60	0.55	6128.27	0.00	3535.60	0.00	16.60
	Pectin	0.05	0.00	0.22	0.40	4793.28	4.02	0.07	0.00	1.82	21.70	7578.42	769.93	1.09	1672.49	0.09	6190.36	0.00	4883.93	0.00	17.06
		0.10	0.00	0.16	0.43	4860.86	4.51	0.04	0.00	2.52	20.41	7945.19	789.21	1.15	1811.73	0.64	6429.47	0.00	3821.95	0.00	17.88
		0.15	0.00	0.22	0.34	5150.44	6.79	0.08	0.00	1.70	21.49	8528.83	797.48	1.12	1928.60	0.19	6645.38	0.00	3791.73	0.00	17.13
		0.20	0.00	0.14	0.41	5066.84	4.76	0.06	0.00	2.16	19.75	8170.13	782.75	1.21	1993.63	1.35	6392.06	0.00	3669.74	0.50	18.77
		0.25	0.00	0.23	0.43	5370.82	5.10	0.07	0.00	2.29	24.16	8572.40	771.19	1.25	2124.70	1.04	6282.34	0.00	3616.31	0.00	23.72
	Carrageenan	0.05	0.00	0.30	0.14	4369.98	4.65	0.01	0.00	2.05	24.76	7403.78	705.29	1.14	1504.65	0.21	6300.03	0.00	4267.32	0.00	16.46
		0.10	0.00	0.26	0.33	5117.61	4.56	0.11	0.00	1.76	18.06	8991.73	840.33	1.12	1789.75	1.20	6662.86	0.00	4976.57	0.00	18.37
		0.15	0.00	0.09	0.41	6464.64	5.10	0.11	0.00	2.15	20.24	10626.32	911.96	1.19	2171.10	0.90	6864.14	0.00	5088.97	0.00	21.02
		0.25	0.00	0.20	0.27	5745.74	4.22	0.05	0.00	2.39	22.21	9536.60	725.58	1.08	1914.73	1.44	5606.85	0.00	4336.54	0.09	19.34
	Control	-	0.00	0.28	0.27	4432.25	4.23	0.09	0.00	1.93	19.87	6772.09	613.88	1.13	1469.60	1.34	5545.01	0.00	3318.11	0.00	15.93

Table 5. Mineral content of liquid phase of yogurt samples (mg/kg)

L PHASE OF YOGURT	Additive	Concent-rations (%)	Al	Co	Mo	Ca	B	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	S	Se	Zn
	Orange	0.05	0.00	0.08	0.07	1404.21	3.10	0.02	0.00	0.36	2.85	1749.79	108.34	0.22	456.09	0.17	651.30	0.00	189.35	0.00	3.59
		0.10	0.00	0.05	0.04	1476.70	3.01	0.02	0.00	0.36	3.50	1871.27	116.24	0.23	482.63	0.14	670.15	0.00	114.20	0.00	3.89
		0.15	0.00	0.06	0.05	1455.69	2.85	0.02	0.00	0.37	3.42	1858.87	114.33	0.23	469.48	0.08	655.06	0.00	87.22	0.00	3.86
		0.20	0.00	0.05	0.00	1369.99	2.61	0.01	0.00	0.34	3.11	1794.22	105.24	0.23	453.59	0.13	611.38	0.01	185.01	0.15	3.38
		0.25	0.00	0.07	0.00	1435.86	2.53	0.01	0.00	0.35	3.00	1888.29	108.91	0.23	469.73	0.00	636.26	0.00	103.98	0.00	3.70
	Grapefruit	0.05	0.00	0.06	0.07	1414.53	2.34	0.02	0.00	0.29	3.39	1864.41	109.43	0.22	461.95	0.06	628.32	0.00	70.84	0.00	3.60
		0.10	0.00	0.04	0.01	1454.01	2.34	0.01	0.00	0.32	3.22	1898.84	111.72	0.22	470.65	0.06	642.31	0.00	112.83	0.00	3.77
		0.15	0.00	0.04	0.07	1476.91	2.32	0.01	0.00	0.25	2.46	1933.17	113.55	0.22	478.07	0.06	650.62	0.00	67.72	0.00	3.63
		0.20	0.00	0.03	0.03	1413.25	2.20	0.02	0.00	0.29	3.34	1864.33	109.10	0.22	461.02	0.01	628.64	0.00	85.88	0.00	3.47
		0.25	0.00	0.05	0.00	1468.80	2.11	0.02	0.00	0.27	2.37	1921.35	113.59	0.21	468.52	0.05	648.18	0.01	194.80	0.00	3.67
	Lemon	0.05	0.00	0.05	0.06	1408.20	1.82	0.02	0.00	0.34	3.35	1850.93	111.33	0.22	462.58	0.09	626.58	0.00	210.41	0.00	3.47
		0.10	0.00	0.03	0.07	274.50	1.53	0.01	0.00	0.29	3.22	406.56	15.12	0.21	136.68	0.02	178.08	0.00	43.05	0.00	0.96
		0.15	0.00	0.05	0.08	1356.03	1.98	0.02	0.00	0.30	3.00	1786.20	105.44	0.22	439.19	0.14	608.84	0.00	65.46	0.00	3.26
		0.20	0.00	0.05	0.04	1471.78	1.97	0.01	0.00	0.30	4.09	1921.49	115.64	0.23	469.86	0.06	643.43	0.00	76.99	0.00	3.52
		0.25	0.00	0.04	0.01	1510.95	1.92	0.02	0.00	0.38	3.48	1981.00	117.22	0.22	480.67	0.00	657.58	0.00	64.72	0.00	3.66
	Pectin	0.05	0.00	0.03	0.00	1406.96	1.84	0.02	0.00	0.31	3.34	1856.68	105.70	0.23	475.20	0.07	614.33	0.00	84.67	0.00	3.45
		0.10	0.00	0.04	0.04	1489.40	1.74	0.03	0.00	0.41	3.99	1979.16	113.57	0.23	529.99	0.07	652.01	0.00	80.34	0.00	3.58
		0.15	0.00	0.04	0.00	1471.70	1.77	0.01	0.00	0.35	3.97	2008.88	114.71	0.24	550.76	0.03	657.65	0.00	63.28	0.00	3.55
		0.20	0.00	0.05	0.03	1386.67	1.73	0.02	0.00	0.34	3.73	1901.67	106.48	0.22	539.51	0.08	622.78	0.00	72.11	0.00	3.27
		0.25	0.00	0.07	0.03	1433.81	1.53	0.02	0.00	0.32	3.31	2008.90	112.37	0.22	593.82	0.03	656.39	0.00	450.27	0.00	3.39
	Carrageenan	0.05	0.00	0.06	0.01	1359.32	1.61	0.02	0.00	0.36	3.54	1804.97	106.10	0.22	437.19	0.00	593.94	0.00	74.80	0.00	3.41
		0.10	0.00	0.05	0.05	1384.08	1.65	0.01	0.00	0.32	3.03	1891.10	107.38	0.23	449.21	0.03	605.36	0.00	110.85	0.03	3.30
		0.15	0.00	0.04	0.00	1365.61	1.67	0.03	0.00	0.36	3.31	1967.87	109.04	0.23	456.93	0.05	618.07	0.00	62.72	0.00	3.36
		0.25	0.00	0.05	0.02	1366.19	1.59	0.02	0.00	0.31	2.87	2025.40	114.54	0.22	457.06	0.03	623.14	0.00	116.69	0.06	3.49
	Control	-	0.00	0.06	0.02	1423.05	1.49	0.01	0.00	0.41	2.78	1877.14	116.24	0.22	451.56	0.05	620.25	0.00	80.06	0.00	3.63

Conclusion

The yoghurts with added 0.05% orange and 0.05% grapefruit also showed nearly same tendency to syneresis than plain yoghurt whilst increased amount of grapefruit increased syneresis level. The addition of different amounts of pectin caused more syneresis when compared with plain yoghurt. Generally, syneresis level of yoghurt contained orange skin at the different contentrations were found partly low compared with other samples. Viscosity values decreaset at increasing rpm levels. Generally, mineral contents of liquid phase decreased according to solid phase of yoghurt samples.

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DETERMINATION OF PROTEIN, FAT AND FATTY ACID CONTENTS OF WALNUT GENOTYPES IN BESNI AND GÖLBAŞI DISTRICTS (TURKEY)

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Abstract

Anatolia is one of the centers of the germplasm of nuts and this fruit species is grown in almost every region there. There are a lot of seedling walnut populations with wide genetic variation in Turkey. This provides a very important potential for walnuts breeders. Walnuts are a functional food ingredient rich in nutrients such as protein, vitamins, and minerals. The most important characteristic of walnut oil is the plenty of polyunsaturated (PUFA) fatty acids such as Omega-3 and Omega-6, which makes it a unique food because of the high amount of linoleic acid. Unsaturated fatty acids play an important role in human nutrition, so walnuts become an indispensable part of human nutrition. Therefore, walnut production and consumption in World and Turkey are increasing every year. In this study, some chemical compositions were determined for 14 promising walnut genotypes in Besni and Gölbaşı (Adıyaman Province) in Turkey. The protein rates of walnut genotypes were determined to vary between 13.69% and 19.85%. We have also found that the oil content of these walnut genotypes varies between 49.44% and 60.87%. Oleic acid contents of walnut genotypes ranged from 14.434% to 30.052%. In addition, linoleic and linolenic acids contents of walnut oils were found between 49.647% and 63.534%, and 7.731% and 15.097%, respectively. This study has confirmed the presence of high percentage of oleic, linolenic and linoleic acids in walnut.

Keywords: W*alnut, fat, protein, fatty acids.*

Introduction

Walnut is one of the oldest fruits species cultured both in Turkey and in the world (Şen, 1986). Anatolia stands for also a germplasm of walnut and walnut trees are extremely plenty within almost all regions in Turkey. Walnut trees are cultivated in Turkey mainly for their nutritious nuts, which are used as a food, in the chocolate industry, for baked foods, as well as in the pharmaceutical and cosmetic industry. The trees are also valuable as timber (Ercisli *et al*., 2012). Turkey has significant walnut populations in its distinct ecological regions. These populations are generally composed of seedling trees, but the cultivation of promising walnut genotypes obtained from breeding programs is increased to nut quality standard and quantity in walnut production in Turkey over the last ten years (Koyuncu *et al*., 2004). Walnut is a fruit species well-adapted to the various ecological regions of Turkey. Walnut trees are widely distributed throughout Turkey in the valleys of big rivers and on the hillside. Therefore, Turkey has many promising genotypes cultivated in the various ecological region of Turkey (Muratoglu *et al.,* 2010). The human body basically needs three main nutrients: fats, proteins, and carbohydrates. For healthy life and to maintain it, these three essential nutrients must be taken in a balanced manner. Walnuts are a very rich fruit species of these important nutrient elements. However, the values of these nutrients are different in each of the walnut genotypes. Therefore, it is of utter importance to identify the composition of walnuts and their nutritional values in order to reveal its position in a balanced diet (Beyhan *et al*., 1995). The kernel of a walnut can be considered as a type of concentrated food due to its high rate of fat and protein. In other words, as a hard-shelled fruit, walnut is an excellent untouched and wrapped "food

store". It abounds in two of three basic nutritional elements, which are necessary for humans. Furthermore, it can be kept without losing any nutritional value under relatively bad conditions for more than one year (Şen, 2011; Sutyemez and Kaska, 2005). Fatty acids in the walnut oil are primarily unsaturated. When confronting other hard-shelled fruits containing chiefly monounsaturated fatty acids (MUFA), walnut is pretty rich in terms of polyunsaturated fatty acids (PUFA) such as Omega-6 and Omega-3. Polyunsaturated fatty acids (PUFA) are crucial for human nutrition (Amaral *et al*., 2003; Zwarts *et al*., 1999). In this study, some promising selected walnut genotypes in Besni and Gölbaşı region in Adıyaman were examined in terms of fat, fatty acid and protein contents in their kernels. We were carried out to bring new information on protein, fat, and some fatty acid contents of 14 walnut genotypes from Turkey.

Material and Methods

Mature walnut shelled-nuts of walnut genotypes were harvested from Besni and Gölbaşı, located in the Adıyaman provinces of Turkey. Nut samples of 14 walnut genotypes were randomly selected with three replicates for each tree. Kernels of walnut were analyzed after drying under incubator at 30°C for 24 hours. According to the Kjheldal method, nitrogen was determined and protein ratio was calculated by multiplying the amount of nitrogen determined by 6.25 (Bayraklı, 1987). Walnuts were separated their kernel using nutcracker and after the separation immediately homogenized and oil extraction of kernel powder (25 g) were done using automatic Soxhalet equipment Bligh and Dyer (Bligh and Dyer, 1959). Automatic Soxhlet equipment (Gerhardt Soxtherm) was used for oil extraction with hexane organic solvent. Triplicate analysis was done for each variety. Methylation was done using Boron trifluoride/methanol (AOAC, 1990; Ichihara et al., 1996). The fatty acid analysis was done using a GC (Gas Chromatography)/FID (Flame Ionization Detector) (Perkin Elmer, Clarus 500, USA) equipped with a and SGE column (100 m × 0.32 mm, ID 0.25 μm). As for the GC condition; the oven temperature was held at 140˚C for 5 min, and then increased to 200˚C at a rate of 4˚C min^{-1} and to 220˚C at a rate of 1˚C min^{-1}. FAME mix standard including 37 components were used for fatty acid identification and quantification.

Results and Discussion

In this study, walnut genotypes grown in Adıyaman region were characterized in terms of protein, total fat, and fatty acids. In addition, genotypes were analyzed in terms of Oleic acid, Linoleic acid, and Linolenic acid. Protein contents of the genotypes ranged from 13.69% to 19.85%. The results of the protein values are presented in Table 1. As shown in the table, the lowest protein level (13.69%) was observed in Genotype 1, it was highest (19.85%) in Genotype 15 among the 14 walnut genotypes investigated in the present study. The results of the total fat ratio of the genotypes grown Besni and Gölbaşı were presented in Table 1. Total fat contents of the walnut genotypes ranged from 49.44% (Genotype 14) to 60.87% (Genotype 16). There are different data about fat ratio of walnut kernel, growing in different places. The fat ratio of walnut kernel varied between 49.04% and 78.68% in these studies (Pereira *et al.*, 2008; Yerlikaya *et al.*, 2012; Gharibzahedi *et al.*, 2014*;* Uzunova *et al.*, 2015; Kafkas *et al*., 2017; Matthäus *et al.*, 2018). In our study, fat rates of walnut kernels were lower than those studies. This is probably due to the fact that the walnut genotypes in our study are composed of genotypes that grow naturally and don't have cultural practices. Walnut has all the time been consumed throughout the history owing to its high nutritional value. It is a suitable fruit for healthy diets because it contains fat (50-80%) and protein (12-15%) (Mitrovic *et al.*, 1997). Table 1 showed the fatty acid contents of the walnut kernel samples. As can be seen, oleic acid concentrations changed between 14.434% and 30.052%. In a study conducted in Tunisia, the chemical composition (total oil content, fatty acids) of six walnuts (*Juglans regia* L.) cultivars ('Lauzeronne', 'Franquette', 'Hartley', 'Local pt', 'Local

gd' and 'Parisienne') gathered from Mateur was evaluated. Oleic acid ingredient of these cultivars were found 19.94%, 16.73%, 14.27%, 13.21%, 13.68%, and 16.49%, respectively (Bouabdallah *et al*., 2014). In a study conducted by Zwarts *et al*., (1999), two US commercial cultivars (Tehama and Vina), three European commercial cultivars (Esterhazy, 139, G120) and five New Zealand walnut cultivars (Rex, Dublin's Glory, Meyric, McKinster, and Stanley) were used. The oleic acid content of walnut cultivars varied between 12.7% to 20.4% of the total fatty acids, while 18:2 content varied between 57.0% to 62.5% and the 18:3 content varied between 10.7% to 16.2%. Also in another study, oleic acid values of monounsaturated fatty acids ranged from 14.73% to 24.17% (Beyhan *et al*., 2017). The oleic acid values obtained in our walnut genotypes showed similar results with these studies.

In the present study, the highest linoleic acid was observed in Genotype 13 as 63.534% and the lowest linoleic acid was in Genotype 9 as 49.647%. In addition to, values belonging to other genotypes were included in the intermediate group. In the present study, linolenic contents of these genotypes were determined and the results are given in Table 1. The lowest value of linolenic contents was detected in the Genotype 12 with 7.731% the highest value was in the Genotype 3 with 15.097%. In a recent study, the fatty acid content and antiradical activity of different walnut (*Juglans regia* L.) genotypes grown in Kolyaei region located in Kermanshah Province (Iran) were investigated and it was reported that linoleic acid and linolenic acid contents of the walnut genotypes ranged from 46.9% to 56.8%, and from 10.8% to 13.9% respectively. Also, it is stated that the distinction might have resulted from ecological, nutrition and genetically factors. The variation in the fatty acid composition of the nuts from varied genotypes may affect the end use of the product (Akbari *et al.*, 2015).

The consequence obtained were compared with similar studies in walnut. Its differences could be attributed to the harvesting year and environmental condition, with different temperatures, rainfall, and light, which can affect the chemical composition of fruits (Parcerisa *et al.*, 1995).

Table 1. Protein, fat and fatty acids ratios of walnut genotypes.

Genotypes	Protein (%)	Total Fat (%)	Fatty Acids (%)		
			Oleic acid	Linoleic acid	Linolenic acid
Genotype 1	13.69	55.22	16.806	60.906	11.822
Genotype 2	17.41	51.29	24.308	57.649	7.750
Genotype 3	15.35	58.53	15.382	59.837	15.097
Genotype 5	18.63	54.00	22.540	56.597	10.509
Genotype 6	18.60	56.57	22.056	54.699	11.732
Genotype 8	17.31	57.88	19.897	60.993	9.326
Genotype 9	18.25	56.58	30.052	49.647	8.729
Genotype 10	15.38	60.64	17.242	59.193	11.797
Genotype 12	17.72	52.88	21.407	61.164	7.731
Genotype 13	19.66	57.25	14.434	63.534	11.207
Genotype 14	16.76	49.44	16.689	62.284	11.461
Genotype 15	19.85	51.53	21.122	58.114	10.600
Genotype 16	15.19	60.87	20.108	57.963	10.458
Genotype 17	17.35	50.26	16.041	62.037	10.628

Conclusions

According to the obtained results, 14 walnut genotypes have important values in terms of protein, total fat and some fatty acids content which importance for human health and nutrition are very. Protein and total fat contents of the walnut genotypes varied between 13.69% and 19.85%, from 49.44% to 60.87%, respectively. Oleic acid contents of walnut oils ranged from 14.434% to 30.052%. In addition, linoleic and linolenic acids contents of walnut oils were found between 49.647% and 63.534%, and 7.731% and 15.097%, respectively. This study has confirmed the presence of high percentage of oleic, linolenic and linoleic acids in walnut.

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THE EFFECT OF DIFFERENT WEED CONTROL METHODS ON CHICKPEA YIELD COMPONENTS IN KIRSEHİR

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Abstract

The study was carried out in order to determine the effects of weeds on chickpea yield factors such as biological yield, number of main branches, plant length, the first pod height, number of pods per plant, number of seeds per plant, seed yield per plant, harvest index, 100-seed weight. Experiments were created according to randomized block design in Kirsehir Ahi Evran University, Agricultural Practice and Research Center, Turkey between 2015 and 2017 years. In the field experiments, 9 different treatments were included - control, weed free, one-time hoeing, two-time hoeing, three-time hoeing, poste-emergence herbicide application, one-time hoeing with herbicide application, two-time hoeing with herbicide application and three-time hoeing with herbicide application. According to the results, there are significant differences between chickpea yield and yield components depending on different weed control methods. According to results, it was determined yield of 159.7 kg when no weed struggle conditions occurred. On the other hand, three-time hoeing of weed revealed the best yield performance as 847.8 kg per ha?. It has been determined that even the single shearing process increases the yield of chickpea about 2.5 times, 3 hoeing operations increased 6 times according to an average. As the number of chickpea hatchings increased in Kirsehir conditions, the result of the study proved that the yield is positive. As the number of chickpea hoeing increased in Kirsehir conditions, the result of the study proved that the yield is positive.

Keywords: *Kirsehir, Aksu, weeds, yield, yield components*

Introduction

Chickpea is widely grown in East Mediterranean, Africa and Central America. As the case worldwide, it holds an important place in closing the food deficit in Turkey. Its grains are used as human food and its stalk and straw can be used as a valuable animal feed. Turkey ranks fifth after India, Pakistan, Mynmar and Australia in chickpea production (FAO, 2016). Despite some recent setbacks in the area, Turkey still stands its ground as one of the major producers of chickpea. Especially the relatively more successful means of controlling newly developed varieties and weeds contribute to the success in chickpea production.

In a more general sense, the control of weeds for chickpea production is not a common practice in Turkey. Since the plants grow rapidly after the initial growing period and can partially suppress weeds, the producers do not prefer to assume additional expenses to control weeds. However, studies carried out in domestic and other countries have shown that this was not the case and weeds had yield-reducing effects in chickpea production. Ahlawat *et al*. (1981) reported that *Cenepodium album* and *Asphodelus tenouifolius* considerably increased the weed amount in chickpea fields in irrigated or rainy areas. Solh and Pala (1990) stated that the lack of weed control in chickpea growing caused significant losses in yield. Tahabi *et al*. (1994) showed that in chickpea fields with no weed control, the yield loss was 81%.

The study investigates the gains and losses obtained by growing non-irrigated chickpea plants under conditions with weed control and no weed control in the Central Anatolia Region.

Material and Methods

The study was carried out between 2015 and 2017 in the area of Kırşehir Ahi Evran University Research, Turkey. The investigation was conducted to obtain whether weed control is necessary in chickpea growing and if necessary, which method is more successful in weed control. The study was conducted in a randomized block design with three replications. The field was not irrigated and instead, the plants were grown using the natural precipitation occurring during the growing season. The Aksu chickpea variety was used in the experiment. The soil was determined to be clayey, loamy, slightly alkaline (pH:7.8), organic matter-poor (1.26), non-saline, and moderately rich in plant-available phosphorus.

The study were divided into nine treatments:

1) no weed control (control),
2) permanent weed control (weed free),
3) one-time hoeing,
4) two-time hoeing,
5) three-time hoeing,
6) herbicide application after crop emergence,
7) one-time hoeing with herbicide application,
8) two-time hoeing with herbicide application,
9) three-time hoeing with herbicide application,

The application doses and times of the herbicides were determined in accordance to Thomson (1997) and Anonymous (1999). Planting was carried out manually with a row spacing of 30 cm and intra-row spacing of 10 cm.

Diammonium phosphate was distributed to the study area in the amount of 150 kg per hectare. The yield and yield values affecting chickpea were analyzed (count all measured parameters). The SPSS 23V statistical package program was used for the analysis of variance and the Duncan multiple comparison tests ($p<0.05$) of the data.

Results and Discussion

Weeds compete with culture plants for CO_2, O_2, light, and nutrients and some release phytotoxic compounds and negatively affect the growth of plants.

Table 1 shows the differences in the weed control methods with respect to branch number, plant length, first pod height, and number of pods per chickpea plant. The control had the highest branch number (3.11 branches), while the branch number of the weed free treatment, one-time hoeing and the twice-hoed group was considerably low. The treatment that received the herbicide after emergence and the treatment with herbicide+one-time hoeing, herbicide + two-time hoeing and herbicide + three-time hoeing were in the same group. In general, the difference between the branch numbers was around 2 or 3 branches and the variation, albeit statistically significant, was not dramatic. The high rate of branching in the non-controlled area was attributed to the competition with weeds to survive. In a competitive environment, the plants are compelled to develop both their aerial and subsurface parts as much as possible to have access to lighting and nutrients, etc. Mohammadi *et al*. (2005) have reported that competition with weeds occurred in the first three to seven weeks and reduced levels of competition were observed before and after these periods. The results obtained in this study revealed clear signs of competition.

Table 1. Chickpea morphological parameters as a result of different weed control methods

Weed control methods	Chickpea parameters			
	Branch Number	Plant Length (cm)	The First Height (cm)	Number of Pods per Plant
Control	**3.11 a***	40.11 b	17.00 b	14.11 b
Weed free	2.00 b	39.44 bc	21.22 ab	16.33 ab
One-time hoeing	1.88 b	42.55 ab	**25.66 a**	13.11 bc
Two-time hoeing	1.99 b	38.88 bc	20.00 ab	14.66 bc
Three-time hoeing	2.55 ab	39.11 bc	19.22 b	**22.22 a**
Herbicide after emergence	2.55 ab	**45.77 a**	22.44 ab	19.44 ab
Herbicide + one-time hoeing	2.77 ab	44.11 ab	21.22 ab	17.44 ab
herbicide + two-time hoeing	2.44 ab	37.00 c	17.66 b	8.44 c
Herbicide + three-time hoeing	2.88 ab	41.11 bc	18.88 b	14.77 b

* Means in the same column by the same letter are not significantly different to the test of Duncan ($p<0.05$).

The results for the plant lengths showed that the herbicide application after emergence had yielded the longest plants (45.77 cm), followed by the herbicide+one-time hoeing (44.11 cm) and one-time hoeing (42.55 cm) applications. The lowest plant length was obtained in the herbice+two-time hoeing application (37.00 cm).

The results for the first pod height showed that the highest first pod (25.66 cm) was obtained in the one-time hoeing application. The substantial difference in the first pod height values is noteworthy. A difference of about 17.66-25.66 cm indicates that the plants reacted to the applications in an extreme fashion.

The variation in the pods per plant was relatively higher compared with other properties. The highest pods per plant value was 22.22 cm and obtained in the three-time hoeing application, while the lowest pods per plant value was 8.44 cm and obtained in the herbicide+two-time hoeing application.

Table 2 shows the differences in the control methods with respect to yield per plant, biological yield, harvest index, 100-seed weight, and yield. As seen in the Table 2, yield per plant varied between 2.55 g and 7.41 g. This indicates a high variation. The applications had affected the yield per plant. The highest yield per plant was obtained in the herbicide application after emergence and three-time hoeing application.

The highest biological yield was 3.11 kg and obtained in the control group. Considering the high branching rate of the treatment, obtaining a high biological yield was expected. The high rate of branching and a growing habitus increased the biological yield. In the harvest index, the results obtained by the applications, except for the herbicide+two-time hoeing application, were not statistically significant. Although the reason behind the reducing-effect of herbicide+two-time hoeing application on the harvest index was not discerned, the results obtained in this study agree with the results obtained in the study carried out by Mahoney (1981) and Yadav *et al*. (1983) in which the phytotoxic effects of herbicides were determined to reduce the grain yield and yield-affecting characteristics of the chickpea plants.

Table 2. Change of difference between constructed struggle methods according to some characters

Weed control methods	Examined parameters				
	Yield per Plant (g)	Biological Yield (kg)	Harvest Index (%)	100-seed weight (g)	Yield (kg ha^{-1})
Control	5.14 ab*	**3.11 a**	45.72 a	37.23 a	159.7 e
Weed free	5.36 ab	2.00 b	47.19 a	35.89 ab	597.2 c
One-time hoeing	4.38 ab	1.89 b	46.16 a	35.35 ab	395.7 d
Two-time hoeing	4.84 ab	1.99 ab	**49.58 a**	37.58 a	593.5 c
Three-time hoeing	6.89 a	2.56 ab	47.51 a	37.59 a	**847.8 a**
Herbicide after emergence	**7.41 a**	2.56 ab	48.18 a	38.25 a	841.2 a
Herbicide + one-time hoeing	5.54 ab	2.77 ab	45.44 a	35.05 ab	689.7ab
Herbicide + two-time hoeing	2.55 b	2.44 ab	39.72 b	33.36 b	773.8 b
Herbicide + three-time hoeing	5.24 ab	2.88 ab	45.90 a	**38.49 a**	572.3 c

* Means in the same column by the same letter are not significantly different to the test of Duncan ($p<0.05$).

One hundred-seed weight values varied between 33.36 g and 38.49 g but the variation was not high. The results obtained for yield revealed a high variation. The highest yield was 847.8 kg per hectare and obtained in the three-time hoeing application, followed by the herbicide application after emergence with 841.2 kg. The difference between the two groups was determined to be statistically significant. The lowest yield was 159.7 kg and obtained in the application with no weed control, while in the parcels in which weeds were completely removed by constant weed control, a yield of 597.2 kg was obtained, which was below the expected value. In their study carried out under the conditions of Erzurum, Turkey, Kantar *et al*. (1999) determined that, compared with weedy control, herbicide applications increased the chickpea yield at a considerably higher level. The results of this study revealed that only the post-emergence herbicide application resulted in a significantly increased yield and the results obtained with other herbicide applications did not make significant difference between treated and untreated variant. Zimdahl (2004) has stated that the effect of weeds and herbicides on yield depended on the timing of the applications. Application dose and time may reduce the yield.

Conclusions

The herbicide application after emergence and three-time hoeing application were deemed suitable for use in weed control in chickpea cultivation. However, considering the costly nature of three-time hoeing, the herbicide application after emergence is believed to be more appropriate. Surely, the weed-intensive structure of the study area may also have contributed to this effect.

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THE EFFECT ON CHICKPEA (AZKAN VARIETY) YIELD COMPONENTS OF DİFFERENT WEED CONTROL METHODS IN KIRSEHIR CONDITIONS

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Abstract

The study was carried out in order to determine the effects of weeds on yield factors for Azkan cultivar. In this context biological yield, number of main branches, plant height, the first pod height, number of pods per plant, number of seeds per plant, seed yield per plant, harvest index, 100-seed weight were evaluated. Experiments were created according to randomized block design in Kirsehir Ahi Evran University, Agricultural Practice and Research Center between 2015 and 2017 years. In the field experiments, 9 different applications were given as - control, weed free, one-time hoeing, two-time hoeing, three-time hoeing, poste-emergence herbicide application, one-time hoeing with herbicide application, two-time hoeing with herbicide application and three-time hoeing with herbicide applicationcontinuous weed control, continuous without weed, one-time hoeing, two-time hoeing, three-time hoeing, herbicide application after emergence, one-time hoeing with herbicide application, two-time hoeing with herbicide application and three-time hoeing with herbicide application. According to the results, there are significant differences as statistically between chickpea yield and yield components depending on different weed control methods.
According to results, it was determined when no weed struggle occurred conditions yield 211.3 kg. On the other hand, three times shearing of weed revealed the best yield performance as 1315.1 kg. It has been determined that even the single shearing process increases the yield of chickpea about 4 times according to an average. As the number of chickpea hatchings increased in Kirsehir conditions, the result of the study proved that the yield is positive. As the number of chickpea shearing increased in Kirsehir conditions, the result of the study proved that the yield is positive.

Keywords: *Kirsehir, Azkan, weeds, yield, yield components*

Introduction

Chickpea is an important edible legume grown worldwide. It is especially important because of its cheap and ample availability as a protein source for which the need grows incessantly (Bhan and Kukula, 1986). Turkey is an important chickpea producer. Thus, the studies focusing on the improvement of chickpea growing are intensively carried out but they have yet to reach the desired levels, which is mainly attributable to precipitation and weed control Kantar et al., 19999). The chickpea plant is not only a human food but also a valuable animal feed (Tepe *et al.*, 2011).
Weeds compete with culture plants and negatively affect the available moisture and nutrients in soils and light causing yield losses (Rao, 2000). In addition, some weeds may inhibit the growth of culture plants by releasing phytotoxic compounds, which is known to result in severe yield losses. Therefore, the control of weeds is an absolute necessity.
The study aims to determine the control methods for weeds that are reportedly observed when growing chickpea plants under non-irrigated dry conditions. Determining the control methods will also economically contribute to the minimization of yield losses.

Material and Methods

The study was carried out in Kırşehir, Turkey, between 2015 and 2017 in the lands of Kırşehir Ahi Evran University Research and Application to investigate weed infestation and determine which method is more successful in the control of weeds in chickpea growing.

The study was conducted in a randomized blocks design with three replications. The field was not irrigated and instead, the plants were grown using the natural precipitation occurring during the growing season. The Aksu Azkan chickpea variety was used in the experiment. The soil was determined to be clayey, loamy, slightly alkaline (pH: 7.8), organic matter-poor (1.26), non-saline, and moderately rich in plant-available phosphorus.

The study samples were divided into nine groups as:

1) no weed control (control group),
2) with constant weed control (weed free),
3) one-time hoeing,
4) two-time hoeing,
5) three-time hoeing,
6) herbicide application after emergence,
7) one-time hoeing with herbicide application,
8) two-time hoeing with herbicide application,
9) three-time hoeing with herbicide application,

The application doses and times of the herbicides were determined in accordance with Thomson (1997) and Anonymous (1999). Planting was carried out manually with a row spacing of 30 cm and intra-row spacing of 10 cm.

Diammonium phosphate was applied in experiment according to the calculations made for 150 kg fertilizer per hectare. In the study, the yield and yield values affecting chickpea were analyzed. The SPSS 23V statistical package program was used for the analysis of variance and the Duncan multiple comparison tests ($p<0.05$) of the data.

Results and Discussion

Table 1 shows the differences in the control methods with respect to branch number, plant length, the first pod height, and number of pods per plant. As shown in Table 1, the difference between the branch numbers of the plants was between 1.87- 2.30 branches and it was not significant. In other words, the applications did not have an effect that can change the branch number of the plants.

The plant lengths varied between 30.25 cm and 41.64 cm. The tallest plant (41.64 cm) was obtained in the three-time hoeing application, while the shortest plant was obtained in the application with no weed control. The highest plants lengths were obtained in the herbicide+two-time hoeing (40.21 cm) application and the application with constant weed control (39.68 cm). This indicates that hoeing positively affected the plants growth in length.

Table 1. Change of difference between constructed struggle methods according to some characters

Methods of Struggle	Examined Characters			
	Branch Number	Plant Length (cm)	The First Height (cm)	Number of Pods per Plant
Control	1.87 a	30.25 c	20.62 a	3.00 c
Weed free	**2.68 a**	**39.68 ab**	20.00 a	**22.68 a**
One-time hoeing	2.43 a	37.50 b	22.25 a	11.50 bc
Two-time hoeing	2.21 a	36.00 b	**25.71 a**	8.42 bc
Three-time hoeing	2.58 a	41.64 a	23.52 a	19.47 ab
Herbicide after emergence	1.93 a	31.20 bc	20.60 a	6.60 c
Herbicide + one-time hoeing	2.28 a	36.14 b	22.57 a	11.35 bc
herbicide + two-time hoeing	1.94 a	40.21 ab	20.36 a	16.21 b
Herbicide + three-time hoeing	2.30 a	36.50 b	21.00 a	16.30 b

* Means in the same column by the same letter are not significantly different to the test of Duncan ($p<0.05$).

As the case for branch numbers of the plants, the results for the first pod height revealed statistically not significant differences in the first pod heights of the groups. The first pod height values varied between 20 cm and 25 cm and this variation was deemed not significant. In contrast, the variation in the pods per plant was considerably high. The highest pod number was 22.68 and obtained in the weed control application with no weed, followed by the three-time hoeing (19.47) application. The lowest pod number was 3.00 and obtained in the application with no weed control, followed by the herbicide application after emergence (6.60). The reason behind the reducing effect of the herbicide application after emergence on the pods per plant was not discerned. Although the results obtained by Mahoney (1981) and Yadav *et al*. (1983) who argued that the phytotoxic effects of herbicides resulted in decreases in the grain yield and yield-affecting characteristics of chickpeas are acceptable, they fall short in explaining the degree of the decreases caused by other herbicide applications. However, it can be argued that the herbicide application after emergence negatively affected the plant growth cells. Table 2 shows the differences in the control methods with respect to yield per plant, biological yield, harvest index, 100-seed weight, and yield. As revealed by Table 2, the yield per plant varied between 1.01 g and 7.18 g, which indicates that the variation was high. High variation increased the significance levels. The applications affected the yield per plant values. The highest yield per plant was obtained in the application with constant weed control, while the lowest yield per plant was obtained in the application with no weed control. According to the results, the lack of weed control causes an 85.93% loss in the yield per plant in areas with no weed control compared with the weed-controlled areas. The results obtained for biological yield was similar to those obtained for yield per plant. The highest yield was obtained in the application without weed (15.33 kg), while the lowest yield was obtained in the application with no weed control (2.12 kg). The applications did not have any effect in terms of harvest index. The harvest index values varied between 33.92 and 47.10. Although the variation seems considerably high, it was statistically not significant.

Table 2. Change of difference between constructed struggle methods according to some characters

Methods of Struggle	Examined Characters				
	Yield per Plant (g)	Biological Yield (kg)	Harvest Index (%)	100-seed weight (g)	Yield (kg da^{-1})
Control	1.01 d	2.12 cd	44.48 a	34.53 b	211.3 g
Weed free	**7.18 a**	15.33 a	46.56 a	41.21 a	993.9 c
One-time hoeing	2.90 bc	7.67 bc	39.18 a	34.03 b	884.3 d
Two-time hoeing	2.19 cd	6.32 bc	33.92 a	35.07 b	996.8 c
Three-time hoeing	6.07 ab	**13.82 ab**	43.28 a	38.46 ab	905.7 d
Herbicide after emergence	2.06 c	4.29 c	**47.10 a**	37.45 ab	843.3 e
Herbicide + one-time hoeing	3.21 bc	7.76 bc	41.80 a	34.14 b	781.1 f
Herbicide + two-time hoeing	5.87 ab	12.41 ab	46.96 a	**42.98 a**	1085.2 b
Herbicide + three-time hoeing	5.16 b	11.08 b	46.21 a	36.79 ab	**1315.1 a**

* Means in the same column by the same letter are not significantly different to the test of Duncan ($p<0.05$).

The 100-seed weight of the groups varied between 34.03 g and 42.98 g, which resulted in a statistically significant difference. The highest 100-seed weight value was obtained in the herbicide+two-time hoeing application, while the lowest yield was obtained in the one-time hoeing application.

The results obtained for yield revealed a high variation between the applications. The yield varied between 211.3 kg and 1315.1 kg per hectare. This considerably high variation increased the significance levels. The highest yield was 1315.1 kg per hectare and obtained in the herbicide+three-time hoeing application, followed by the herbicide+two-time hoeing application in which a yield of 1085.2 kg per hectare was obtained. The lowest yield value was 211.3 kg and obtained in the application with no weed control. In the application in which weeds were completely removed by constant weed control, the yield was 993.9 kg and below the expected value. In their study carried out under the conditions of Erzurum, Turkey, Kantar *et al*. (1999) determined that, compared with weedy control, herbicide applications increased the chickpea yield at a considerably higher level. The results of this study revealed that only the herbicide application after emergence resulted in a significantly increased yield and the results obtained with other herbicide applications were not significant. Zimdahl (2004) has stated that the effect of weeds and herbicides on yield depended on the timing of the applications. Application dose and time may reduce the yield.

Conclusion

In conclusion, the herbicide application after emergence and three-time hoeing application were deemed suitable for use in weed control in chickpea growing. However, considering the costly nature of three-time hoeing, the herbicide application after emergence is believed to be more appropriate. Surely, the weed-intensive structure of the study area may also have contributed to this effect.

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DETERMINATION OF *PSEUDOMONAS SAVASTANOI* FROM OLEANDER IN TEKIRDAG OF TURKEY

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Abstract

Pseudomonas savatanoi is the causal agent of bacterial knot disease. These bacteria is thought to result from abnormal plant cell multiplication at the infection site in response by the host to large amount of indole-3-acetic acid (IAA) or cytokinin produced by pathogen. Bacterial knot has a lot of host range such as olive, oleander, jasmine, fontanesia, privet, myrtle and pomegranate plants. Bacterial knot disease is one of the most important and common disease. The aim of study was isolation and identification with pathogenicity and biochemical tests of disease agent *Pseudomonas savastanoi* from oleander. So in spring 2017, we obtained some samples of oleander leaves and branches with small ground galls in Tekirdağ province of Turkey. To investigate the variability of *Pseudomonas savastanoi* population, twenty one strains of the bacterium were isolated in Tekirdağ from oleander. The strains were exposed to Gram reaction, levan production on 5% saccharose nutrient agar (SNA), oxidase, pectolytic activity, arginine dihydrolase reaction, hypersensitive reaction on tobacco leaves and pathogenicity test on oleander plant. As result of tests twenety one bacterial strains from oleander produced knots on host species were tested, all strains were pathogenic on oleander plants. They were negative for levan, oxidase, arginine dihydrolase, pectolytic activity and positive for hypersensitive reaction on tobacco leaves. According to results of this study, bacterial knot disease determined and all strains were allocated in the LOPAT 1b with biochemical tests.

Key words: *Nerium oleander, oleander, bacterial knot*

Introduction

Pseudomonas savastanoi pv. *savastanoi* is a pathogenic bacterium inducing galls or knots on stem, twigs, and leaves of several host plants belonging to the family Oleaceae and olive, oleander, jasmine, fontanesia, privet, myrtle and pomegranate plants (Janse, 1981). According to current classification, three different pathovars of *P. savastanoi* denoted savastanoi, nerii and fraxini, elicit hyperplastic growth formations (galls or knots) on young stems and branches of olive (*Olea* spp.), oleander (*Nerium oleander*), and ash (*Fraxinus excelsior*), respectively (Young et al., 1996). The presence of bacterial knot in Turkey was observed on olives growing in the Aegean (Azeri, 1993; Tatli and Benlioglu, 2004) and western Mediterranean (Basim and Ersoy, 2000) regions of the country and on oleander in Sanliurfa (south-eastern Anatolia) (Kavak and Ustun, 2009). Bacteria isolated from different host plants differ in host specificity. Oleander strains infect oleander and plants of Oleaceae, whereas olive strains infect Oleaceae. Strains from ash specifically infect only ash plants (Janse, 1982; Iacobellıs et al., 1998).

The aim of the study was to characterize the population of twenty one *P. savastanoi* pv. *nerii* isolates from leaves and branches of oleander in Tekirdag of Turkey.

Materials and methods

Survey and isolation

In spring 2017, oleander leaves and barnches with small ground galls were collected from different park, garden and roadside in Tekirdag of Turkey. Samples were placed in paper and

transported to the laboratory. Isolations of the pathogen was caried out from diseased branches and leaves. Totaly 21 bacterial strains were isolated fom 53 diseased plants on King's medium B.

Screening bacteria pathogenicity

Hypersensitive reaction on tobacco leaves (HR): Pathogenicity of the obtained isolates were tested on tobacco plants according to Klement (1963). Bacterial suspension of each isolate (10^6 cfu/ml) was infiltrated in tobacco leaf. HR was observed after 24 h.

Pathogenicity tests

Pathogenicity tests were carried out as described by Surico et al. (1984). A total of 21 strains were inoculated into 1-year-old shoots of olive and oleander. Bacterial suspensions (10^8 CFU mL^{-1}) of selected bacterial strains were injected in three replicates into the bark of each test plant using a sterile needle. Swelling at the inoculation sites are evident 20-30 days after inoculation, depending on the plant reaction but virulence assays should last for at least 60 days (Janse, 1982). Sterile distilled water and reference *P. savastanoi* pv. *savastanoi* strain Akçay 9 from olive (provided by Prof.Dr Mustafa Mirik, NKU,TURKEY) were used as negative and positive controls, respectively.

Biochemical and physiological tests

Morphological properties on KB (King et al., 1954) media and biochemical and nutritional tests were used to identify the bacteria. The bacterial cultures were tested for, Gram reaction, levan production on 5% saccharose nutrient agar (SNA), oxidase and pectolytic activity, arginine dehydrolase reaction, and HR test on tobacco leaves (Schaad et al., 2001). All tests were repeated with three replicates. The reference strain Akçay 9 was used as positive control.

Results and discussion

Totaly twenty-one bacterial strains were isolated from fifty three diseased plants on King's medium B in Tekirdag. These strains produced knots on braches of both olive and oleander plants. So twenty one strains were re-isolated and used in identification tests. According to test results, the bacterial cultures were Gram-negative, did not produce levan on SNA, were negative for pectolytic activity, arginin dihydrolase and oxidase, therefore were allocated in the LOPAT 1b group (Lelliott and Stead, 1987).

All samples were collected on oleander plants in Tekirdag. Two different host plants as olive and oleander plants used for Pathogenicity test. The aim of these determined whereas *P. savastanoi* pv. s*avastanoi* or *P. savastanoi* pv. *nerii*. Because olive strains (*P. savastanoi* pv. *savastanoi*) only cause knots in olive plants. Otherwise oleander strains (*P. savastanoi* pv. *nerii*) cause knots on both oleander and olive (Iacobellis et al., 1998).

Conclusion

According to result of this study, bacterial knot disease determined as *P. savastanoi* pv. *nerii* and all strains were allocated in LOPAT 1b group with pathogenicity and biochemical tests.

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OLFACTORY RESPONSE OF THE GENERALIST PREDATOR *ORIUS LAEVIGATUS* (FIEBER) TO SOME VEGETABLE AND FRUIT PUREE

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Abstract

Natural enemies use information cues, mainly plant odors, in their foraging behaviour. The predatory bug *Orius laevigatus* is one of the effective predators against many herbivores in greenhouse vegetables. In mass rearing programs, a diverse variety of natural food stuffs have been employed in artificial diets. For this purpose we analyzed the response of *O. laevigatus* to tomato, carrot, zuchinii and mixed vegetable puree and plum, peach, pear and mixed fruit puree. These products were offered to *O. laevigatus* adult females in 1g quantities in experiments involving a dual choice Y-tube olfactometer. Female predatory bug showed the highest preference for carrot puree to clean air (control) in one replicate (100%). In three replicates female showed high preference for carrot puree (90%), too. *O. laevigatus* did not show a preference in one replicate of zuchinii (30%), but at all replicates preference were 57%. The predatory bug oriented similar preference to tomato puree in three replicates (67%). Mixed vegetable puree attracted *O.laevigatus* in three replicates (83%). However in one replicate predatory bug showed high preference (90%). The predatory bug was attracted to peach, plum, and pear puree 73%, 67%, 40%, respectively. *O. laevigatus* showed high preference (80%) in one replicate for peach and plum puree. Additionally, predatory bug showed 67% preference for mixed fruit puree. However, *O. laevigatus* showed 63% preference when we mixed carrot and peach puree. A better knowledge of the role of olfactory response of predatory bug to different diets will improve the efficiency and utilization of the food in mass rearing programs.

Keywords: *Orius laevigatus, Fruit puree, Vegetable puree, Y-tube olfactometer, Attraction.*

Introduction

The omnivorous predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) is widely used as a commercial biological control agent in protected cultivation (Chambers *et al*., 1993; Van Lenteren, 2012). This bug feeds on aphids, whiteflies, mites, and thrips, with a distinct preference for the latter group of pests. It can also consume plant food, such as pollen and sap; this alternative diet helps the bug to maintain its population in the absence of protein-rich food (Van de Veire and Tirry, 1999; Saprykin and Pazyuk, 2003). Although *Orius* spp. are effective predators in protected cultivation, they are relatively expensive to mass rear. In commercial mass production systems, this anthocorid is mainly produced on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), in some cases in a mixture with *Artemia* cysts or eggs of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) (De Clerq *et al*., 2005; Nishimori *et al*., 2016). Several authors have described alternative foods and artificial diets for *Orius* bugs (Bonte and De Clercq, 2008; Riddick, 2009). Several workers proposed artificial moisture sources, and living and oviposition substrates for *Orius* spp. (Richards and Schmidt, 1996; Shapiro and Ferkovich, 2006). However a lot of work is needed on different artificial diet formulation in mass rearing of entomofagous insect.

Fruits and vegetables are an important part of human and animal diet. They provide, not only the major dietary fiber component of food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds, such as carotenoids and polyphenols (Augspole *et al*., 2014). Tomato juice was found to contain higher levels of 3 sweet/fruity

related aroma compounds: 6-methyl-5-hepten-2-one, linalool, and β-ionone (Zhu *et al*., 2018). When tomatoes undergo thermal treatment, the pectin methylesterase and endopolygalactuonase are inactivated (Hsu, 2008). A total of 148 volatile compounds were identified as plum fruit constituents, esters were by far the dominant volatiles in plum among them (Pıno and Quijano, 2012). More than 300 volatile compounds have been identified in pear fruit (*Pyrus pyrifolia* Nak.) (Rapparini and Predieri, 2003). The possibilities of using olfactory responses of entomophages to various volatile compounds have been actively studied in the recent years with the view of improvement of biological plant protection methods.

The goal of this work was to study the behavioral responses of *O. laevigatus* to vegetable and fruit puree volatiles with the purpose of selecting the most attractive ones.

Material and Methods

Orius laevagatus were purchased from Koppert Biological Systems Company located in Antalya. Carrot, zuchinii, tomato and mixed vegetable puree (carrot 32%, tomato 25%, zucchini 12%, eggplant 3%, onion 4%, pepper 3%) were used in tests (Fig. 1). 1g of puree put in every replicate in jars. Pear, peach, plum and mixed fruit puree (peach+ plum+ pear) and mixed carrot and peach puree were also used in tests (Fig. 2). Olfactometer experiments were carried out by using a Y-tube olfactometer to test whether *O.laevigatus* can discriminate vegetable and fruit puree volatiles versus clean air (control). Females of *O. laevigatus* (1-2 day old) were put in a glass tube and starved for 2 hour. A single female to be tested was introduced into the Y-tube olfactometer and observed until she had walked 5 cm up one of the arms or until 5 minutes had elapsed (Takabayashi and Dicke, 1992; Cakmak et al., 2006). After every 10 insect responses, the Y-tube was cleaned with alcohol and dried. Each experiment was replicated three times.

Statistics: Differences in the proportions of *O. laevigatus* adult' attraction by moving toward one of the puree sources or toward the clean air control were analyzed using the replicated goodness of fit test at a 5% critical level (Sokal and Rohlf, 1995). The null hypothesis was that the predator would exhibit a 1:1 distribution across the two odor sources for each replicate. Insects that did not make a choice were excluded from the statistical analysis.

Figure 1. Vegatable puree in petri dishes (carrot, zucchini, tomato, mix vegetable).

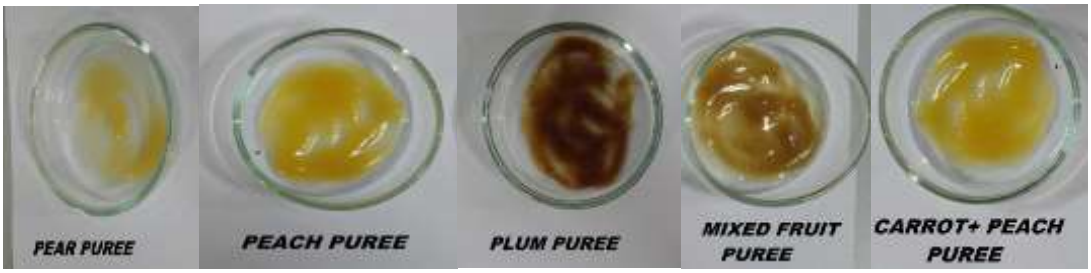

Figure 2. Fruit puree (pear,peach, plum, mixed fruit puree) and mixed carrot and peach puree in petri dishes.

Results and Discussion

The response of *O.laevigatus* to different vegetable and fruit puree and mixed of them were shown in Figure 3. Female predatory bug showed the highest preference for carrot puree to clean air (control) in two replicates (100%, 90%) (P> 0.01, Fig. 3) and one replicate of mixed vegetable puree (90%). In three replicates female show high preference in carrot (90%) and mixed vegetable puree (83%), too. *O. laevigatus* did not show a preference in one replicate of zucchini (30%) and pear puree (20%), but at all replicate preference were 57%, 40%. The predatory bug oriented to tomato and mixed fruit puree similar preference in three replicates (67%). Mixed vegetable puree attracted *O. laevigatus* in three replicates (83%), but in one replicate predatory bug showed high preference (90%) (Fig. 3). The predatory bug attracted to peach and plum puree 73% and 67%, respectively. *O. laevigatus* show high preference (80%) in one replicate for two of them. However, *O. laevigatus* showed 63% preference when we mixed carrot and peach puree. According to these results, the best orientation was found in carrot puree and mixed vegetable puree (Table 1).

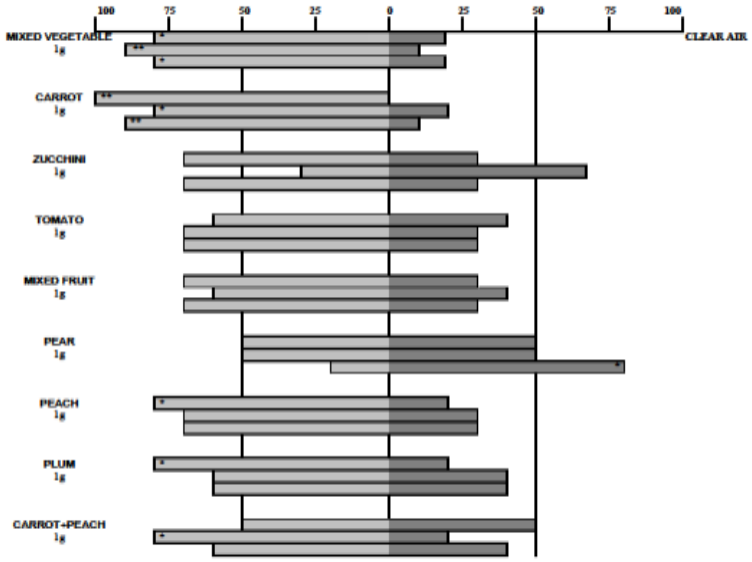

Figure 3. The response of *Orius laevigatus* when offered 1g doses of fruit and vegetable puree; *,**, significant P-values of 0.05 and 0.01, respectively, based on replicated goodness of fit test for G per replicate and GP.

Our results suggest that vegetable and fruit puree volatiles play a role in the attraction of *O. laevigatus*. The best orientation was seen in vegetables. Fruits were in second place with attracting predators. When the best vegetable and fruit puree were mixed together, the attractive effect appears to be falling. Some authors have studied with anthocorid species' orientation to herbivore induced plant volatiles (e.g.: methyl salicylate, methyl isonicotinate) (Lee, 2010; Stephanycheva *et al*., 2014). However, *Orius* predators were also known faculatatively phytophagous (Coll, 1996; Lattin, 1999). For example, *Orius insidiosus* feeds on xylem and mesophyll cells, ingesting water, small amount of sugars, starches and amino acids from the plants (Armer *et al.*, 1998). Also *Orius* species eat pollen (Xu and Enkegaard, 2009). Different

Table 1. The results of replicated goodness of fit test on response of *Orius laevigatus* to fruit and vegetable puree

Vegetable and Fruit Puree	Bases	n	GH, df=2	GP, df=1
			P	P
Mixed Vegetable Puree	1	30	0.772	0.05 0.006664 0.05
Carrot Puree	1	30	0.224	0.000187 0.05 0.006664
Zucchini Puree	1	30	0.414	0.2 0.2 0.2
Tomato Puree	1	30	0.863	0.526 0.2 0.2
Mixed Fruit Puree	1	30	0.862	0.526 0.2 0.2
Pear Puree	1	30	0.266	1 0.05 0.05
Peach Puree	1	30	0.838	0.005 0.2 0.2
Plum Puree	1	30	0.532	0.05 0.526 0.526
Carrot+Peach Puree	1	30	0.36	1 0.05 0.526

Fruit and Vegetable puree replicated goodness of fit test; GH, G for heterogeneity; GP, pooled G; GT, total G; P, probability; df, degree of freedom; n, 3 replicates x 15 individuals; *,**, significant P-values of 0.05 and 0.01, respectively. diets composed of prey or plant materials may influence developmental time, survival and reproduction of *Orius* species (Bonte and De Clerq, 2011; Tommasini *et al*., 2004). In order to stimulate the use of augmentative biological control, increasing effort is being put into the reduction of production costs. This involves developing economically viable and nutritionally adequate alternative foods or artificial diets (Cohen, 2004; De Clercq, 2008). *O. laevigatus* also can complete its development on certain plant materials, such as fresh sweet pepper pollen (Vacante *et al*., 1997). Additionally, this study showed that carrot puree had significantly more attractiveness for *O.laevigatus*. Besides, comparing to other vegetables, carrots can provide in the human diet significant amount of vitamin A due to the high bioavailability of carrot carotenoids (Van het Hof *et al*., 2000). Also, insects need vitamin for growth and in general they can not synthesize them (Chapman, 1998). Only vitamin A and E are known to be required in insects, where they play a role in the synthesis of pigments and in reproduction, respectively (Chapman, 1998). Carotenoids were reported in many physiological functions, with beneficial effects on survival, growth and immunity (Park *et al*., 2010). Carrot diet decreased developmental time in yellow mealworm, *Tenebrio molitor* L. (Coleoptera:Tenebrionidae) (Oonincx *et al*., 2015). Dhinaut *et al*. (2017) stated that prevention of immunopathology relies on endogenous antioxidant enzymes and the consumption of dietary antioxidants, including carotenoids such as astaxanthin on *T. molitor*. Also mixed vitamin (B6, E, C, etc.) was added in artificial diet (Bonte and De Clerq, 2010). The current study demonstrated that fruit puree was also attractive for *O. laevigatus* females than the control.

Conclusions

In conclusion, we demonstrated that the omnivorous *O. laevigatus* could be reared with vegetable and fruit puree in their artificial diet. A diverse variety of undefined natural foodstuffs, including butter fat, wheat germ, royal jelly, honey, liver extract, yeast products and plant material (pollen, leaf extracts) have been employed for rearing predators. A better knowledge of the role of the different ingredients in these oligidic diets will improve the efficiency and utilisation of the food. More research is needed to develop artificial diets. Also, the attractiveness of the oviposition substrate for the females could be improved by incorporating plant extracts as cues to elicit oviposition behaviour. For instance, Constant *et*

al. (1996) reported that spraying their substrate with leaf extract of *Inula viscosa* (L.) increased oviposition by *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) from 1.2 to 1.6 eggs/female/day. In future we can use carrot puree volatiles to attract predatory bug. The most popular odor sources of *O. laevigatus* can be used by participating in their diets in mass rearing programmes and also these odors can be used to attract predators to their release sites.

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RAINBOW TROUT EGG AS A BIOACTIVE COMPOUND DELIVERING VEHICLE: DETERMINATION OF FOOD COLORANT (BRILLANT BLUE) INTAKE CAPACITY

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Abstract

Drug delivery systems are carriers that allow for the protection of the bioactive compound from physicochemical factors such as oxidation, denaturation, ultraviolet radiation (UV), high temperature, pH, enzymes, and biochemical interactions. Rainbow trout fish (*Oncorhynchus mykiss*) is one of the main commercial fish species widely farmed in Europe. Rainbow trout eggs contain high content of omega-3 polyunsaturated fatty acids in phospholipids form, essential amino acids, vitamins and valuable minerals. A wide variety of encapsulating materials has been used to protect bioactive ingredients. Brilliant blue is a most common food colorant used in food industry. Brilliant blue (BB) can soluble easily in water and gives blue color to food materials. It is hypothesized that BB could be used as an indicator to determine bioactive compound encapsulating capacity of rainbow trout egg. Thus, the aim of this research was to determine water-soluble compound loading capacity of trout fish egg by using BB. BB colorant was selected as a model bioactive compound for loading and encapsulating trial. Among the all concentrations of BB colorant tested, sample BB750 and BB1000 showed the highest bioencapsulating capacity. It resulted that trout fish egg could be successfully used as a bioactive compound carrier vehicle for water-soluble compounds.

Keywords: *Rainbow trout egg, Encapsulating, Brilliant blue colorant, Loading capacity*

Introduction

Rainbow trout fish (*Oncorhynchus mykiss*) is one of the main commercial fish species widely farmed in Europe. Native to the Pacific coast of the United States, it was brought to Europe at the end of the 19 th century and today it is farmed in nearly all European countries (Hardy, 2002) . Fish eggs, commonly referred to as roe, are one of the valuable foodstuff of aquatic sources. Fish roes are obtained as a by-product collected from female fish before spawning time like farmed rainbow trout (Bledsoe, Bledsoe and Rasco, 2003). In recent decades, significant advances in drug delivery systems have enabled more effective drug and bioactive compound administration. To deliver drugs to specific organs, a range of organic systems (e.g., micelles, liposomes, and polymeric nanoparticles) have been designed (Barbe et al., 2004). Contrary to common belief, humans have been developing drug delivery strategies since ancient times, beginning with the chewing of leaves or roots and the inhalation of smoke particles from the burning of medicinal plants. Over centuries, the delivery mechanisms have been refined through the development of more reliable dosage forms, such as tablets and capsules (Lee, Park and Robinson, 2000). In this study, water soluble compound intake capacity of rainbow trout egg was investigated. It is hypothesized that a colorant, brilliant blue (BB), could be used as an indicator to determine bioactive compound encapsulating capacity of rainbow trout egg. BB water soluble food colorant was selected as a model bioactive compound for loading and encapsulating trial.

Material and Methods

Rainbow trout (*Onchorhynchus mykiss*) eggs, with a diameter of 5-7 mm, were freshly obtained from Selina Aquaculture Co. Ltd., Fethiye, Muğla-Turkey and transported in cold chain system to the laboratory. Eggs were washed thoroughly with distilled water and passed to filter paper for 10 min to remove excessive water on surface. Food colorant Brillant Blue was purchased from KRK Gida Ltd. Şti. (İstanbul Turkey).

Bioencapsulating trials

Rainbow trout eggs were divided into seven groups prior to encapsulating trials. Trout fish eggs (10 g) were immersed in BB loading (100 ml) solutions containing 0, 50, 100, 250, 500, 750 and 1000 mg/kg of BB for 30 minutes. After the immersing process, excessive BB solution on eggs surface was removed by the paper towel. Trout eggs containing BB solution were passed to the deep freezer (Dairei Europe, ULTF 80) and stored at -80 ºC for 24 hours. Frozen eggs were dried in a freeze dryer (Telstar, Lyoquest 50) at -50 °C, below 0.4 mbar up to water activity of 0.35±1 (*aw*:0.35)for 30-32 hours.

Color analysis

A CR-400 Minolta chroma meter (Minolta, Osaka, Japan) instrument was used to determine the color of trout egg samples. Four readings on the surface of eggs were done using the CIE Laboratory *L* (*lightness), *a** (redness), *b** (yellowness) system in day light conditions.

LC-MS/MS analysis of Brillant Blue colorant

BB colorant analysis was performed in LC-MS/MS (Thermo TSQ Quantum Access Max LC-MS/MS) according to the instrumental method described by according to the method of (Li, Xia, Ma, Zhao, & Pidatala, 2017) with a slight modification. About 50 mg of egg powder was weighted and transferred to 2 ml tubes. Nine volumes of methanol/water (20:80, v/v) with 10 µM trypan blue was added and mixed thoroughly. The mixture was then sonicated for 10 min at room temperature and centrifuged at 16000g for 10 min at 4 °C. The supernatant was collected and stored at -80 °C until analysis. Chromatographic separation was performed on a Hypersil C18 column (100 mm × 2.1 mm, 1.9 µm). The mobile phase consisted of a gradient of water and methanol, both containing 20 mM ammonium acetate. Extract (10 µl) was injected onto column held at 30 °C for chromatographic separation. All of the samples were kept in an autosampler with the temperature controlled at 4 °C prior to injection during analysis.

Statistical analysis

All experiments were conducted in duplicate, and all analyses were done at least in duplicate. Statistical analysis was conducted according to the statistical analysis software of SAS institute (Statistical Analysis System, Cary, NC, USA). Differences among the mean value of samples were tested by Duncan's Multiple Range Test and significance was defined at $P<0.05$.

Results and Discussion

Color analysis of rainbow trout fish eggs containing brilliant blue colorant

Color analysis of trout egg was performed in daylight and *L*, *a*, *b* values were given in Table 1. *L* value reflects the white-black color of the surface. Highest *L* color value was determined in control group trout fish egg (BB0), whereas lowest was found in BB1000. *L* values of egg samples significantly ($P<0.05$) decreased in parallel with the increase in BB concentration up to the 750 ppm. There was no significant difference *L* values of BB750 and BB1000. *a* value reflects the red-green color of surface. Positive *a* color (redness) value was determined in control group trout fish egg (BB0), whereas negative *a* colors (greenish) were found in BB50-BB1000. It means that the BB solution turned the egg colors from red to greenish. *a* value of egg samples significantly ($P<0.05$) increased in parallel with the increase in BB concentration up to the 1000 ppm. *b* value reflects the yellow-blue color of the surface. Positive *b* color

(yellowness) values were determined in *the* control group (BB0), BB50 and BB100 of trout fish egg, whereas negative *b* colors (bluish) were found in BB250-BB1000. Color alteration from positive *b* values to negative *b* values means that the BB solution turned the egg colors from yellow to a bluish color. Negative *b* values of egg samples significantly ($P<0.05$) decreased in parallel with the increase in BB concentration up to the 1000 ppm.

Table 1. Colour (*L*, *a* and *b*) values of rainbow trout fish egg containing brillant blue.

Samples*	*L (*lightness)	*a* (redness)	*b* (yellowness)
BB0	59.74±2.81	5.47±0.13	21.07±0.41
BB50	31.86±1.23	-9.72±0.92	2.19±0.68
BB100	24.35±2.18	-8.24±0.18	3.00±0.77
BB250	25.20±1.38	-7.92±0.25	-10.08±0.54
BB500	19.06±1.29	-4.01±0.28	-11.95±0.32
BB750	17.72±1.01	-2.57±0.17	-16.42±0.54
BB1000	17.23±1.25	-1.86±0.09	-18.07±0.65

* BB0-BB1000: Eggs immersed in 0-1000 ppm of BB solution.

Optic surface image analysis of rainbow trout fish egg containing brilliant blue colorant

Images of freeze-dried rainbow trout eggs containing brilliant blue colorant were given in Figure 1. As seen in Figure 1, the yellow color of eggs was turned from yellow to blue. Lower concentrations (50 and 100 ppm) of BB solution gave green color to eggs. The color of eggs immersed in 250 ppm BB solution (BB250) were greenish-bluish color. Higher concentrations of immersing solution (500-1000) changed egg colors from light bluish to dark blue. Colors of BB750 and BB1000 samples were almost the same (dark blue).

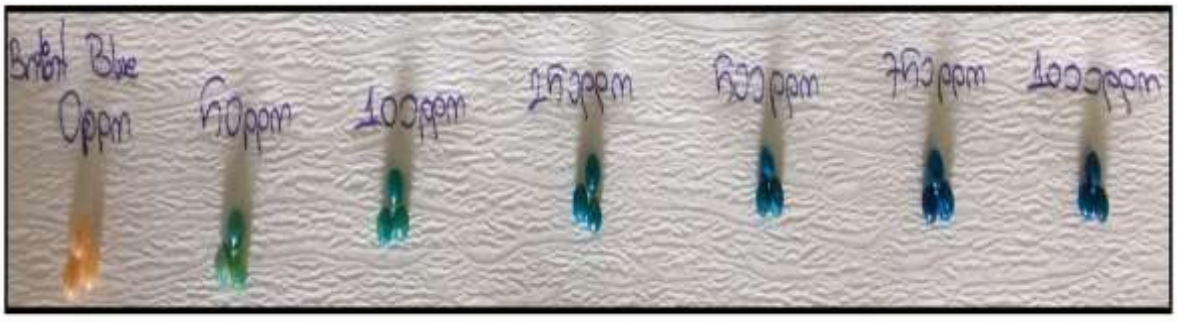
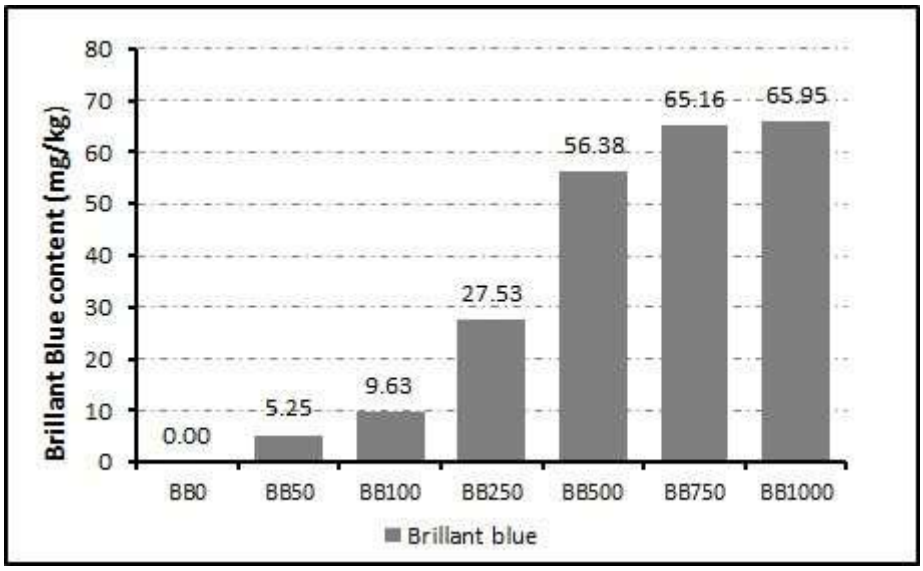


Figure 1. Images of rainbow trout eggs containing brillant blue colorant

Brilliant blue analysis of freeze-dried rainbow trout fish eggs

The brilliant blue content of freeze-dried rainbow trout fish eggs were given in Figure 2. Producing a bioencapsule for envelopment and protection of bioactive substances starts with the selection of an adequate capsulation material (de Vos et al., 2009). Rainbow trout fish eggs could be delivering vehicle to uptake of bioactive compounds in to body with its bioencapsulation property. Thus brilliant blue, water-soluble food colorant, was selected to determine trout eggs bioencapsulation capacity. As seen in Figure 2, BB content of freeze dried eggs samples significantly ($P<0.05$) increased in parallel with the increase in BB concentration up to the 750 ppm of BB solution (BB750). There was no significant difference between BB contents of BB750 and BB1000. It can be postulated that maximum 65-66 mg/kg BB could be intake into the trout egg. Aquatic organisms such as artemias (Ates et al., 2015),

daphnia (Embregts et al., 2018), rotifers (Manickam et al., 2019) are served as bioencapsulation vehicles for delivering of bioactive compounds.



* BB0-BB1000: Eggs immersed in 0-1000 ppm of BB solution.

Figure 2. Brillant Blue content of rainbow trout fish eggs containing brilliant blue colorant

Conclusions

The results of this study reveal the rainbow trout fish egg could be used in bioencapsulation and delivering of water-soluble bioactive compounds. In this study brilliant blue colorant successfully used as a marker for the determination of bioencapsulation capacity of trout egg. Immersing in trout eggs into the solution containing water-soluble bioactive compound and applying of freeze-drying method to eggs could be a novel method for encapsulating and delivering of bioactive compounds.

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PROPETIES OF SOME PLANTS USED AS FOLK MEDICINE BY TYPE 2 DIABETES MELLITUS PATIENTS

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Abstract

Diabetes mellitus (DM) is described by World Health Organisation as "Chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves". Oral antidiabetic agents, insulin, diet and lifestyle changes are conventional therapy in DM. However, patients also prefer complementary therapies including plants. In literature, there are various studies on this subject. Culture, geographic location, religion etc. affect the type of plants commonly used. According to the studies, the most widely used plants are *Nigella sativa, Cinnamomum sp, Prunus Amygdalus amara, Thymus vulgaris,* and *Olea europaea L.* folium. Bioactive compounds (thymoquinone, cinnamaldehyde, catechin, epicatechin, cyaniding, procyanidin etc.) found in these plants have anti-diabetic properties. In vitro and experimental animal studies have shown these plants improve fasting glucose, HbA1c and insulin sensitivity, stimulate insulin secretion, and inhibit carbohydrate digestion. There are also interventional studies indicating the beneficial effects of the folk medicine plants on DM patients but due to the lack of strong research evidence, there is no primary recommendation for usage in the management of diabetes.

Keywords: *Type 2 diabetes mellitus, plants, folk medicine.*

Introduction

Diabetes mellitus (DM) is described by World Health Organisation (WHO) as "Chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves". According to global report 422 million people have diabetes and 1.6 million people die due to diabetes (WHO, 2018 Treatment of type 2 DM consist of lifestyle intervention (education of patients, nutrition, exercise, self-monitoring of blood glucose and pharmacotherapy (insulin sensitizers with predominant action in the liver, insulin sensitizers with predominant action in peripheral insulin-sensitive tissues, insulin secretagogues, carbohydrate absorption inhibitors, incretin-based therapies, sodium glucose co-transporter 2 inhibitors and insulins) (Dungan, 2016). Treatment failure causes high blood glucose levels. This situation increases the risk of some complications like cardiovascular events, nephropathy (Domingueti et al., 2016).

In recent years, the number of studies on the use of plants in the treatment of diseases has increased. And patients also tend to use natural products. In Turkey, there are various studies on this issue. Prevalence of using folk medicine is 26.9-88.1%. The most widely used plants are *Nigella sativa, Cinnamomum sp, Prunus Amygdalus amara, Thymus vulgaris*, and *Olea europaea L.* Folium (Candar et al., 2018; Ceylan et al., 2009; Ilhan et al., 2016). The aim of this review is to provide detailed information about folk medicine used in diabetes mellitus.

Material and Methods

The paper is based on an extended review of "*Nigella sativa, Cinnamomum s.p, Prunus Amygdalus amara, Thymus vulgaris*, and *Olea europaea L.* folium and type 2 DM". The literature search was done in the PubMed database. The search terms were the following combination of keywords: "*Nigella sativa*" AND "Type 2 diabetes mellitus", "C*innamomum*

sp" AND "Type 2 diabetes mellitus", *"Prunus Amygdalus amara*" AND "Type 2 diabetes mellitus", "*Thymus vulgaris*" AND "Type 2 diabetes mellitus" and "*Olea europaea L.* folium" AND "Type 2 diabetes mellitus". The data search was restricted to the last 10 years from 2008 to 2018.

Results and Discussion

***Nigella sativa* (black seed or black cumin):** It belongs to the Ranunculacea family. Black seeds and their oil have been used for more than 2000 years especially in the Indian and the Arabian civilizations for treatment of a variety of diseases in the respiratory system, digestive tract, kidney, liver cardiovascular system, and immune system (Tavakkoli et al.,2017). Hazrat Rasool Allah (PBUH) said, "Let all the black seed upon you, these contain cure of all diseases except death" Chemical composition of *Nigella sativa*: 32-40% fixed oil, 0.4-0.45% volatile oil, 16-19.9% protein, 33.9% carbohydrates, 5.5% fiber, 6% water and 1.79-3.74% minerals (Ijaz et al., 2017).

Nigella sativa contains many active compounds: nigellicine, nigellidine, thymoquinone (TQ), dithymoquinone, thymol, and carvacrol (Gholamnezhad et al., 2016). According to Table 1, 2 g/day *Nigella sativa* supplementation with anti-diabetic medication has improved fasting blood glucose, HbAlC etc. in type 2 DM patients. And in the studies, no adverse effects were recorded.

It has been determined that TQ has the main role to improve glucose metabolism in type 2 DM. The possible mechanisms of TQ and other active compounds on type 2 DM: improves body antioxidant defense, increases insulin secretion, decreases gluconeogenesis, and reduces glucose absorption and body weight (Heshmati and Namazi, 2015).

Cinnamomum sp: Cinnamon has been used as both a spice and a flavoring agent. In Asian countries, it has had popularity as an herbal medicine for the treatment of DM (Necyk and Zubach-Cassano, 2017). There are main varieties; *Cinnamomum zeylanicum and Cinnamon cassia* (also known as *Cinnamomum aromaticum*/Chinese cinnamon). *Cinnamomum zeylanicum* is also known as Ceylon cinnamon (the source of its Latin name, zeylanicum) or 'true cinnamon'. One important difference between Cinnamon cassia and *Cinnamomum zeylanicum* is their coumarin content. The levels of coumarins in *Cinnamon cassia* appear to be very high and pose health risks when consumed regularly in higher quantities. Coumarins are secondary phytochemicals with strong anticoagulant, carcinogenic and hepato-toxic properties. And it has been reported *Cinnamomum zeylanicum* contains 'hardly any' coumarin (Ranasinghe et al., 2013). The European Food Safety Authority advocates against the regular, long-term use of *Cinnamon cassia* as a supplement due to its coumarin content (EFSA, 2008). Cinnamaldehyde, cinnamic acid, eugenol, coumarin, and procyanidins are major components of cinnamon (Azimi, Ghiasvand, Feizi, Hariri, & Abbasi, 2014). Cinnamon polyphenols display insulin-like properties and stimulate glucose uptake in skeletal muscle and adipose tissue (Akilen et al., 2012). According to Table 2, clinical studies on Cinnamomum sp. and Type 2 diabetes mellitus do not have standard features (sample size, dosage, duration etc.). And results are conflicting. When 120 mg/day and 1 g/day cinnamon supplementation have improved HbAlC and fasting blood glucose, 3 g/day cinnamon supplementation has shown no beneficial effect. In the Cochrane review, it has been reported there is insufficient evidence to support the use of cinnamon for type 1 or type 2 DM (Leach and Kumar, 2012).

***Prunus Amygdalus amara (*bitter almond):** Almonds are the good dietary source of unsaturated fatty acids, fiber, vegetable protein, α-tocopherol, manganese, magnesium, copper, phosphorus. Almonds also contain an array of phytonutrients including phenolic acids, phytosterols, and polyphenolic compounds such as flavonoids and proanthocyanidins (Kamil and Chen, 2012). Clinical studies about bitter almond and type 2 DM are limited. However, almond supplementation (20% of energy intake) during 12-24 weeks has improved

glycemic control and lipid profiles in patients with type 2 DM (Gulati et al., 2017; Li et al., 2011). Incorporation of almonds in a well-balanced healthy diet could lead to beneficial effects in patients with type 2 DM.

***Thymus vulgaris* (thyme):** It is commonly used as a culinary herb and different medicinal purposes (treatment of disorders affecting the respiratory, digestive, cardiovascular, and nervous systems) (Salehi et al., 2018). It has been reported thyme has the antioxidative activity which better than α-tocopherol and BHA. Thyme is rich in phenolic acids and flavonoids (Roby, Sarhan, Selim, & Khalel, 2013). However, clinical studies about thyme and type 2 DM are limited.

***Olea europaea L. folium*:** *Olea europaea* is a traditional Mediterranean plant and its fruit and oil are frequently consumed for the healthy diet. It's also known as the symbol of abundance, glory, and peace. Olive oil contains monounsaturated fatty acids, phenolic compounds etc., so most people prefer to consume it (Uylaser and Yildiz, 2014). Oleuropein is the most abundant bioactive component and its quantity can reach up to 60-90 mg per gram of dry olive leaf (Ghanbari et al., 2012; Sahin and Bilgin, 2018). In a clinical study with type 2 DM patients, it has been shown 500 mg oral olive leaf extract supplementation during 14 weeks improved HbA1c and fasting plasma insulin levels. According to the results obtained from the study of experimental animals olive leaf extract without olive leaf reduced digestion and absorption of starch(Wainstein et al., 2012).

Table 1. Clinical studies on Nigella *sativa* and Type 2 diabetes mellitus

Participants	Intervention duration/Doses	Control	Outcomes	Adverse Effect	Reference
94 patients with type 2 DM treated with anti-diabetic medication	3 months Group 1: 1 g/day Group 2: 2 g/day Group 3: 3 g/day *Nigella sativa* capsule	-	<u>In group 2</u> Fasting blood glucose↓ HbAlC↓ Insulin resistance B-cell function↑ <u>In group 1 and 3</u> No significance improvement	No record of any side effect	(A. O. Bamosa, Kaatabi, Lebdaa, Elq, & Al-Sultanb, 2010)
114 patients with type 2 DM treated with anti-diabetic medication	3 months (n:57) 2 g/day *Nigella sativa* capsule	3 months (n:57) activated charcoal	<u>In intervention group</u> Fasting blood glucose↓ HbAlC↓	No record of any side effect	(Kaatabi et al., 2015)
60 patients with type 2 DM treated with standard oral hypoglycemic drugs	12 months (n:30) 2 g/day *Nigella sativa* capsule	12 months (n:30) activated charcoal	<u>In intervention group</u> HbAlC↓	No record of any side effect	(A. Bamosa et al., 2015)

Table 2. Clinical studies on Cinnamomum sp and Type 2 diabetes mellitus

Participants	Intervention duration/Doses	Control	Outcomes	Adverse Effect	Reference
59 patients with type 2 DM	3 months 1.2 g/day cinnamon capsules	Microcrystalline cellulose	Compared to control and baseline no improvement in fasting blood glucose HbA1c	No information	(Wainstein, Stern, Heller, & Boaz, 2011)
66 patients with type 2 DM treated with oral hypoglycemic agents	3 months Group 1: 120 mg/day Group 2: 360 g/day cinnamon tablets	Placebo tablets	Compared to baseline in group 1 and 2 HbAlC↓ fasting blood glucose↓	No record of any side effect	(Lu et al., 2012)
105 patients with type 2 DM treated with oral hypoglycemic agents	3 months Group 1: 1 g/day cinnamon capsules Group 2: 1 g/day whortleberry capsules	Starch	In group 1 HbAlC↓ Body mass index↓	No information	(Mirfeizi et al., 2016)
44 patients with type 2 DM treated with oral hypoglycemic agents	2 months 3 g/day *Cinnamomum zeylanicum* capsules	Microcrystalline cellulose	No improvement in fasting blood glucose HbA1c HOMA-IR carboxymethyl lysine levels	No record of any side effect	(Talaei et al., 2017)

Conclusions

There are interventional studies indicating beneficial effects of the folk medicine plants on DM patients but lack of strong research evidence of herbal medicines on diabetes outcomes does not allow for their inclusion by American Diabetes Association as the primary recommendation in the management of diabetes (ADA, 2018). It is the right approach to ensure and encourage compliance with medical treatment, diet, and exercise for diabetic patients.

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EFFICIENCY OF SOME BACTERIAL ISOLATES AGAINST GREEN SHIELD BUG (*PALOMENA PRASINA* L., HEMIPTERA: PENTATOMIDAE)

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Abstract

Green shield bug (*Palomena prasina* L., Hemiptera: Pentatomidae) is a quite common pest species in Turkish hazelnut orchards. *P. prasina* causes severe loss of yield by damaging quality and quantity in the edible parts of the hazelnut. Usually, chemical control methods are applied against this pest. Due to the adverse effects of insecticides on the environment and human health, alternative control methods are gaining importance, and microbial control methods are taking importance in field control actions. For this purpose, four different entomopathogenic bacterium isolates 271a (*Bacillus thuringiensis*), Akçakoca2 (*Bacillus megaterium*), HMA5 *(Bacillus megaterium*), FPN2 (*Pseudomonas putida*) were applied against nymphs and adults of *P. prasina* under laboratory conditions (24° C, 70% humidity). The study was conducted in two iterations and three replications, and each replication was composed of 5 individuals of the same age and was observed for 12 days. All isolates were used at 10^8cells ml $^{-1}$ concentration. Per cent effects were calculated via the Abbott formula. In the study, Akçakoca2 and FPM2 isolates were found to be 100% effective, followed by HMA5 isolate at 96% and 271a isolate at 73%. When the effects of entomopathogenic bacterial isolates against the adults were examined, it was found that Akçakoca2 isolate was 75% effective, followed by 271a isolate at 70%, FPM2 isolate at 50%, and HMA5 isolate at 41%. As a result, Akçakoca2 isolate was identified as the most effective against both adults and nymphs of *P. prasina*, having an effectiveness close of that of the chemical. All of the isolates used were found to be more active on nymphs than on adults.

Keywords: *Biological control, Palomena prasina, Bacillus thuringiensis, Bacillus megaterium, Pseudomonas putida*

Introduction

With its 600,000 hectares of production area and approximately 600,000 tons/year production, hazelnut is one of Turkey's most important export products that sums an annual average exchange input of about 2,5 billion dollars. Turkey covers 65-75% of world hazelnut production and 70-75% of total exports. There are about 400,000 families, in other words, about 2 million persons, who takes an income from hazelnuts production (Bozoğlu, 2001; Anonymous, 2006). Some of the insect species that are harmful to hazelnuts indirectly affect the product by harming different plant tissues, while others have a more direct effect like feeding on the fruits. Species belonging to the Pentatomidae (Hemiptera) are also included in the fruit-damaging group. These pests cause the total yield and product quality to decrease by feeding on nuts (Kurt, 1975; Tuncer et al., 2002). The green shield bug [*Palomena prasina* L. (Heteroptera: Pentatomidae)] is the most critical fruit-damaging species, and it dramatically surpasses other pests because the damage level that inflicts in all hazelnut orchards. (Işık et al., 1987; Tuncer et al., 2005). Feeding on the fruit's early stages causes empty shells, deformed fruits and shedding while feeding on the fruit after its inner bonding period causes spotting. The spotting damage is sometimes up to 20% in hazelnuts and creates significant problems in export (Tuncer et al., 2005). The green shield bug is reported as the most harmful hazelnut pest in Italy also, where it builds-up high populations (Tavella et al., 2002). *P. prasina* is a polyphagous species and is found in many regions in Turkey, especially in

hazelnut production areas. Its harm to other plants is insignificant and has 1 generation per year (Lodos, 1982; Saruhan, 2004).

Biological methods can be an alternative in the control of *P. prasina*. Several antagonist microorganisms have been successfully used to control pests in agricultural areas, as *Bacillus thuringiensis, B. megaterium and Lysinibacillus sphaericus*. *Bacillus megaterium* is considered an important entomopathogen in the control of insects (Aksoy ve Ozman-Sullivan 2008) also because of its extensive habitat (Gu et al. 2007, Patricia et al. 2007). Although bacteria are used by many researchers in the biological control against many pests (Sezen et al., 2004, Bursova et al., 2006, Gökçe et al. 2010, Özşahin et al. 2014), the application against *P. prasina* if overlooked. Four different isolates of *B. megaterium* were used against *P. prasina* and at the end of day 12, some of the isolates were found to be 98% effective (Aksoy et al., 2018).

This study was conducted to determine the effect of some bacterial isolates on *P. prasina* adults and nymphs, in laboratory conditions.

Material and Methods

Palomena prasina adults and nymphs used in the experiment were collected from different hazelnut production areas of Samsun province and breeding were managed in the laboratory on fresh beans. The four isolates of the bacteria used in the experiment were obtained from the bacteriology laboratory in the Plant Protection Department of the Faculty of Agriculture in Ondokuz Mayis University (Table 1).

Table 1. Isolates of entomopathogenic bacteria used in this study.

Isolate denomination	**Entomopathogenic bacteria isolates**
271a	*Bacillus thuringiensis*
Akcakoca2	*Bacillus megaterium*
HMA5	*Bacillus megaterium*
Fpin2	*Pseudomonas putida*

The study was carried out in laboratory conditions (24°C, 70% humidity) and in 10x10x10 cm plastic containers, making sure that the nymphs and adults used in the study were of the same age. The study was conducted in 3 iterations and repeated twice. Each iteration contained five insects. These insects were fed with fresh beans. The study was carried out by spraying suspensions (2 ml) prepared from entomopathogenic bacterial isolates at a dose of 10^8 cells/ml. Containers in which only water was sprayed were considered as the control group. The study was checked daily for 12 days, and mortality numbers were noted.

Results And Discussion

When Akçakoca2 and Fpin2 isolates were applied against *Palomena prasina* nymphs in the study, mortality started from the second day and reached 100% at the end of day 12. HMA5 isolate followed Akçakoca2 and Fpin2 efficacy, at 96.67%. The less effective isolate against nymphs was 271, summing a 73.3% mortality. All isolates used against the *P. prasina* nymphs in laboratory conditions were found to be highly effective (Figure 1).

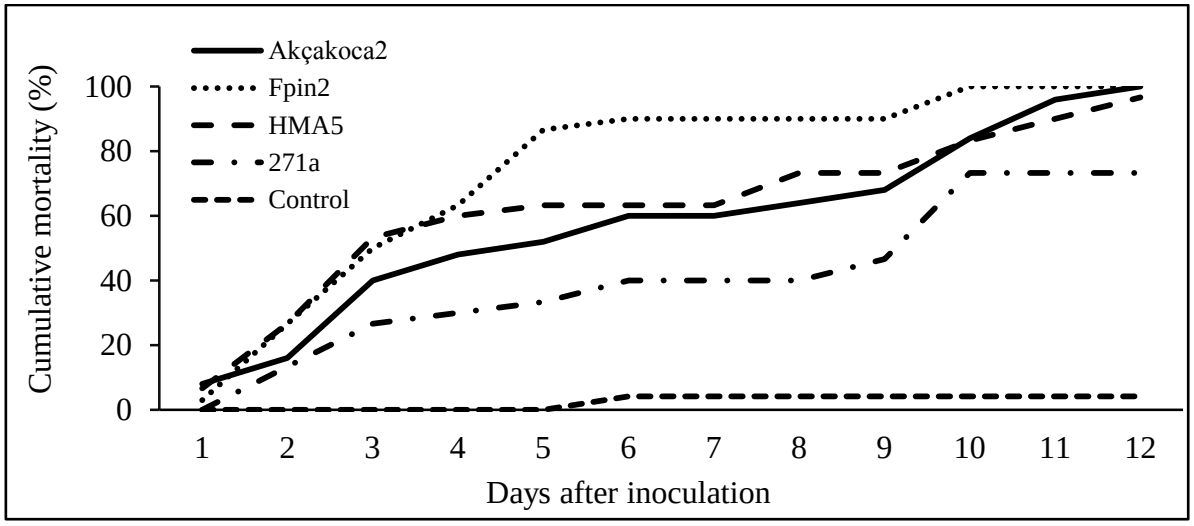

Figure 1. Cumulative mortality of nymph of *Palomena prasina* after inoculation with different isolates of entomopathogenic bacterial isolates.

Several studies have been carried out on the insecticidal and nematocidal effects of *B. megaterium*. Khyami-Horani et al. (1999) reported that *B. megaterium* populations were highly toxic to the 4th instar larvae of *Culiseta longiareolata* (Diptera: Culicidae). Aksoy and Ozman-Sullivan (2008) reported that *B. megaterium* isolates were effective against *Aphis pomi* (Hemiptera: Sternorrhyncha: Aphididae) causing 92% -100% mortality five days after application. This bacterium has also been used against nematodes. In another study, *B. megaterium* SAkc-2 isolate was shown to be highly pathogenic for the 4th period nymphs of *P. prasina.* SAkc-2 isolate showed 98% efficacy after 12 days of application (Aksoy et al. 2018).

The same isolates were tested against the adults of *P. prasina* and had a lower effect than on the nymphs. At the end of day 12, the highest effect was observed with Akçakoca2 isolation at 75%, followed by 271a isolate at 70.83%, Fpin2 isolate at 50.00% and HMA5 isolate at 41.67% (Figure 2).

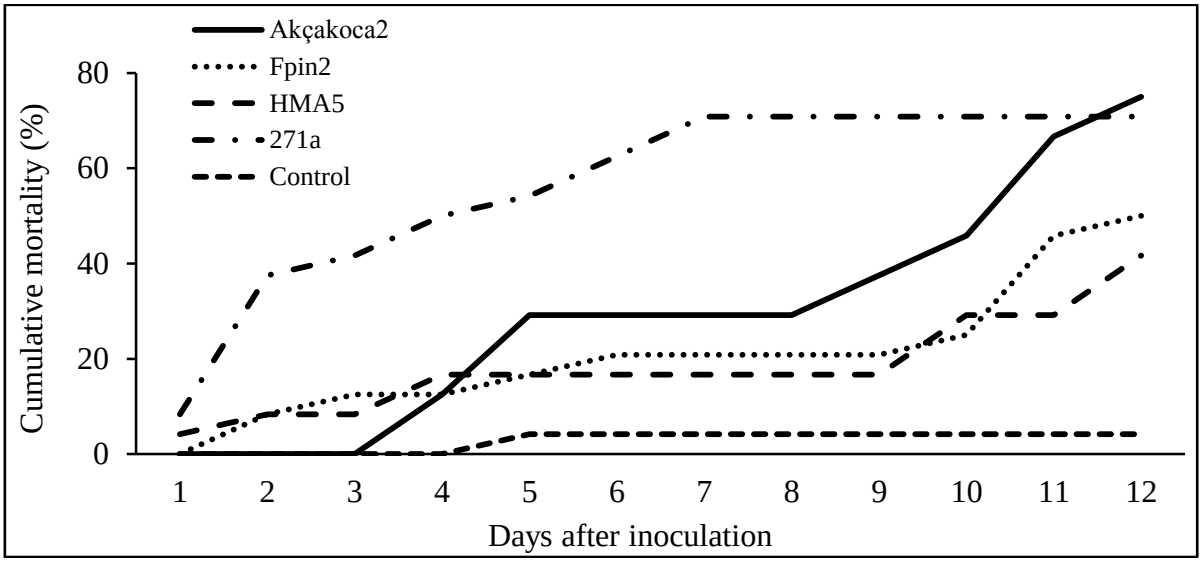

Figure 2. Cumulative mortality of adults of *Palomena prasina* after inoculation with different isolates of entomopathogenic bacterial isolates.

Conclusions

Our results are consistent with the effect of some *B. megaterium* isolates used as entomopathogenic bacteria against different insects and nematodes (Padgham, Sikora 2007; Aksoy and Ozman-Sullivan 2008; Huang et al. 2010). We suggest that Akçakoca2 isolate will be a potential biocontrol agent against the adults and nymphs of *P. prasina*. However, isolates Fpin2 and HMA5 have been identified as a potential biological control agent in the control of pest nymphs.

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DETERMINATION OF THE EFFECTIVENESS OF COLOR AND PHEROMONE TRAPS IN APPLE FLOWERS FEEDING HAIRY BEETLE (*Tropinota hirta* Poda) CONTROL

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Abstract

Tropinota (*Epicometis*) *hirta* Poda (Coleoptera: Scarabaeidae) damages reproductive parts of flowers of several fruit trees, some vegetables, and many ornamental bushes. The hairy beetle overwinters as larvae and ecloses in soil. Adult beetles emerge in time for spring fruit trees blooming. This beetle is active during the day, concealing near soil to overnight. The control of hairy beetle is complicated, and insecticides cannot be used due to the impact on the honeybees, pollinators, and other beneficial insects. Traps, as chromotropic traps, alone or in combination with a pheromone lure, are a method for the control of hairy beetles while adult. We used picasso blue (#0000FF) traps lured by pheromone in several combinations: 1) clear/no pheromone; pheromone clear; colour/no pheromone, and) and colour/pheromone. Each traps were assembled with a 22 cm wide funnel, eventually painted with Picasso blue, connected with the neck of a 5-litre colourless plastic bottles filled with water (1 litre). The combination of Picasso blue funnel + pheromone lure was the most effective in hairy beetle control. Total caught hairy beetle counts: 351.33 for Picasso blue + pheromone treatment, 282.67 for Picasso blue treatment, 13.67 for pheromone treatment and 2.33 for the control. The maximum of hairy beetle catching was in the fifth week (17-24.04.2017); number of hairy beetles caught between 3rd and sixth weeks was high. Use of the funnel traps is an important method of harmful keep under control.

Keywords: *Apple, Konya, Blue, Attractant trap, Biotechnical control.*

Introduction

Turkey is among the world's leading fruit producers with a total of 20.321.777 tons (TUIK, 2016). Because Turkey has different climate and soil characteristics, fruit production is performed in almost all regions. According to the data from 2016, annual fruit production in Konya, where the study was conducted is about 314.873 tons. Apple takes the most significant production tonnage (101.081 tons) compared to other fruits (TUIK, 2016). In Konya, although fruit growing is an essential agricultural production sector, the producers have problems with cultivar selection, irrigation, harvesting, preservation, and marketing. In addition to these factors, many plant diseases and pests also affect production negatively (Eşitken et al., 2012)

Scarabaeids is a significant group of insects that contain many pests. Adults of many species are phytophagous and fed with leaves, flowers and roots of cultivated plants (Hurpin 1962). *Tropinota hirta* Poda is a member of this group and is one of the most important pests causing yield loss by feeding with flower parts of plants. This beetle damage the reproductive parts of flowers of several fruit trees, some vegetables, and many ornamental bushes (Endrödi, 1956; Homonnay and Homonnayne-Csehi, 1990). In a study conducted in Hungary, it was determined that *T. hirta* adults cause significant yield losses by feeding with flowers (Rackskō et al., 2007). The pest also causes damage to forest trees and cereals. (Hurpin, 1962; Zashev, Keremedchiev, 1968**).** Hairy beetle survives to overwinter as larvae and matures under the soil. Hairy beetle caterpillars usually live alone, and in this period they are fed with wood,

dead leaves and annual plant roots **(**Demir, 2005). Arslan and Aslan (2015) found that the population density of *Epicometis hirta* adults increased from the end of March to the middle of April in the almond trees of Kahramanmaraş province. Mature beetles emerge with the bloom of fruit trees in spring. Adult hairy beetles cause the most damage to plants at the end of the spring (Özbek et al., 1998; Özbek, 2008).

Adults of the beetle begin to come out of the soil in the spring and are fed by the flowers of fruit trees, ornamental plants, grains, and forest trees. They feed with male and female organs of flowers, and the damaged flowers cannot produce fruit. As a result of a study in the cherry garden in Bulgaria, adult hairy beetles were found to be fed with flowers of cherry trees and cause significant crop loss (Kutinkova and Andreev, 2004). Control of the hairy beetle is complicated, and insecticides cannot be used due to the damage on honeybees and other beneficial insects. In a study which determines the density of hairy beetle in peach, apple, cherry and apricot orchards in Çanakkale, blue funnel, pit traps, sticky plates, and cinnamyl alcohol + trans-anethole (1:1) mixture were used. As a result of the study, the most number of hairy beetle adults were caught in traps where the blue bowl + attractant was used (Gezer and Özpınar, 2015).

Toth et al. (2003; 2009), used an attractive mixture (cinnamyl alcohol and anethole) with blue and white colour traps in their study. As a result of the study, adult hairy beetles were caught in the cinnamyl alcohol and anethol traps. However, Kara (1995), reported that it was challenging to control this pest because it damages the flower. In such cases chemical control can be used.

In the study conducted on the apple and cherry gardens in the Sultandağı district of Afyonkarahisar province; it was determined that the pest could be kept under control mostly when the blue funnel and the attractant were used over the flowering period of the plants (Sağdaş, 2010). The catch rate of adult hairy beetles seemed to increase with white traps before and after blooming and with blue traps during blooming (Aydın, 2011). A study was conducted in Bulgaria to determine the seasonal population change of *Tropinata hirta*. A colour tone similar to the Picasso blue was used in the study (VARb3k). As a result of this study, it has been demonstrated that this colour is a method to control adult hairy beetles (Mitko et al., 2011). Uysal (2011), used Picasso blue as a trap in a study on plum and apricot trees in Isparta and reported the colour to be a critical biotechnical control to catch adult hairy beetles.

This study was conducted to determine the rate of hairy beetle adult catching using blue funnels and pheromone traps.

Material and Methods

This study was carried out on fruit orchard of Braeburn apple cultivar grafted on M9 and MM106 clonal rootstocks at Research and Application Orchard of Department of Horticulture, Faculty of Agriculture at Selçuk University in 2017. In the study, Picasso blue and pheromone (against hairy beetles) were used as traps. Funnels (14 cm top-wide, 9 cm height, 3 cm hole-wide) were painted with Picasso blue and placed on 5-litre colourless bottles with water inside. Large holes were opened in the bottle to prevent water from overflowing in case of rain and loss of beetles caught. The pheromones used in the study were connected to the funnels by wire and placed in the middle of the funnel. The pheromones in the trap have regularly been changed on a monthly basis. The bottles were checked once a week. The adult hairy beetles in the water were removed from the water using a strainer and counted. Then captured adult hairy beetles were removed from the orchard.

The study was set up according to randomised complete blocks trial design with three replications. In the study, control (no colour, no pheromone), pheromone (no colour), Picasso blue (no pheromone) and Picasso blue + pheromone treatments were tested. The experimental

data were collected and subjected to analysis of variance (ANOVA) using SPSS v.20 (IBM Corp. 2011. Armonk, NY) statistical software. The means were separated by Duncan's multiple test at $p \leq 0.05$ level.

Results and Discussion

Traps in the study were established at Selçuk University apple orchard on 13.03.2017. Traps were checked regularly, once a week. The first hairy beetle adults were caught on 23.03.2017. The study showed that blue + pheromone traps are more catching over the whole week than other traps. During all weeks, a total of 1950 hairy beetles were caught. 1054 of this number were caught in traps where blue + pheromone was used. Toth et al. (2003) reported that the number of captured hairy beetle adults increased significantly in traps used with attractant. Besides, Vuts et al. (2009) reported that the attractant they used (trans-anethole) had a remarkable effect in catching adult hairy beetles. In the study; a small number of hairy beetles were caught in all treatments in the first weeks. Climatic conditions have a relevant influence on the emergence of this situation. Because studies have shown that the population density of hairy beetle adults decreases during rain, low temperature or closed weather (Kara, 1995). After that, most of the adults were caught in traps where only blue colour was used (848 hairy beetle adults). This shows that adult hairy beetles can be caught using blue traps without using any attractors. The maximum number of hairy beetle was caught in the fifth week (17-24.04.2017) in all treatments. The number of hairy beetles caught between 3rd and sixth weeks was the highest and beetle caught in other weeks can be ignored. It is thought that most of the beetles were caught in this period because the apples had the most bloom. Also, in the control groups, the number of hairy beetles caught in all weeks of the study was less than the other traps. It has been determined that the differences in the number of beetles caught between the practices be statistically significant up to the 10th week ($p \leq 0,05$). At weeks 11 and 12, the difference between treatments at all traps was insignificant he low catches rate over the last weeks may be due to a limited number of flowers in apple trees (Table 1.).

Table 1. Statistical analysis of trapped hairy beetle numbers

	Control	**Pheromone**	**Blue**	**Blue+Pher.**	**P**
20.03.2017	$0,00 \pm 0,00^{b}$	$0,00 \pm 0,00^{b}$	1,67 ± 1,53b	4,0 ± 1,00a	0,002
27.03.2017	$0,00 \pm 0,00^{d}$	2,00 ± 1,00c	11,67 ± 1,53b	15,00 ± 1,00a	0,000
03.04.2017	$0,00 \pm 0,00^{b}$	1,00 ± 1,00b	54,00 ± 2,65a	57,00 ± 3,00a	0,000
10.04.2017	$0,33 \pm 0,58^{c}$	1,00 ± 1,00c	83,33 ± 7,64b	95,67 ± 3,06a	0,000
17.04.2017	0,33 ± 0,58c	1,67 ± 2,09c	90,00 ± 3,00b	111,00 ± 7,55a	0,000
24.04.2017	0,67 ± 1,15c	1,33 ± 1,53c	32,00 ± 9,85b	47,67 ± 6,43a	0,000
02.05.2017	$0,00 \pm 0,00^{c}$	1,67 ± 1,53c	5,33 ± 2,89b	$9,00 \pm 0,58^{a}$	0,001
08.05.2017	0,67 ± 0,58b	2,33 ± 1,16b	2,67 ± 0,53b	$5,00 \pm 0,58^{a}$	0,010
15.05.2017	0,33 ± 0,58b	1,67 ± 1,15b	1,67 ± 1,15b	4,00 ± 1,00a	0,013
22.05.2017	$0,00 \pm 0,00^{b}$	0,67 ± 1,16ab	0,00 ± 0,00b	1,67 ± 0,58a	0,040
29.05.2017	$0,00 \pm 0,00^{a}$	0,00 ± 0,00ab	0,33 ± 0,58a	1,00 ± 1,00a	0,193
05.06.2017	$0,00 \pm 0,00^{a}$	0,00 ± 0,00ab	0,33 ± 0,58a	0,33 ± 0,58a	0,596
Total	2,33 ± 2,31c	13,67 ± 5,51c	282,67 ± 12,74b	351,33 ± 14,84a	0,000

In the present study, the number of adult hairy beetles started to appear towards the end of March, reaching the highest level in the middle of April. In other studies, these months were

the period when the hairy beetle population was at the highest level (Hurpin,1962). There were significant differences between treatments in the 4th and fifth weeks. As we already mentioned, this situation is thought to be due to plants being in the flowering period. Compared with other treatments (Control, Pheromone, Picasso blue), many adults in traps when blue + pheromone used was at the highest level in the mentioned weeks. There has been a decrease in the adult population after the 6th week. Moreover, the 11th and 12th weeks, the population of adults was at a level that can be ignored (Fig.1.).

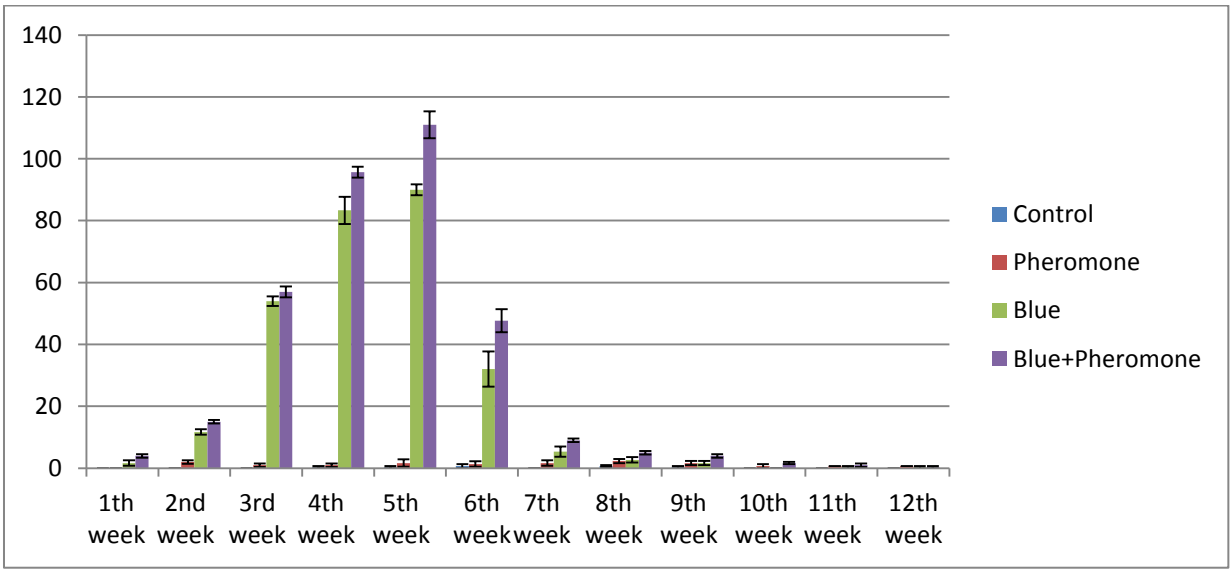

Fig.1. Number of adult *T. hirta* captured in traps

Uysal (2011) also used an attractant with a bottle filled with water and a blue funnel in his work in Isparta. He has reported that the use of attractant with blue colour is an effective method of catching adult hairy beetles. Similarly, Sağdaş (2010) has also used a blue colour (parliament blue) and attractant in his work and reported that this method is useful in insect catching. The blue colour used in these studies is a colour close to the colour we used. In this study, the most significant number of adult hairy beetles were caught in blue color+pheromone treatment as in the other studies mentioned above.

Mitko et al. (2011), used the colour referred to as 'deep royal blue' to study the seasonal fluctuation of *Epicometis hirta* Poda in Bulgaria, which is similar to the colour we used in this study. They reported that the colours they used were an effective method for catching pest. Also, Aydin (2011) used different tones of blue in his study to capture the pests. As a result of the study, 6,57 individuals in 'dark blue' trap and 10,53 individuals in 'light steel blue' trap were caught. However, the dark blue colour, which is the closest colour to Picasso blue in the -study mentioned above, caught less adult hairy beetles.

Conclusions

In this study, blue color+pheromone treatments were found to be more effective in catching adult hairy beetles compared to all other treatments (Blue, Control, Pheromone). Besides, it has been determined that only blue colour treatments have more effective results than control.

In the present study has shown that blue traps are an effective biotechnical method in the control of adult hairy beetles. According to the results obtained, it is possible to keep the adult hairy beetles under pressure by using only blue color without using any attractant or any chemical material. Thus the natural balance would be preserved.

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PROSPECTIVE USE OF BACTERIOPHAGE ISOLATES AS AGENTS OF PHAGE THERAPY AND PLANT PROTECTION FROM PHYTOPATHOGENIC BACTERIA

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Abstract

Bacteriophages, as natural antagonists, have considerable potential for developing methodological approaches to control the spread of bacterial pathogens. Bacteriophages have a number of advantages over other control: they are highly specific to host bacteria, non-toxic to macro organisms, non-pathogenic for common microflora of plants and soil. Therefore, the main objective of the study was to isolate and identify bacteriophages that were specific to phytopathogenic bacteria. Samples of beets, potatoes, apples, garlic and mandarins with symptoms of rot were selected from vegetable storehouses. The main symptoms of bacterial lesions observed in fruits were areas of mild decay, softening of the tissues, and the extraction of the exudate. As a result of this work we isolated phages specific to Pseudomonas syringae lachrymans 7591, P. fluorescens 8573, P. savastanoi pv. *phaseolicola* 4013 and Serratia marcescens sp. Bacteriophages led to the formation of negative colonies which were separated into three groups based on plaque size: large (6 mm), medium (3 mm) and small (1 mm). To identify host range specificity of isolated phages, spectrum of lytic activity was studied against 9 strains of phytopathogenic bacteria. Our research revealed that among 14 phage isolates, five expressed lytic activity against different strains of phytopathogenic bacteria. Obtained data indicate that isolates with a broad spectrum of lytic activity can be used as promising biological agents in control of bacteriosis. Consequently, isolated bacteriophages can be considered as phage therapeutic agents.

Keywords: *plant pathogenic bacteria, morphological features, bacteriophages.*

Introduction

Bacteriophages, the viruses of bacteria, have received increased research interest in recent years as a genuine environmentally friendly means of controlling bacterial diseases. Phage biocontrol possesses advantages over chemical controls since tailor-made phage cocktails can be adapted to target specific disease-causing bacteria (Buttimer et al., 2017). They show specificity and affect only certain groups of bacteria, preventing the spread of infectious diseases in plants. It is possible to apply phage-based product at bacteriophage concentration from 10^6 to 10^8 pfu/ml. The introduction of a new ecologically sound system in plant protection is an important scientific task that will allow obtaining competitive agricultural products without residues of pesticides in it. Therefore, the main objective of the study was to isolate and identify bacteriophages specific to phytopathogenic bacteria in order to develop basics for reduction of pathogen populations below the economic threshold levels (Sovinska et al., 2015).

Materials and Methods

Samples of plants with symptoms of bacterial lesions were investigated. In our research we used test bacteria: *Pseudomonas syringae lachrymans* 7591, *P. fluoresc*ens 8573, *P. savastanoi pv. phaseolicola* 4013 and *Serratia marcescens* sp. Phages were isolated by direct seeding. Phage titers were determined as plaque forming units (pfu/ml) using the standard double layer agar plate method. Pure bacteriophage lines were acquired by 6-times passaging

with subsequent accumulation on sensitive bacteria cultivated in commercial nutritional broth with additional aeration at 25ºC. The morphological features of viral particles were studied using electron microscope JEOL-1400 (Japan) (Faidiuk et al., 2015).

Results and Discussion

Plant diseases caused by bacteria are a serious problem in the cultivation and storage of agricultural products. Among such pathogens the most important are bacteria related to the *Pseudomonas* and *Serratia* genera (Sovinska et al., 2017). Using these species as test bacteria, the selection of specific phages was carried out. The selected phages possessed a wide range of lytic activity against these bacterial species and virtually eliminated them. That is why one of the alternative approaches for controlling bacterial diseases is the application of bacteriophages as natural bacterial antagonists (Clokie et al., 2011). Due to the high specificity of phages against host bacteria, such therapy will destroy pathogenic bacteria without affecting useful microorganisms. In addition, the ability of phages to self-replicate indicates that low doses of phage preparations can be used to protect plants. Thus, the use of phages led to a reduction of peach morbidity caused by *Xanthomonas pruni* at 86 – 100%. In the US, a grant was received in order to patent the right to produce phage-based antimicrobials to control legume disease caused by *Pseudomonas syringae* (Kutter et al., 2004; Ramos et al., 2011). For our research samples of beets, potatoes, apples, garlic and mandarins with symptoms of rot were selected from vegetable storehouses. The main symptoms of bacterial lesions observed in fruits were areas of mild decay, softening of the tissues, and the extraction of the exudate. After selection of vegetable samples with symptoms of bacterial lesion bacteriophages specific to phytopathogenic bacteria were obtained. As a result of this work we isolated phages specific to Pseudomonas syringae lachrymans 7591, P. fluorescens 8573, P. savastanoi pv. ***phaseolicola*** 4013 and Serratia marcescens sp. All selected phages were reproduced with the same efficiency on the bacteria strains used in this study. Bacteriophages led to the formation of negative colonies with large (6 mm), medium (3 mm) and small (1 mm) sizes depending on the test bacterium (fig.1).

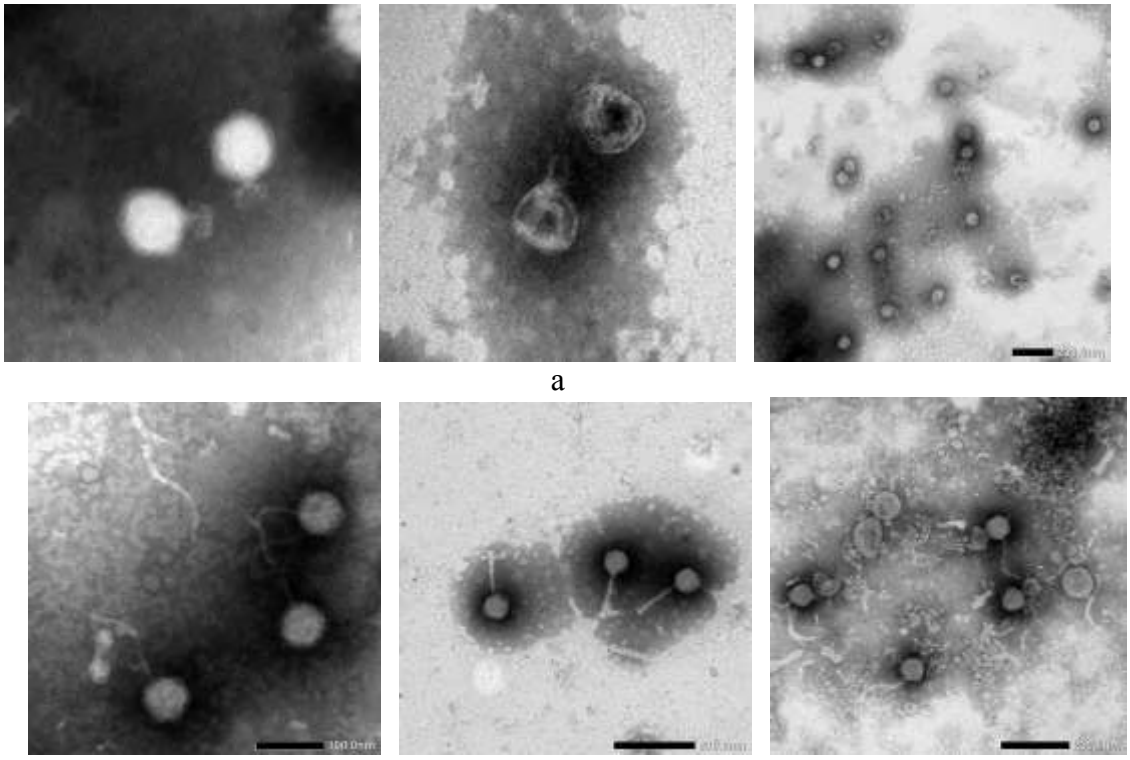
Fig. 1. Phage plaque morphology on test culture *Serratia marcescens sp.* (plaques diameter = 3 mm)

To identify host range specificity of isolated phages spectrum of lytic activity was studied against 9 strains of phytopathogenic bacteria. Our research revealed that among 14 phage samples five expressed lytic activity against different strains of phytopathogenic bacteria. It shows that the selected phages possessed a wide range of lytic activity (i.e., polyvalent bacteriophages) and could be used as perspective biologic agents in control of bacterial diseases.

a b

Fig. 2. The spectrum of phages' lytic activity against test bacteria: a - *Pseudomonas fluorescens 8573*, b - *Serratia marcescens sp.*

Analysis of electron microscopic observations showed that investigated bacteriophages had different morphology and size. Phage particles revealed short-tailed icosahedral structures with a head of approximately 43±1 nm in diameter and a short tail of 1 ± 0,5 nm in length, confirming them as belonging to the morphotype C1 of family *Podoviridae* of *Caudovirales* order. Beside these, electron microscopy analysis showed another group of bacteriophage particles with typical features of *Siphoviridae* family, *Caudovirales* order (icosahedral head with long tail, size – head diameter 67±2 nm, tail length 120±3 nm) (fig. 3).



a

b

Fig.3. Electron microscopy images of phage isolates: a – virions of phages belonging to the family *Podoviridae*, b – virions of phages belonging to the family *Siphoviridae*

Activity of selected phages indicated that they can be used as a biological plant protection product in disease management against phytopathogenic bacteria. This product had a selective effect on phytopathogenic bacteria, did not pollute the environment, increased profitability of

production and provided environmentally friendly agricultural production. Its application could reduce the exposure of toxic chemicals on food products. Biologically active preparation was designed for protection against bacterial infections of sugar beet plants and reduction of rot during the storage of agricultural products. The active ingredients of the preparation were naturally occurring bacteriophages that selectively and specifically killed only certain strains of microorganisms.

Conclusions

The results of this research create possibility for developing biologically active product based on specific bacterial viruses (bacteriophages). Laboratory tests in order to preliminary assess efficacy of plant protection product are used for making selection of agents which will be used as biological product in order to manage bacterial diseases in plants. These products are proven to be nontoxic, environmentally safe and effective for significantly reducing or eliminating plant pathogenic bacteria.

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FIRST RECORD OF THE BROWN-BANDED COCKROACHES, *SUPELLA LONGIPALPA* (F.) IN NEW VALLEY, EGYPT

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Abstract

The tropical cockroach, *Supella longipalpa* (Fabricius) (Blattodea: Ectobiidae) was detected as pest for the first time in El-Kharga, New Valley, Egypt. The city is the capital of the New Valley Governorate and located in the south-west of the western desert in Egypt (24°32'44" N, 27°10'24" E, altitude 32 m) and has a hot desert climate with annual average relative humidity 37.9%. These special conditions in this region are very suitable for proliferation the brown-banded cockroach, *Supella longipalpa*. The complaint led to the inspection of infested houses, using glue traps and the brown-banded cockroaches were found in every room where they showed a preference for high places, often around furniture and sometimes in the kitchen. In addition, they were found in subsequent surveys, confirming the establishment of this species in this region. The brown-banded cockroaches lately have invaded the houses, restaurants and hospitals in this region. Many of adults, nymphs and oothecae were obtained after their rearing under room conditions. The aim of this study was to recognize current information gaps about this pest in the New Valley area to acquire more knowledge about health-related effects and prospective management of *Supella longipalpa* in Egypt. This paper reports the first detection of tropical roach, *S. longipalpa*, as an urban pest in New Valley, Egypt. For enhanced management of *S. longipalpa* an intelligent pest management program is required. Sanitation, harborage removal, glue traps and insecticide baits are advised to be used in an integrated manner.

Keywords: *Blattodea, Furniture roach, Tropical roach, urban pest, Egypt*

Introduction

Cockroaches are primitive tropical and subtropical insects that have existed for over 200 million years. There are approximately 4600 species of cockroaches that have been identified worldwide, divided into eight families: Cryptocercidae, Blattidae, Blattellidae (Ectobiidae), Blaberidae, Polyphagidae, Nocticolidae, Tryonicidae and Lamproblattidae and about 445 genera (Wu *et al.*, 2014). Originally, all cockroaches were creatures of the outdoors. They lived in trees and vegetation in a region where warm temperatures favoured their development. Some species (about 1 %) of these roaches adapted to take the food, water, warmth and shelter related to man's lifestyle. The association with man has improved at different rates within the numerous species of cockroaches considered to be pests. Some cockroaches are thought to be pests because they invade houses, hospitals, hotels and food institutions (Kassiri *et al.*, 2014; Pomes and Arruda, 2014). They contaminate food and food preparation areas with their saliva, faces and body parts that play a role as allergens and vectors of organisms causing food poisoning and infectious illnesses (Rivault *et al.*, 1993; Kopanic *et al.*, 1994). *Supella longipalpa* is considered as one of the important sources of diseases in carrying many different microorganisms such as *Bacillus cereus, Escherichia coli, Salmonella typhimurium, Shigella dysenteriae* (Vazirianzadeh *et al.*, 2014), eggs of *Ascaris lumbricoides*, cysts of *Entamoeba coli*, cysts of *Entamoeba histolytica*, and eggs of *Taenia* spp. (Illgen-Wilcke *et al.*, 1992; Kinfu and Erko, 2008). Also, its cast skins and excrement have been reported as a source of allergens (Gore and Schal, 2007; Wirtz, 1980).

Material and Methods

On 23 July 2016, some females, males and nymphs of brown-banded cockroaches were collected in some houses in the city of El-Kharga, New Valley, Egypt and reared under room conditions (average temperature 29.5 °C ± 2 °C and relative humidity 29% ± 3%), using the method of Gilbert (1964). The cockroaches were reared in a glass cage (30 × 25 × 20 cm) of which the top was greased with medical, petroleum jelly, 2.5 cm width, to prevent the escape of cockroaches. Small wood boxes were supplied for shelter. The diet consisted of dry bread and rabbit-food pellets. All food was treated with heat before use to control a white mite, *Caloglyphus* sp. The water was supplied with a small water bottle (Piquett and Fales, 1952), with twisted medical cotton wool to provide a moist surface. The cages were covered with screen net (18 mesh) for ventilation and were cleaned every week. Sticky traps were used as a tool for surveillance cockroach.

Results and Discussion

This paper reports the first detection of the tropical roach, *S. longipalpa*, as an urban pest in the New Valley, Egypt. Adults, nymphs and oothecae of the cockroach were identified using the cockroach dichotomous key based on morphological characters (Choate, 2013). They were found to belong to the species *S. longipalpa*. The presence of two light-coloured pale stripes across the middle of the nymph body and the two cross bands on the closed wings of the adults are characteristic (Fig. 1). The most common pest cockroaches in Egypt are *Blattella germanica* (German cockroach or croton bug) followed by *Periplaneta americana* L. (American cockroach) and *Blatta orientalis* (Oriental or shad cockroach) (William 2005). *Supella longipalpa* is usually referred to by its common names of brown-banded cockroach, spotted cockroach, furniture cockroach, TV cockroach and the tropical cockroach. (Houseman 2004, Schal 2011). It was known previously as *S. supellectilium* (Serv.) (Gurney 1970). Tsai and Chi (2007) suggested that the origin of the brown-banded cockroach is the Nile Valley region of Africa. Rehn (1945) also indicated that *S. longipalpa* is native to Africa. A few overviews indicate that the distribution range and abundance of this species is declining. Kinfu and Erko (2008) led a study in Ethiopia, which is believed to be the root territory of *S. longipalpa* but did not collect specimens of this species among 2 240 cockroaches gathered in Addis Ababa. Furthermore, it represented only 10% of the 4,240 cockroaches collected in Ziway. The brown-banded cockroach is associated with human residences and prefers to live in a dry area with the favourite places being under tables, electrical devices, inside television sets and behind the pictures in rooms. This wide range of suitable habitats results in *S. longipalpa* being distributed through various zones of apartments and structures, which makes their control difficult (Hassan 2016).

The present works expands knowledge of brown-banded, *S. longipalpa* that is the first record for the brown-banded cockroach, as a pest in New Valley, Egypt in July 2016, a new species to the Egyptian fauna as urban pests in areas at relatively high temperature, where they have found a suitable environment due to for living and spreading in this region.

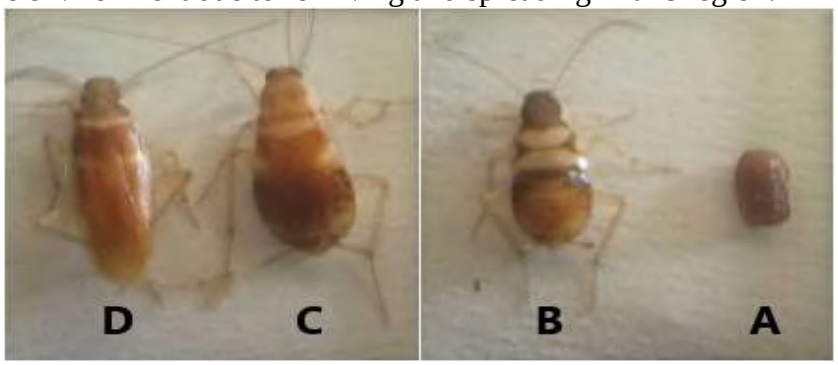

Fig. 1. The morphological characters of *Supella longipalpa* stages
(A) Oothecae (B) Nymph (C) Female (D) Male

Conclusion

New detection of brown-banded cockroach, *Supella longipalpa* in El-kharga, New Valley Governorate, Egypt is reported. This region has a special climate with dry conditions which is very suitable for this species that adapted to these conditions more than other cockroaches. *Supella longipalpa* is known as the spreader of foodborne diseases, and our report documents a new distribution region of this species in Egypt which may be a future problem in the field of public health.

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THE MICROBIOLOGY OF GREEK/CYPRUS TRAHANAS AND OF TURKISH TARHANA: A PRESENTATION OF SOME LITERATURE DATA

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Abstract

Greek and Cyprus Trahanas are the most popular fermented milk-cereal products of Greece and Cyprus, and are produced during summer from fresh ewes', goats' milk or a mixture of them. Broken wheat is then added to the fermented milk and heated to cook the mixture and then a thick paste is left to cool and cut into small pieces and left to dry (sun or oven drying). In Greek trahanas, fermentation of the lactic acid bacteria *Streptococcus lactis, Streptococcus diacetylactis, Leuconostoc cremoris, Lactobacillus lactis, Lactobacillus casei, Lactobacillus bulgaricus* and *Lactobacillus acidophilus* plays the major acid- and aroma -producing roles. A great biodiversity of microorganisms was observed during Cyprus trahanas fermentation. Lactic acid bacteria (LAB) were the predominant group, followed by yeasts. *Lactococcus*, *Lactobacillus*, and yeast species contribute greatly to its fermentation. Turkish Tarhana is the dry form of yoghurt-cereal mixture that is produced by mixing cereal flour, yoghurt, baker's yeast (*Saccharomyces cerevisiae*) and cooked vegetables, salt and spices followed by fermentation for one to seven days. The fermented slurry is then air-dried and used in soup making. LAB species found in Tarhana fermentation vary depending on the raw materials, fermentation time and techniques used for its production and play an important role in lactic acid and aromatic compounds formation. *Lactococcuslactis* spp. *lactis, Leuconostoc mesenteroides, Lactobacillus acidophilus, Enterococcus durans, Pediococcus* spp., *Lactobacillus delbrueckii* ssp. *lactis* and *Lactobacillus paracasei* bacteria played a role during the fermentation of Tarhana dough. Yeasts were mainly represented by *S. cerevisiae*.

Keywords: *Trahanas, Tarhana, Microbiology.*

Introduction

Fermented foods are an important part of diets in many parts of the world and are known from ancient times. Traditional fermented cereal foods are widely used in the diet of people in the Middle East, Asia, Africa and some parts of Europe. 'Trahanas' in Cyprus and Greece and 'Tarhana' in Turkey are two of the oldest traditional fermented milk/cereal foods and very nutritive foods due to the nutritional properties of wheat and milk/ yogurt. These products have also interesting organoleptic characteristics. There are similar products with different names such as 'Kishk' in Egypt, Syria, Lebanon and Jordan, 'Kushuk' in Iraq, 'Tarhonya/Talkuna' in Hungary and Finland. Methods for preparation of such products vary from place to place but cereals and fermented milk are always the major components.

Trahanas is one of the most popular fermented milk-cereal products of Greece which is produced during summer mainly from whole fresh ewes', goats' milk or a mixture of them. Sometimes instead of milk, a pulp of vegetables is used and then the product taken is called 'nistisimos trahanas'. For trahanas making, fresh milk is allowed to acidified for some days' either spontaneously or by adding a culture of yogurt while it is stirred every day until it reaches the desired acidity. Broken wheat is then added to the fermented milk and heated to cook the mixture and the thick paste formed is left to cool down and cut into small pieces (finger-sized pieces) which subsequently are left to dry (sun or oven drying). When trahanas is dry enough, it is stored in a cool place. Sometimes eggs are also added in the production of

trahanas and the final product is called 'sour trahanas with eggs'. The ratio of milk to wheat used in trahanas production is usually 3:1 or 4:1. Sometimes instead of sour milk, fresh milk is used for trahanas production and then the product is called 'sweet trahanas'. Greek 'Trahanas' is a very nutritive food as it is a good source of proteins, minerals etc. and is used largely for feeding people and elderly people.

Tarhana is the dry form of a yogurt-cereal mixture and is one of the oldest and most popular fermented cereal foods of Turkey representing an important part of the diets of many Turkish people. Production method and recipe of tarhana may differ from place to place to place. Tarhana has an acidic and sour taste with a yeasty flavor and is mainly used for soup making. It can also be consumed as a snack after being dried to thin layer or nugget, not to be ground. Tarhana is produced by mixing cereal flour (mainly wheat flour), yogurt, baker's yeast (*Saccharomyces cerevisiae*) and a variety of cooked vegetables (tomatoes, onions, green peppers and red peppers), salt and spices (mint, thyme, dill, tarhana herb etc.) followed by lactic and alcoholic fermentation for one to seven days. The fermented slurry is then air-dried and used in soup making, giving a product with high nutritional contents of proteins, vitamins etc. making tarhana healthy for children, the elderly and medical patients.

Results and Discussion

This work is a review on some literature data on the microbiology of the Greek/Cyprus fermented milk/cereal product named 'Trahanas' and of the Turkish fermented milk/cereal food named 'Tarhana'.

Lazos et al (1993) studied the microbiological changes that occur during the fermentation period of Greek Trahanas (a milk-wheat flour combination prepared in Greece) made with or without prior boiling of milk. Both boiled and un-boiled milk was fermented for 35 hours, a time that is required for the curd separation. Afterward, the curd-wheat flour mixtures were fermented for 15 hours. In a fermentation time longer than 15 hours the microbial population grew in sufficient numbers in order to convert milk into a fermented product. In un-boiled milk preparation (MAF) the microorganisms *Streptococcus* reached their maximum values at the 23 hours of fermentation. However, it was observed that from 30 hours of fermentation and afterward their growth was limited as pH value dropped to 4.36 and acidity expressed as the lactic acid was 0.82%. In the case of boiled milk preparation (MBF) the microorganisms *Streptococcus* reached their maximum values after 30 hours of fermentation while their numbers were lowered as the pH failed to 4.42 and the acidity was 0.72% (expressed as lactic acid). Total counts of microorganisms and Lactobacillus counts increased continuously with the fermentation time while streptococci increased up to 23 hours of fermentation and reduced thereafter as the pH value dropped to values lower than 5.0-4.5. Similar behavior showed the coliform bacteria which were found in lower counts. The yeasts and molds counts were low and their amount remained constant during fermentation and increased only if enough acid (0.4-0.7% lactic acid, pH 4.4-5.5) was produced. In unboiled milk preparation (MAF preparation) the microorganisms *S. lactis* and *S. diacetylactis* predominated among all streptococci. In comparison, *S. cremoris* was found in low counts. Initially, in the fermentation process, cultures of the microorganisms *Lactobacillus casei* and *Lactobacillus lactis* were predominated while later on cultures of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* were isolated. When the numbers of *L. bulgaricus* increased, the numbers of *L. acidophilus* dropped. In comparison to unboiled milk preparation (MAF), no counts of the microorganisms *Streptococcus faecalis*, *Leuconostoc cremoris,* and *Lactobacillus acidophilus* were found in the boiled milk preparation (MBF). *L. casei* and *L. bulgaricus* were the only lactobacilli isolated, while *S. lactis* and *S. diacetylactis* were the major cocci found. The main aroma producing microorganisms isolated during trahanas fermentation were *Streptococcus diacetylactis* and *Leuconostoc cremoris*. Also, the main acid

producing microorganisms were *Streptococcus lactis*, *Lactobacillus casei* and *Lactobacillus bulgaricus*. Concluding, Lazos et al. (1993) refer that the fermentation process that takes place in the production of Greek Trahanas food is a lactic acid bacterial fermentation that last about 50 hours and in which *Streptococcus lactis*, *Streptococcus diacetylactis*, *Leuconostoc cremoris*, *Lactobacillus lactis*, *Lactobacillus casei*, *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* paly the major acid and aroma producing role.

Bozoudi et al. (2017) studied the microbiological characteristics of Cypriot Trahanas, a traditional fermented ewe's milk product. A great biodiversity of the authentic microbiota of the traditional Cypriot Trahanas was found. Lactic acid bacteria (LAB) were the predominant group of microorganisms, followed by yeasts. During a 5-day fermentation period lactococci, lactobacilli and yeasts were found to be the predominant species that contribute greatly to the completion of the fermentation stage. *Lactococcus lactis* and *Lactococcus* spp. displayed their higher numbers on the 3rd day of fermentation time. However, there were not found any *Lactococcus* species at the end of fermentation time (day 5) probably due to the acidic conditions formed in fermented milk (pH 3.8 at day 5) and the cell lysis. Concerning *Lactobacillus* species there were found in low numbers during the first two days but they highly increased at the 5th day (end) of the fermentation period. Yeasts were found throughout the fermentation period with *Saccharomyces unisporus* to be the predominant one from the start to the end of the fermentation time. Also, *Lactobacillus* spp. seemed to predominate throughout the fermentation period. LAB on MRS agar (pH 6.2) and LAB on M-17 agar reached their higher numbers on day 4 of the fermentation process. Also, total aerobic counts and LAB on MRS agar (pH 5.7) reached their higher numbers on day 3 of fermentation. A significant decrease in their population was observed at the end of fermentation time due to the acidic environment created by the significant reduction of pH that NSLAB cause. However, LAB on M-17 agar and yeasts were found in high levels until the end of fermentation time.

Ibanoglu et al. (1999) studied the effect of different ingredients on the fermentation activity of tarhana. Three different tarhana formulations were prepared: a) tarhana with a yoghurt to wheat flour ratio of 0.5 with 80.0 g salt kg^{-1} wheat flour used (standard tarhana, named S1), b) tarhana with a yoghurt to wheat flour ratio of 1.0 with 80.0 g salt kg^{-1} wheat flour used (tarhana named S2) and c) tarhana with a yoghurt to wheat flour ratio of 0.5 without salt (tarhana named S3). The total and lactic acid bacteria count of tarhana made with increased yogurt was higher than that of the rest tarhana samples as more yogurt was added to the sample. At the end of fermentation period, the tarhana sample without salt had a higher lactic acid bacteria count than the rest two tarhana samples. So, it is shown that the addition of salt before fermentation resulted in a lower fermentation activity and lactic acid concentration. The increase of yeasts number showed that yeast fermentation was involved in tarhana fermentation. The microbial populations of all tarhana samples increased significantly during the first day of fermentation and decreased gradually thereafter.

Erbas et al. (2005) studied and compared the microbiological properties of wet and dry tarhana during a storage period of 6 months. Also, the changes of some microbiological properties of tarhana dough at its fermentation stage of production were studied. The *Lactobacillus* spp. the count was decreased from 6.47 to 5.44 $\log_{10}$ CFU/g during tarhana fermentation and the total mesophilic aerobic bacteria decreased from 6.43 to 5.95 $\log_{10}$ CFU/g with the increase of tarhana acid content. The reduction of the above microorganisms continued through the storage period of tarhana product. The TMAB, LAB and yeasts and molds counts were approximately equal at the beginning of tarhana fermentation. TMAB and LAB increased on the first day of fermentation but decreased thereafter as a result of the increase of the acid content. The yeasts and molds populations decreased continuously during the tarhana fermentation period. No coliforms were detected in any of the tarhana samples

during the whole fermentation and storage periods. This showed no contamination either from the environment or from the raw materials.

Sengun et al. (2009) studied for the first time by molecular-based methods the predominant lactic acid bacteria (LAB) isolated during the processing of Tarhana. Tarhana samples were collected from eight different regions of turkey. By using the molecular-based methods the bacteria Pediococcus acidilactici was found to be the 27% of the microbial isolates while 19% of the microbial isolates were identified as *Streptococcus thermophilus*, 19% as *Lactobacillus fermentum*, 12% as *Enterococcus faecium*, 7% as *Pediococcus pentosaceus*, 5% as *Leuconostoc pseudomesenteroides*, 4% as *Weissella cibaria*, 2% as *Lactobacillus plantarum*, 2% as *Lactobacillus delbrueckii* spp. *bulgaricus*, 2% as *Leuconostoc citreum*, 1% as *Lactobacillus paraplantarum* and 0.5% as *Lactobacillus casei*. The bacteria *P. acidilactici* and *Streptococcus thermophilus* were found to be isolated from the majority of the Tarhana samples examined. Yoghurt added for tarhana production was found to be the main source of the bacteria P. acidilactici and S. thermophiles. One hundred and fifty Gram-positive, catalase negative isolates were obtained from different steps of Tarhana dough. However, 16 isolates were taken from dried tarhana samples. From yoghurt and broken wheat that were added for tarhana production, 50 isolates and 10 isolates were obtained, respectively.

Settanni et al. (2011) studied the evolution of lactic acid bacteria (LAB) and yeasts during the fermentation of tarhana that was produced with some pasteurized ingredients and carried out at 30^0C and 40^0C. During tarhana fermentation LAB and yeasts were found to be in the range of 10^7-10^8 colony forming units (CFU)g^{-1}. However, there were not detected any coliform bacteria. The lactic acid bacteria that were isolated during tarhana fermentation were grouped according to their phenotypic and polymorphic characteristics and the species identified were the Pediococcus acidilactici, Lactobacillus plantarum, and Lactobacillus brevis. The pasteurization that was applied to the tarhana vegetable ingredients (except wheta flour) improved the hygienic conditions of tarhana while it did not influence the common LAB evolution during fermentation. Concerning the LAB counts, no statistically significant differences were found between the two tarhana production that took place under 30^0C or 40^0C fermentation temperature.

Kivanc and Funda (2016) studied the changes in bacteria and yeast number during the fermentation period of Tarhana dough. At the initial part of fermentation, the number of yeasts, molds, TMAB (total mesophilic aerobe bacteria), LAB (lactic acid bacteria) were found to be similar. The lactic acid bacteria (LAB) and yeasts which are responsible for the flavor and taste of tarhana as they produce lactic acid, ethanol, carbon dioxide, increased within the 5 days of fermentation. The lactic acid bacteria increased from $1.32x10^2$ to $4.20 x10^4$ CFU/g and the yeasts content increased from $3.45x10^1$ to $2.40x10^5$ CFU/g in the content of tarhana dough. The TMAB count increased rapidly within 3 days of fermentation. A total of 640 bacteria were isolated through tarhana dough fermentation and from them 623 were identified as lactic acid bacteria. Also, a total of 540 yeasts were isolated. The bacteria *Lactococcus lactis* spp. *lactis, Leuconostoc mesenteroides, Lactobacillus acidophilus, Enterococcus durans, Pediococcus* spp., *Lactobacillus delbrueckii* ssp. *lactis* and *Lactobacillus paracasei* bacteria were found to play a role during the fermentation of Tarhana dough. Also, the yeasts *Kluyveromyces marxianus, Yarrowia lipolytica, Pichia membranaefaciens, Pichia mexicana, Pichia angusta, Debaryomyces hansenii, Candida sorboxylosa, Candida fluviatilis, Saccharomyces cerevisiae* were identified during the Tarhana fermentation. The various strains of yeasts determined and the lactic acid bacteria (LAB) were the dominating strains found in the natural Tarhana fermentation. The microorganisms *E. durans, L. plantarum, S. cerevisiae, K.marxianus* and *Y. lipolytica* dominated at the final stages of tarhana fermentation.

Conclusion

A great biodiversity of microorganisms was observed during the fermentation period of the two fermented milk/cereal products, the Greek/Cyprus Trahanas and the Turkish Tarhana.

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ENTOMOPHAGOUS FAUNA - PREDATORS OF PEARS IN EAST SARAJEVO AREA (BOSNIA AND HERZEGOVINA)

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Abstract

Entomophagous fauna – predators in the region of East Sarajevo (Republic of Srpska, Bosnia and Herzegovina) was examined in 2011 and 2012 in intensive orchards in the locations Vojkovići and Kula, in semi-intensive orchards in locations, Tilava and Petrovići, and in the extensive orchard in the location Kasindo. Sampling by direct trees scrutiny, branches shaking, the collection of infested plants organs, and the collection of predators adults and juveniles weekly and biweekly during vegetation season by entomological nets and meshes, aspirators & collecting vacs restituted the predators and instars which lived freely on the infested and free parts of the plants. In the area of East Sarajevo, in orchards of pears, in 2011 and 2012 year, eight predators species were found. Larvae and imaginal stages of these insects were reared from colonies of aphids (*Dysaphis pyri* Boyer de Fonscolombe, *Aphis pomi* De Geer) and psyllid species (*Cacopsylla pyri* Linne, *Cacopsylla pyrisuga* Forster, *Cacopsylla pyricola* Forster). Examinations revealed the presence the following predators species: *Anthocoris nemorum* Linne (Hemiptera: Anthocoridae), *Adalia bipunctata* Linnaeus, *Coccinella septempunctata* Linnaeus, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), *Episyrphus balteatus* DeGeer, *Syrphus ribesii* Linne (Diptera: Syrphidae), *Chrysoperla carnea* Stephens and *Chrysopa septempunctata* Wesmael (Neuroptera: Chrysopidae).

Keywords: Beneficial insects, Pears, City of East Sarajevo.

Introduction

In addition to harmful species, many beneficial insects live on pear; the predators represent the most important regulators of pest populations. During their life and all stages of development (Mihajlović, 2008), they can kill a significant number of preys. Among predators of pear insect pests, there are many available species from different orders: Hemiptera, Dermaptera, Neuroptera, Coleoptera and Diptera (Maceljski 2002; Almaši et al., 2004).

Predators from the order Hemiptera - the most significant order - belong to Anthocoridae, Miridae and Nabidae and feed on aphids and scale insects, psyllid and miners caterpillars. From family Anthocoridae the most important are *Anthocoris nemoralis* (F.), *A. nemorum* (L), *Orius majusculus* Reuter, *O. minutus* (L.), *O. vicinus* (Ribaut) (Staubli et al., 1992; Beers et al. , 1993; Horton et al., 1997; Solomon et al., 2000). Particularly significant is *Anthocoris nemoralis* F. which is regularly present in the demes of psyllid species (*C. pyri, C. pyrisuga and C. bidens*) and aphids (Souliotis, 1999; Shaltiel and Coll, 2004).

Predators from the order Neuroptera - that are well-known predators of aphids, scale insects, psyllid species, and smaller caterpillars in orchards, comprehend the Family Chrysopidae with a large number of species in Genus *Chrysopa: perla* Linnaeus, *Ch. septempunctata* Wesmael, *Ch. ventralis* Curtis, *Ch. nigricostata* Brauer, *Chrysoperla carnea* Stephens, *Hemerobius micans* Olivier (Vuković, 1986). Adults feed pollen or honeydew, despite adul*ts of Ch. perla* can also prey leaf aphids (Hodek and Honek, 1988) and psyllid species (*C. pyri, C. pyrisuga and C. pyricola)* (Vilajeliu et al., 1998; Souliotis, 1999; Jerinić-Prodanović, 2010), is considered among the most efficient species (Vuković, 1986).

Coleoptera predators belong to Coccinellidae with a number of species that feed on leaf aphids, scale insects and psyllid species: *Adalia bipunctata* Linnaeus, *Coccinella septempunctata* Linnaeus, *Adonia variegata* Goeze, *Propylea quatuordecimpunctata* Linnaeus, *Synharmonia conglobata* Linnaeus and *Hippodamia tredecimpunctata* Linnaeus (Vuković, 1990), and in recent years *Harmonia axyridis*, which was found in the area of East Sarajevo (Tešanović et al., 2011).

Predators from the order Diptera are common antagonists of aphids and psyllid, belonging to Syrphidae or Cecidomyiidae. Females lay eggs on plants infested with aphids, and their larvae feed on haemolymph from aphids body (Nijveldt, 1988). The most important species are: *Episyrphus balteatus* De Geer, *Syrphus ribesii* Linnaeus, *Metasyrphus colorae* Fabricius, *Sphaeroforia scripta* Linnaeus, *S. menthastri* Linnaeus (Vuković, 1990). In the area of East Sarajevo, the most common from Syrphidae was *Episyrphus balteatus* and *Aphidoletes aphidimyza* (Tešanović et al., 2011) from Cecidomyiidae.

Material and Methods

The scrutiny was realised in 2011 and 2012 in the orchards (locations Vojkovići, Kula, Tilava, Petrovići, Kasindo) and the laboratory of the Faculty of Agricultural in East Sarajevo. Visual examination of randomly selected trees restituted presence of insects and their number. Observing 10 (Kula and Kasindo) or 20 (Petrovići, Tilava and Vojkovići) trees weekly or biweekly during host plant growing season restituted collection data on all instars of insects free-living on plant organs by shaking branches, using entomological nets and aspirators meshes and vac.

Sampling refereed 100 different plant organs (leaf and flower buds, leaves) within a randomly selected tree. All sampled infested plant material with predators was scrutinised in the laboratory. All collected eggs, larvae and pupa of entomophagous insects, were reared in the laboratory to adult in plastic flax or Petri dishes with prey-infested plant parts. Collected and reared insects were fixed in 70% alcohol; some of them were mouted, in slide or different preparations for microscopy or pinned/carded and labelled for preservation in the collection. Adults & juveniles morphology comparison by keys and appropriate entomological literature (Balachowsky 1966) allowed the identification to species level.

Results and Discussion

In pear orchards, eight entomophagous species of insects - predators were collected. Larvae and adult instars of these antagonists were identified in demes of aphids and psyllid. The following species were found: *Anthocoris nemorum* Linne (Hemiptera: Anthocoridae), *Adalia bipunctata* Linne, *Coccinella septempunctata* Linne, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), *Episyrphus balteatus* DeGeer, *Syrphus ribesii* Linne (Diptera: Syrphidae), *Chrysoperla carnea* Stephens and *Chrysopa septempunctata* Wesmael (Neuroptera: Chrysopidae). Table 1 summarises their presence.

Table 1. Findings of insect predators in the area of East Sarajevo, 2011 and 2012

	Order	Familia	Species of insects	Locations				
				Vojkovići	Kula	Tilava	Petrovići	Kasinso
1.	HEMI-PTERA	Anthocoridae	*Anthocoris nemorum*	+	-	-	-	-
2.	COLEO - PTERA	Coccinellidae	*Adalia bipunctata*	+	+	+	+	+
3.			*Coccinella septempunctata*	+	+	+	+	-
4.			*Harmonia axyridis*	+	+	+	+	+
5.	DIP-TERA	Syrphidae	*Episyrphus balteatus*	+	+	+	-	-
6.			*Syrphus ribesii*	+	-	+	-	-
7.	NEURO - PTERA	Chrysopidae	*Chrysoperla carnea*	+	+	+	+	-
8.			*Chrysopa septempunctata*	+	+	+	+	-
			Total number of insects	**8**	**6**	**7**	**5**	**2**

Intensive orchards in Vojkovići host eight species of predators. Two species, *A. bipunctata* and *H. axyridis*, were found in all locations. Three species of predators, *Coccinella septempunctata*, *Chrysoperla carnea* and *Chrysopa septempunctata* were present in all pear orchards, except in extensive orchards in Kasindo which host two species *only: A. bipunctata* and *H. axyridis. E. balteatus* was identified both in Vojkovići and Kula intensive orchards, and semi-intensive Tilava orchard, while the *S. ribesii* was found in Vojkovići intensive and Tilava semi-intensive orchards. *A. nemorum* was present only in the Vojkovići intensive orchard. Our data sustain the literature (Maceljski, 2002, Almaši et al., 2004; Jerinić-Prodanović, 2010; Tamaš, 2012;), that describe an abundant predatory guild, build-up by Hemiptera, Neuroptera, Dermaptera, Coleoptera and Diptera that are devoted to controlling invasive insects pests. During our examination, we identified predatory species from the same orders. Order Coleoptera and fam. Coccinellidae contributes with *Adalia bipunctata* L. and *Coccinella septempunctata* L. both psyllid-oriented predators (Jerinić- Prodanović, 2010), and *Harmonia axyridis* Palla, reared from *Dysaphis pyri* Boyer de Fonscolombe demes, as also reported by Vuković (1990). All these species have already been identified in the area of East Sarajevo as predators of aphids on apple (Tešanović et al., 2010). Besides these species, from order Neuroptera and fam. Chrysopidae, two species were found: *Chrysoperla carnea* Stephens and *Chrysopa septempunctata* Westmael as predators of aphids and psyllid species, in all locations except the Kasindo, also by Vuković (1986, cit. Tamaš, 2012). The critical predatory species are from Diptera and fam. Syrphidae (*Episyrphus balteatus* De Geer, *Syrphus ribesii* L., *Metasyrphus colorae* Fabricius, *Sphaeroforia scripta* L., *S. menthastri* L., *Scaeva pyrastri* L., *Syrphus latifasciatus* Macquart (Vuković, 1990). Our examination showed the presence of *E. balteatus and S. ribesii* in Vojkovići and Tilava, while E. balteatus only *exists* in Kula. Their larvae were feeding on *D. pyri* demes, as also observed by Vuković (1990) and Tamaš (2012). In Vojkovići also the Hemipteran *Anthocoris nemorum* L., exists and is one of the most relevant species, feeding on *C. pyri* and *C. pyrisuga*, as reported by Jerinić-Prodanović (2010).

Conclusion

In the area of East Sarajevo, during 2011 and 2012 and in intensive, semi-intensive and extensive orchards of pears, eight antagonists species were found: *Anthocoris nemorum, Chrysoperla carnea, Chrysopa septempunctata, Adalia bipunctata, Coccinella septempunctata, Harmonia axyridis, Episirphus balteatus* and *Syrphus ribesii.* Entomophagous species were most numerous in the intensive orchard in Vojkovići, that may depend by the of selective insecticides with a minor side-effect on the predatory guild. The lowest number of predatory exists in Kasindo extensive orchards.

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GROWING AGARICUS BISPORUS ON COMPOST MIXTURES BASED ON CHICKEN MANURE AND BANANA RESIDUES

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Abstract

The current study evaluated the potential use of two widely available agricultural wastes at local level: chicken manure (chic) and banana wastes (ban) in the production of *Agaricus bisporus* mushroom in order to assess the possibility of alternating the traditionally imported substrate (based on horse manure). Therefore, the trial consisted on growing the mushroom on substrates formed by several mixtures of both wastes subjected to composting and mixed in various proportions (0%, 30%, 50%, 70% and 100% at volume basis) compared to a control substrate (based totally on horse manure). Fruit formation in the substrate chic-ban:100-0 was earlier by 1 to 3 days compared to remaining substrates. Average yield was the highest (350.9 g/box) in control substrate and the lowest in substrates based totally on chicken manure or banana wastes composts. Individual mushroom weight ranged between a minimum of 36.8 g in chic-ban:0-100 and a maximum of 58.5 g in control substrate. In the substrates chic-ban:70-30 and chic-ban:50-50 yields were comparable to control (283.9 g/box and 294.2 g/box respectively). Fruit number did not vary significantly among all substrates. Cap diameter was the highest in chic-ban: 50-50 (6 cm), stipe diameter was the highest in control (2.25 cm), and mushroom length was superior in both substrates compared to others. All substrates produced marketable fruits except chic-ban:0-100. Consequently, it was possible to totally substitute the traditional compost by the mixtures chic-ban:70-30 and chic-ban: 50-50 making of this type of cultivation a less expensive one at the level of Lebanon.

Keywords: *A. bisporus, agricultural wastes, composting, substrate, yield.*

Introduction

Innovation in managing agricultural wastes or biomass is a continuous challenge and recent trends favor the utilization of this biomass for value added purposes (Rosentrater *et al.,* 2009). Mushroom cultivation is a direct utilization of their ecological role in the bioconversion of solid wastes generated from agriculture into edible biomass (Savoie *et al.,* 2011). Button mushroom (*Agaricus bisporus* (Lange) Sing.) is one of the most commonly consumed mushrooms in the world (Manzi *et al.,* 2001). It is highly nutritious and it is being traditionally grown on a substrate based on composted horse manure and wheat straw. Physical and chemical properties of substrates are prerequisite for providing essential nutritive compounds and adequate texture for mycelia growth (Chang and Miles, 2004).

In Lebanon, mushroom sector could promote the economic and social development of rural areas (Karam *et al.,* 2010), however the scarcity of horse manure has been obliging local growers to import the traditional compost at a high cost from foreign countries. Consequently, there is a need to find alternative compost mixtures based on more available resources (Sassine *et al.,* 2005). Millions of wastes are generated each year from agricultural activities (El Zein *et al.,* 2015). Previous local trials have recommended the utilization of various waste types such as chicken manure (Sebaaly *et al.,* 2018a) and winery wastes (Sebaaly *et al.,* 2018b) as additives for the traditional compost during button mushroom production. Additionally, the banana cultivation sector is also generating huge amounts of wastes that are

normally unutilized or burned. In fact, banana cultivated areas are in continuous expansion mainly in Southern Lebanon (Kamthan and Tiwari, 2017). The potential use of banana wastes was earlier reported on *Pleurotus* spp. (Sturion, 1994). Moreover, chicken is one of the most produced livestock products (USDA, 2016) and chicken manure generated by local farms is being used as soil amendments. It is well known that properties of agricultural wastes differ globally due to variation in agricultural activities, animal feed and climate conditions. Consequently, the current work investigated the potential use of local types of wastes as a method to provide easily available and less expensive substrates for *A. bisporus* production in Lebanon. Therefore, deep litter chicken manure was mixed with composted banana wastes in different ratios aiming to compare the productivity of the various formulations formed to the one of the traditional compost.

Materials and methods

Composting and pasteurization

The experiment started by preparing three types of composts: the first was based on straw-bedded horse manure, the second on deep litter chicken manure and the third on banana leaves and pseudo-stems (Table 1). It was an outdoor process where composts were turned and wetted until they turned dark-brown and consistent. Nitrogen-rich supplements like urea, ammonium nitrate and protein supplements were added to the compost pile for accelerating the composting process. This phase was achieved in 45 days after obtaining an odorless product and a compost temperature of 50°C (Demirer *et al.,* 2005). It was followed by 8 days of pasteurization (Peker *et al.,* 2007) which consisted on steam pasteurization for the preparation of pathogen-free and selective compost with zero ammonia level.

Table 1: Composition of compost formulations based on agricultural wastes

Amounts of components added to 400 Kg of each waste type	Straw-bedded horse manure	Deep litter chicken manure	Banana wastes (leaves and pseudo-stems)
Wheat straw (Kg)	200	200	200
Urea (Kg)	1.2	1.7	2.3
Ammonium nitrate (Kg)	1.2	1.5	2.5
Gypsum (Kg)	2	2	5
Protein supplements (Kg)	-	-	5

Treatments

Chicken and banana composts were mixed in various percentages based on volume (0 %, 30 %, 50 %, 70 % and 100 %) to form five different substrates: chic-ban:100-0, chic-ban:70-30, chic-ban:50-50, chic-ban:30-70 and chic-ban:0-100. Control consisted on the traditional compost totally based on horse manure. The Table 2 shows the physical and chemical properties of the various tested substrates.

Table 2: Properties of various substrates used for growing *A.bisporus*

	control	chic-ban (100-0)	chic-ban (70-30)	chic-ban (50-50)	chic-ban (30-70)	chic-ban (0-100)
M.C (%)	54.8	62.7	65.9	66.7	69.8	72.4
O.M (% d.w)	65.5	54.7	62.8	70.1	76.8	80.9
Total N (% d.w)	1.9	2.3	2.2	2	1.7	1.6
Total C (% d.w)	34.2	39.1	39	40.5	37.4	38.4
C:N ratio	18:1	17:1	18:1	20:1	22:1	24:1
pH	5.9	5.1	5.4	6	6.3	6.8

M.C: moisture content, O.M: organic matter content, N: nitrogen, C: carbon

Spawning

"Agaricus A15" strain was imported from Sylvan farm Hungary. Ten boxes were used for each substrate. One kilogram of spawn was used per substrate type, thus 100 g per box. A layer of 5 cm of casing soil (peat) was added at the surface of spawned composts. Inside the growing room, temperature was maintained at around 25°C and relative humidity at 90 %. Regulations of air temperature, compost temperature and watering were adopted from Le Lion (1998). Compost scratching was done five days after spawning. In day seven, CO_2 was liberated through ventilation and room temperature was decreased to 16°C as triggers for pin head formation. Mushrooms were harvested in three consecutive flushes over a period of 30-36 days depending on substrate type.

Measured indicators

Number and fresh weight of mushrooms were recorded daily in order to determine the average yield per box relative to each mixture. In addition, mushroom length (considering the stipe length and cap thickness), cap diameter and stipe diameter were measured on harvested fruits using a sliding caliper. Timing of fruit flushes was recorded as soon as mushrooms became well-developed and was expressed as number of days after spawning. For the evaluation of shelf-life, three representative mushrooms of each substrate were subjected to bagging (paper bags) and/or refrigeration as follows: First group was kept at ambient temperature (23°C) with no paper bags; second group was kept at ambient temperature (23°C) in paper bags; third group was refrigerated (4°C) with no paper bags and fourth group was refrigerated (4°C) after being disposed in paper bags.

Experimental design and statistical analysis

A Complete Randomized Design (CRD) with 6 treatments and 10 replications (10 boxes per treatment) was used. Analysis of variance (ANOVA) was adopted for evaluating the effect of substrates on tested indicators and consisted of means ± SE compared by Fishers' least-significant differences test (LSD). Statistical analysis was done using STATISTICA10 program.

Results and discussion

Substrate effect on mushroom production

The type of substrate has variously affected the timing of fruit formation (Table 3). The earliest first flush of mushrooms was obtained in chic-ban:100-0 and the latest in chic-ban:30-70 and chic-ban:0-100. All substrates produced mushrooms over three consecutive flushes with different frequencies. The whole cropping cycle was achieved first in chic-ban:100-0 and the last in chic-ban:30-70.

Table 3: Timing of mushroom flushes in experimental substrates

Mixtures	**Flush 1**	**Flush 2**	**Flush 3**
Control	19 DAS	26 DAS	31 DAS
chic-ban:100-0	18 DAS	24 DAS	30 DAS
chic-ban:70-30	19 DAS	26 DAS	33 DAS
chic-ban:50-50	20 DAS	26 DAS	35 DAS
chic-ban:30-70	21 DAS	28 DAS	37 DAS
chic-ban:0-100	21 DAS	27 DAS	36 DAS

On the other hand, substrate effect was statistically significant on averages of mushroom weight (P_{value}=0.00) and yield (P_{value}=0.00) while it was not significant (P_{value}=0.19) on average mushroom number per box.

Average mushroom number (Fig. 1a) ranged between a minimum of 3.4 mushrooms per box in chic-ban:0-100 and a maximum of 6 mushrooms per box in control. Average mushroom weight (Fig. 1b) was significantly higher in control compared to remaining substrates except chic-ban:50-50 (58.5 g in control and 55.5 g in chic-ban:50-50). It did not differ significantly between the substrates chic-ban:100-0 and chic-ban: 30-70 (46.8 g and 45.4 g respectively) and was the lowest in chic-ban:0-100 (36.8 g). Average yield (Fig. 1c) was significantly higher in control substrate compared to chic-ban:100-0, chic-ban:30-70 and chic-ban:0-100 (350.9 g/box compared to 187.5 g/box, 190.7 g/box and 125.2 g/box respectively). Substrates based totally on composted chicken manure or banana wastes had a lower productivity compared to the traditional substrate based on horse manure (chic-ban:100-0: 1.3 Kg/m^2 and chic-ban:0-100: 0.8 Kg/m^2 compared to control: 2.3 Kg/m^2). However, average yield, thus productivity in chic-ban:70-30 and chic-ban:50-50 were comparable to control (1.9 Kg/m^2 and 2 Kg/m^2 respectively compared to 2.3 Kg/m^2).

Substrate effect on mushroom characteristics

There was a significant effect of the substrate type on averages of mushroom length (P_{value}=0.00), cap diameter (P_{value}=0.00) and stipe diameter (P_{value}= 0.00). Average mushroom length (Fig. 1d) was not significantly different in the mixtures chic-ban:50-50 and control where it was around 6.7 cm. On the other hand, it was significantly higher in control, chic-ban:70-30 and chic-ban:50-50 compared to chic-ban:100-0, chic-ban:30-70 and chic-ban:0-100 (6.7 cm, 6 cm, 6.7 cm compared to 5.5 cm, 5.2 cm and 4.3 cm respectively). Average cap diameter (Fig. 1e) was not significantly different among control, chic-ban:50-50 and chic-ban:70-30 (5.9 cm, 5.7 cm and 5.5 cm respectively) as well as between chic-ban:100-0 and chic-ban:30-70 (5 cm in both substrates). The lowest average of this indicator was recorded in chic-ban:0-100 (3.7 cm). Moreover, the addition of composted banana wastes in proportions of 30 % or 50 % has induced the formation of mushrooms with higher stipe diameter (Fig. 1f) compared to higher proportions (70 % and 100 %).

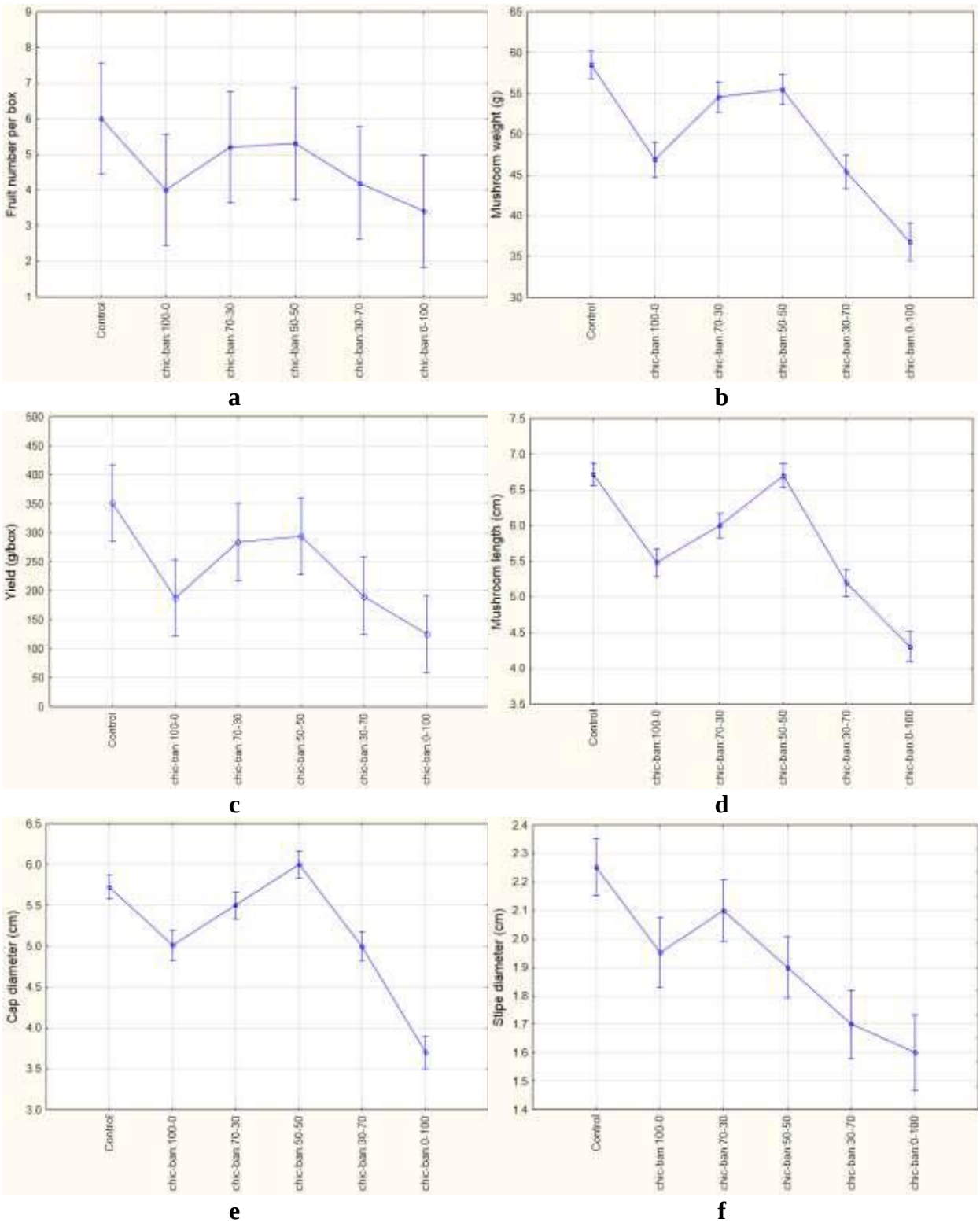

Figure 1: Averages (middle markers) and their 95% limits of confidence (±2 x standard Error: SE) (vertical bars) of indicators

Mushroom shelf-life

Despite the effect of post-harvest treatments (no bagging, no refrigeration), mushrooms of the substrate based totally on composted banana wastes had the longest shelf-life (Fig. 2). With no post-harvest treatments shelf-life was extended with increasing proportions of banana wastes in substrates. It was also extended by bagging and refrigeration. For instance, shelf-life of non-bagged mushrooms kept at room temperature ranged between 1.5 to 3 post-harvest days, while the one of bagged and refrigerated mushrooms ranged between a minimum of 7 post-harvest days in control substrate and a maximum of 10 post-harvest days in chic-ban:0-100.

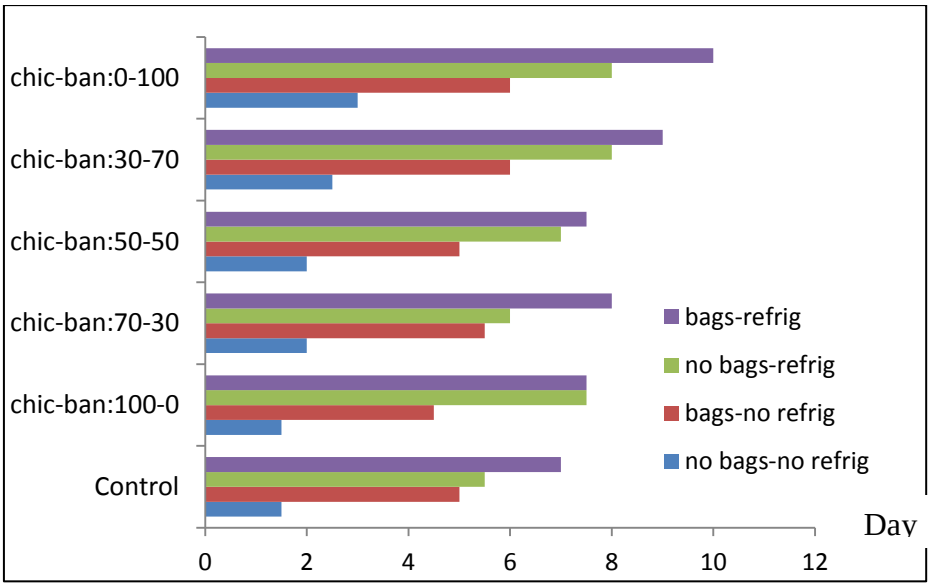

Figure 2: **S**helf-life (number of days after harvest) of mushrooms collected from experimental substrates

Under the specific experimental conditions, it was possible to grow *A. bisporus* on composted banana wastes and composted chicken manure although productivity of both substrates was low compared to that of the traditional compost. On the other hand, when chicken manure compost was dominant in the substrate (chic-ban:70-30) or equally mixed to banana wastes compost (chic-ban:50-50) productivity was comparable to that of traditional substrate.

The amount of nitrogen in the compost is directly related to mushroom growth ensuring an energy supply for the micro-organisms (Baar and Paradi, 2004). Normal nitrogen concentration in the compost is approximately of 1.5 % (Chang and Miles, 2004). Sözbir *et al.* (2015) found that the highest *A. bisporus* yield was obtained from substrates containing maximum nitrogen amounts. Our results showed that mushroom yield was high with nitrogen concentrations ranging between 1.9-2.2 % and was negatively affected by lower or higher N concentrations. In addition, the earliest primordial initiation, thus the earlier first flush was observed in the substrate chic-ban:100-0 due to the high nitrogen content (2.3 %) which has induced the shortest pre-harvest time (mycelia growth rate, spawn running time and primordial initiation). On the other hand, according to Huang *et al.* (2010) and Carmona *et al.* (2012) a nitrogen content exceeding 2 % affects negatively mushroom production which explains the low productivity of the substrate chic-ban:100-0. In general, when N is high in the substrate the compost temperature increases due to high mycelia growth, however, high compost temperature may damage the mycelium and eliminates the possibility of maximum productivity (Royce and Beelman, 2007). Moreover, variations in substrates productivity can

be attributed their C:N ratios. Best yields were obtained from substrates with C:N ratios of 18:1 and 20:1. They were in confirmation with findings of Zhen *et al.* (1995). In addition, C:N ratios higher or lower than those values (17:1, 22:1 and 24:1) have negatively affected yield with the lowest productivity obtained at C:N ratio of 24:1 in the substrate chic-ban:0-100. Huang *et al.* (2010) and Carmona *et al.* (2012) have noted earlier that a high C:N ratio affects negatively mushroom growth and Sözbir *et al*. (2015) have observed that low C:N ratio increased yield with best results obtained at C:N ratio of 19 and 22.

The presence of banana wastes in the mixtures has increased their organic matter content, thus their C:N ratios. Banana wastes are rich in cellulosic materials (Mahalakshmi and Linnett Naveena, 2016) and leaves are a good source of lignin, which is higher than in banana pseudo-stems (Mohapatra *et al.,* 2010). Edible mushrooms are known to be a good agent in the degradation of cellulose (Padam *et al.*, 2014). However, the longer growth cycle reached when banana wastes were present in the mixture in proportions higher than 30 % may be attributed to the more complex structure of banana wastes compared to chicken manure requiring longer time for degradation by mycelia. In fact, organic matter in banana compost was greatly higher than that of chicken manure compost. Cellulose and lignin contained in such substrates could have played an important role deciding yield. According to Sivaprakasam (1980) mushroom yield was positively related with cellulose content and negatively related with lignin content of the substrate. Mushroom physical characteristics were influenced by the various substrates confirming the findings of Sözbir *et al*. (2015). In general, mushrooms obtained from all substrates were marketable except those produced by the mixture chic-ban:0-100. They had a cap diameter falling in the range determined by EC (2002) and FAO (2004): 2.5-7 cm together with a normal stem length/cap diameter ratio (EC, 2002). Furthermore, the positive effect of increasing proportions of banana wastes in the mixture on mushroom shelf-life could be attributed to the lower water content in mushrooms grown in such substrates. In general, the short shelf-life of mushrooms is due to their high water content (Marshall and Nair, 2009) and according to Maheshwari (2013) storing mushrooms in paper bags or subjecting them to cold refrigeration prevents moisture loss.

Conclusion

The traditional substrate could be supplemented by banana wastes when extended mushroom shelf-life and fresh market are targeted or by chicken manure when an earlier cropping is seeked. In addition, the total substitution of traditional compost is possible through the use of substrates chic-ban:50-50 or chic-ban: 70-30, the use of which has maintained quantity and quality of the product. Since both types of agricultural wastes are widely available in Lebanon, mushroom growers could benefit from a reduced substrate cost. Finally, in order to optimize the benefits from banana wastes, composting for more than one year is recommended prior to their utilization in mushroom production.

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BIOACTIVE COMPOUNDS CONTENT OF NEW ZEALAND SPINACH (TETRAGONIA TETRAGONOIDES (PALL.) O. KUNTZE SYN. (TETRAGONIA EXPANSA)) AT DIFFERENT NITROGEN CONCENTRATIONS

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Abstract

The aim of study was to determine the influence of different nitrogen concentrations (75, 140 and 205 mg L^{-1} NH_4NO_3) on the bioactive compounds content in New Zealand spinach (*Tetragonia tetragonoides* (Pall.) O. Kuntze syn. (*Tetragonia expansa*)) grown in floating hydroponic systems. Also, under the same conditions spinach (*Spinacia oleracea* L.) was grown and mentioned species were compared. Spinach as well as New Zealand spinach are nutritionally valuable leafy vegetables with high vitamin and mineral content. Both species are specific for the tendency of nitrogen accumulation, so it is necessary to be careful when consuming and processing it. New Zealand spinach is less known and mostly un-researched species in our region. By comparing the analyzed species significantly higher content of vitamin C (46 100 g^{-1} FW), phenols (108.04 mg L^{-1} FW), flavonoids (70.77 mg L^{-1} FW), non-flavonoids (37.27 mg L^{-1} FW), total chlorophyll (0.88 mg g^{-1} FW), carotenoid (0.17 mg g^{-1} FW) and antioxidant capacity (2172.63 μmol TE L^{-1}) were determined in spinach compared to New Zealand spinach. In spinach, nitrogen had a significant negative impact on the bioactive compounds content significantly reduced vitamin C content for 36.35 %, total phenols for 23.38 %, flavonoids for 26.08 %, non-flavonoids for 47.84 % and pigment compounds. In New Zealand spinach, with an increase of nitrogen in nutrient solution the opposite trend was determined. Namely, by increasing the nitrogen concentration significantly higher amounts of vitamin C even twice and total phenols for 5 % was determined in New Zealand spinach, while content of pigment compounds did not significantly change.

Keywords: *Floating Hydroponics, Leafy Vegetables, Vitamin C, Total Phenol, Antioxidant Activity.*

Introduction

Leafy vegetables become an important part of nutrition due to high nutritional and low energy value (Fatema *et al.*, 2013). To ensure the continuous production of leafy vegetables for the market throughout the year hydroponic techniques suitable for the production of vegetables with a short vegetation cycle are used (Nicola *et al.*, 2006; Toth *et al.*, 2012). The most widely used hydroponic techniques suitable for leafy vegetables cultivation are Nutrient Film Technique (NFT) and floating hydroponics. In order to achieve a higher yield and quality for each vegetable species it is necessary to determine the optimum composition of nutrient solution, the sowing density and choose appropriate cultivars depending on the cultivation period and purpose (Toth *et al.*, 2012). In floating system, cultivation of spinach (*Spinacia oleracea* L.) is already well developed, while possibilities of hydroponics cultivation of New Zealand spinach (*Tetragonia tetragonoides* (Pall.) o. Kuntze syn. (*Tetragonia expansa*)) are still being researched. Both mentioned leafy vegetables species have tendency to accumulate higher levels of nitrates (nitrophilic plants) which can potentially become harmful for human health because of nitrification process transferring to nitrites. Except nitrates and nitrites,

leaves of both mentioned species also contains oxalates, therewith scientific studies cites that accumulation of mentioned compounds is less in New Zealand spinach leaves compared to spinach (Kawashima *et al.*, 2003; Jaworska, 2005a; Jaworska, 2005b). Mentioned leafy vegetables species are rich in nutritional composition containing a numerous antioxidants such as vitamin C, E, minerals, polyphenols, chlorophylls and carotenoids (Jaworska *et al.*, 2001; Barzegar *et al.*, 2007). The aim of study was to determine the influence of different nitrogen concentrations (75, 140 and 205 mg L^{-1} NH_4NO_3) on the bioactive compounds content in New Zealand spinach (*Tetragonia tetragonoides* (Pall.) O. Kuntze syn. (*Tetragonia expansa*)) and spinach (*Spinacia oleracea* L.) grown in floating hydroponic system..

Material and Methods

Plant material

Experiment was conducted at experiment station of Department of Vegetables Crops (University of Zagreb Faculty of Agriculture, NW Croatia, Zagreb, 45°49'N, 16°02E). New Zealand spinach 'Nuova zelanda' and spinach 'Matador' were grown in heated greenhouse in floating system. The floating system consisted of 4 pools dimensions 70 cm width x 115 cm length. The sowing of New Zealand spinach was carried out on October 17, 2017, and spinach on October 30, 2017. Prior to sowing, New Zealand spinach seeds were treated with potassium nitrate (KNO_3, 4.000 ppm) solution for 24 hours in in order to achieve better germination. Polystyrene containers (30 cm width x 50 cm length) with 40 pots filled with seedling substrate (Potgrond H Klasman) were used for cultivation. During the production cycle minimum (min. 16 °C) and maximum air temperature (max. 27 °C) and air humidity (min. 42%; max. 70%) were recorded. Spinach harvest was carried out on December 19, while New Zealand spinach harvest on January 22. Immediately after harvest fresh vegetable leaves were transported to the Department of Agricultural Technology, Storage and Transport (University of Zagreb Faculty of Agriculture) where healthy, undamaged leaves were separated for further analysis.

Nutrient solutions for cultivation of leafy vegetables were prepared with 4 different concentrations of ammonium nitrate (NH_4NO_3). As control solution (samples Sk and Nzk), the nutrient solution adjusted for spinach cultivation following composition was used (Cukrov *et al.*, 2017): 46.6 g $Ca(NO_3)_2$, 56.70 g KNO_3, 30.08 g KH_2PO_4, 13.61 g NH_4NO_3, 10.23 g K_2SO_4, 3.36 g $MgSO_4$, 1.008 g Fe-EDDHA, 0.045 g H_3BO_4, 0.1561 g $MnSO_4$, 0.0112 g $CuSO_4$ and 0.0056 g Na_2MoO_4 prepared in total volume of 70 L. In the next three treatments concentrations of NH_4NO_3 were: 75 mg L^{-1} (samples SN 1, NzN 1), 140 mg L^{-1} (samples SN 2, NzN 2) and 205 mg L^{-1} (samples SN 3, NzN 3). pH, EC value and amount of dissolved oxygen of nutrient solutions were measured daily for each pool and amounted average: pH value 5.98 (1. pool), 6.33 (2. pool), 6.00 (3. pool), 6.24 (4. pool); EC values 2.69 dS m^{-1} (1. pool), 2.76 dS m^{-1} (2. pool), 3.09 dS m^{-1} (3. pool) and 3.42 dS m^{-1} (4. pool) while amount of total dissolved oxygen varied daily and ranged from 3.7 mg L^{-1} to 11.4 mg L^{-1}.

Determination of chemical composition and antioxidant capacity by ABTS method

The basic chemical composition of fresh leaves was carried out by standard methods (AOAC, 1995) and included: total dry matter content (%), total acid content (%) and pH value. The content of following biologically active compounds was also analyzed: vitamin C (mg 100 g^{-1} FW) (AOAC, 2002), total phenols (flavonoids and non-flavonoids) (mg L^{-1}) according to Ough & Amerine (1988) and pigment compounds, total chlorophylls and carotenoids content by Holm (1954) and Wettstein (1957). The absorbance of pigment extracts was measured spectrophotometrically (Shimadzu UV 1650 PC) at 662, 644 and 440 nm with acetone (p.a.) as a blank probe. The absorbance values were recalculated according to the Holm-Wettstein equations (mg L^{-1}) while the final result of the pigment content was expressed in mg g^{-1}:

(1)
$$chlorophyll\ a = 9.784\ \times A_{662} - 0.990\ \times A_{644}[mg\ L^{-1}],$$
$$chlorophyll\ b = 21.426\ \times A_{644} - 4.65\ \times A_{662}[mg\ L^{-1}],$$
$$chlorophyll\ a+b = 5.134\ \times A_{662} + 20.436\ \times A_{644}[mg\ L^{-1}\ ],$$
$$carotenoids = 4.695 \times A_{440} - 0.268 \times (klorofil\ a+b)[mg\ L^{-1}].$$

Antioxidant capacity by ABTS assay was performed according to Miller et al. (1993). ABTS, potassium persulfate and standard Trolox were obtained from Sigma-Aldrich. As stock standard Trolox (2.5 mM) was prepared in ethanol (80 %). To prepare ABTS radical solution (ABTS•1) the 5 mL of ABTS solution (7 mM) and 88 mL of potassium persulfate (140 mM) solution were mixed and allowed to stand in the dark at room temperature for 16 h. On the day of analysis 1 % ABTS•1 solution (in 96 % ethanol) was prepared. Ethanol extract prepared for the purposes of total phenol content was used. A total of 160 µL of ethanol extract were directly injected in the cuvette and mixed with 2 mL 1 % ABTS•1, while absorbance at 734 nm was measured (Shimadzu 1650 PC, Germany). The final results was calculated based on calibration curve and expressed as mmol TE L^{-1}.

Statistical analysis

Statistical analysis was performed using the SAS (2010). The experiment was set as randomized block design with two containers of each vegetable species in each pool. The experiments were performed in triplicate. Data were subjected to the one-way analysis of variance (ANOVA). Mean values were compared by the t-test (LSD) and were considered significantly different at $p \leq 0.0001$. In tables different letters indicate significant differences between mean values within each column. Also standard deviation (±SD) was expressed.

Results and Discussion

Bioactive compounds content of spinach and New Zealand spinach grown in floating

Significant statistical differences for all analyzed bioactive compounds and antioxidant capacity of spinach and New Zealand spinach between treatments were determined (Tab 1). In spinach cultivated in control solution (Sk) the highest vitamin C content (46.70 mg 100 g^{-1} FW) was determined, while the lowest vitamin C content (28.67 mg 100 g^{-1} FW) was determined in treatment with 75 mg L^{-1} NH_4NO_3 (SN 1). The vitamin C content in New Zealand spinach leaves varied from 10.19 mg 100 g^{-1} FW (NzN 1; 104 mg L^{-1} NH_4NO_3) to 22.44 mg 100 g^{-1} FW (NzN 3; 205 mg L^{-1} NH_4NO_3). According to the obtained results, in New Zealand spinach leaves even twice lower vitamin C content was determined compared with spinach regardless of the treatment. Also, in spinach, vitamin C content significantly decreases due to the increase of NH_4NO_3 concentration during which in treatment SN 3 (205 mg L^{-1} NH_4NO_3) even 36% less vitamin C was determined compared to the control treatment (Sk). But in New Zealand spinach samples such trend was not determined, respectively the increase of NH_4NO_3 concentration stimulated the increase of vitamin C content. Numerous scientific data cites that increased nitrogen concentration negatively affects on the vitamin C content (Mozafar, 1993; Radman et al., 2015). Except vitamins, significant bioactive compounds are also phenols. In spinach cultivated in control solution (Sk) the highest total phenol content (108.04 mg L^{-1}) were determined while the lowest content (87.57 mg L^{-1}) in treatment SN 3 (205 mg L^{-1} NH_4NO_3). In New Zealand spinach samples the opposite results were obtained, i. e., the the highest NH_4NO_3 concentration (NzN 3) resulted with the highest total phenol content (43.91 mg L^{-1}). Cultivation of New Zealand spinach in solutions with lower NH_4NO_3 concentrations (NzN 2, NzN 1) and in control solution (Nzk) resulted with significantly lower total phenol content. Stowe & Osborn (1980) and Radman et al. (2015) cite that by increase of nitrogen concentration content of phenolic compounds are significantly reduced. The main reason for the opposite effect of higher nitrogen concentration on bioactive compounds content in samples of New Zealand spinach might be due to the

lower tendency of nitrates, nitrites and oxalates accumulation in New Zealand spinach compared to the spinach (Jaworska and Kmiecik, 1999). But also the content of bioactive compounds depends on the plant part (Jaworska, 2005). Significantly higher values, average 29%, of antioxidant capacity were determined in spinach compared to the New Zealand spinach regardless of the treatment. Obtained results are expected given that significantly higher values of bioactive compounds were determined in spinach leaves. Also, given that higher NH_4NO_3 concentrations affected on the reduction of bioactive compounds in spinach leaves, lower antioxidant capacity were determined in treatments SN 1, SN 2 and SN 3. Contrary, in New Zealand spinach treatment with the highest NH_4NO_3 concentration (NzN 3) the highest antioxidant capacity was determined.

Table 1. Bioactive compounds of spinach and New Zealand spinach

Treatment	VIT C (mg 100 g^{-1})	TPC (mg L^{-1})	TFC (mg L^{-1})	TNFC (mg L^{-1})	ANT_CAP (mmol TE kg^{-1})
Spinach					
Sk	46.70a±2.92	108.04a±1.75	70.77a±1.44	37.27a±0.04	2.17a±2.74
SN 1	28.67b±3.95	94.75c±1.21	62.30b±1.26	32.46b±0.37	2.16b±1.37
SN 2	38.34ab±8.61	98.92b±1.64	73.71a±1.79	31.44c±0.15	2.13c±2.06
SN 3	34.25ab±4.99	87.57d±0.36	56.13c±0.95	25.21d±0.59	2.16b±0.90
Pr≤F	0.0200	0.0001	0.0001	0.0001	0.0001
New Zealand spinach					
Nzk	10.71c±1.65	41.62b±0.58	29.07b±1.25	12.55a±0.30	1.62b±33.76
NzN 1	10.19c±1.87	42.33ab±0.95	33.81a±1.46	8.53b±1.07	1.64b±25.22
NzN 2	15.86b±1.22	38.10c±0.96	25.53c±1.25	12.57a±1.68	1.41c±12.08
NzN 3	22.44a±1.90	43.91a±0.71	31.53ab±0.67	12.38a±0.71	2.02a±23.46
Pr≤F	0.0001	0.001	0.001	0.0390	0.0001

VIT C- vitamin C; TPC- total phenol content; TFC- total flavonoid content; TNFC- total non-flavonoid content; ANT_CAP- antioxidant capacity; SN 1- spinach 75 mg L^{-1} NH_4NO_3; SN 2- spinach 140 mg L^{-1} NH_4NO_3; SN 3- spinach 205 mg L^{-1} NH_4NO_3; NzSk- New Zealand spinach control solution; NzN 1- New Zealand spinach 75 mg L^{-1} NH_4NO_3; NzN 2- New Zealand spinach 140 mg L^{-1} NH_4NO_3; NzN 3- New Zealand spinach 205 mg L^{-1} NH_4NO_3. Different letters indicate significant differences between mean values within each column.

Pigment compounds of spinach and New Zealand spinach grown in floating

The presence of nitrogen in the soil and chlorophylls in plants is directly related, whereby the content of chlorophyll increases by increasing the nitrogen concentration (Jones et al., 2007; Nemadodzi et al., 2017). However, the results of total chlorophyll content (Tab 2) obtained in this research are not in agreement with above mentioned. In both researched species the lower total chlorophyll content was determined due to the increase of NH_4NO_3 concentration in nutrient solution. The same trend was also determined for total carotenoids which content decreased by increasing NH_4NO_3 concentration, whereby the negative effect was less pronounced in New Zealand spinach treatments (NzN 1, NzN 2, NzN 3). Also, in spinach, higher values of pigment compounds content was determined compared to the New Zealand spinach, in average 59% more of total chlorophylls and 48% more of total carotenoids.

Tab 2. Pigment compounds of spinach and New Zealand spinach

Treatment	CHa (mg g^{-1})	CHb (mg g^{-1})	TCH (mg g^{-1})	TC (mg g^{-1})
Spinach				
Sk	0.54a±0.001	0.33b±0.005	0.88a±0.0003	0.17a±0.0007
SN 1	0.45c±0.002	0.37a±0.001	0.83b±0.004	0.12c±0.0001
SN 2	0.44c±0.001	0.32b±0.003	0.77c±0.0006	0.13c±0.0003
SN 3	0.47b±0.002	0.30c±0.001	0.77c±0.005	0.14b±0.003
Pr≤F	0.0001	0.0001	0.0001	0.0001
New Zealand spinach				
Nzk	0.39a±0.002	0.22a±0.0112	0.60a±0.032	0.10a±0.005
NzN 1	0.26c±0.0016	0.11d±0.003	0.37c±0.004	0.08b±0.0004
NzN 2	0.34b±0.0003	0.15c±0.0003	0.49b±0.0005	0.10a±0.0001
NzN 3	0.37a±0.0002	0.20b±0.0006	0.57a±0.0007	0.10a±0.0001
Pr≤F	0.0001	0.001	0.001	0.0390

CHa- chlorophyll a; CHb- chlorophyll b; TCH- total chlorophyll content; TC- total carotenoid content; SN 1- spinach 75 mg L^{-1} NH_4NO_3; SN 2- spinach 140 mg L^{-1} NH_4NO_3; SN 3- spinach 205 mg L^{-1} NH_4NO_3; NzSk- New Zealand spinach control solution; NzN 1- New Zealand spinach 75 mg L^{-1} NH_4NO_3; NzN 2- New Zealand spinach 140 mg L^{-1} NH_4NO_3; NzN 3- New Zealand spinach 205 mg L^{-1} NH_4NO_3. Different letters indicate significant differences between mean values within each column.

Conclusions

Based on the obtained results can be concluded that increase of the NH_4NO_3 concentration in nutrient solution in spinach cultivation significantly reduces the content of analysed bioactive compounds (vitamin C, total phenols, chlorophylls and carotenoids and also antioxidant capacity). In samples of New Zealand spinach opposite trend was determined. Also, it is important to emphasize that both tested leafy vegetable species cultivated in floating system are rich in nutritional composition and further studies in optimization of cultivation by hydroponics techniques especially of New Zealand spinach are necessary.

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INFLUENCE OF CROP DIVERSIFICATION ON POPULATION AND DAMAGE OF MILLIPEDES IN CASSAVA CULTIVATION

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Abstract

Millipedes are common pests of cassava and often cause serious economic losses. Pesticide application remains the first option that farmers choose to minimize the damage caused by the myriapods. The result is high health risk, increased cost of production and also environmental pollution. A field experiment involving false horn plantain (Apantu) as component crop, an improved cassava (Ampong) and landrace cassava (Afia Abaayaa), base crop was conducted on farm at two hot spot areas in 2015 and 2016. Plantain based intercrops and sole cassava were tested for their ability to reduce the population and damage caused by millipedes to cassava and the effect on root yield of cassava. The results followed a similar pattern in both locations. Plantain intercropped with both improved and local cassava proved the most effective intercrops. In these two intercrops, the incidence of the millipedes and the extent of damage to cassava roots were the lowest ($P<0.05$). The intercropping could not influence the yield of cassava however, the yield of plantain was negatively affected ($P< 0.05$) in the intercrop plots recording 50% low yields compared to the sole plantain. A total of 44 contact farmers were sensitized on using intercropping as a strategy to manage the activity of millipedes in cassava cultivation.

Keywords: *Cassava, incidence intercropping, millipedes, monocultures, pesticides, plantain.*

Introduction

Cassava *(Manihot esculenta* Crantz) is the fifth largest food crop after maize, rice, wheat and potato in the world (FAO, 2014). It provides the main source of carbohydrates to a large proportion of the population of the tropics, especially the inhabitants of the wetter tropics, including the western region of Ghana. It can be grown on a variety of soils and generally requires or receives little inputs. Cassava is therefore relatively cheap to produce. Again the potential yields are not realized because of a myriad of pest problems. The Research Extension-Farmer Linkage Committee (RELC), a body set up as a link between researchers and farmers in their report for 2010-2011 identified millipede infestation as a major contributing factor to low root yield of cassava and other root and tuber crops in the Western Region of Ghana. Accordingly, the estimated annual average for cassava production in metric tons decreased from 722,162 in 2003 to 707,894 in 2008 (MoFA 2008). Millipede infestation in January 2012 caused a panic situation for officials of the national agricultural ministry, researchers and politicians. Reconnaissance survey to the area by a team of research scientists from CRI identified three hot spots in Wassa Amenfi East, Prestea-Huni Valley and Wassa East districts. Their pest status has been documented (Fening *et al.*, 2016).

Millipedes normally live in and feed on rotten leaves and wood and other kinds of moist decaying plant matter and are therefore useful as scavengers. However, when conditions are favourable they develop high populations and invade farms and dwellings (Baker, 1979). There are reports that millipedes cause economic damage to several crops in Ghana (Peters, 2000; Fening *et al*., 2014) and elsewhere (Atukunda *et al*., 2003; Ebregt *et al*., 2004; Epidi and Njoku, 2010). These include root and tubers such as cassava, sweet potato, cocoyam and yam. Other affected crops are guinea corn, cotton, millet, groundnut, watermelon and

cabbage. With the current world population increasing by an estimated 97 million per year (Saravi and Shokrzadeh, 2011) the implication is that many more mouths would have to be fed. This therefore necessitates a growing need for increased agricultural production. Cultivation of arable crops to maximize crop productivity has attained the simplified status of monocropping resulting in artificial ecosystems. Most arable, cash and plantation crops such as rice, maize, pineapple, cassava and plantain are currently produced in monocultures worldwide. Consequently, this system has resulted to increased vulnerability of such crops to pests and diseases pressure and ultimately leading to agro pesticide approach of managing these biotic stresses. Besides the heavy investments in agro chemicals coupled with its varied effects on food poisoning and environmental pollution, crop losses due to pests currently stand at an average of 35% globally (Oerke, 2006). About 3 billion kilograms of pesticides is applied globally each year with an annual spending of nearly $38 billion (Pan-Germany, 2012). Environmental and social costs associated with current level of pesticide use have been estimated at $10 billion per year (Pimentel, 2005). According to Carriger *et al*. (2006), only about 0.1% of the pesticides reach the target organisms and the bulk of the remnant contaminates the surrounding (Kumar *et al*., 2015). Annually, cases of pesticide poisoning affects 5 million people, most of them in developing countries and accounts for 20,000 unintentional deaths (WHO, 2004). Although Africa accounts for less than 4% of global agrochemical use, its farmers face the greatest barriers in equipping themselves against pesticide hazards (Williamson, 2003). As a result, the continent which uses the least of world agro chemicals, suffers over 90% of pesticide effects (Eddleston *et al.,* 2002).

An alternative approach based on sound ecological principles which conforms to indigenous farming practice to ensure efficiency and sustainability must be adopted. This is needed to take advantage of the immense benefits of diversifying crop ecosystems and can be achieved by promoting polycultures such as mixed cropping and intercropping. Intercropping is a common phenomenon in Ghana's indigenous agriculture but mostly recognized as insurance against crop failures. Intercropping of cassava with plantain in field trials has been found to reduce the population of millipedes (Braimah *et al*., 2012). In general, Intercropping with cassava is vital in weed, pest and disease reduction (Wolfe, 2000). Ibeawuchi (2004) also reported that intercropping effects through its diverse crop species and morphology, keeps low its attack and destructive tendencies since some of the intercrop species may be resistant to the pest and or disease. The objectives of the study therefore were; to evaluate the incidence of millipedes and their damage in plantain based intercropping system in comparison with sole crops of cassava and to sensitize and train farmers on impact of intercropping on millipedes.

Materials and Methods

Experimental site description

Field experiments were conducted on-farm in June 2015 to October 2016 in two communities of two districts of the Western region of Ghana. These are Nsuopun (5.862°N, 1.985°W) within an elevation of 181m above sea level (a.s.l) in the Amenfi East District and Tebe (5.391°N, 2.033°W) at an elevation of 91m (a.s.l) in Prestea-Huni Valley district identified as endemic areas of millipede infestation of root and tuber crops in the Western region. The area falls within the high forest vegetation zone and lies in the equatorial climate zone that is characterized by moderate temperatures and receives bimodal pattern of high rainfall with an average of 1,600mm per annum. Dominant crops cultivated in the area include cassava, cocoyam, plantain, cocoa, oil palm rubber and vegetables.

Intercropping treatments and field layout: A land space of 35 x 31 m^2 (1.09 ha) was prepared by slashing and burning and marked out into plots of 6m x 9m. Treatments comprised; Sole Plantain (SP), Plantain + improved cassava (PCI) Sole improved cassava (SCI), Plantain +

local cassava (PCL) and Sole local cassava (SCL). The cassava varieties were improved variety (Ampong) and a landrace (Afia Abaayaa). The Apantu variety of plantain was used. The treatments were arranged in a Randomized Complete Block Design (RCBD) and replicated thrice. Weeding was done at 3, 6, and 9 months after planting.

Data collection: Parameters measured included number of millipedes per plant, number of roots damaged by the millipedes per plant, number of holes per plant and yield in tons per hectare. Data were collected at 6 months after planting and at harvest. During sampling, 5 cassava plants were uprooted in the inner rows and 5kg of soil was scooped and assessed for soil arthropods on all plots. Roots were also counted, visually assessed for damage holes and weighed

Selection of contact farmers for sensitization and training exercise: Purposive sampling procedure was used to select participating contact farmers. Farmers who have cropped cassava for 5 years continuously and were ready to participate were selected from the two millipede endemic communities. In all 44 farmers comprising 16 from Nsuopun and 38 from Tebe registered. They were made up of 17 and 27 females and males respectively. All husbandry activities were carried out with the contact framers. On each activity day farmers were sensitized on intercropping as a measure to manage millipedes on cassava fields.

Data analysis: To test the effects of the cropping system (sole cropping and intercropping) on millipede population, root damage and yield, the field data collected was subjected to analysis of variance (ANOVA). The significance of differences in mean values was analyzed by Tukey's Studentized Range (HSD) Test method of SAS (Proc GLIMMIX, SAS 9.3; Cary, North Carolina, USA). Quantitative data recorded for beneficiary farmers' sensitization was analyzed with descriptive statistics.

Results and discussion

Assessment of intercropping effects on millipede incidence and damage 6MAP

Intercropping plantain with cassava significantly affected millipede population 6 MAP in both locations (Table 1). However, no damage to roots was recorded 6 months after planting. In both locations, the intercrop of the landrace harboured significantly higher millipede population than any of the intercrop and sole cropping. Generally, millipedes' population was higher at Tebe compared to Nsuopun. Lower millipede population was recorded for sole plantain at Tebe but in sole cassava (improved) at Nsuopun.

Table 1. Mean number of millipedes at 6 months after planting (MAP), Nsuopun and Tebe 2015

Treatments	Millipedes (Nsuopun)	Damaged roots	Millipedes (Tebe)	Damaged roots
Sole Plantain (SP)	1.25±0.63	0	4.50± 0.29	0
Plantain+ Improved Cassava(PCI)	1.50±0.65	0	3.75±0.85	0
Plantain+ Local Cassava (PCL)	3.50±0.95	0	5.75±0.75	0
Sole Improved Cassava (SCI)	1.75±0.48	0	2.50±0.29	0
Sole Local Cassava (SCL)	2.50±0.87	0	4.25±0.95	0
F value P<0.05	1.53 0.2425		2.96 0.0548	

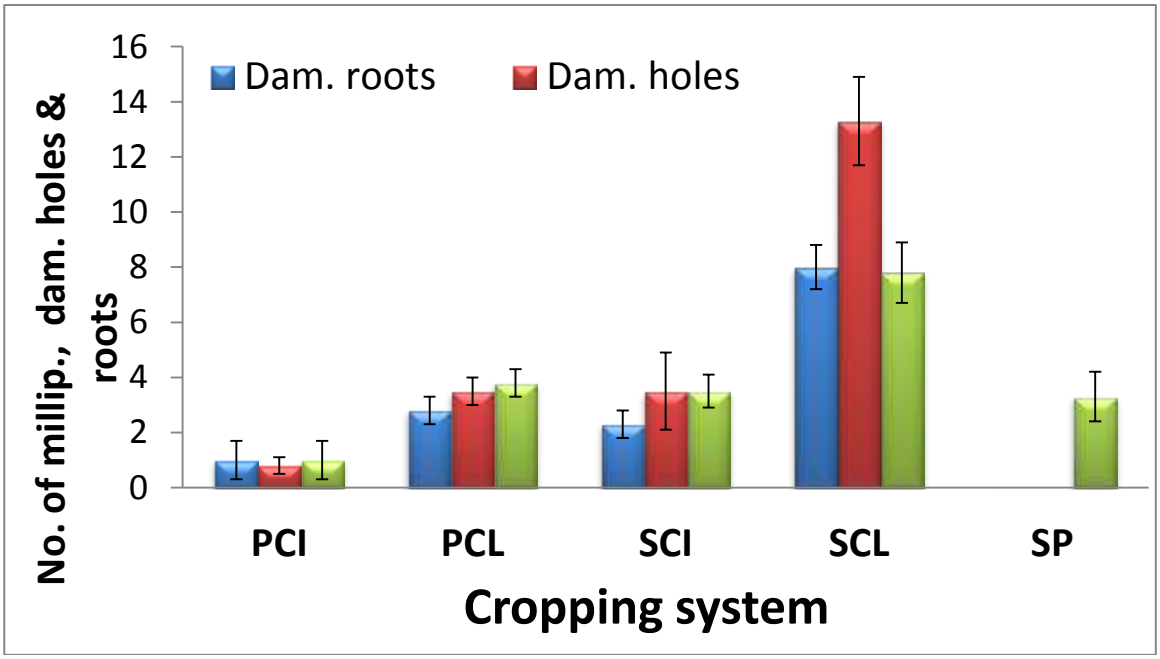

Figure 1. Effect of intercropping plantain with cassava on millipede population and damage at Tebe, 2016

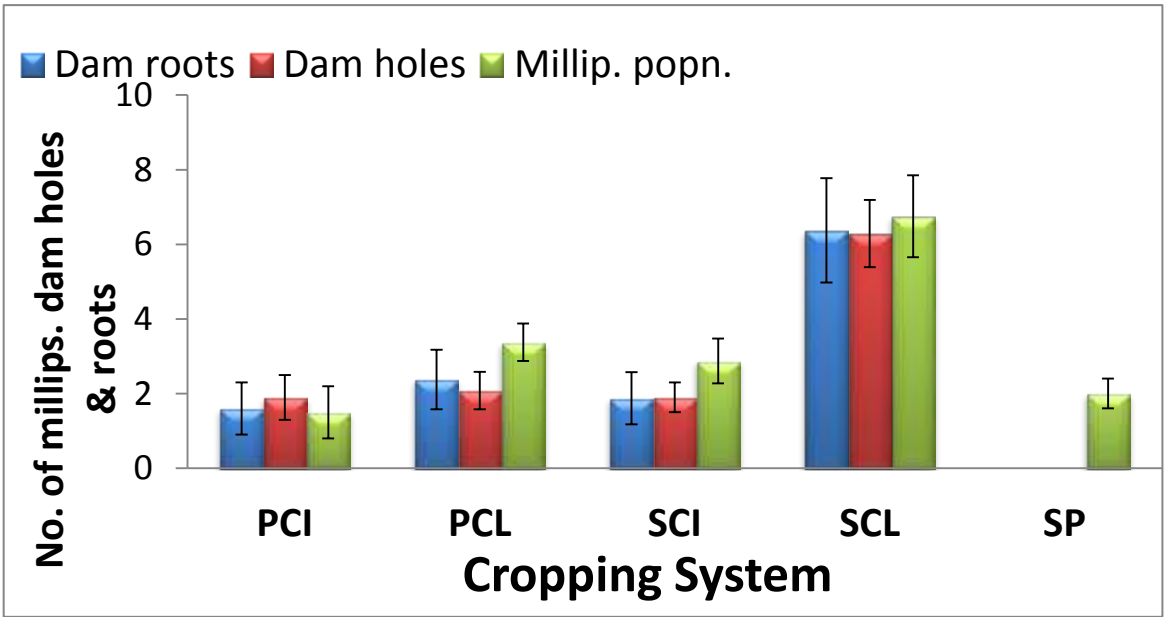

Figure 2. Effect of intercropping plantain with cassava on millipede population and damage at Nsuopun, 2016

Assessment of intercropping effects on millipede incidence and damage at harvest: At harvest, the population and damage records followed a similar pattern at both locations with intercropping plots recording lower population and damage levels compared to sole plots (Figure 1). At Tebe more millipedes as well as their damage were recorded than at Nsuopun. Millipedes showed some preference for local cassava than the improved materials. The plantain and improved cassava intercrop recorded significantly low number of millipedes as well as their damage among all the treatments. No differences ($P<0.05$) were shown in plantain and local cassava as well as sole cropping of improved cassava. Millipede population and damage was significantly highest in the landrace as sole crop

Assessment of intercropping effects by millipede incidence and damage on yield: The intercropping could not significantly affect yield of cassava figure 2(a). The result is expected as improved variety significantly (P< 0.05) out-yielded the landrace. On plantains however, there were significant differences in yield with the intercropping of improved cassava recording 4 tons/ha while local cassava intercrop recorded about 6 tons/ha. Yields of plantain in the intercropping system was 50% lower than the sole plantain.

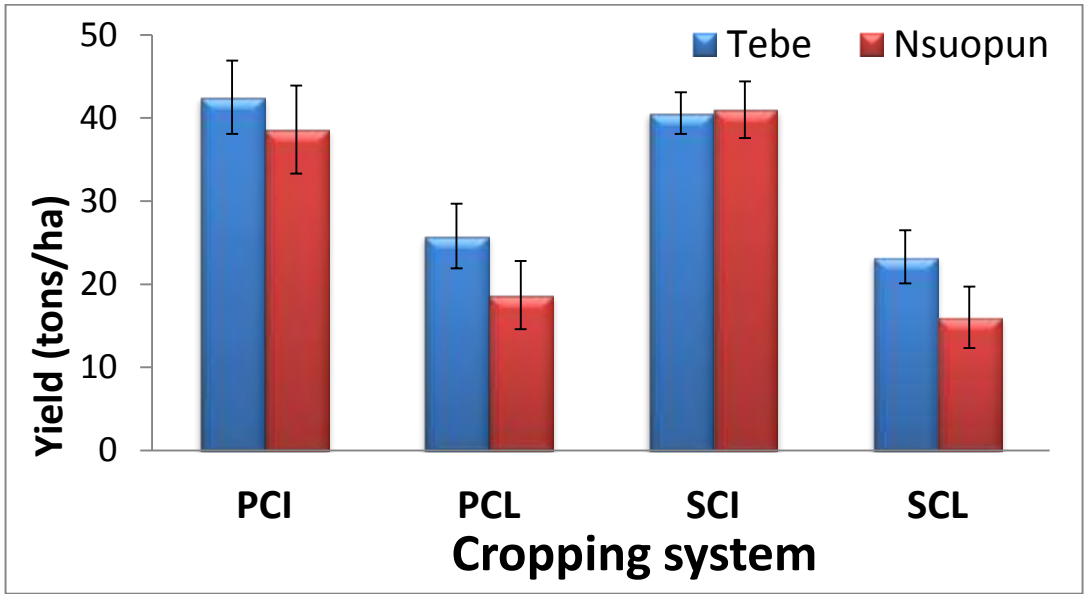

Figure 3. Yield of cassava in plantain based cassava intercrop at Tebe and Nsuopun, 2016

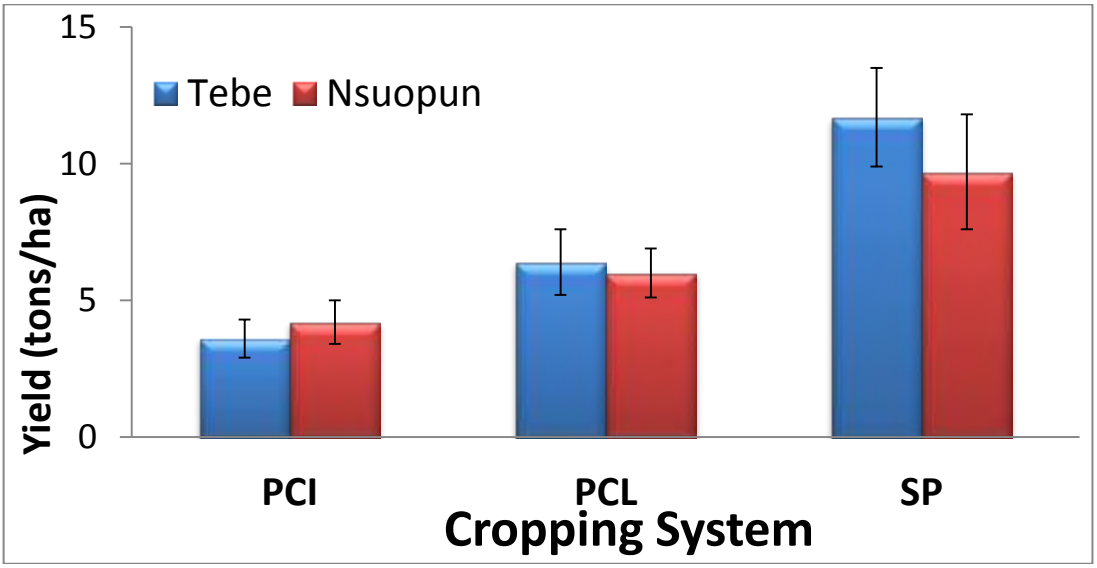

Figure 4. Yield of plantain in plantain based cassava intercrop at Tebe and Nsuopun, 2016

Beneficiary farmer sensitization on effects of intercropping to manage millipedes

Table 2. shows details of contact farmers sensitized on how to use intercropping technology to mitigate the deleterious effects of millipedes on cassava fields. In total, 44 farmers were sensitized 28 of which were from Tebe. Females constitute 39% much the same for youthful farmers.

Table 2. Beneficiary farmers sensitized on effects of intercropping plantain with cassava at Nsuopun and Tebe 2016

Location	**Estimated Area (Ha)**	**Beneficiaries**			**Youth *(up to 35yrs)***	**% Youth**
		Male	Female	Total		
Nsuopun	0.2ha	10	6	16	5	31
Tebe	0.2ha	17	11	28	12	43
Total	0.4ha	27	17	44	17	39

The ever increasing scarcity of cultivable land in the developing world necessitates the practice of intercropping. Plantain and cassava intercrop is commonly practiced in Africa (Dowiya *et al*., 2009; Akinyemi *et al*., 2010; Ngeleza *et al*., 2011). Plantain and cassava intercrop is advocated from this study to manage the deleterious effect of millipedes in cassava fields. This assertion falls in line with Ennin *et al.*, (2002) who stated that the practice of intercropping increase efficiency of resource utilization. Assessment at 6 MAP showed millipede presence in intercrops and sole cropping fields of plantain and cassava as observed by Fening *et al*. (2016). The millipede presence however did not manifest any damage on the developing tuberous roots probably due to their low numbers or the short dry spell experienced around the time of assessment which could have reduced millipede activities. The higher millipede numbers recorded at Tebe as compared to Nsuopun support the findings of Fening *et al*., 2016 which could probably be as a result of the high elevation of Nsuopun. Lowest millipede population recorded in sole plantain at Tebe was expected since plantain was found to be unattractive to millipedes (Braimah *et al*., 2012)

At harvest, the trend was not different. Activities of millipedes were minimal in the intercropping plots as compared to the sole plots. The unattractiveness of plantain to millipedes coupled with the non preference of improved cassava varieties was exhibited in this study and corroborates the findings of (Braimah *et al.*, 2012; Fening *et al.* 2016). This resulted in the least millipede populations and damage to roots in the intercropping of the improved cassava. The performance of plantain and local cassava as well as sole improved cassava was similar, controlled millipede population and damage lower than sole local cassava. Sole local cassava therefore recorded very high millipede population and damage which confirms the assertion of Fening *et al*. (2016) that millipedes have particular preference for local cassava and highly susceptible to pests and diseases (Okechukwu and Dixon, 2000).

The yield of cassava in both intercrop and sole cropping were not influenced by intercropping, this corroborate the findings of (Prabhakar and Nair, 1992) noting that neither spatial arrangement of cassava nor change in plant density of intercropped groundnut had any substantial effect on tuber yield of cassava. Yields of intercrops were generally reduced as compared to the potential of individual crops but the reduction is often compensated by utilization of resources such as weeds smothering, reduction in runoff and soil losses, additional income to farmers, provision of food security and reduction in pests' population and their damage (Ennin *et al.*, 2002; Akinyemi *et al*., 2010). Reduction in yield of plantain in intercropping as against the sole crop is supported by (Karikari, 1981; Adetiloye, 2003) and could probably be due to the high nutrient requirements of cassava.

The approach of sensitizing farmers by use of evidence-based interventions in formulating training according to Ssekyewa (2005), Mariyono (2007) and Pophiwa (2012) ensure success by promoting results oriented innovations which are visible and significant enough to convince farmers to try the new practice themselves. Training of farmers to improve their knowledge of new technologies and increase their level of productivity **(**Rosegrant and Cline,

2003). Forty per cent of farmers who participated in the sensitization exercise belong to the youthful bracket, an indication that the innovation could be practiced for a long time and adopted by many farmers in future.

Conclusion

We conclude that for effective and sustainable management of millipedes as pests of root crops, plantains intercropped with improved cassava is best although yields of plantains could be compromised and sensitizing farmers to try a new practice on their own ensures sustainability of the innovation.

Acknowledgements

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Authors' contributions

AI, MMB AWB and BH conceived and designed the research and data collection protocols and participated in drafting the manuscript. AI and ADA collected the data, participated in data analyses and wrote the manuscript. MMB performed data analyses. BH revised the first draft. All authors read and approved the final manuscript.

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APHICIDAL ACTIVITY OF AQUEOUS EXTRACTS OF *EPHIDRA SINICA* MEDICINAL PLANT AGAINST THE PEACH TRUNK APHID, *PTEROCHLOROIDES PERSICAE* (CHOLODKOVSKY) (HOMOPTERA: LACHNIDAE), ON CHERRY

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Abstract

The peach trunk aphid is an important insect pest attacking stone fruits in Jordan and worldwide. It attacks almond, cherry, apricot and peach. Insecticides represent the main solution to control this insect pest, but they proved to pose many environmental problems and cause toxicity to human. In addition, many insects developed resistance to insecticides that lead farmers to raise their application rates and increase their frequency. So, it is not recommended to use these chemicals to control this insect particularly at home gardens. Using botanical insecticides proved to have success in controlling insect pests. The purpose of this research project was to test the aphicidal activity of the aqueous extracts of *Ephidra sinica* medicinal plant on the peach trunk aphid using the shoot-cut bioassay technique and different concentrations prepared to determine the Median Lethal Concentration rate (LC_{50}) of the plant extract. Results revealed that high concentration of aqueous extract of *Ephidra sinica* medicinal was toxic as Imidacloprid to the peach trunk aphid, *Pterochloroides persicae.*

Key words: *Botanical insecticide, Peach trunk aphid, Integrated pest management, Botanical insecticide.*

Introduction

Stone fruits are grown in Jordan both commercially and as part of home gardens (Ateyyat, 2008). In 2014, about 91671 dunums were under productive stone fruit cultivation in Jordan that produced 117,326 tons (Statistical Year Book, 2014). Stone fruits in Jordan are attacked by several insects including *Capnodis* spp., ***Zeuzera Pyrina, Scolytus rugulosus*** and different aphids that threaten their produce (Mustafa & Al-Momany, 2007) . Three aphid species are recorded to colonize stone fruits in Jordan from which peach trunk aphid, *Pterochloroides persicae* represent the most destructive one (Ateyyat, 2008). Sever infestations result in branch decline due to aphid colonies sucking that may induce tree decline if infestation continued to a few years. As other aphids, it excretes huge amount of honeydew which encourage sooty mold growth on upper plant parts and affect physiological operations (Ateyyat, 2008). Mustafa (1991) studied the reproductive biology of the peach trunk aphid in Jordan. Also, Ateyyat (2008) evaluated the efficacy of three insecticides and two insect growth regulators against this insect pest. Farmers mainly depend on chemicals to control this insect; the chemical insecticides pose negative effects on environment and human health particularly when using them in home gardens. Botanical insecticides proved to provide an alternative safe control methods for aphids (Ateyyat, 2009; Ateyyat et al., 2012a; Ateyyat et al., 2012b; Ateyyat et al., 2012c; Ateyyat and Abu Darwish, 2009; Al-Mazra'awi and Ateyyat, 2009; Hakimi et al., 2015).

Ephedra plants are members of the Ephedraceae family. They have medicinal properties as they are used as part of the tradition medicine to treat hay fever and common cold (Janet et al., 2003). Methanolic Extracts of *Ephedra pachyclada* showed to have an antimicrobial

Activity on Some Enteric Gram-Negative Bacteria that known to cause Nosocomial Infections (Sadeghi Dosari et al., 2016). Many negative impacts were recorded because of the extensive and misuse of chemical insecticides application. These include; environmental pollution, acute and chronic toxicity to agricultural workers and consumers, development of resistance, resurgence and outbreaks in insect pests. The present research project provides an alternative safe solution to reduce these negative impacts as botanicals could be use as part of the integrated pest programs and/or organic agriculture. The insecticidal activity of Ephidra was not tested as far as the data bases show.

So, the purpose of this study is to study the aphicidal activity of the aqueous extracts of the leaves of *Ephidra sinica* against the peach trunk aphid.

Materials and method

Plant extract

Ephedra leaves will be collected from Al-Tafila city (220 km south of Amman) in Jordan. Leaves will be thoroughly washed with tap water to avoid unwanted materials accumulated on the leaves then they will be left to air-dry under shade at room temperature. Powder will be prepared from the dried material using an electrical home grinder. Ten grams of the powdered material will be dissolved in 90 ml of distilled water and will be kept in a reciprocating shaker for continuous agitation for 24 h for thorough mixing. After that, filtration for the extract will be made using vacuum pressure pump. The resulted amount will be completed to 100 ml by adding extra distilled water to get a stock solution of 10 000 mg/L concentration. More concentrations (dilutions) will be prepared as required.

Bioassay

The aqueous extract of Ephidra leaves will be tested against the peach trunk aphid. Insects will be collected from infested stone fruit trees and the excised cut-shoot bioassay technique will be used in which about 25 adults or immatures will be moved to 10-cm long shoot of healthy stone fruit. After ensuring that the insects settle on the healthy shoots, they will be exposed to the required treatment. Two controls will be used, the negative control in which distilled water will be used and the positive control in which imidacloprid insecticide will used at the lower recommended rate of application. Each treatment will be replicated at least three times. Treatments will be applied using aerosol sprayers (Preval, Chicago Aerosol, Coal City, Illinois) under a fume hood. Mortalities will be recorded after 24, 48 and 72 hr of treatment applications.

Statistical Analysis

For calculating the Median Lethal Dose (LC_{50}) and the Lethal Concentration 90 % (LC_{90}), mortalities will be corrected using Handerson-Tilton's formula, after which SAS (2014) will be used to get the LC_{50} and LC_{90} of the extract. Also, the same program will be used to obtain the one-way ANOVA and mean separation to compare the best tested concentration of the aqueous extract of Ephidra with both negative and positive controls at the three recorded times.

Results and discussion

About 15, 37 and 96 grams per liter of *Ephidra sinica* aqueous extracts are required to achieve the thirty lethal dose (LC30), the median lethal dose (LC_{50}) and the ninety lethal dose (LC_{90}), respectively (Table. 1).

Table 1. Concentration-mortality data for peach trunk aphid treated with *Ephidra sinica* aqueous extracts

Slope ±SE	LC_{30} (gm/l)	95% CL	LC_{50} (gm/l)	95% CL	LC_{90} (gm/l)	95% CL
0.0373± 0.0103	14.555	-25.802 – 47.292	37.274	11.338- 10.1763	96.189	58.137 – 292.531

Using Ephidra at high concentration of 100 grams per liter gave high mortalities to PTA immatures, which is not significantly different with that obtained using the positive control treatment through the use of imidacloprid insecticide after one day, two days and three days of applications (Figs 1,2,3). Decreasing the concentration of Ephidra to 10 g/l resulted in 85% mortality after 72 days of application (Fig. 3) though it was 58 % after 24 h of treatment (Fig. 1). Even though all the tested concentrations of aqueous extracts of Ephidra gave significant mortalities to PTA compared with negative control, but its use at 1 g/l and below resulted in mortalities below 40 % after 24 h of application (Fig. 1).

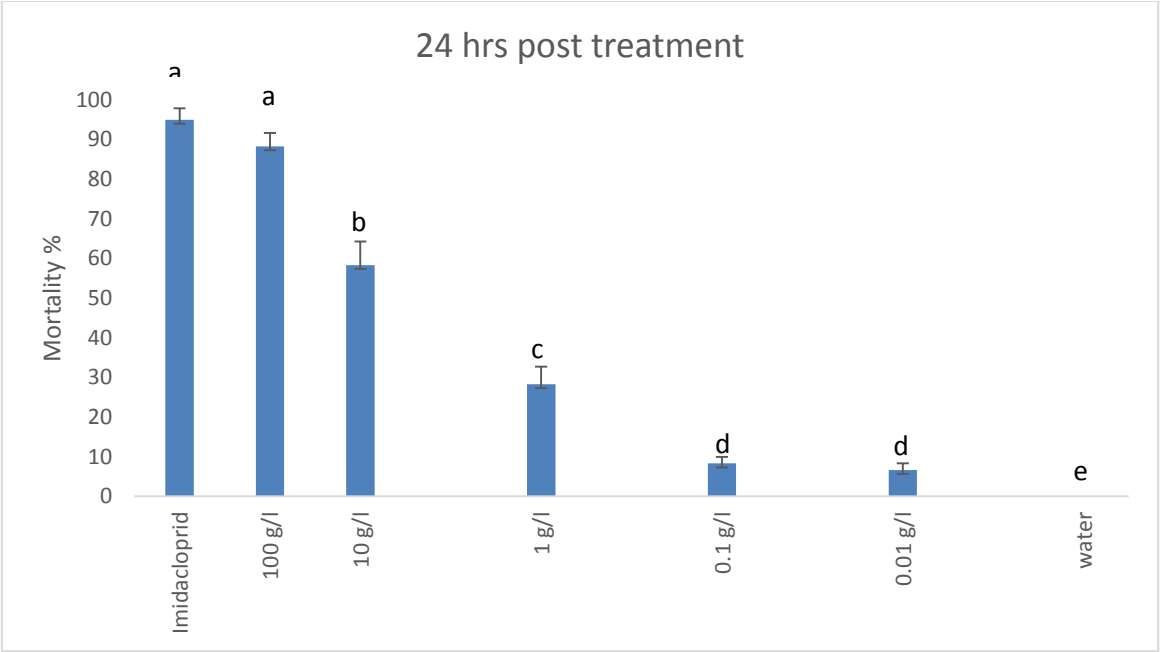

Figure 1. Percentages of mortality of peach trunk aphid exposed to different concentrations of *Ephidra sinica* aqueous extracts 24 hrs post treatment. Means with different letters are not significantly different using LSD at 95% CL after arcsine transformation.

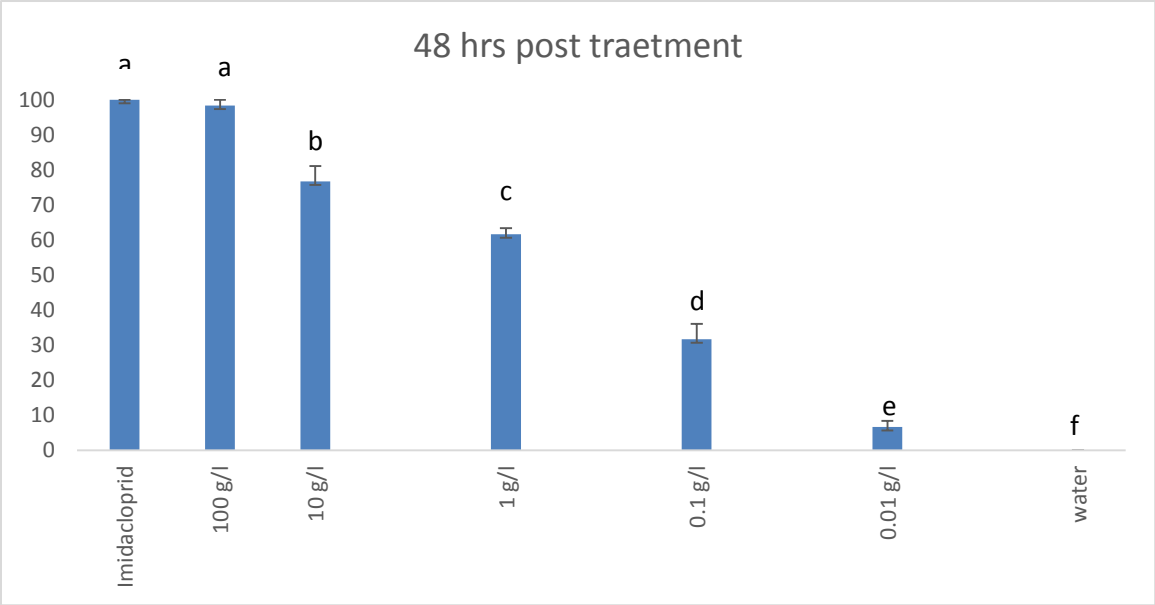

Figure 2: Percentages of mortality of peach trunk aphid exposed to different concentrations of *Ephidra sinica* aqueous extracts 48 hrs post treatment. Means with different letters are not significantly different using LSD at 95% CL after arcsine transformation.

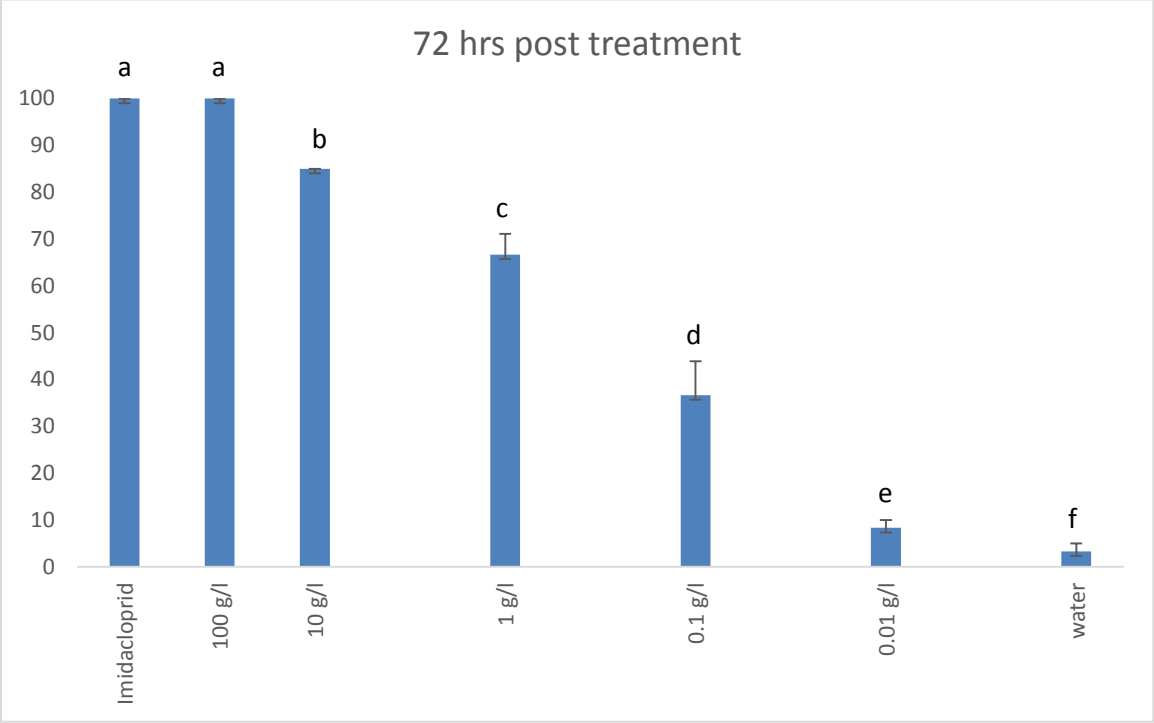

Figure 3: Percentages of mortality of peach trunk aphid exposed to different concentrations of *Ephidra sinica* aqueous extracts 72 hrs post treatment. Means with different letters are not significantly different using LSD at 95% CL after arcsine transformation.

Results show the requirement of huge amounts of Ephidra plants to be extracted to achieve a valuable control of PTA that may be related to the large size of this insect pest. This decrease its chance to use plant as botanical insecticide. However, the case may be different with other insects or pests. No studies on the insecticidal activity of this plant are available as far as databases show. Therefore, its mode of action on insects is not available, but Gurley et al. (1998) identified various alkaloid and non-alkaloid compounds, particularly ephedrine-type

alkaloids (ephedrine, pseudoephedrine, norephedrine, and methylephedrine). Mortality of PTA may be ascribed to these alkaloids.

Conclusion

Though ephedra used as a medicinal plant but the FDA in 2003 recommended consumers stop using ephedrine-alkaloids' products (FDA, 2007) due to registered cases of severe disability or sudden cardiac death caused by ephedra use (Haller, 2000). This opens the door for extended research to divert the attention of using this plant from medicinal plant to botanical insecticide as part of integrated pest management programs.

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FUNGISTATIC AND FUNGICIDAL EFFECT OF AQUEOUS PLANT EXTRACTS AGAINST SOME PHYTOPATHOGENIC FUNGI

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Abstract

Every year a lot of crop damage is caused by various diseases and among them fungal diseases are very common. The fungistatic and fungicidal effect of aqueous plant extracts were evaluated on several phytopathogenic fungi (*Fusarium oxysporum, Rhizoctonia solani, Alternaria alternata, Botrytis cinerea* and *Plasmopara viticola).* Fresh aerial parts of *Pinus nigra* J. F. Arnold, *Pinus sylvestris* L and *Juniperus communis* L. were collected for the preparation of aqueous extracts. The poisoned food method was used in the preliminary screening of aqueous extracts for their antifungal properties evaluation (for 20 and 10% of aqueous extracts of each plant), and broth-microdilution method was used to determine the minimal inhibitory concentrations (MICs) and the minimal fungicidal concentrations (MFCs) of the extracts (with 10% of starting test concentrations of aqueous extracts of each plant). This study showed that all the aqueous extracts obtained from the aerial parts of the tested plants had strong fungistatic and fungicidal activities against the phytopathogenic fungi. Anyway, the extract from *Juniperus communis* L. showed the strongest activity against *Alternaria alternata* with MICs of 2.5% and MFCs of 5%, and *Alternaria alternata* was shown as most sensitive fungus against tested plant extracts. The antifungal properties of *Pinus nigra* J.F. Arnold, *Pinus sylvestris* L and *Juniperus communis* L. make these plants of potential interest for the control of fungi affecting plant yield and safety. These preliminary results, obtained from *in vitro* experiments, may be supplemented by other more comprehensive studies *in vivo*, both in controlled greenhouse conditions and in open field to practically evaluate the use of these extracts.

Key words: *plant extracts, phytopathogenic fungi, poisoned food method, broth-microdilution method.*

Introduction

More than 800 million people in developing countries do not have adequate food supplies and at least 10% of food is lost due to plant diseases (Strange and Scott, 2005). In a hungry world with an increasing population, efforts are made at reducing crop loss due to pathogen fungi. Compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses. In recent time, the application of synthetic fungicide proved effective in inhibiting growth of plant-pathogenic fungi and this increases the use tremendously, thereby causing a lot of harmful effects such as health and environmental problems. In the attempt to combat this, many countries have made rules and regulations in the use of fungicides. The uses of phytochemicals in the treatment of plant microbes have been found to be the better alternative. Increased public concern about the accumulation of pesticide residues in the biosphere and the development of resistance among pathogens against conventional antibiotics has led scientists toward the development of alternative strategies for plant disease suppression. Also, recently, public pressure to reduce the use of synthetic fungicides in agriculture has significantly increased (Shafique et al., 2007).

Biological control is a nature friendly approach that uses different bioactive compounds produced by other organisms that may act as suppressors or/and inhibitors in the

developments of phytopathogens. Natural product-based fungicides have the ability to decompose rapidly, reducing risk to the environment with the added advantage in that they have both unique modes of action and low mammalian toxicity (Duke et al., 2003; Ortiz et al., 2008). Plants have the ability to synthesize secondary metabolites highly active against the pathogen. These metabolites show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms.

Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases (Varma and Dubey, 1999; Gottlieb et al., 2002).

Considering these, in this work, we reported the *in vitro* antifungal activity of extracts from three plants against five important phytopathogenic fungi.

Materials and methods

Plant material

The needles of 50 year old *Pinus nigra* J.F. Arnold, *Pinus sylvestris* L. and *Juniperus communis* L. were collected during October 2013 (autumn season) from Berovo region in Macedomia. The needles from lower third of crown of ca. 30 randomly selected trees in each of the populations were collected. The plant parts were air-dried at room temperature for three weeks in dark conditions. The dried plant parts were milled to a fine powder in a mill.

Preparation of the plant aqueous extracts

First, stock aqueous extracts were obtained by soaking 20 and 40 g of air-dried and milled plant material in 100ml of distilled water (20% and 40% w/v) at room temperature (20 ± 2^0C) for 24 hours with occasional shaking. Then, the mixtures were filtered through two layers of cheesecloth and centrifuged for 20 min at 5.000 rpm to remove particulate materials. Finally, the extracts were stored in the refrigerator at 4^0C to be used in the future.

Test organisms

The target fungi were chosen to represent potential pathogenic fungi causing plant diseases. The fungi samples, *Botrytis cinerea* FNS- FCC 23, *Fusarium oxysporum* FNS- FCC 103, *Plasmopara viticola* FNS- FCC 65, *Alternaria alternata* FNS- FCC 624, *Rhizoctonia solani* FNS- FCC 218 were provided from the Culture Collection of the Department of Microbiology and Microbial Biotechnology, Faculty of Natural Sciences and Mathematics, Skopje, Macedonia. Fungal cultures of phytopathogenic strains were kept on Sabouraud Dextrose Agar (SDA) at 4^0C.

Inoculum disc

Seven days old culture of the test fungi were used for the preparation of inoculum disc of 5 mm in diameter.

The poisoned food method

This technique involves the poisoning of the fungal growth medium using antifungal agent and then measuring the reduction of growth of the organism on the medium. The decrease in mycelial growth indicates the inhibition of fungal growth by the antifungal substance (Ali-Shtayeh and Abu Ghdeib, 1999; Mukherjee and Raghu, 1997; Kumar et al., 2013). The extracts were incorporated into the molten Sabouraud Dextrose agar at a desired final concentration (20 and 10% of aqueous extracts of each plant) and mixed well. Then, the medium was poured into Petri dishes. After overnight pre-incubation, the inoculation was by a mycelia disc of 5 mm, which was deposited in the center of the plate. After further incubation under suitable conditions for the fungal strain tested (25 ± 1°C/ five days) the diameters of fungal growth in control and sample plates were measured, and the antifungal effect was estimated by the following formula:

$$\text{Antifungal activity}(\%)=((Dc-Ds)/Dc)\times 100$$

Where Dc was the diameter of growth in control plate and Ds was the diameter of growth in the plate containing tested antifungal agent.
Five replications were maintained for each isolate and the mean values were calculated.

Broth-microdilution assays

Antimicrobial activity was tested using broth microdilution method, where minimum inhibitory concentrations (MIC) determination was performed by a serial dilution method in 96 well microtitre plates. Cultures of fungal species were maintained on Sabouraud Dextrose agar at appropriate optimal temperature.
After 5 days of cultivation, fungal suspensions were made in Sabouraud Dextrose broth and their turbidity was standardized using McFarland 0.5. Each well contained the final fungal concentration of 10^6 CFU/mL.
Stock solutions of the extracts was 20% (w/v) and than serially diluted. The tested concentrations of extracts were within 10- 0.078125% (w/v). After making dilutions of the extracts, the inoculum was added to all wells and the plates were incubated at 25±1°C for seven days. To ensure medium sterility, each plate contained one non-inoculated well without extract. Experiments were performed in triplicate against each fungus. MIC was defined as the lowest concentration of extracts at which microorganisms showed no visible growth after seven days. Determination of minimal fungicidal concentrations (MFC) was performed by inoculation of broth taken from all clear wells on Sabouraud Dextrose agar, which were further incubated for seven days at 25±1°C. The MFC was defined as the lowest concentrations of the extracts at which inoculated microorganisms were killed.

Results and discussion

Phytopathogenic fungi are the most problematic pests of agricultural crops worldwide (Savary et al., 2006). *Aspergillus flavus*, *Alternaria alternata* and *Botrytis cinerea* are among the prevalent pathogens causing diseases in many economic crop species.
Plants produce many kinds of low-molecular-mass secondary metabolites that are generally non-essential for the basic metabolic processes of the plant.
In the current study, we evaluated the antifungal activity of plant extracts on some phytopathogenic fungi. The effect of 20 and 10% of aqueous extracts of each plant (*Pinus nigra* J. F. Arnold, *Pinus sylvestris* L and *Juniperus communis* L.) on the radial growth rate of the tested fungal species is presented in Table 1.

Table 1. Fungal growth inhibition (in percentage) by the extracts of the tested plants on phytopathogenic fungi.

		Botrytis cinerea FNS- FCC 23	*Fusarium oxysporum* FNS- FCC 103	*Plasmopara viticola* FNS- FCC 65	*Alternaria alternata* FNS- FCC 624	*Rhizoctonia solani* FNS- FCC 218
Pinus nigra J. F. Arnold	10%	68.2	70.4	45.4	72.1	54.5
	20%	78.5	74.6	54.9	84.3	62.3
Pinus sylvestris L	10%	66.3	69.1	52.2	76.3	65.9
	20%	74.2	77.3	61.9	84.8	72.1
Juniperus communis L.	10%	75.9	78.3	63.3	87.3	75.8
	20%	81.2	82.0	71.5	97.8	88.4

This study showed that all the aqueous extracts obtained from the needles of *Pinus nigra* J.F. Arnold, *Pinus sylvestris* L. and *Juniperus communis* L. have antimicrobial properties. All the

extracts showed inhibition effects on the growth of the test fungui (Table 1). Among the tested plant species, *Juniperus communis* L. markedly inhibited the fungus growth even at a 10% of concentration. The inhibition of the mycelial growth of the test fungi was found to be 63.3 to 97.8 percent by the extract of *Juniperus communis* L.; 52.2 to 84.8 percent by the extracts of *Pinus sylvestris* L; and 45.4 to 84.3 percent by the extracts of *Pinus nigra* J. F. Arnold at the concentration of 10 and 20%. The extracts of *Juniperus communis* L. was found to be most effective for the control of the test fungi.

The inhibition in the radial growth rate was concentration dependent, and *Alternaria alternata* FNS- FCC 624 was shown as most sensitive fungus against tested plant extracts, with growth inhibition of 97.8% by 20% of extract from *Juniperus communis* L.

The MICs of aqueous extracts of plants were determined using a broth dilution micromethod in 96-well round-bottomed polystyrene microtiter plates. Broth- microdilution method is the most appropriate one for the de termination of MIC values, since the method offer the possibility to estimate the concentration of the tested antimicrobial agent or extract in each well in 96-well microtitration plate. Microbial growth was determined by visual readings and the MIC was considered as the lowest concentration of each extract at which there was no microbial growth after seven days of incubation.

Table 2. Minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC) of tested plant extracts against phytopathogenic fungi.

		Pinus sylvestris L.	*Juniperus communis* L.	*Pinus nigra* J.F. Arnold
Alternaria alternata FNS- FCC 624	MIC	2.5%	2.5%	5%
	MFC	10%	5%	10%
Fusarium oxysporum FNS- FCC 103	MIC	5%	5%	5%
	MFC	10%	10%	10%
Botrytis cinerea FNS- FCC 23	MIC	5%	2.5%	5%
	MFC	10%	10%	10%
Plasmopara viticola FNS- FCC 65	MIC	5%	5%	5%
	MFC	10%	10%	10%
Rhizoctonia solani FNS- FCC 218	MIC	5%	5%	5%
	MFC	10%	10%	10%

The *in vitro* evaluation for antifungal activity of aqueous extracts of tested plants at different concentrations revealed that 10% concentration completely killed all tested phytopathogenic fungi, and 5% concentration inhibited the growth of fungi. The results from Table 2 showed that *Alternaria alternata* FNS- FCC 624 with MICs of 2.5% and MFCs of 5% was most sensitive fungus against tested plant extracts and the extract from *Juniperus communis* L. showed the highest antifungal activity among all three plants. The fungitoxic effects of the extracts indicate the potential of these plant species as a natural source of fungicidal material.

Ortiz et al. (2007) found that the ethanolic extracts from the aerial parts of *Juniperus lucayana* Britton exhibited antifungal activity against *Botrytis cinerea*. This fungus is a serious pathogen that attacks economically important crops (Aleu et al., 2001; Daoubi et al., 2005). *Juniperus* species have been extensively investigated as a source of natural products with potential antimicrobial, acaricidal and insecticidal activities (Karaman et al., 2003; Schmidt, 2004; Barrero et al., 2005).

Also, reports are available on antifungal activity of pine (*Pinus sylvestris* L.) and spruce (*Picea abies* L.) extracts and essential oils against a wide range of fungi: Fusarium culmorum, F. poae, F. solani (Krauze-Baranowska et al., 2002). Natural substances of plant origin, like plant extracts and essential oils may be promising, because plant extracts are effective against phytopathogens (Albouvette et al., 2006; Koul et al., 2008), their extraction is not complicated and time-consuming and extracts do not pollute the environment (Zarins et al., 2009).

Conclusion

The aqueous extracts obtained from the needles of *Pinus nigra* J.F. Arnold, *Pinus sylvestris* L. and *Juniperus communis* L. exhibited good antifungal activities and were capable of reducing growth of tested phytopathogenic fungi. The tested plant aqueous extracts could serve promising and potential raw materials in industry for producing natural antifungal agents with potential applications in agriculture for controlling the pathogenic fungi.

These preliminary results, obtained from *in vitro* experiments, may be supplemented by other more comprehensive studies *in vivo*, both in controlled greenhouse conditions and in open field to practically evaluate the use of these extract in the frame of an Integrated Pest Management System. Also, it is of great interest to deepen our knowledge about the molecular mechanisms of action of plant aqueous extracts not only against microorganisms, but even on plant biology.

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EVALUATION OF THE DEGREE OF SURFACE COVERAGE DURING SPRAYING WITH SELECTED DOUBLE-STREAM NOZZLES

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Abstract

The use of plant protection products has been the most effective way to protect crops against pests. The basic aim of the spraying operation is to achieve the highest possible effectiveness in combating pests. This effectiveness, as well as the level of pesticide consumption and safety during the treatment, depends largely on the quality of spraying usable liquid. One of the three determinants of the quality of the treatment is the degree of coverage of the sprayed objects. The quality of the treatment is influenced by many technical and technological factors, among them nozzles used. This study about the degree of coverage on sprayed objects using selected nozzles assessed which of the tested sprays had the best properties in terms of covering both horizontal and vertical surfaces on sprayed plants. The tests were carried out in laboratory conditions. The object was an artificial plant, to which water-sensitive papers were attached vertical and horizontally and sprayed surfaces were determined. After the experiment, the probes were subjected to computer image analysis. On the basis of the analysis of the obtained test results, it was found, that the characteristic feature for tested nozzles was the range in the degree of coverage of surfaces marked as vertical and horizontal.

Keywords: *degree of coverage, nozzle, quality of spraying*

Introduction

Users of sprayers have a problem to find appropriate nozzles for the spraying procedure. The right choice of nozzles such as the type and size determines a highly effective spraying. On the basis of the research, it was shown that the nozzles have a direct influence on the quality of the treatment (Nuyttens et al. 2009; Őzkan 2008; Szewczyk et al. 2013). It is also important to adopt the appropriate technical and technological parameters in order to achieve a successful treatment. One of the three indicators to assess the quality of the procedure is the degree of coverage (Abbaspour-Fard et al. 2008, Cunha i in. 2012, Fox i in. 2003, Lipiński, Lipiński 2009, Thomson, Lyn 2011). The authors emphasized in their work that it is a practical and accurate method.

Therefore, at the Institute of Agricultural Engineering of the University of Environmental and Life Sciences in Wrocław, tests were carried out to assess the degree of coverage when spraying with double-stream nozzles.

Materials and methods

The experiments were executed in 2018 in real conditions. Coverage tests were carried out on a test stand, which scheme is presented on Figure 1.

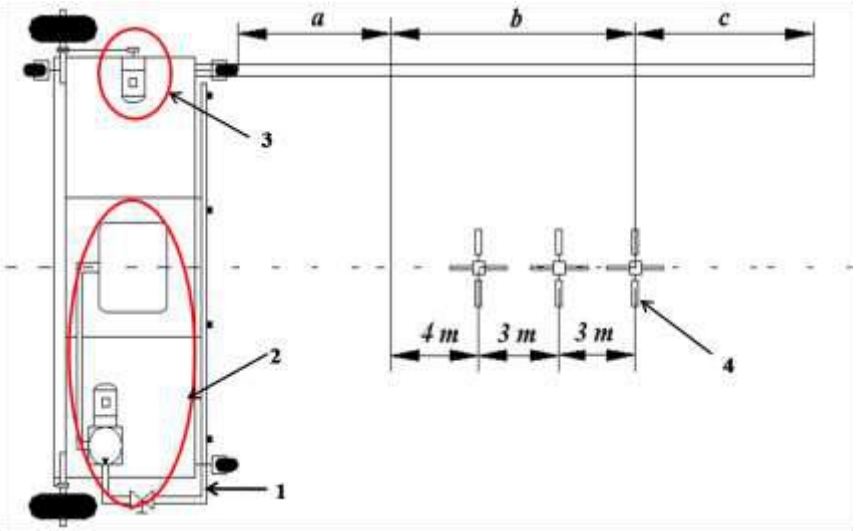

Fig. 1. Scheme of the test position for the degree of coverage for the sprayed objects a – acceleration section, b – measurement section, c – final section 1 –carrier nozzles, 2 – driving system of carrier nozzles, 3 – liquid system of carrier nozzles 4 – artificial plant

The primary element measurement stand was a nozzle carrier, which is equipped with both driving and liquid spraying systems. The carrier nozzles was moved in a straight line on the designated route. The road that the carrier traveled was divided into three parts, the run-up section, on which the carrier obtained the set speed, the measuring section, and the ending section, where the carrier lost speed and stopped. Three artificial plants were placed in the measurement section, to which water-sensitive papers were attached on horizontal (top and bottom) and vertical (approach and leaving) surfaces (fig. 2).

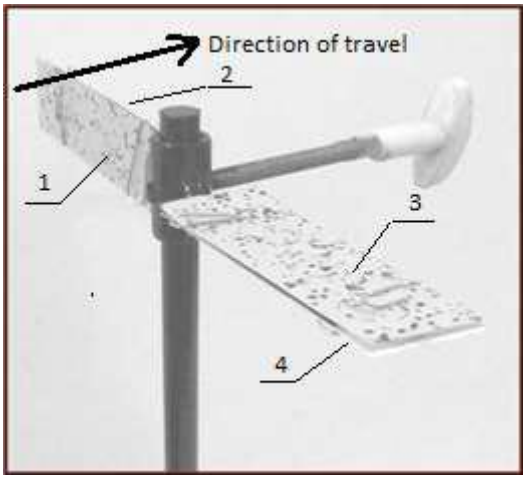

Fig. 2. Water sensitive papers were attached to four collector plant zones: 1 - the vertical transverse approach surface, 2 - the vertical transverse leaving surface, 3 - the horizontal upper level surface, 4 - the horizontal bottom level surface

The research involved the use of ejector double-stream nozzles, which differ in the way of liquid emission, asymmetrical AI 3070 11003, symmetrical AITTJ 60 11003 and TTI 60 11003 (Fig. 3).

AI 3070 11003 AITTJ 60 11003 TTI 60 11003

Fig. 3. Nozzles used in research

The following parameters and working conditions of the sprayers were adopted for the tests:
• Set speed - 6 km · h^{-1}
• Liquid pressure - 0.2; 0.3; 0.4 MPa.
During the tests, the wind speed was approx. 2 m · s^{-1}. In order to assess the degree of coverage, water-sensitive papers subjected to computer image analysis. Three fragments with a surface area of 1 cm^2 were randomly selected on the scanned paper, randomly sized and the surface covered with working liquid was read in Adobe PhotoShop®. The degree of coverage was determined as the ratio of the surface of the paper covered with working liquid to the total surface area tested (1 cm^2).

Results and discussion

The test results are presented in figures 4 - 6. During the measurements there were no traces of coverage of the horizontal lower objects, therefore there are only shown the results of the three remaining surfaces. Raising the working pressure of the nozzles resulted in an coverage of individual surfaces. In all analyzed cases, the highest cover value was noted for the TTI 60 nozzle with symmetrical spray angles, and the lowest for the AI3070 nozzle with asymmetrical spray angles. Taking into account the vertical surfaces, it should be stated that the highest values of the driveway surface were obtained for the TTI60 nozzle, while the lowest for the AITTJ60 nozzle. However, in the case of covering the leaving surfaces, it can be noticed that similar values were noted for the TTI60 and AI3070 nozzle, while slightly lower values were obtained for the AITTJ60 nozzle.

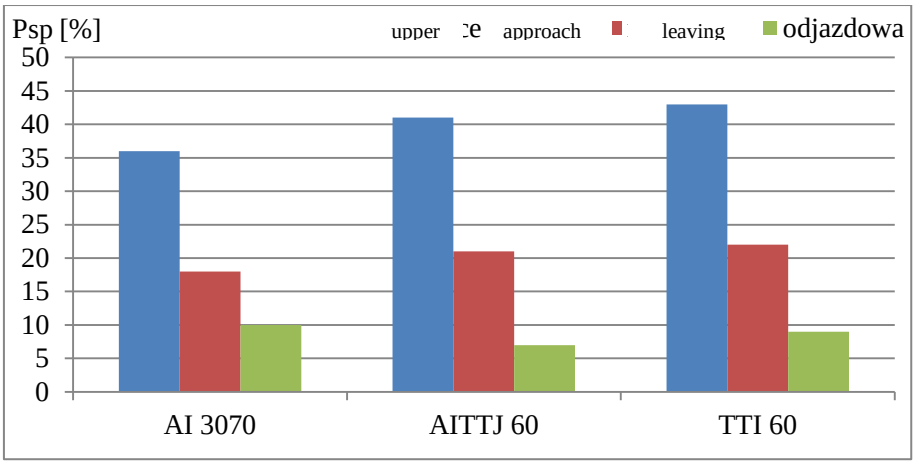

Fig. 4. The value of coverage degree of sprayed objects during spraying with selected nozzles at a pressure of 0.2 MPa
upper

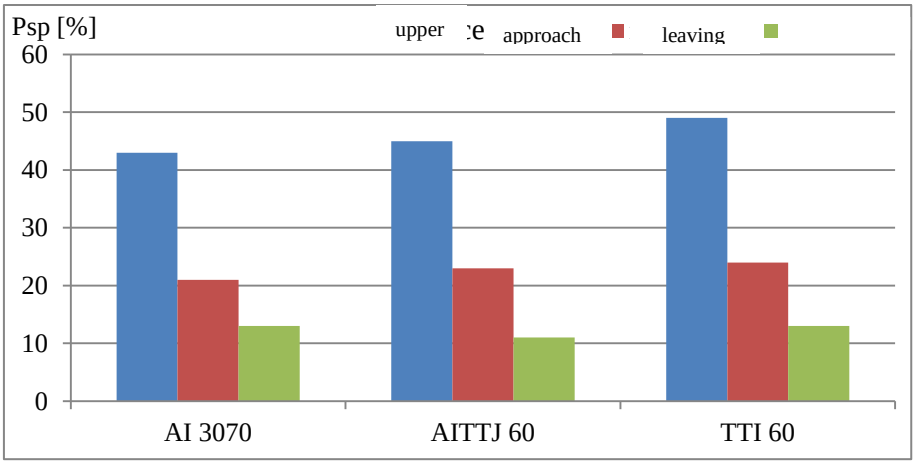

Fig. 5. The value of coverage degree of sprayed objects during spraying with selected nozzles at a pressure of 0.3 Mpa

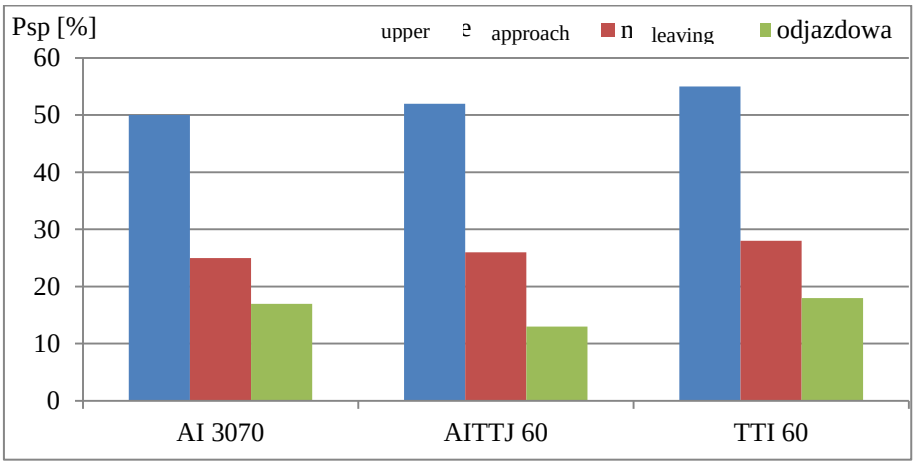

Fig. 6. The value of coverage degree of sprayed objects during spraying with selected nozzles at a pressure of 0.4 MPa

Interpretation of the obtained results is in line with what many researchers emphasize in their publications in relation to the importance of selecting the right nozzle for spraying as an important factor of work quality performed during plant protection treatments (Guler et. al. 2007).

Conclusions

On the basis of the conducted experiments and their analysis, the following conclusions were formulated:

1. The increase in pressure caused an increase in coverage of the analyzed surfaces.
2. The highest values of the degree of coverage on of the upper horizontal surface were obtained with the use of the ejector nozzle TTI 60, with symmetrical spray angles.
3. The highest values of degree of coverage of vertical surfaces were noted for the asymmetrical atomizer AI3070, with asymmetrical spray angles.
4. No traces of coverage were found on the horizontal bottom surface.

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ALLELOPATHYC EFFECT OF ESSENTIAL OIL OF CANNABIS SATIVA L. ON SELECTED VEGETABLE SPECIES

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Abstract

Examination of pesticidal effect of essential oil in agriculture is increasingly important due to tendency to reduce use of synthetic pesticides. The aim of this study is to determine allelopathic effect of *Cannabis sativa* L. essential oil on the initial development of selected vegetable species. Applied concentrations are 200□l/l, 400□l/l, 600□l/l i 800□l/l. Used vegetable species are *Allium cepa* L., *Solanum lycopersicum* L. and *Daucus carota* L., while observed parameters are length of hypocotyl and epicotil. Experiment was carried out in laboratory conditions, each variant of experiment had two repetitions, control variant used distilled water. By processing data, all applied concentrations attach inhibitory effect to monitored parameters. Highest inhibitory effect on average length of hypocotyl *A. cepa* L. of 2,73mm had highest applied concentration of essential oil of 800□l/l, as opposed to 9,97mm in control. Length of epicotil *A. cepa* was 13,6mm in control, while the highest inhibitory effect gave the highest concentration of essential oil with a length of 2,4mm. Length of hypocotyl *S. lycopersicum* L. was 30mm in control while in variant with 600□l/l it recorded the highest inhibitory effect with a length of 10,67mm. Length of epicotil *S. lycopersicum* L. in control was 13,03mm while in variant with 600□l/l was 3,2mm. Length of hypocotyl *D. carota* L. in control was 8,73mm while concentraion of 200□l/l gave the highest inhibitory effect with length of 5,97mm. Length of epicotil *D. carota* was 16,3mm in control but varianth with 200□l/l gave the highest inhibitory effect with 11,07mm.

Key words: *essential oil, Cannabis sativa L., Allium cepa L., Solanum lycopersicum L., Daucus carota L.*

Introduction

According to some estimates, weeds can reduce the yield of world production by 10-15%, which amounts to about 300 milion tons of food annually (Kojić i Janjić, 1994). In addition to unwashed weed plants that cause losses to agricultural production and certain cultivated plants in other crops, they can significantly reduce the yield of crops grown (Kojić et al., 1996). *Cannabis sativa* L. except that the cultivated species can also be found on roderal habitats, on arable land in crops, perennial fodder plants, orchards, but also in vegetable crops (Vrbničanin, 2015). Among the many negative effects that the weed species have on cultivated crop, they can cause allelopathic effects with their secondary metabolites. Allelopathy refers to the relationship between the two organisms where one organism influences positively or negatively on another with its secondary metabolites (Farooq et all, 2013). Tests of allelopathic relations between weeds and cultivated plants are very important for sustainable agricultural production (Marinov-Serafimov, 2010). Plant species in which allelopathic effects have been shown to a large number of cultivated species are: *Ambrosia artemisiifolia* L., *Avena* sp., *Amaranthus retroflexus* L., *Cyperus esculentus* L., *Chenopodium*

album L., *Helianthus tuberosus* L., *Rumex crispus* L., *Sorghum halepense* (L.) Pers., *Xantium strumarium* L. (Šćepanović et al., 2007). The repellant and pesticidal effects of certain *Cannabis sativa* L. compound (McPartland, 1997) have also been reported, and since it can also be reported as crop in crops, it is necessary to determine its allelopathic effect on cultivated plants. For the examination of allelopathic effects, biotests derived in laboratory conditions were used, since they are of high sensitivity and are performed in a relatively short time (Mahmoodzaden et all, 2015).

The aim of this paper is to determine the allelopathic effect of *Cannabis sativa* L. essentia oil on the initial development of vegetable species: *Allium cepa* L., *Solanum lycopersicum* L. and *Daucus carota* L. in laboratory conditions.

Material and method

An overgrown part of the plants, stems, leaves, fruits of *Cannabis sativa* L. harvested during the period of ripening in 2016, was collected. Work in the laboratoryws carried out during 2017, with the plant material being first crushed by a blender. In the process of hydro-distillation, 60g of ground plant material was boiled in 800ml distilled water for 2h and 30min. *Cannabis sativa* L. essential oil obtained by this process was used to treat seeds of examined vegetable species in concentrations of □□□□l/l, □□□□l/l, □□□□l/l i □□□□l/l (Rahimi et all, 2013). Fifteen seeds of vegetable species (*Allium cepa* L., *Solanum lycopersicum* L., *Daucus carota* L.) were placed in Petri dishes (diameter 6cm) with filter paper in two repetitions. Then dishes were packed with 5ml of dilution from each concentration of essential oil while distilled water was used in control (Rahimi et all, 2013). Upon completion of the experiment, the length of hypocotyl and epicotilles of seeds in each Petri dish were measured.

Results and discussion

Influence of *Cannabis sativa* L. essential oil on *Allium cepa* L.

Inhibitory effect of esential oil was observed when monitoring perameters of initial development of plant species *Allium cepa* L.. Increasing concentration of essential oil increased inhibitory effect on length of hypocotile and length of epicotile. Control variant yielded an average length of hypocotil of 9,97mm while variation with a concentration of essential oil of 800□l/l was 2,73mm. The smallest inhibitory effect was in appplication of 400□l/l where average length was 7,03mm. Average length of epicotill of *Allium cepa* L. also indicates inhibitory effect of essential oil. Average length of epicotill of 2,4mm was recorded in a variant with the use of 800□l/l of essential oil as opposed to control in which was 13,6mm. The weakest inhibitory effect was recorded by variant with use of 400□l/l essential oil where an average length of 8,3 was recorded. Average length of hypocotyl and epicotil *A. cepa* L. in different variant experiment are shown in Table

Table 1. Average length of hypocotyl and epicotil (expressed in mm) of *Allium cepa* L., *Solanum lycopersicum* L. and *Daucus carota* L. depending on concentration of *Cannabis sativa* L. essential oil used.

Plant species	Length of hypocotyl					Length of epicotyl				
	□□l/l	□□□□l/l	□□□□ l/l	□□□□ l/l	□□□□ l/l	□□ l/l	□□□□l/l	□□□□l/l	□□□□l/l	□□□□l/l
Allium cepa L.	9,97	6,13	7,03	3,23	2,73	13,6	7,2	8,37	3,67	2,4
Solanum lycopersicum L.	30	16,83	18,97	10,67	18,63	13,03	7,43	6,4	3,2	5,7
Daucus carota L.	8,73	7,27	8,3	6,27	5,97	16,3	12,27	14,1	11,1	11,07

Pavlovski et al. (2013) examined effect of essential oil of plant species *Schinus lentiscifolius* March. on germination and initial development of *A. cepa* L. and showed an inhibitory effect on parameters monitored.

Influence of Cannabis sativa L. essential oil on *Solanum lycopersicum* L.

Essential oil in all variants showed an inhibitory effect on *Solanum lycopersicum* L.. The greatest inhibitory effect was observed in variation in which 600□l/l was applied were average length of hypocotile was 10,67mm while in control was 30mm. The smallest inhibitory effect had a variant with 400□l/l with an average length of hypocotil of 18,97mm. Average length of epicotile *S. lycopersicum* L. in control variant was 13,03mm, while in variant with 600□l/l of essential oil it was 3,2mm. The smallest inhibitory effect was observed in variant with use of 200□l/l essential oil and amounted to 7,43mm. Average length of hypocotil and epicotile of *S. lycopersicum* L. in different variant experiment are shown in Table 1..

Ali and all (2016) investigated effect of *Rosmarinus officinalis* L., *Thymus vulgaris* L. and *Pimpinella anisum* L on *S. lycopersicum* L. essential oils and conclude that different allelopathic effects on tested germination parameters and initial development of investigated species.

Influence of *Cannabis sativa* L. essential oil on *Daucus carota* L.

Applied concentrations of *Cannabis sativa* L. essential oil to *Daucus carota* L. showed an inhibitory effect on both of investigated initial development parameters. Average length of hypocotil *D. carota* L. in control was 8,73mm while the lowest concentration of essential oil produced the strongest inhibitory effect, with average lengthin that case of 5,97mm. The weakest inhibitory effect was the concentration of essential oil of 600□l/l and length of hypocotil in that variant was 8,3mm. The same effect was observed when monitoring second parameter, length of epicotile. Average length of epicotil in control variant treated with distilled water was 16,3mm, while in variant with 200□l/l was the strongest inhibitory effect and length of 11,07mm. The weakest inhibitory effect on average length of epicotil *D. carota* L. in different variant experiment are shown in Table 1..

In 2014, Aslani and all proved inhibitory effect of the methanol extract of *Tinospora tuberculata* L. on *Daucus carota* L..

Conclusion

Based on conducted experiment, inhibitory allelopathic effect of *Cannabis sativa* L. essential oil on initial development of selected vegetable species is clearly observed. All ivestigated concentrations led to inhibition of growth of hypocotil and epicotill. Obtained results indicate ability of *C. sativa* L. species to reduce development of examined vegetable species *Allium cepa* L., *Solanum lycopersicum* L. and *Daucus carota* L. with their secondary metabolites if they are found in their crop as a weed. The results of the experiment should also be taken into account when organizing crop, if *C. sativa* L. sowing is planned and the vegetable species tested on the same plot.

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IDENTIFICATION OF LEPTOSPHAERIA MACULANS AND LEPTOSPHAERIA BIGLOBOSA ISOLATES BASED ON PATHOGENICITY AND PRODUCTION OF SIRODESMIN PL

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Abstract

The aim of this study was to identify isolates within the pathogenic species of *Leptosphaeria maculans* and *Leptosphaeria biglobosa*, based on the pathogenicity and phytotoxin production. The fungus cultures were isolated from rapeseed plants showing the symptoms of stem canker. They were collected from nine locations in rapeseed growing region of northern Serbia. In the study were used 119 local isolates from Serbia and two reference isolates from the Center for Agricultural Research, Rothamsted, Great Britain, (L. m. for *Leptosphaeria maculans*, and L. b. for *Leptosphaeria biglobosa*). The pathogenicity of the isolates was examined on the Westar, Glacier and Quinta rapeseed cultivars. Eight isolates caused small necrotic spots on cotyledons in all tested cultivars, while 111 caused large necrotic spots in which the formation of pycnidia was observed. Four local isolates (K-113, C-3, St-5 and S-11) and reference isolate L.m. were used for the extraction and isolation of phytotoxins, based on their morphological and pathogenic characteristics. Isolates C-3, St-5, S-11 and L. m. did not produce a yellow brown pigment in the Czapek medium but produced sirodesmin PL while the K-113 isolate produced a yellow brown pigment and did not produce sirodesmin PL.

Keywords: *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, rapeseed, phytotoxin**.**

Introduction

Leptosphaeria maculans (Desm.) Ces and De Not (anamorph: *Phoma lingam* (Tode ex Fr.) Desm.) cause dry (black leg) on oil rapeseed worldwide. Desease is economically very important in Europe, Australia and Canada (West *et al.*, 2001). The appearance of the disease to a greater or lesser intensity depends on climatic factors, cultural practices and varieties resistance (Howlett, 2004; Aubertot *et al.*, 2006; The population of *L.maculans* can be divided into at least two subgroups,which are termed as virulent or avirulent (McGee & Petrie 1978 Koch *et al.*, 1989 Badawy & Hoppe 1989),Tox^{+} or Tox^{0} (Balesdent *et al.*, 1992),A or B (Johnson and Lewis 1994). Aggressive isolates (group A or Tox^{+}) induce grey-green,sporulating lesions on leaves and destructive stem canker while non-aggressive isolates (group B or Tox^{0}) cause leaf spot and superficial stem lesions (Koch *et al.*, 1989; Badawy *et al.*, 1991; Williams, 1992; Thürwächter *et al.*, 1999). Brun *et al.*, (1997) and Williams and Fitt (1999) indicated that group A is highly virulent, while group B is slightly virulent. Koch *et al.*, (1991), Mengistu *et al.*, (1991) & Kutcher *et al.*, (1993) have studied the virulence of different isolates of *L. maculans* on three varieties of oil rapeseed (Westar, Quinta and Glacier) and classified them into four pathogenic groups. Group B was then isolated and identified as a new species called *Leptosphaeria biglobosa* (Shoemaker and Brun, 2001). A number of plant pathogens produce secondary metabolites (toxins) in order to obtain nutrients from plant cells. In some fungi, the toxins have potential toxicity or carcinogenic properties that could endanger the health of humans, animals and plants.(Bohnert *et al.*, 2010) In some cases, toxins cause death of plant cells to release nutrients or disrupt plant metabolism in favour of the pathogenic fungi. (Howlett, 2006). Many selective toxins play a role in the virulence of pathogenic fungi(Howlett, 2006; Wolpert, 2002) The role of non-selective toxin

in virulence is complex and the production of toxins is not always correlated with virulence (Elliott *et al.,* 2007). Sirodesmin PL, the product of the pathogenic fungus *Leptosphaeria maculans*, belongs to the class of epipolythiodioxopiperazines (ETPs), and is characterized by the presence of disulphide bridges (Gardiner *et al.,* 2004; Gardiner *et al.,* 2005) The diketopiperazine ring originates from cyclic dipeptides and sulphur bridges are responsible for all the known toxic effects of these molecules (Mullbacher *et al.,* 1986). Gardner *et al.,* (2005) stated that disulphide bridges are a key structural element for the creation of a variety of reactive forms of oxygen and for connection with the cysteine residues of proteins. It is assumed that the toxicity of ETPs could be explained by these reactions. Sirodesmin PL is a non-selective toxin that causes chlorosis and necrosis, inhibits root growth and leads to the extinction of plant cells (Badawy *et al.,* 1989; Fox and Howlet, 2008). In addition, these compounds have antibacterial and antiviral properties. (Rouxel *et al.,* 1988). The aim of this study was to identify isolates within the pathogenic species of *Leptosphaeria maculans* and *Leptosphaeria biglobosa*, based on the pathogenicity test and to perform crude separation of fungal toxins using preparative TLC on silica-gel plates, and to identify individual toxins using HPLC cupled with a tandem mass-spectrometric detector (LC–MS–MS).

Materials and Methods

Isolation of fungi and obtaining monospore culture

Infected plants of oilseed rape were collected during 2009/10 in the region of Vojvodina, Serbia. Diseased plant organs (root, basal and upper stem, leaf, flower, pod and seed) with clearly defined symptoms of the disease were used for the isolation of the fungi. Diseased tissue fragments were soaked in a 3 % solution of sodium hypochlorite for 5–10 min and then washed with sterile water and naturally dried under controlled conditions. After drying, the fragments of diseased tissue were applied to the culture medium of potato dextrose agar (PDA) (Difco, Detroit, USA) that had previously been poured into petri plates. To prevent bacterial growth in the medium, 50 mg of streptomycin sulphate (Galenika, Belgrade, Serbia) was added per litre. The inoculated petri plates were incubated at 25±1°C. After 5 to 10 days, the formation of pycnidia and pycnidiospores was observed under a stereo microscope. Pure cultures were obtained by the following procedure: pycnidiospores, which were released from pycnidia serving as a single droplet originating from the culture media, were transferred with the tip of a spear needle into plastic tubes to which 2 mL of sterile water had previously been added. The prepared suspension of conidia was applied onto the aqueous agar medium, which had previously been poured into petri plates. After 48 h, germination of the conidia was observed under a stereo microscope. The germinated conidia, together with fragments of the substrate, were transferred onto PDA medium in petri plates and placed in an incubator at 25°C in order to develop monospore fungal isolates. In this way, 119 isolates of fungi were obtained. All isolates were analysed at the morphological and molecular level. Based on morphological(colony appearance, shape, size and colour of the pycnidia and picnospores (Punithalingam and Holliday, 1972; Fitt *et al.,* 2006) and molecular characteristics (Polymerase Chain Reaction – Restriction Fragment Lenght Polymorphism, PCR–RFLP Balesdent *et al.,* 1998) it was determined that 111 isolates belonged to *L. maculans* and 8 to *L. biglobosa* (data not shown). Using the method of random selection, four isolates (three of L. maculans, St-5, C-3 and S-11, and one of *L. biglobosa*, K-113) were taken for further research.

Phytopathogenic test

The pathogenicity of the isolates (total of 119 iolates from serbia and two isolates from the Centre for Agricultural Research, Rothamsted, UK designated L.m. - *Leptosphaeri maculans* and L.b - *Leptosphaeri biglobosa*) hes been examined by the method of Koch *et al.,* (1991), Chen and Fernando (2006). Seeds were disinfected by dipping in 3% solution of sodium-

hipohlorite (NaOCl) for 3 to 5 min. and then washed with tap water and dried at room temperature under controlled conditions. The success of disinfection was tested on PDA nutrient medium. Disinfected seeds of varieties Westar, Quinta and Glacier were applied in two Petri dishes, and not disinfected seeds of listed varieties were used as controls. In each box was placed 5 seeds. Thus prepared Petri dishes were placed in a thermostat at 25°C ± 1°C in the dark. After 15 days was carried out visual and microscopic examination of the seeds and substrate, after which the seeds were placed in a germination chamber. Germinated seeds were sown in plastic containers with a diameter of cells 4 × 5 cm, which were previously filled with sterile substrate. Each cell was seeded by one germinated seed. After germination (6-7 days), when the cotyledons separated, inoculation was performed as follows: sterile needle was used to injure one cotyledon, while the other cotyledon was not injured. Injured part was inflicted by micropipette with 5 μl (10^6) suspension of pycnospores. After inoculation, plants were transferred to controlled conditions at a temperature of 20ºC, 95% RH and 12 h photoperiod. After 48 h the plants were placed in greenhouse conditions. During follow-up of symptoms true leaves were removed several times. Symptoms were rated after 15 days. using the scale – (no symptoms), + (slight lesions), ++ (moderate lesions), +++ (severe lesions). As a negative control, a variety of glacier was used where distilled water was applied to cotyledons.

Extraction and isolation of phytotoxins

Four isolates of L. maculans were used for the extraction and isolation of the phytotoxins. Three isolates (C-3, St-5 and S-11) originated from Vojvodina and the fourth, designated L.m. (*L. maculans*) and serving as the reference isolate, was received from the Centre for Agricultural Research, Rothamsted, UK. In addition, an *L. biglobosa* culture (designated K113) was prepared as a known negative. All five isolates were sown in Czapek liquid medium, 16 which had previously been poured into tubes. The tubes were placed in a climate chamber at 20°C with 12 h photo period. After 30 days, the cultures were filtered to separate the fungus mycelium from the liquid medium.Culture extracts were prepared and purified according to previously published procedures, (Koch *et al.*, 1989, Pedras and Bisesenthal, 2001) using a thin layer chromatography (TLC) fractionation, with double elution. The culture filtrate was first extracted with ethyl acetate (6 mL of ethyl acetate per 5 mL of filtrate). The organic extract was dried with anhydrous sodium sulphate. After removal of sodium sulphate by filtration through a qualitative filter paper, the samples were evaporated under a stream of nitrogen. The residue was redissolved in 100 μL of chloroform at room temperature. The chloroform solution was applied on an analytical TLC plate (Macherey-Nagel, Silicalgel G-25 UV_{254}, glass plates, 20 × 20 cm × 0.25 mm) using a glass capillary and the plate was developed using ethyl-acetate : chloroform (1:1) as the eluent. After drying, the plate was examined under UV light et 254 nm (Mitrović et al., 2012). According to the literature data, (Koch *et al.*, 1989, Badawy and Hoppe, 1989) spots that can be visually observed under the UV light at 254 nm, are unambiguously identified as phytotoxins Based on the preliminary results, a larger-scale experiment (with a greater amount of media) was set up with the purpose of preparative isolation of the observed phytotoxins (for the analysis of their chemical properties and phytotoxicity.). The final chloroform solution of the dry extract was applied to a preparative (prep.) TLC plate: (Macherey-Nagel, Silicagel G-200 UV254, glass plates, 20 × 20 cm × 2 mm), and the plate was developed in the binary eluent system, ethyl-acetate : chloroform (1:1). Three spots were identified under UV light at 254 nm, and designated as 1 (with retention time, Rf = 0.15), 2 (with retention time, Rf = 0.44) and 3 (with retention time, Rf = 0.60). The spots were removed from the TLC plate by scratching the silica gel layer, and individually extracted in absolute ethanol (20 mL) at room temperature with viguorously shaking for 2 h. The extract of TLC spot, designated as 2, was evaporated under a nitrogen stream, and then again applied on a preparative TLC plateand eluted,

according to the above mentioned procedure, yielding fractions 2a (Rf = 0.49), 2b (Rf = 0.40), 2c (Rf = 0.32) and 2d (Rf = 0.22). Spots were removed and ectracted according to the same procedure as described earlier. All subsequent extracts are were purified on a Sephadex column (SPE Bakerbond Sephadex G-25) previously conditioned with 10 mL of ethanol. The collected ethanolic filtrate was evaporated under a nitrogen-stream, and the dry residue was used in the identification of phytotoxins using the LC–MS–MS method.

LC–MS–MS characterization of phytotoxins

Chemical composition of isolated fractions was determined by reversed-phase high-performance liquid chromatography (Agilent Technologies Series 1200 Rapid Resolution liquid chromatograph) coupled with tandem mass spectrometric detection (Agilent Technologies Series 6410A Triple-Quad mass spectrometer with an electrospray ion source). 1 µl of undiluted sample was injected into the system. The components were separated using a Zorbax Eclipse XDB-C18 rapid resolution column 50 mm × 4.6 mm, 1.8 µm (Agilent Technologies), held at 40 °C. The samples were eluted using the gradient mode: 0 min 30 % B, 7–10 min 100 % B (phase A being 0.1 % aqueous formic acid and phase B – 0.1 % formic acid in acetonitrile) with a post time of 2.5 min. The mobile phase flow was 1 mL min^{-1}. The eluent was forwarded into the electrospray ion source (ESI) without flow splitting. The ESI parameters were as follows: nebulizer pressure 40 psi, drying gas temperature 350°C, drying gas flow 9 L min^{-1}, capillary voltage 4000 V and fragmentor voltage 100 V. All samples were analyzed in the MS2Scan mode (MS1 experiment), using positive polarity, in the m/z range 150–900. Afterwards, the representative sample (St-5/2b) was analyzed in the Product Ion Scan mode (MS2 experiment), using [M+H]+ of suspected sirodesmins peaks as precursor ions, and a collision voltage of 0–30 V (in 10 V increments). All the acquired data were processed using MassHunter Workstation-Qualitative Analysis software, ver. B.03.01 (Agilent Technologies).

Results and Discussion

Isolates of L.m, St-5 and S-11 were caused by chlorotic spot (+ slight phytotoxicity) after 5 days, while no change in cotyledones was observed in the K-113 isolates compared to the control variant. After 10 days, isolates L.m, St-5 and S-11 showed moderate phytotoxicity (++) except for K-113. After 15 days, isolates of L.m, St-5, S-11 caused the most severe lesions (+++) on cotiledons while K-113 was assessed as slight virulent (+) compared to the control variant (Figure 1). Expressed aggressiveness of the isolates in the conducted studies indicates that L.m, St-5 S-11 belong to the species *L.maculans* while K-113 *L.boglobosa* (Fitt et al 2006, West et al. 2001, Brun et al. (1997)

Figure 1. Pathogenicity of isolates on varieties Westar, Glacier and Qiunta

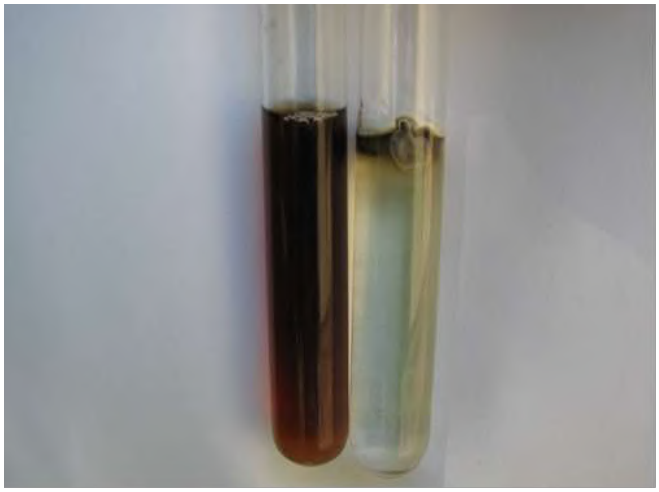
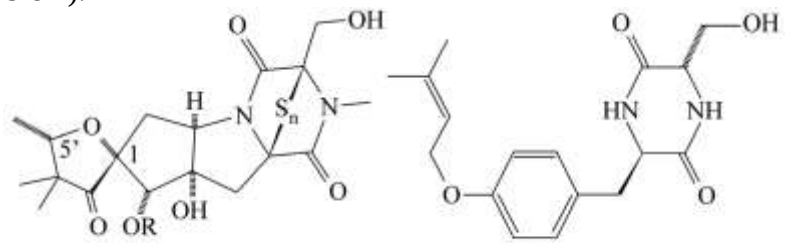


Figure 2: Production of pigment in Liquid Czapek medium

Isolates L.m., St-5, C-3, S-11 do not produce yellow pigment in Czapek liquid medium, compared to K-113 (Figure 2) The absence of yellow pigments in the examined isolates (L.m., St-5, C-3, S-11) classified them as a group of virulent (aggressive) isolates relative to K-113 which form pigments, which is in line with allegations (Williams & Fitt, 1999).

Chemical profile of L. maculans extracts

Since reference standards for sirodesmins were not available, the absolute concentrations of the detected compounds in the samples could not be determined. However, it was possible to compare the differences in content of each compound throughout the fractions. It was observed that the bulk of the identified sirodesmins were contained within the fraction (according to the spot from preparative TLC) designated 2b (Mitrović *et al.*, 2012). For the majority of compounds, only a small percentage was present in fractions 2a and 2c, with the exception of compounds with the strongest signals (sirodesmin PL, sirodesmin C and P444), that diffused into 2c spot to a greater extent. Another exception is P318 (phomamide), which occurs exclusively in fraction 1. Fractions 2d and 3 were practically devoid of sirodesmins (Figure 3, Table 2).



n=1, R=Ac sirodesmin H
n=2, R=H sirodesmin J, 1-*epi*-sirodesmin J
n=2, R=Ac sirodesmin A, sirodesmin PL
n=3, R=H de-*O*-acetylsirodesmin C/F
n=3, R=Ac sirodesmin C/F
n=4, R=H de-*O*-acetylsirodesmin B/E/K
n=4, R=Ac sirodesmin B/E/K

phomamide

Figure 3: Structures of the detected epipolythiodioxopiperazines.

Table 1. Relative abundances of sirodesmin congeners, given as peak areas calculated from extracted ion chromatograms. For each compound, all abundant adduct and fragment ions were taken into account

Extract, fraction	Peak areas, arb. units										
	P318	P444	P454	P476	P486a	P486b	P508	P518a	P518b	P518c	P550
St-5/1	324	0	46	8	0	45	12	7	1	160	181
St-5/2a	0	16	16	53	0	24	82	5	1	73	89
St-5/2b	0	485	79	290	338	2086	56	119	34	1423	461
St-5/2c	0	121	10	30	38	855	2	9	2	286	22
St-5/2d	0	0	0	0	0	0	0	0	0	0	0
St-5/3	0	0	0	0	0	3	0	0	0	2	0
C-3/1	106	0	17	5	0	15	6	1	0	56	56
C-3/2a	0	35	22	124	4	30	182	10	0	80	104
C-3/2b	0	232	87	164	261	1706	39	138	35	1307	524
C-3/2c	0	80	12	18	41	802	2	10	2	238	18
C-3/2d	0	0	0	0	0	0	0	0	0	0	0
C-3/3	0	0	0	0	0	3	0	0	0	4	1
S-11/1	103	0	6	0	0	5	3	0	0	16	18
S-11/2a	0	12	15	33	2	26	41	1	6	75	79
S-11/2b	0	370	35	159	263	1739	23	76	18	1014	232
S-11/2c	0	137	6	22	50	966	2	9	2	232	12
S-11/2d	0	0	0	0	0	1	0	0	0	2	1
S-11/3	0	0	0	0	0	0	0	0	0	1	0
L.m./2a	0	443	4	136	131	383	0	32	1	90	5
L.m./2b	0	252	31	353	185	924	129	227	19	971	476
L.m./2c	0 24	1	109	13	53	156	30	1	165	200	
L.m./2d	0	44	0	121	2	19	121	7	1	45	38
L.m./3	0	141	0	103	10	218	23	8	1	150	29
K113	0	0	0	0	0	0	0	0	2		

Due to differences in the response factors, which are to be expected when using ESI-MS, the peak areas (given in Table 1) can only be treated as a rough approximation of relative abundances within a sample. However, in our opinion, it is safe to assume the protonation constants of the sirodesmin congeners are sufficiently comparable to be able to state that the dominant ETP components of the investigated L. maculans cultures were sirodesmins PL and C, and their deacetylated derivatives, which is in agreement with previous results (Badawy and Hoppe, 1989b), and also supported by our results obtained by analyzing TLC plates (Mitrović *et al.*, 2012).

While it is known that a polysulphide bridge in the diketopiperazine ring is responsible for the observed toxic effects (while the nature of side groups does not affect toxicity), and the reduction thereof leads to complete loss of activity, the exact mechanism of toxicity is still a matter of debate(Gardiner *et al.*, 2004; Gardiner *et al.*, 2005).

Regardless of the mechanism (or a combination thereof) that is responsible for the phytotoxic effects of ETPs, activity could be expected for all detected polysulphide-bridge containing

sirodesmins, although it is likely that only the dominant components contribute significantly, as indicated by Badawy and Hoppe, (1989b).
Referring to our previously published results (Mitrović *et. al.*, 2012), we came to the conclusion that there was no significant difference between investigated *L. maculans* extracts. No sirodesmins were found in the reference extract K113 prepared from an *L. biglobosa* culture (Mitrović *et. al.*, 2012). This indicates that sirodesmins could be employed as markers for the differentiation of two species – *L. maculans* and *L. biglobosa*.

Conclusions

The results obtained by the phytopathogenic test, developed in the liquid Czapek medium, show that the investigated isolates of St-5, S-11 and C-3 originate from Serbia, cause severe symptoms in cotyledones in all varieties (Westar Glacier, Quinta) and do not form yellow-brown pigments while K-113 isolate forms a yellow-brown pigment and causes slight virulence.Isolates C-3,St-5, S-11 in thin-layer chromatography and high-performance liquid chromatography with mass spectrometric detection demonstrated the presence of sirodesmins except for K-113. It was found that the dominant epipolythiodioxopiperazines in the investigated L. maculans isolates were sirodesmin PL, sirodesmin C, and their deacetylated derivatives.Based on the conducted studies, the isolates C-3, St-5, S-11 belong to the species *Leptosphaeria maculans* while the K-113 type of *Leptosphaeria biglobosa.*

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CONTROL OF *CERATITIS CAPITATA* (WIEDEMANN) (DIPTERA:TEPHRITIDAE) WITH MASS TRAPPING ON WASHINGTON ORANGE IN ANTALYA PROVINCE OF TURKEY

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Abstract

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is one of the important pests of citrus in Turkey. The Medfly is a polyphagous tropical fruit fly which attacks more than three hundred and fifty botanical species from sixty five different families. The females puncture the fruits and lay eggs below the skin of the host fruits, which are destroyed by larval feeding. The study was conducted to control of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) with mass trapping on washington orange in Antalya province of Turkey. Fourty Eostrap® invaginada traps baited with 95% trimedlure impregnated in a polymeric plug-type dispenser were used in both years. After two years of the study, the average number of the catches per trap varied in the sampling period. In the first year, a total of 894 adults were caught by traps the average number of the catches per trap was recorded between 1 and 2 percent. The highest number of catches per trap was recorded on 1st November, followed by 6th September and 15th November 2016. In the second year, a total of 408 adults were caught by traps. The average number of the catches per trap was recorded between 1 and 2 percent. The highest number of catches per trap were recorded on 11th October, followed by 13th September, and 6th September 2017.

Keywords: *Ceratitis capitata, Washington orange, pheromone traps, Antalya province.*

Introduction

The washington orange, *Citrus sinensis* (L.) Osbeck (Rutaceae: Sapindales) is one of the main cultivated *Citrus* variety in Turkey. The washington orange production is consisting of approximately 375.435 ha with a total produce of 1.358.100 tons of fruit per annum in Turkey, and Antalya province's share is 100.912 ha and 410.035 tons (Anonymous, 2016). The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is the most destructive pest among economically important fruit fly species (IAEA, 2003; Thomas *et al.*, 2007). The medfly is a highly adaptive polyphagous tropical fruit fly attacking more than three hundred and fifty plant species (Weems, 1981; Liquido *et al*., 1991). The female flies lay eggs below the skin of fruits which are destroyed by larval feeding (Christenson and Foote, 1960). Protein bait sprays mixed with malathion or low toxicity insecticides, spinosad, lambda-cyhalothrin are successfully used to control medfly populations (McQuate *et al.*, 2005; Urbaneja *et al*., 2009). Traps baited with trimedlure are important tools for detection, monitoring and controlling of the medfly (IAEA, 2003). The mass trapping has proved to be effective in suppression of the Medfly and advantage of reduced environmental impact when comparing with toxic bait sprays (Navarro-Llopis *et al.*, 2008; Martinez-Ferrer *et al.*, 2010). In addition, mass trapping has been developed in several countries using traps baited with trimedlure, hydrolysed proteins or food-based attractants (Epsky *et al.*, 1999; Katsoyannos *et al*., 1999; Cohen and Yuval, 2000; Miranda *et al*., 2001; Heath *et al*., 2004; Ortu *et al.*, 2005; Alemany *et a*l., 2006). This method has been conducted on different fruit trees like peach (Sastre, 1999], cherimoya (Ros *et al*., 2000), citrus (Miranda *et al.*, 2001; Alonso Muñoz *et al*., 1999; Llorens *et al*., 2004), fig (Escudero *et al*., 2005), persimmon and coffee (McQuate *et al.*, 2005) and apple (Ortu and Prota, 1988). The purpose of the current

study was to evaluate mass trapping for the control of Medfly in washington orange in Antalya province of Turkey.

Material and Methods

The study was conducted in 2016-2017 at washington orange orchard in Antalya province of Turkey. The study was carried out using the Eostrap® invaginada traps (Sanidad Agricola Econex, Santomera, Murcia, Spain) baited with 95% Trimedlure, (formulated in a polymeric plug-type dispenser) (Sanidad Agricola Econex, Santomera, Murcia, Spain) and dichlorvos or 2.2- dichlorovinyl dimethyl phosphate (DDVP) tablet (Sanidad Agricola Econex, Santomera, Murcia, Spain). In the first year, the study was conducted with 40 traps/1.8 ha and placed in the experimental site from 26 July to 15 November. In the second year, the study was carried out with 40 traps/1.8 ha and placed in the same site from 17 August to 22 November. The traps were placed 1.5 m above ground and checked weekly, trapped medfly adults were counted and removed from the traps. The trimedlure and DDVP tablet in traps were replaced with the new ones in every 90 days.

Results and Discussion

The population density of *C. capitata* varied in each of the sampling year. In the first year, the mass trapping was conducted with 40 traps/1.8 ha in washington orange orchard and a total of 894 medfly adults were caught by traps (Figure 1). The first adults were caught by the traps on 2 August, and the population density of this pest was gradually low during the sampling periods. The largest mean of catches per trap were recorded on 1 November (1.75), followed by 6 September (1.70), 15 November (1.68) and 18 October (1.63).

In the second year, the mass trapping was conducted with 40 traps/ha at the same orchard and a total of 408 medfly adults were caught by traps (Figure 2). The population density of medfly was very low comparing with previous year. The first adults were caught by the traps on 24 August, and the population density of this pest was significantly low during the sampling periods. The largest mean of catches per traps were recorded on 11 October (1.33), followed by 13 September (1.20), 6 September (1.08) and 8 November (0.95).

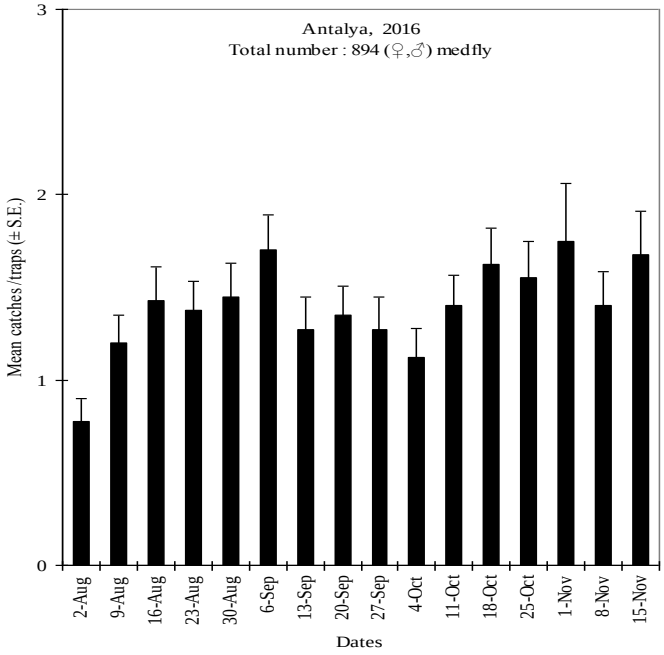

Figure 1. Mean (±SE) catches of medfly adults in traps baited with trimedlure (26 July to 15 November) at washington orange orchard in Antalya province.

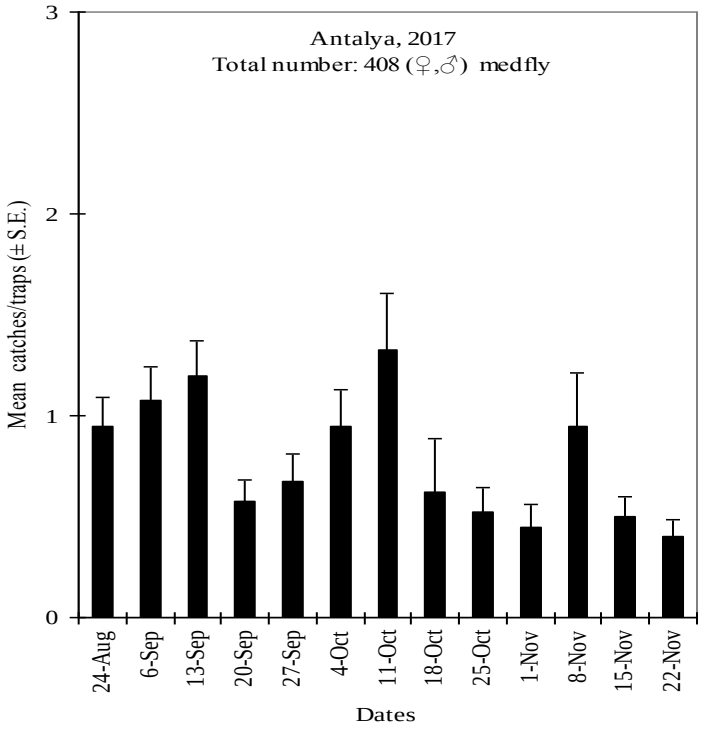

Figure 2. Mean (±SE) catches of medfly adults in traps baited with trimedlure (17 August to 22 November) at washington orange orchard in Antalya province.

The mass trapping has shown significantly effective pest management tool for the Medfly and developed in several countries using traps baited with trimedlure, hydrolysed proteins or food-based attractants (IAEA, 2003; McQuate *et al.,* 2005; Navarro-Llopis *et al*., 2008; Katsoyannos *et al.,* 1999; Cohen and Yuval, 2000; Miranda *et al*., 2001; Heath *et al.,* 2004; Ortu *et al.,* 2005; Alemany *et al*., 2006; Avery *et al*., 1994; Sastre *et al.,* 1999; Ros *et al*., 2000; Garcia *et al*., 2003; Jemâa *et al.,* 2010; Ros *et al*., 1997).

A various number of traps were used with mass trapping to control medfly on different host plants. Martinez-Ferrer *et al.* (2010) suggested that 25 trap per ha (Maxitrap Model baited with Ferag. CC D TM® attractant) can be a good stand-alone control method against the Medfly in citrus groves in Spain. In addition, IAEA (2003) suggested that 20-25 traps per ha density was required by using a mass trapping technique for medfly. Martinez-Ferrer *et al.* (2010] reported that for the Clemenules variety, a 25 traps per ha were enough to capture adults flying within the grove and attract the foraging medflies, as low percentage of fruits were attacked. A number of researchers reported that if the population density of medfly increase, the number of trap per ha will be increased. Therefore, during September-October Medfly population is high, a 50 trap per ha density has been widely accepted as appropriate for citrus orchards (Navarro-Llopis *et al.,* 2008; Primo, 2004; Navarro-Llopis *et al.,* 2004; Leza *et al*., 2008; Martinez-Ferrer *et al.,* 2012; Martınez-Ferrer *et al.,* 2006). Martinez-Ferrer *et al.* (2010) reported that for the early-season varieties, 50 traps per ha captured as many adults as did 75 and 100 traps per ha, but not enough to diminish the adult medflies foraging in the grove under accepted levels. The percentage of the total caught over this period varied for each of the sampling month in both years. The distribution of the total caught of this pest according to the months as percentages were 27.85 (August), 25.05 (September), 25.50 (October) and 21.59 (November) in 2016 (Figure 3). These percentages were 9.31 (August), 34.56 (September), 33.58 (October) and 22.55 (November) in 2017 (Figure 4).

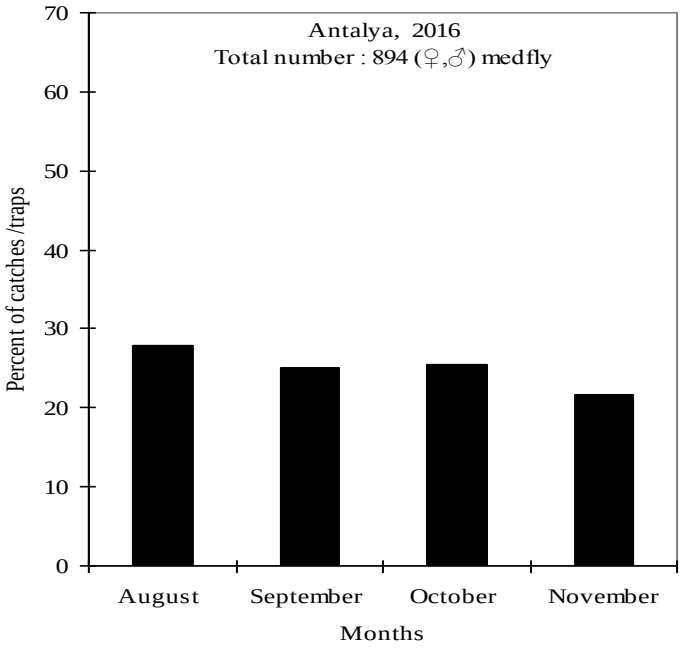

Figure 3. Percentage of the total medfly adults caught over the sampling period at washington orange orchard in Antalya province.

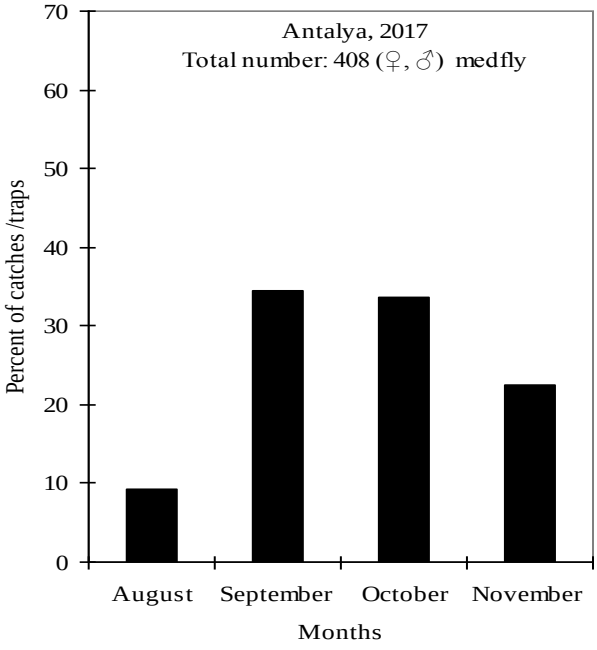

Figure 4. Percentage of the total medfly adults caught over the sampling period at washington orange orchard in Antalya province.

Several studies were conducted by many researchers to evaluate the population density of medfly on various host plants. The population density of medfly was significantly high in September and October due to high temperatures in citrus- producing area (Martinez-Ferrer *et al.,* 2010; Martınez-Ferrer *et al.,* 2007; Boulahia-Kheder *et al.,* 2012). The present results indicated that the population density of medfly was high in August in 2016 and September and October in 2017.

Conclusions

The present study was conducted by traps baited with trimedlure to evaluate of mass trapping for the control of Medfly in washington orange in Antalya province of Turkey. As a result of two-year investigations, the population density of this pest varied in each of the sampling year. In the first year, 40 trap per 1.8 ha were used with mass trapping to control of the medfly. A significant number of medfly were caught by traps, keeping population density under 2 mean catches per traps during the sampling periods. In the second year, 40 trap per 1.8 ha were used with mass trapping to control of this pest. The population density of medfly was signifacantly low comparing with that of the previous year.

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EVALUATION OF POPULATION DENSITY OF EUROPEAN GRAPEVINE MOTH, *LOBESIA BOTRANA* (DENIS & SCHIFFERMÜLLER) (LEPIDOPTERA: TORTRICIDAE) ON DIFFERENT VARIETIES OF VINEYARDS IN HATAY PROVINCE (TURKEY)

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Abstract

The grape berry moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), is one of the important pests of vineyards in Hatay province of Turkey. The current study was conducted to determine the population density of grape berry moth at the different varieties of vineyards in Hatay province of Turkey. The study was carried out in 2016-2017 at ten varieties: Arra 4, Arra 13, Arra 15, Arra 16, Arra 18, Arra 19, Arra 30, Redglop, Vitroblack 25, and Crimson varieties, located in Reyhanlı district of Hatay province. The grape berry moth pheromone (10 mg, (E, Z) -7,9-dodecadienyl acetate) with delta type traps were used in this study. One pheromone trap was placed in each of the vineyard. The pheromone traps were weekly checked and captured grape berry moth adults were counted and then traps were cleaned. Pheromones in the delta traps were replaced with the new ones in every 40 days. After two years of the study, the average number of the catches per trap varied in the sampling period. In the first year, the highest number of the the grape berry moth were recorded in June, followed by July, May, August and April. In the second year, the highest number of the grape berry moth were recorded in June, followed by July, May and August.

Keywords: *Grape berry moth, Lobesia botrana, vineyards, pheromone traps, Hatay*

Introduction

The grape berry moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), is one of the most important pest of grapevine in Turkey. The grape berry moth is a major pest of grapevines responsible for great economic losses and frequent insecticide applications. The grape berry moth is a polyvoltine species (Bovey, 1966; Roehrich and Boller, 1991) and adaptive polyphagous attacking many plant species (Savopoulou-Soultani *et al*., 1990, Stavridis and Savopoulou-Soultani, 1998). The grape berry moth larvae cause damage to grapes by feeding on fruit, resulting in direct damage and secondary infection indirect damage caused by increased susceptibility of the berries to gray mold, *Botrytis cinerea* (Bovey, 1966; Roehrich and Boller, 1991; Fermaud and Le Menn, 1989). The main sex pheromone component of *L. botrana*, (E)-7,(Z)-9-dodecadienyl acetate (E7,Z9-12:Ac), was identified three decades ago (Roelofs *et al*., 1973; Buser *et al*., 1974). The main pheromone component has been used to monitor the flight activity and population dynamics (Anshelevich *et al*., 1994), as in mating disruption for pest control (Arn *et al*., 1988; Sauer and Karg, 1998, Charmillot and Pasquier, 2004; Kast, 2001; Varner *et al*., 2001; Ioriatti *et al*., 2005). The purpose of the current study was to evaluate the population density of grape berry moth at the different varieties of vineyards in Hatay province of Turkey.

Material and Methods

The study was conducted in 2016-2017 in Reyhanlı district of Hatay province. In the first year, the study was conducted from 20 April to 24 August 2016 at ten vineyards consiting of Arra 4, Arra 13, Arra 15, Arra 16, Arra 18, Arra 19, Arra 30, Redglop, Vitroblack 25, and Crimson varieties. In the second year, the study was conducted from 28 April to 4 August

2017 at ten vineyards consiting of Arra 4, Arra 13, Arra 15, Arra 16, Arra 18, Arra 19, Arra 30, Redglop, Crimson and Vitroblack 25 varieties. In both studies, the grape berry moth pheromone (10 mg, (E, Z) -7,9-dodecadienyl acetate) with delta type traps were used. One pheromone trap was placed 1.5 m above ground grapes, checked weekly and captured grape berry moth were counted and cleaned. Pheromones in the delta traps were replaced with the new ones in every 40 days.

Results and Discussion

The population density of *L. botrana* varied in each of the sampling year. In the first year, the pheromone trapping was conducted at ten different vineyards and a total of 595 grape berry moth were caught by pheromone traps (Figure 1). The first moth was caught by the traps on 27 April, and the population density of this pest was gradually varied during the sampling periods. The largest mean of catches per trap were recorded on 6 July (5.8), followed by 1 June (5.50), and 29 June (5.30). The highest number of *L. botrana* moth were recorded in June (235), followed by July (169), May (116), August (49) and April (26).

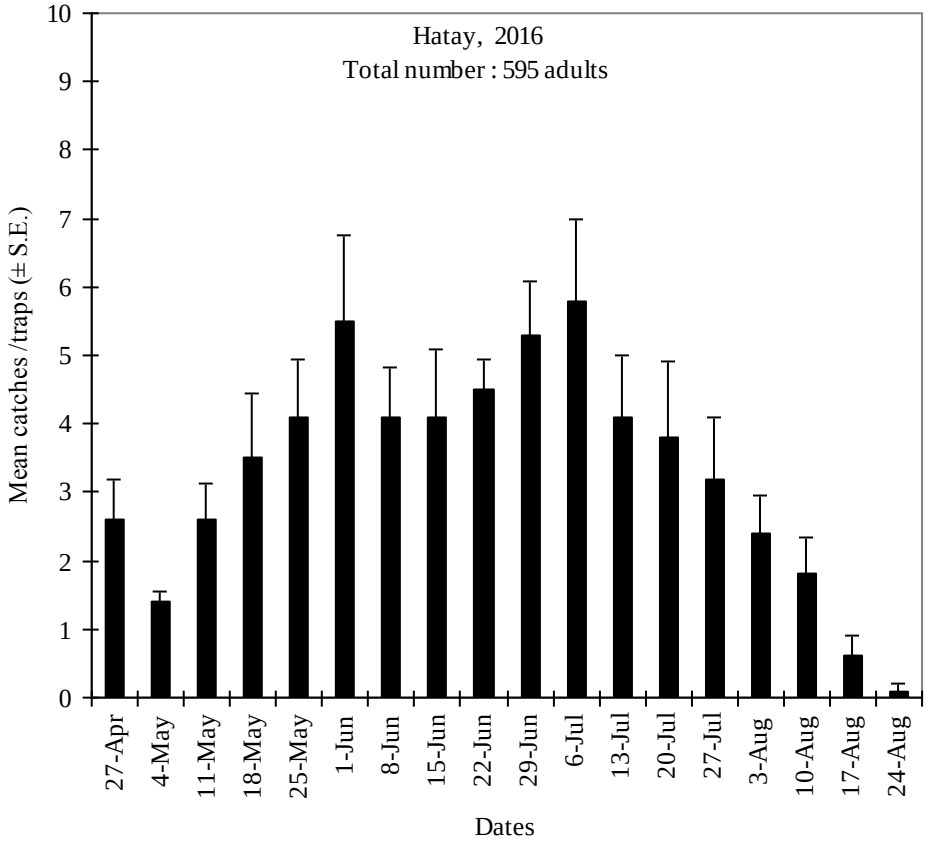

Figure 1. Mean (±SE) catches of grape berry moth in traps baited with pheromone (20 April to 24 August 2016) at ten vineyards in Hatay province.

In the second year, the pheromone trapping was conducted at ten different vineyards and a total of 401 grape berry moth were caught by pheromone traps (Figure 2). The first moth was caught by the traps on 5 May, and the population density of this pest was gradually varied during the sampling periods. The largest mean of catches per trap were recorded on 23 June (5.20), followed by 16 June (4.60), and 30 June (4.50). The highest number of *L.botrana* moth were recorded in June (221), followed by July (95), May (82), and August (3).

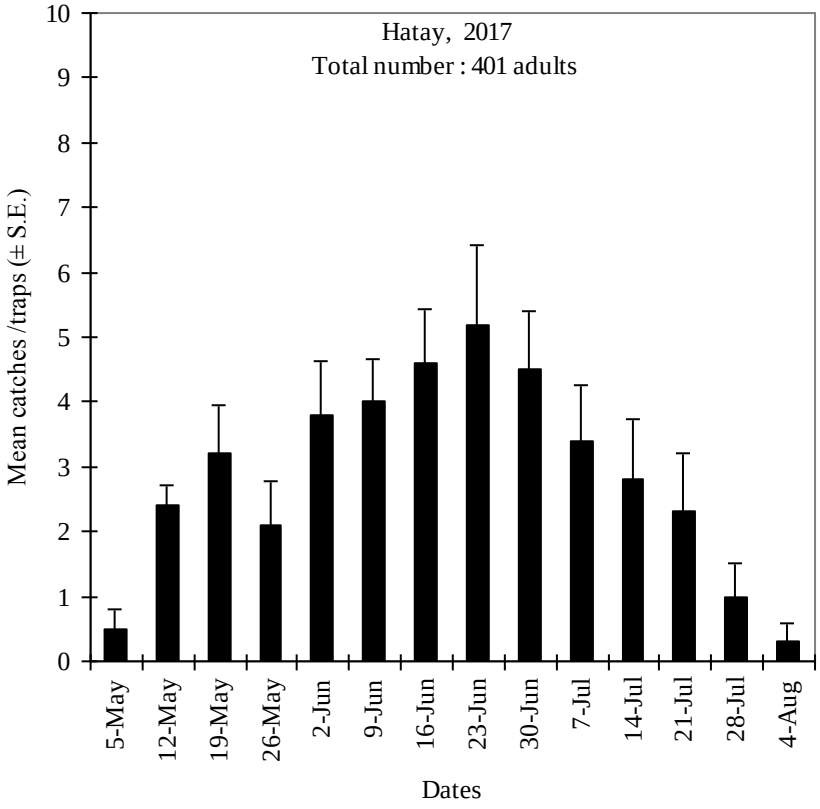

Figure 2. Mean (±SE) catches of grape berry moth in traps baited with pheromone (28 April to 4 August 2017) at ten vineyards in Hatay province.

Karabulut and Demirel (2014) reported that population density of the grape berry moth differed for sampling locations and years. In the first year, a total of 844 grape berry moth adults were caught by the pheremone traps. The first adults were caught on 27th May. The highest number of adults caught was on 15th July, followed by 22nd and 29th July. The ratios of the grape berry moth adults caught by the pheremone traps were, in descending order, 49.4% in July, 22.39 % in June, 17.65 % in August, 6.99% in September and 3.55 % in May. In addtion, in the second year, a total of 846 grape berry moth adults were caught by the pheremone traps. The first adults were caught on 26th May. The highest number of adults caught was on 28th July, followed by 2nd June and 7th July. The ratios of the grape berry moth adults caught by the pheremone traps were, in descending order, 39.83% in July, 31.56 % in June, 19.26 % in August, 5.31 % in September and 4.01% in May.

Conclusions

The present study was conducted by traps baited with pheromone to evaluate the population density of grape berry moth at the different varieties of vineyards in Hatay province of Turkey. As a result of two-year investigations, the population density of this pest varied in each of the sampling year. In the first year, a total of 595 grape berry moth were caught by pheromone traps. The largest mean of catches per trap were recorded on 6 July, followed by 1-29 June. The highest number of *L.botrana* moth were recorded in June, followed by July, May, August and April. In the second year, a total of 401 grape berry moth were caught by pheromone traps. The largest mean of catches per trap were recorded on 23 June, followed by 16-30 June. The highest number of *L.botrana* moth were recorded in June, followed by July, May and August.

Acknowledgement

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EVALUATION OF THE POPULATION DENSITY OF EUROPEAN GRAPEVINE MOTH, LOBESIA BOTRANA (DENIS & SCHIFFERMÜLLER) (LEPIDOPTERA: TORTRICIDAE) AT WINE VINEYARDS IN HATAY PROVINCE (TURKEY)

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Abstract

The grape berry moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), is one of the important pests on wine vineyards in Hatay province of Turkey. The study was conducted to determine the population density of grape berry moth on the wine vineyards in Hatay province (Turkey). The study was carried out at four different wine vineyards located in Belen district of Hatay province. The wine vineyard contained Syrah, Sangiovese, Cabetnet sauvignon and Barbari varieties. The grape berry moth pheromone (10 mg, (E, Z) -7,9-dodecadienyl acetate) with delta type traps were used in this study. Two pheromone traps were placed in each of the wine vineyards. The pheromone traps were weekly checked and captured grape berry moth adults counted, and then the traps were cleaned. Pheromones in the delta traps were replaced with the new ones every 40 days. A total of 1786 grape berry moth adults were caught by the pheromone traps. The number of captured grape berry moth adults was changed during the sampling period. The highest number of the grape berry moth adults captured by pheromone traps was in 11 July, following in 25 July, 18 July, the lowest number of the grape berry moth adults caught by pheromone traps was on 15 August.

Keywords: *Grape berry moth, Lobesia botrana, wines vineyards, pheromone traps, Hatay.*

Introduction

The grape berry moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), is one of the most important pest of grapevine in Turkey. The grape berry moth is a polyvoltine species (Bovey, 1966) and adaptive polyphagous attacking many plant species (Savopoulou-Soultani *et al*., 1990). The grape berry moth larvae cause damage to grapes by feeding on fruit, resulting in direct damage and secondary infection indirect damage caused by increased susceptibility of the berries to gray mold, *Botrytis cinerea* (Bovey, 1966; Roehrich and Boller, 1991). The main sex pheromone component of *L. botrana*, (E)-7,(Z)-9-dodecadienyl acetate (E7,Z9-12:Ac), was identified three decades ago (Roelofs *et al*., 1973). The main pheromone component has been used to monitor the flight activity and population dynamics (Anshelevich *et al*., 1994). The purpose of the current study was to evaluate the population density of grape berry moth at the different varieties of wine vineyards in Hatay province of Turkey.

Material and Methods

The study was conducted in 2017 in Reyhanlı district of Hatay province. In the first year, the study was conducted from 04 July to 15 August 2017 at five wine vineyards consiting of Syrah, Sangiovese, Cabetnet sauvignon and Barbari varieties. The grape berry moth pheromone (10 mg, (E, Z) -7,9-dodecadienyl acetate) with delta type traps were used. Three pheromone traps for Syrah, Sangiovese + Syrah varieties, two pheromone traps for Sangiovese, Barbari varieties and one pheromone trap for Cabernet sauvignon variety were placed 1 m above ground on grapes. All pheromones traps were checked weekly and captured grape berry moth were counted and cleaned . Pheromones in the delta traps were replaced with the new ones in every 40 days.

Results and Discussion

The pheromone trapping was conducted at Syrah variety and and a total of 791 grape berry moth were caught by pheromone traps (Figure 1). The population density of this pest was gradually varied during the sampling periods. The largest number of catches per trap were recorded on 11 July (560), followed by 25 July (117), 18 July (65), 1 August (35), 8 August (11) and 15 August (3).

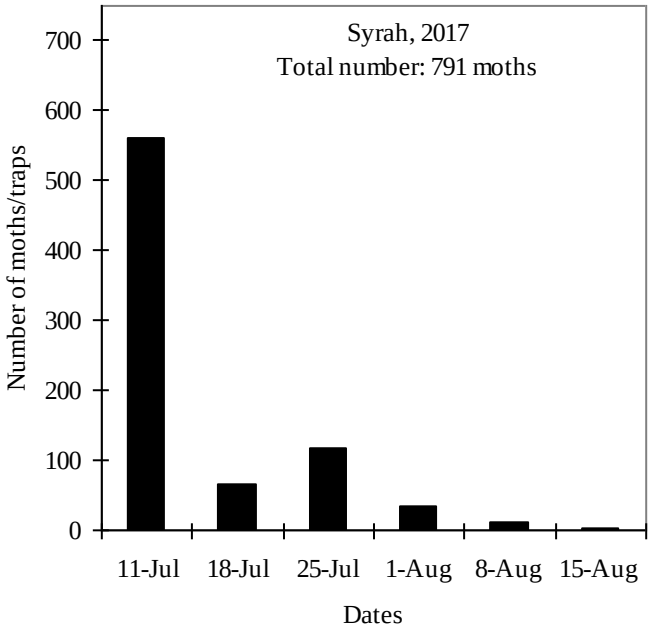

Figure 1. Number of catches of grape berry moth in traps baited with pheromone (04 July to 15 August 2017) at Syrah wine vineyards in Hatay province.

The pheromone trapping was conducted at Sangiovese+Syrah varieties and and a total of 427 grape berry moth were caught by pheromone traps (Figure 2). The population density of this pest was gradually varied during the sampling periods. The largest number of catches per trap were recorded on 11 July (312), followed by 25 July (82), 1 August (16), 8 August (8), 18 July (7) and 15 August (2).

The pheromone trapping was conducted at Sangiovese variety and and a total of 119 grape berry moth were caught by pheromone traps (Figure 3). The population density of this pest was gradually varied during the sampling periods. The largest number of catches per trap were recorded on 11 July (78), followed by 25 July (24), 18 July (13), 1 August (4), 8 August (0) and 15 August (0).

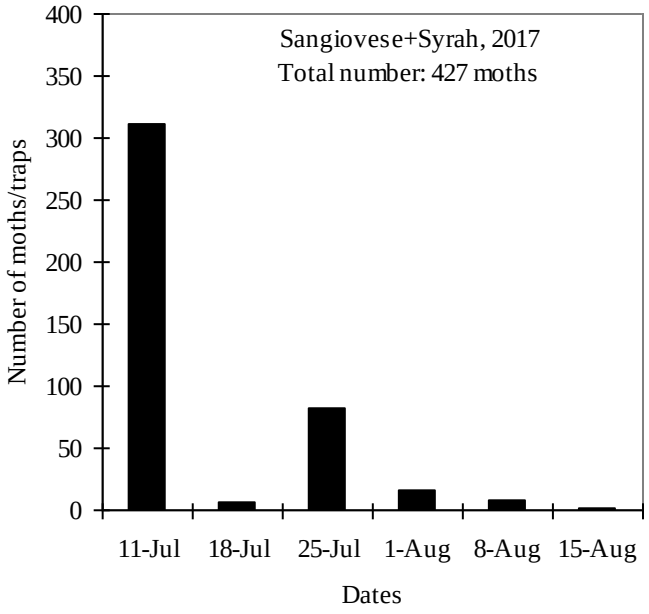

Figure 2. Number of catches of grape berry moth in traps baited with pheromone (04 July to 15 August 2017) at Sangiovese + Syrah wine vineyards in Hatay province.

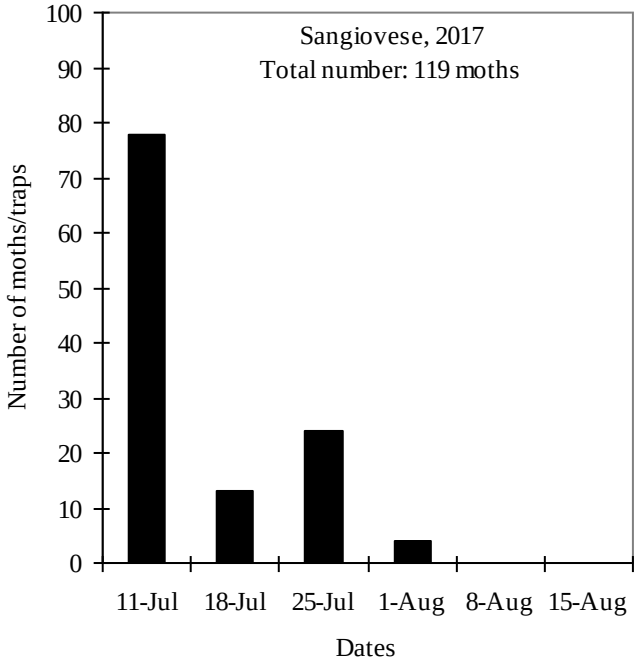

Figure 3. Number of catches of grape berry moth in traps baited with pheromone (04 July to 15 August 2017) at Sangiovese wine vineyards in Hatay province.

The pheromone trapping was conducted at Cabernet sauvignon variety and and a total of 403 grape berry moth were caught by pheromone trap (Figure 4). The population density of this pest was gradually varied during the sampling periods. The largest number of catches per trap were recorded on 11 July (285), followed by 25 July (87), 18 July (16), 1 August (10), 8 August (4) and 15 August (1).

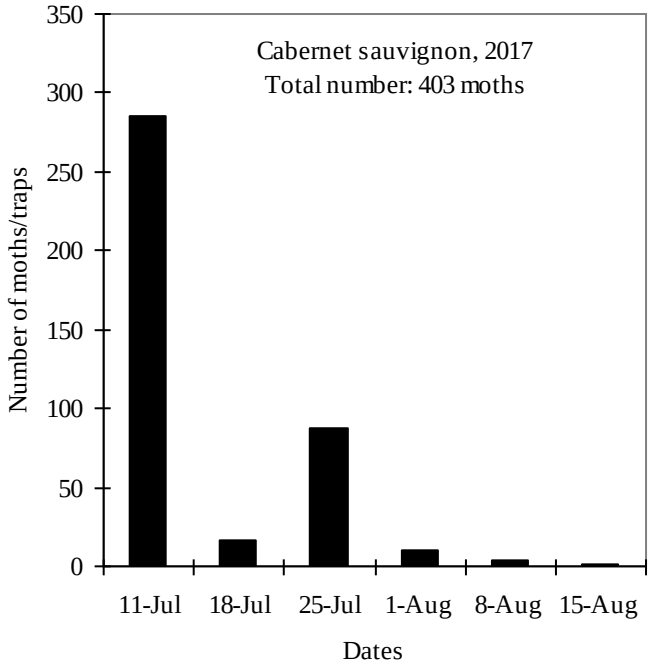

Figure 4. Number of catches of grape berry moth in traps baited with pheromone (04 July to 15 August 2017) at Cabernet sauvignon wine vineyards in Hatay province.

The pheromone trapping was conducted at Barbari variety and and a total of 46 grape berry moth were caught by pheromone traps (Figure 5). The population density of this pest was gradually varied during the sampling periods. The largest number of catches per trap were recorded on 11 July (29), followed by 1 August (7), 25 July (3), 18 July (3), 8 August (3) and 15 August (1).

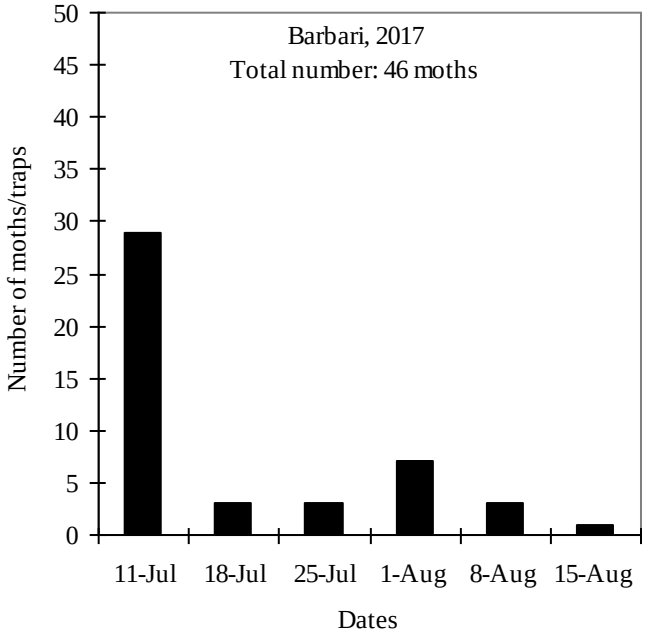

Figure 5. Number of catches of grape berry moth in traps baited with pheromone (04 July to 15 August 2017) at Barbari wine vineyards in Hatay province.

Karabulut and Demirel (2014) reported that population density of the grape berry moth differed for sampling locations and years. In the first year, a total of 844 grape berry moth

adults were caught by the pheremone traps. The first adults were caught on 27th May. The highest number of adults caught was on 15th July, followed by 22nd and 29th July. In the second year, a total of 846 grape berry moth adults were caught by the pheremone traps. The first adults were caught on 26th May. The highest number of adults caught was on 28th July, followed by 2nd June and 7th July.

Conclusions

The present study was conducted by traps baited with pheromone to evaluate the population density of grape berry moth at the different varieties of wine vineyards in Hatay province of Turkey. As a result of investigations, the population density of this pest varied in each of the sampling varieties. The highest number of the grape berry moth adults captured by pheromone traps was in 11 July, following in 25 July, 18 July, the lowest number of the grape berry moth adults caught by pheromone traps was on 15 August.

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USE OF EDIBLE FILMS AND COATINGS IN MEAT AND MEAT PRODUCTS

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Abstract

Meat and meat products are commonly consumed foods around the world due to their high nutrients availability. However, these foods also provide a suitable environment for growth of pathogenic and spoilage microorganisms. In addition, lipid oxidation causes a reduction in the nutritional value and the formation of undesirable flavor, color, odor and toxic compounds in the meat and meat products. Therefore, some natural or synthetic food additives are used in meat and meat products. However, the effectiveness of these additives which are added to the products by using traditional application methods decreased over time and the meat industry suffers some problems in maintaining product quality. In addition, nowadays consumers demand high quality foods with an extended shelf life and without chemical additives. Edible films and coatings obtained from vegetable or animal sources and applied to the food surfaces by various methods to improve the quality characteristics of food and extend the shelf life. Besides, edible films and coatings can prevent the undesirable color formation, lipid oxidation and microbiological deterioration when they are combined with antioxidant and antimicrobial compounds. Edible films and coatings applied to meat and meat products have some advantages such as the reduction of moisture losses during the storage period, the confinement of water in ready-to-eat meat, the limitation of lipid and myoglobin oxidation, the elimination of spoilage and pathogenic microorganism contamination, the reduction of volatile flavor loss and the prevention of undesirable taste and odor formation. In this review study, research about edible films and coatings prepared with antioxidant and antimicrobial compounds are reviewed.

Keywords: *Edible films and coatings, antimicrobial, antioxidant, meat and meat products.*

Introduction

Adequate and balanced diet is one of the most important contributing factors for achieving or maintaining a good state of health. It is generally accepted that balanced diets of meat and plant food are most effective for human nutrition. Meat and meat products are widely distributed and consumed around the world due to their high nutrients availability. Meat and meat products are better sources of protein than plant foods (except soy bean products). In meat, the essential amino acids are available in well-balanced proportions and concentrations. As well, meat and meat products have B12 deposits for children and particularly rich in iron to prevent anemia. However, they are highly perishable products due to their high moisture contents, nitrogenous nutrients, minerals, fermentable carbohydrates, suitable pH and other growth factors, therefore they can be possibly dangerous to health through microbial growth and chemical changes (Sanchez-Ortega et al., 2014).

Lipid oxidation is reduced the nutritional value of the meat and meat products as far as the development of undesirable taste, color, smell and toxic compounds (Vital et al., 2016; Noor et al., 2018). On the other hand, microorganisms that grow in meat products may cause changes in appearance, flavor, odor, and other features of the meat products. Also, they can threaten human health by causing food infections and intoxications (Ozbay-Dogu and Saricoban, 2014). Many strategies are implemented in meat industry to prevent lipid

oxidation, inhibit undesirable changes, increase shelf life and protect human health (Mor-Mur and Yuste, 2010). Use of edible films and coatings that among these protection methods has increasing trend. Edible films and coatings obtained from vegetable or animal sources and applied to the food surfaces by various methods improve the quality characteristics of food and extend the shelf life. Besides, edible films and coatings can prevent the undesirable color formation, lipid oxidation and microbiological deterioration when they are combined with antioxidant and antimicrobial compounds (Umaraw and Verma, 2017).

Edible Films and Coatings

Edible films and coatings are defined as a non-synthetic materials produced by vegetable or animal origin natural resources (polysaccharide, protein and lipid) alone or in combination. They form a thin layer on the surface of food or between food components and can be consumed with food (Pavlath and Orts, 2009). Edible films and coatings have been used for centuries to prevent loss of moisture during storage and transportation. First recorded use of biopolymer was in China around twelfth century where citrus fruits were preserved by coating them with wax. Later in the sixteenth century, in England larding (coating with lard) was practiced to prolong shelf-life of meat (Ortiz et al., 2014). Currently, edible films and coatings are used for various purposes such as protecting the food against chemical and microbiological deterioration and increasing the shelf life of products (Hassan et al., 2018). Packing material is defined as an "edible" packaging when it is an integral part of the food and can be consumed with together. Coatings are a particular form of films directly applied to the surface of materials and are regarded as a part of the final product. On the other hand, edible films are obtained from food suspensions that are usually cast over an inert surface, which after drying can be placed in contact with food surfaces (Salgado et al., 2015). Edible films and coatings are an alternative to extend the shelf life of foods by acting as barriers to water vapor, oxygen, and carbon dioxide and as a carrier of substances to inhibit pathogenic and spoilage microorganisms. In addition, these films and coatings prevent food from physical changes by providing mechanical protection during transportation and storage (Temiz, 2006). In addition, they provide brighter and smoother product appearance (Salgado et al., 2015). These films are generally biodegradable in nature making it an eco-friendly packaging approach. Thus, they contribute to the protection of the environment (Song and Zheng, 2014). Edible films and coatings act as a barrier between muscle foods and the surrounding environment to enhance the quality of products protecting them from physical, chemical and microbiological deterioration. Additionally, they may provide moisture loss reduction during storage of fresh or frozen meats and a decrease in lipid and myoglobin oxidation of the meat (Oguzhan Yildiz and Yangilar, 2016).

Components of Edible Films and Coatings

Edible films and coatings vary in the vast range of hydrophilic and hydrophobic boundaries. Additionally, they need a suitable solvent in order to prepare a film or coating forming the dispersion. Mostly water or alcohol are used as a solvents according to natural solubility characteristics of film forming materials. In addition, functional additives are used to improve protective properties of edible films and coatings.

The main components which are used in the preparation of edible films and coatings are polysaccharides, proteins (hydrophilic) and lipids (hydrophobic) (Salgado et al., 2015). Polysaccharides are hydrocolloids of high molecular weight which in water form intensive hydrogen bonds to form gel. The hydrophilic polysaccharide components are found to have good mechanical properties but the edible films and coatings made up of these components have greater permeability for water vapour and gasses. Among polysaccharides, chitosan derivatives, cellulose, alginate, pectin derivatives, seaweed extract and starch are most

commonly used in the preparation of edible films and coatings (Mellinas et al., 2015). Proteins inherently have interactive forces that provide cohesiveness which is essential for film/coating formation. Protein based edible films and coatings exhibit excellent gas barrier but poor moisture barrier properties. This gas barrier property can be used in preventing oxidative rancidity, enzymatic browning and flavor loss in meat and meat products (Cutter, 2006). Various types of protein such as gelatin, casein, whey protein, corn zein, wheat gluten, soy protein, mung bean protein and peanut protein have been used as edible films (Umaraw and Verma, 2017). Lipids are hydrophobic substances which have excellent moisture barrier property, were the very first kind of coating used on fresh meats as "Larding" in sixteenth century. Nowadays beeswax, paraffin, mineral and vegetable oils are also used (Mellinas et al., 2015). Lipid based edible films and coatings are brittle and have poor strength and elasticity (Dursun and Erkan, 2009). Thus, better form of lipids is composite films/coatings with hydrocolloids or proteins (Umaraw and Verma, 2017). Composite films and coatings can be formed of polysaccharides, proteins and/or lipids. All substances have been combined to create composite barriers with desired mechanical strength and low permeability (Umaraw and Verma, 2017). Renewable agricultural resources or industrial by-products can be used as a main component in the production of edible films and coatings. Starch, cellulose, chitosan, carrageenan, whey protein, gelatin, and keratin are materials that can be used as an alternative in the preparation of edible films and coatings (Reddy et al., 2013). Water or ethanol is usually used as a solvent in the preparation of edible films and coatings to preserve the consumption of the food (Ustunol, 2009). Plasticizers, which are small molecules such as glycerol, propylene glycol, or polyethylene glycol, are used to control the viscosity of the liquid formulation, add flexibility and tensile strength and control surface tension (Dursun and Erkan, 2009; Ustunol 2009). Antioxidants and antimicrobials, emulsifiers, antifungal and flavoring agents, colorants and other functional substances may be added into edible films and coatings for improving the quality of food and protecting consumer health (Oussalah et al., 2004; Quintavalla and Vicini, 2002).

Production Techniques of Edible Films and Coatings

Edible coatings can be obtained by dipping the product into, or by brushing or spraying it with a solution, so as to the film directly on food surface. Edible films can be obtained by creating a standalone film from a solution for the subsequent covering of food surface (Ortiz et al., 2014).

Use of Edible Films and Coatings in Meat and Meat Products

Red Meat and Meat Products

The use of edible films and coatings containing antioxidant and antimicrobial compounds could improve the oxidative and antimicrobial stability of meat. For this reason, their use in meat and meat products has been attracting an interest in recent years and various research on this area has been conducted. In Table 1, some examples of the use of edible films and coatings in red meat and meat products are presented. Previous studies indicated that the application of protein and carbohydrate based edible films and coatings delayed lipid oxidation, inhibited the growth of microorganisms and increased the shelf life of the products during storage of raw or cooked red meats. For example, Shin et al. (2017) studied the effect of apple peel powder based edible films containing tartaric acid on the raw or cooked beef meatball. The authors concluded that the use of edible films prevent lipid oxidation and growth of microorganisms and the process does not affect the sensory properties of raw or cooked beef meatball.

Table 1. Application of edible films and coatings for red meat and meat products preservation

Products Used	Major Component	Additives	Storage Conditions	Effects	References
Ham slices frankfurter	Sodium alginate	Distilled ethanol Tsipouro Raki, Ouzo	4 and 10 ^{0}C 40, 52, 81 d	Reduced *L. Monocytogenes* population	Kapetanakou et al. (2016)
Ground beef	Zein	2% or 3% *Zataria multiflora* essential oil 1% monolaurin	4 ^{0}C 3 d	High phenolic content Increased antioxidant activity Effective in controlling *E. coli* O157:H7 and *L. monocytogenes* growth	Moradi et al. (2016)
Raw or cooked beef meatball	Apple peel powder	0.75% (w/v) Tartaric acid	4 ^{0}C 10 d	Prevented lipid oxidation and microbial growth Enhanced shelf life	Shin et al. (2017)
Bovine meat	*Plantago major* seed mucilage	0%, 0.5%, 1%, 1.5% dill essential oil	4 ^{0}C 18 d	Reduced lipid oxidation Antimicrobial effect	Behbahani et al. (2017)
Cooked beef meatbal	Whey protein isolate	2%, 4% laurel and sage extracts	18 ^{0}C 60 d	Retarded oxidative changes; Enhanced shelf life	Akcan et al. (2017)

Poultry Meat and Meat Products

Various research on the use of edible films and coatings was conducted in the poultry meat and meat products. In Table 2, some studies on these products are presented. These studies stated that antioxidant edible films and coatings could prevent lipid oxidation, the development of off-flavors, and nutritional losses, whereas antimicrobials could prevent spoilage from food-borne bacteria and organoleptic deterioration. For example, Tosati et al. (2017) studied the quality and extension of the shelf life of fresh frankfurter sausage with turmeric starch residue and gelatin based edible coatings. They determined that the edible coatings reduce growth of microorganisms during the storage period. Also, researchers found that the physicochemical properties such as pH, texture, humidity and color remain stable and these coatings may be an alternative substitution for synthetic casing in sausages.

Table 2. Application of edible films and coatings for poultry meat and meat products preservation

Products Used	Major Component	Additives	Storage Conditions	Effects	References
Chicken breast fillets	Whey protein isolate	10 and 20 g/kg oregano or clove essential oil	4 ^{0}C 13 d	Reduced total mesophilic aerobic, *Pseudomonas* spp. and the count of lactic acid bacteria Enhanced shelf life	Fernandez-Pan et al. (2014)
Fresh chicken breast	0.2% c-carragenan, 2% kitosan	Allil izotiyosiyanat Oriental mustard extract EDTA	4 ^{0}C 21 d	Reduced Salmonella level Decreased lactic acid bacteria and the count of aerobic bacteria.	Olaimat and Holley (2015)
Refrigerated chicken fillet	2% chitosan	1%, 2% propolis extract	4 ^{0}C 12 d	Prevented growth of bacteria Inhibited increase of peroxide values, TBA and total volatile nitrogen: Enhanced shelf life and quality	Jafari et al. (2017)
Fresh frankfurter sausage	Turmeric starch residue and gelatin	-	5 ^{0}C and 10 ^{0}C 30 d	Prevented microbial growth pH, texture, humidity and color stability; Substitution of synthetic casing	Tosati et al. (2017)
Chicken breast fillets	Sodium caseinate nanoemulsion	6% ginger essential oil	4 ^{0}C 12 d	Reduced total aerobic psychrophilic bacteria, mold and yeast ; Weak antioxidant potential Strong antimicrobial activity	Noori et al. (2018)

Seafood

The quality of seafood is quickly deteriorated during storage due to chemical and enzymatic reactions. For these reasons, the main objective of using edible films and coatings in seafoods is to prevent the growth of microorganisms and lipid oxidation. The edible film and coating studies about seafoods are presented in Table 3. Previous studies revealed that the application of edible films and coatings not only inhibit the microbial spoilage but also prevent lipid oxidation and total volatile bases during storage of seafood. For example, Jasour et al. (2015) studied the effect of chitosan based edible coating containing lactoperoxidase to enhance quality and shelf life of rainbow trout. The results indicated that the edible coatings have significantly lower numbers of *Shewanella putrefaciens*, *Pseudomonas fluorescens*, and psychrotrophic and mesophilic bacteria. Researchers also concluded that the coating application extend the shelf life of trout fillets by at least 4 days.

Table 3. Application of edible films and coatings for seafood preservation

Products Used	Major Component	Additives	Storage Conditions	Effects	References
Rainbow trout	Chitosan	Lactoperoxidase	4 ^{0}C 16 d	Reduced *Shewanella putrefaciens, Pseudomonas fluorescens* and psychrotrophic and mesophilic bacteria Lower TBA values Enhanced shelf life Improved sensory properties	Jasour et al. (2015)
Fish sausage	Chitosan-gelatin	Shrimp concentrate	4 ^{0}C 45 d	Extended lag phase and prevented to the growth of microorganisms Decreased total volatile bases and pH values	Aleman et al. (2016)
Silver carp fillet	Sodium alginate-carboxymethylcellulose	0%-0.5% *Ziziphora clinopodioides* essential oil 0%-1% apple peel extract 0%-0.5% zinc oxide nanoparticle	4 ^{0}C 14 d	Enhanced shelf life Prevented growth of *L.monocytogenes*	Rezaei and Shahbazi (2018)

Conclusions

The use of edible films and coatings are a very common application in the meat industry and the number of researches on this area has increasing trend. Especially, the use of edible films and coatings prepared with natural antioxidant and antimicrobial materials has been becoming popular due to the consumer awareness. It is believed that the use of this technology reduces the amount of waste and prevents the environmental pollution. However, there are some shortcomings in these studies and they are not commercially viable and acceptable. For this reason, more studies are required about the choice of suitable edible film and coating materials, optimization of the process, provide for industrial suitability and development of low-cost applications for enhancing to the availability of this technology.

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FREE AND BOUND PHENOLICS AND ANTIOXIDANTS IN RAW AND INFRARED STABILIZED IMMATURE RICE GRAIN

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Abstract

Total phenolics, phenolic acid composition and antioxidant capacity of immature rice grains which were stabilized at 1200 W (8.0 and 10.1 min), 1400 W (7.0 min), and 1600 W (4.4 and 5.0 min) with medium wave and 1200 W (10.1 min), 1400 W (6.2 and 7.1 min) and 1600 W (5.0 min) with short wave infrared emitters were investigated and compared with unprocessed immature grains. Although a decrease in the content of phenolics and antioxidant capacity was observed in some stabilized grains, it was concluded that these decreases might not be directly related with stabilization conditions since they are not consistent with radiation intensity or process time. Furthermore, the content of the phenolics and antioxidant capacity were higher in some stabilized grains when compared to unprocessed immature grains. Instead, the variations were attributed to random error. In conclusion, it was found that infrared radiation can be used to stabilize immature rice grains without negatively affecting the content of phenolic compounds and antioxidant capacity.

Keywords: *Rice bran, infrared, stabilization, phenolics, antioxidants.*

Introduction

The phytochemical profile of whole grain rice has been widely studied and found that it includes notable amount of bioactive compounds such as γ-oryzanol, tocopherols, tocotrienols, and phenolics compounds (Guafo and Trindade, 2017). However, there is lacking information related to immature rice grains. Immature rice grains are defined as either thin, chalky kernels or kernels which have green seed coat. Besides, rice grains that have a moisture content exceeding 22% (before harvest) are also considered as immature (Buggenhout et al., 2013).

Immature rice grains are generally utilized as feed since they are susceptible to rancidity and rapidly deteriorates immediately after milling. In a recent study, it has been shown that free fatty acid content of unprocessed immature rice grains increased from 5.49 % to 35.71 % in 3 months of storage at room temperature (Yılmaz et al., 2018). Although it has been widely applied in thermal operations, the use of infrared (IR) radiation in food industry is more recent. However, it has been used in dehydration, baking, frying, pasteurization and sterilization (Krishnamurthy et al., 2008). Recently, Yılmaz et al. (2014a) have shown that IR radiation can be used for stabilization, in other words enzyme inactivation, of rice bran.

The aim of this study was to determine the effects of IR radiation on phenolics and antioxidants of immature rice grains at selected IR stabilization conditions which have already been proven to be effective in terms of retarding free fatty acid increase.

Materials and Methods

Material

Immature rice grains (variety of Osmancık-97), which were yellow-green in color, were provided by a local rice milling factory (Özer Gıda) in Çanakkale, Turkey immediately after milling. Broken grains and foreign matters were separated from the immature grains using a

laboratory-scale trieur (CRM-1252T, Yaşar Makina, Samsun, Turkey) and the cleaned grains were used for stabilization.

Infrared Stabilization

The laboratory-type IR stabilization system was explained in previous studies (Yılmaz et al., 2014a; Yılmaz et al., 2014b). Before stabilization, 500 g of cleaned immature grain was soaked in water for 10 min and allowed to drain for 5 min. Samples were placed on a conveyor system with a Teflon belt (20 cm width and 110 cm length) as a single layer and stabilized using either medium wave (Item no: 09755054) or short wave (Item No: 09751741) IR emitters (Heraeus Noblelight, Hanau, Germany). Distance between the emitters and the sample (belt) was maintained constant at 15 cm throughout the experiments. Stabilization was carried out at IR powers ranging from 800 W to 2200 W for varying process times. As a result of the preliminary analyses, 10 stabilization conditions which were successful in terms of stabilization (retarding the increase in free fatty acid) were selected. These conditions were 1200 W (8.0 and 10.1 min), 1400 W (7.0 min), and 1600 W (4.4 and 5.0 min) for medium wave and 1200 W (10.1 min), 1400 W (6.2 and 7.1 min) and 1600 W (5.0 min) for short wave IR emitters. The effect of these conditions on the phenolics and antioxidants of immature rice grains were investigated.

Extraction of phenolics and antioxidants

Extraction of free and bound phenolics and antioxidants was carried out using the method specified by Tuncel and Yılmaz (2011).

Determination of total phenolic content

Total phenolic content was analyzed spectrophotometrically according to Folin-Ciocalteau assay. Briefly, 100 µL of sample extract was mixed with 900 µL of deionized water, 5 mL of 0.2 N Folin Ciocalteau reagent and 4 mL of sodium carbonate (75 g/ L). The content was incubated in the dark for 2 h and the absorbance was measured at 765 nm with UV-VIS spectrophotometer (Agilent, Waldbronn, Germany). The results were expressed as mg ferulic acid equivalents per gram immature rice seed.

Determination of phenolic acid composition

Phenolic acid composition was determined by reverse phase liquid chromatography according to the method outlined in our previous study (Tuncel and Yılmaz, 2011). Gallic acid (GA), protocathechuic acid (proto-CA), *p*-hydroxybenzoic acid (*p*-hydBA), vanillic acid (VA), caffeic acid (CA), chlorogenic acid (ChA), syringic acid (SA), *p*-coumaric acid (*p*-COU) ferulic acid (FA), *o*-coumaric acid (*o*-COU), rosemarinic acid (RA) and trans-cinnamic acid (tr-CIN) were analyzed in either free or bound form extracts. The mobile phase was consisting of solvent A (methanol: water: formic acid, 10: 88: 2, v / v) and solvent B (methanol: water: formic acid, 90: 8: 2, v / v). Initial condition was 100 % A; 0 -20 min, changed to 80 % A; 25 - 50 min, to 50 % A; 50 - 54 min, followed by isocratic elutions of 50 % A; 54 - 63 min, 0 % A; and 63 - 70 min, 100 %A. Stationary phase was an Agilent Zorbax Eclipse XDB C18 column (150 mm x 4.6 mm x 5 µm) which was operated at 23 ºC. The flow rate was 1 mL/min throughout the analysis and the injection volume was 5 µL. Quantitation was based on external calibration curves built for each of the phenolic acids and the results were expressed as µg/g.

Determination of antioxidant capacity

Trolox equivalent antioxidant capacity (TEAC)

Trolox antioxidant capacity of the immature rice grains was determined according to Re et al. (1999). ABTS stock solution (7mM) was reacted with 2.45 mM potassium persulfate for 14 h at room temperature in a dark cabinet to generate ABTS radical cation. The radical solution was diluted with phosphate buffer saline to an absorbance between 0.680 and 0.720. Three different concentrations of extracts were added to 1 mL of diluted ABTS radical and the percentage inhibition of the radical was monitored for 6 min at 734 nm. All measurements

were performed with three dilutions per extract and in duplicate. The results were expressed as milimolar equivalents of Trolox per gram immature rice grain.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity

DPPH radical scavenging capacity of the extracts was measured according to the method reported by Yılmaz and Tuncel (2015). Briefly, 600 µL of 1 mM methanolic DPPH solution was mixed with three different concentrations of sample extract and brought to 6 mL volume using methanol. Absorbance of the content was measured at 517 nm using UV-VIS spectrophotometer (Agilent, Waldbronn, Germany) after incubation in the dark for 15 min. Percentage inhibitions were plotted against concentration and the results were expressed as EC_{50} values, the efficient concentration (mg) at which 50 % inhibition occurred.

Statistical analysis

Due to the unequal number of observations, one-way ANOVA was carried out to understand the effect of IR stabilization conditions on phenolics and antioxidants of immature rice grain using MINITAB. When significant effects were observed, Tukey's test was used for multiple comparisons. Results were presented as mean ± standard error and means were the average of 3 replicates.

Results and Discussion

Total phenolics content

Total phenol contents of unprocessed and IR stabilized immature rice grains were presented on Table 1. The results of the phenolics acids were presented as ferulic acid equivalents (FAE) since it is one of the most abundant phenolics acid in cereals. It was found that the content of the free phenolics was higher than that of the bound ones. Similarly, Tuncel and Yılmaz (2011) reported higher free phenolics content in rice milling fractions such as immature rice, rice bran, brown rice, unpolished rice, white rice, and chalky rice compared to bound phenolics. Although the effect of IR stabilization conditions was found statistically significant ($p<0.05$) on total phenolics content, it was observed that the results were very close mathematically and the variations might be attributed to random error. Free, bound, and total phenolics content of the IR stabilized samples were ranged between 0.37-0.55 mg FAE/g, 0.06-0.10 mg FAE/g, and 0.48-0.66 mg FAE/g, respectively. On the other hand, free, bound, and total phenolics content of the unprocessed immature rice grains were 0.52, 0.13, and 0.65 mg FAE/g, respectively (Table 1).

Table 1. Total phenol content of unprocessed and infrared stabilized immature rice grains

Wave lenght	Process time (min)	IR Power (W)	Total phenol (mg FAE/g)	
			Free	Bound
Unprocessed immature rice grain			0.52 ± 0.07^{AB}	0.13 ± 0.01^{A}
MW	8.02	1200	0.50 ± 0.02^{AB}	0.10 ± 0.02^{AB}
MW	10.12	1200	0.44 ± 0.01^{AB}	0.07 ± 0.01^{B}
MW	7.01	1400	0.52 ± 0.01^{AB}	0.10 ± 0.02^{AB}
MW	4.01	1600	0.49 ± 0.03^{AB}	0.06 ± 0.01^{B}
MW	4.40	1600	0.55 ± 0.04^{A}	0.10 ± 0.01^{AB}
MW	5.02	1600	0.51 ± 0.01^{AB}	0.09 ± 0.01^{AB}
SW	10.12	1200	0.37 ± 0.02^{B}	0.07 ± 0.01^{B}
SW	6.18	1400	0.40 ± 0.02^{AB}	0.07 ± 0.01^{B}
SW	7.10	1400	0.48 ± 0.02^{AB}	0.08 ± 0.01^{AB}
SW	5.02	1600	0.42 ± 0.01^{AB}	0.10 ± 0.01^{AB}

*Within each column, means followed by different letters are significantly different by Tukey (P <0.05) **MW (Medium wave), SW (Short wave)

Table 2. Free (soluble) phenolic acids (μg/g) profile of unprocessed and infrared stabilized immature rice grains

Wavelength	Process time (min)	IR Power (W)	GA	proto-CA	*p*-COU	FA	*o*-COU	*tr*-CIN
Unprocessed immature rice grain			3.69 ± 0.16	3.56 ± 1.61	1.79 ± 0.05	1.87 ± 0.05	1.27 ± 0.08^{A}	0.89 ± 0.20
MW	8.02	1200	3.91 ± 0.14	3.84 ± 1.13	2.55 ± 0.07	1.95 ± 0.00	1.18 ± 0.00^{AB}	0.95 ± 0.16
MW	10.12	1200	3.65 ± 0.23	2.34 ± 0.10	2.50 ± 0.02	1.94 ± 0.00	1.00 ± 0.00^{BC}	0.66 ± 0.02
MW	7.01	1400	3.47 ± 0.59	4.88 ± 0.50	2.73 ± 0.14	1.94 ± 0.03	1.90 ± 0.01^{ABC}	0.86 ± 0.01
MW	4.01	1600	3.74 ± 0.29	2.15 ± 0.77	1.76 ± 0.11	1.83 ± 0.03	1.15 ± 0.06^{AB}	0.62 ± 0.07
MW	4.40	1600	3.84 ± 0.53	5.06 ± 1.30	2.35 ± 0.23	1.87 ± 0.06	1.11 ± 0.05^{ABC}	1.00 ± 0.10
MW	5.02	1600	4.13 ± 0.47	2.83 ± 0.94	2.63 ± 0.12	1.91 ± 0.04	0.99 ± 0.00^{BC}	0.69 ± 0.11
SW	10.12	1200	3.01 ± 0.12	1.22 ± 0.06	2.29 ± 0.02	1.76 ± 0.02	1.00 ± 0.02^{BC}	0.69 ± 0.11
SW	6.18	1400	3.82 ± 0.31	2.24 ± 0.59	2.02 ± 0.53	1.84 ± 0.24	0.98 ± 0.03^{BC}	0.55 ± 0.00
SW	7.10	1400	4.25 ± 0.19	1.92 ± 0.09	2.68 ± 0.11	1.89 ± 0.00	1.05 ± 0.01^{ABC}	0.62 ± 0.00
SW	5.02	1600	3.93 ± 0.07	1.87 ± 0.03	2.56 ± 0.03	1.86 ± 0.01	0.92 ± 0.01^{C}	0.58 ± 0.01

Table 3. Bound (insoluble) phenolic acids (μg/g) profile of unprocessed and infrared stabilized immature rice grains

Wavelength	Process time (min)	IR Power (W)	GA	*p*-HBA	VA	CA	ChA	SA	*p*-COU	FA
Unprocessed immature rice			1.74 ± 0.02	2.49 ± 0.23	3.65 ± 0.54^{A}	2.68 ± 0.30^{B}	4.73 ± 0.29^{A}	2.58 ± 0.91	71.28 ±5 .69^{AB}	150.90 ± 7.22^{AB}
MW	8.02	1200	1.73 ± 0.12	1.87 ± 0.34	1.51 ± 0.36^{B}	1.57 ± 0.25^{BC}	2.95 ± 0.69^{AB}	1.82 ± 0.35	73.55 ± 7.76^{AB}	156.20 ± 14.40^{AB}
MW	10.12	1200	2.45 ± 0.24	2.19 ± 0.53	1.75 ± 0.36^{B}	1.80 ± 0.15^{BC}	2.58 ± 0.00^{B}	1.63 ± 0.00	54.50 ± 7.74^{BC}	117.40 ± 20.20^{ABC}
MW	7.01	1400	2.60 ± 0.04	1.74 ± 0.10	1.30 ± 0.01^{B}	1.67 ± 0.03^{BC}	2.71 ± 0.01^{AB}	1.49 ± 0.04	59.27 ± 4.43^{ABC}	107.10 ± 11.40^{BC}
MW	4.01	1600	2.69 ± 1.37	2.00 ± 0.92	1.05 ± 0.05^{B}	1.50 ± 0.21^{C}	2.00 ± 0.13^{B}	1.22 ± 0.12	38.44 ± 0.28^{C}	83.19 ± 1.58^{C}
MW	4.40	1600	2.72 ± 0.51	1.91 ± 0.14	1.42 ± 0.01^{B}	1.90 ± 0.00^{BC}	3.09 ± 0.57^{AB}	1.68 ± 0.32	70.25 ± 4.17^{AB}	149.09 ± 5.14^{AB}
MW	5.02	1600	2.33 ± 0.24	1.77 ± 0.07	1.24 ± 0.02^{B}	1.49 ± 0.03^{C}	2.84 ± 0.23^{AB}	1.72 ± 0.14	53.22 ± 0.72^{BC}	117.58 ± 1.17^{ABC}
SW	10.12	1200	1.36 ± 0.02	1.92 ± 0.07	1.29 ± 0.19^{B}	1.04 ± 0.02^{C}	1.72 ± 0.12^{B}	1.12 ± 0.10	33.40 ± 5.67^{C}	68.00 ± 10.50^{C}
SW	6.18	1400	1.34 ± 0.00	1.56 ± 0.04	1.35 ± 0.08^{B}	1.37 ± 0.02^{C}	2.35 ± 0.00^{B}	1.32 ± 0.09	53.63 ± 3.03^{BC}	118.34 ± 3.97^{ABC}
SW	7.10	1400	2.15 ± 0.32	1.75 ± 0.52	1.68 ± 0.07^{B}	1.45 ± 0.46^{C}	2.96 ± 0.67^{AB}	1.58 ± 0.25	83.58 ± 0.79^{A}	170.60 ± 13.00^{A}
SW	5.02	1600	2.46 ± 0.36	2.45 ± 0.12	1.53 ± 0.03^{B}	3.95 ± 0.11^{A}	2.86 ± 0.14^{AB}	2.98 ± 0.21	71.18 ± 3.25^{AB}	161.13 ± 6.93^{AB}

*Within each column, means followed by different letters are significantly different by Tukey (P <0.05) **MW (Medium wave), SW (Short wave)
*** GA: Gallic acid, proto-CA: proto-catechuic acid, *p*-COU: *para*-coumaric acid, FA: ferulic acid, *o*-COU: *ortho*-coumaric acid, *tr*-CIN: *trans* -cinnamic acid, *p*-HBA: *p*-hydroxybenzoic acid, VA: vanillic acid, CA: caffeic acid, ChA: chlorogenic acid, SA: syringic acid, *p*-COU: *para*-coumaric acid

Phenolic acids

Among the analyzed 12 phenolic acids, gallic, proto-catechuic, *p*-coumaric, ferulic, *o*-coumaric and *tr*-cinnamic acids were identified in free, while gallic, *p*-hydroxy benzoic, vanillic, caffeic, chlorogenic, syringic, *p*-coumaric and ferulic acids were detected in bound phenolic extracts (Table 2, Table 3). Gallic, *p*-coumaric and ferulic acids were found either in free or bound form extracts. However, the amounts of *p*-coumaric and ferulic acids were 1.79 and 1.87 µg/g in free and 71.28 and 150.90 µg/g in bound form extracts, which clearly explains the requirement of bound phenolics extraction (acid / alkali hydrolysis) for cereals to understand the real phenolic acid composition.

The effect of IR stabilization conditions was statistically insignificant on the content of all free phenolic acids excluding *o*-coumaric acid ($p>0.05$). *O*-coumaric acid content of immature rice grain significantly decreased with IR stabilization ($p<0.05$). The lowest *o*-coumaric acid content was observed in immature rice grains which were stabilized at 1600 W for 5.02 min using short wave IR emitters. Among the bound phenolics acids, the effect of IR stabilization conditions was significant on the content of vanillic, caffeic, chlorogenic, p-coumaric and ferulic acids ($p<0.05$). The highest bound vanillic acid content was observed in unprocessed immature rice grain while no clear decreasing trend was observed for the other bound phenolics with regard to IR stabilization conditions (Table 3).

Antioxidants

Trolox equivalent antioxidant capacity (TEAC) and DPPH scavenging activity assays were employed to measure antioxidant capacity of either free or bound form extracts and the results were presented on Table 4. DPPH scavenging assay results were expressed as EC_{50} values, the efficient concentration (mg) at which 50 % DPPH radical inhibition occurred. Therefore, higher EC_{50} values correspond to lower antioxidant capacity. In this manner, the results of the 2 assays were compatible. The effect of IR stabilization was insignificant on either TEAC or DPPH scavenging capacity of the free form extracts ($p>0.05$). However, it was significant on bound form antioxidants with respect to both antioxidant capacity assays ($p<0.05$). Nevertheless, variation in antioxidant capacity of the bound form extracts was not consistent with the IR stabilization conditions and therefore could not be related with the power of IR emitters or the duration of the stabilization process.

Table 4. Antioxidant capacities of unprocessed and infrared stabilized immature rice grains

Wavelenght	Process time (min)	IR Power (W)	TEAC (mM Trolox/g)		DPPH scavenging capacity (mg EC_{50})	
			Free	Bound	Free	Bound
Unprocessed immature rice			6.90 ± 0.89	7.04 ± 0.65^{ABCD}	63.50 ± 10.80	210.86 ± 0.35^{ABC}
MW	8.02	1200	7.13 ± 0.53	5.64 ± 0.60^{D}	62.84 ± 9.56	211.35 ± 0.25^{ABC}
MW	10.12	1200	6.52 ± 0.23	6.33 ± 0.40^{CD}	83.34 ± 2.72	229.00 ±15.50^{BC}
MW	7.01	1400	7.37 ± 0.28	7.22 ± 0.20^{ABCD}	58.30 ± 5.56	193.80 ± 10.40^{ABC}
MW	4.01	1600	6.94 ± 0.24	5.70 ± 0.58^{D}	67.20 ± 12.40	227.04 ± 8.40 ^{BC}
MW	4.40	1600	6.82 ± 0.96	8.52 ± 0.19^{ABC}	64.20 ± 4.45	152.14 ± 2.29^{ABC}
MW	5.02	1600	5.72 ± 0.48	6.76 ± 0.63^{BCD}	75.90 ± 9.29	149.13 ± 8.60^{ABC}
SW	10.12	1200	5.41 ± 0.27	6.18 ± 0.46^{CD}	99.15 ± 4.60	227.70 ± 15.90^{BC}
SW	6.18	1400	6.33 ± 0.10	5.83 ± 0.40^{D}	91.59 ± 5.68	132.30 ± 15.20^{A}
SW	7.10	1400	5.99 ± 0.42	9.30 ± 0.01^{A}	87.6 ± 10.00	233.90 ± 44.60^{C}
SW	5.02	1600	5.82 ± 0.15	8.84 ± 0.04^{B}	79.73 ± 5.06	136.94 ± 9.44^{AB}

*Within each column, means followed by different letters are significantly different by Tukey (P <0.05) **MW (Medium wave), SW (Short wave)

Conclusion

In conclusion, it was found that IR radiation can be used to stabilize immature rice grains without negatively affecting the content of phenolic compounds and antioxidant capacity**.**

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BLENDED OILS IN THE VEGETABLE OIL SECTOR

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Abstract

The vegetable oil sector is an industrial sector that brings raw materials from agriculture to more durable and ready-to-use conditions through various preparation, processing, preservation and packaging techniques. Values such as monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, smoke point are of great importance for use in the food industry and industrial vegetable oils. Stability and nutritional characteristics of oils are the most important factors in food technology. There is no pure oil with high functional and nutritional properties and high oxidative stability. For this reason, blended oils are prepared to improve commercial applications and functional properties of vegetable oils. New specific products with desired nutritional properties can be created. Blending oils lead to improved industrial applications. The economic, high nutritional value and strong stability properties of blended oils, which are offered at a more affordable price to the market, have become quite common in recent years. Blended oils have advantages such as low cost, reduced complexity of varieties, ideal and desired performance for applications, high smoke point, helping to establish omega 6/3 balance. The disadvantages are; unfavorable oils and unfavorable blends may cause undesirable results in taste, odor, color and function. In this study, a literature review about blended oils was presented and samples from domestic and foreign markets were showed. The commercial examples of oil blends in the market are generally comprising of olive, canola, sunflower, hazelnut, soybean and grape seed oils, while scientific research are more focused on blends containing palm oil and some specialty oils with functional properties such as rice bran oil, black cumin oil etc. In the future, there will also be many economical and health reasons for the production of new oil blends using new and conventional oil sources to be introduced to the market.

Keywords: *Edible oils, Oil blends, Vegetable oil sector.*

Introduction

Thanks to technological developments and increased production, the vegetable oil sector has also improved considerably. The types of vegetable oil produced have rised and the product variety advanced (Arıoğlu, 2010). Consumers prefer vegetable oil consumption by looking at factors such as taste, price and nutritional characteristics. Without sacrificing nutritional values, tasty and affordable oil consumption is a desire for everyone. In the modern world, it is a difficult and important responsibility to select and consume the oils that are beneficial to health among the many options available. Variety of mono- polyunsaturated and saturated fatty acids and controlling they amount in the vegetable oil of the diet, play a key role in regulating many physiological processes in the body. Studies have suggested that oil consumption provides 20% energy per day of normal nutrition, with 8-10% of saturated fatty acids and 10-12% of polyunsaturated fatty acids. The World Health Organization (WHO) recommends a total oil consumption of 30-35%, with saturated fatty acid <10%, polyunsaturated fatty acid 6-11%, and monounsaturated fatty acid 10-14%. The consumption of 2 or 3 tablespoon per day of vegetable oils with this fatty acid formulation completes a

healthy and balance diet. Polyunsaturated fatty acids contain omega-3 ALA (α-linolenic acid) and omega-6 LA (linoleic acid). These fatty acids are essential and are taken only by diet.
Because of the health effects of the fatty acid composition, blended oils have gained popularity by providing more stable and healthy consumption of vegetable oils. Blending vegetable oils is a viable method for regulating fatty acid composition. Blending two or more oils with different properties is one of the simplest procedures to produce a new specific product (blended oil). Blended oil production not only regulates fatty acid composition but also contributes to high oxidative stability and prolong shelf life, affordability and more nutritional elements (Srikanth and Rajaveni, 2014). Some companies in the vegetable oil sector are only interested in the economy, using the cheap oil, rather than looking at the nutritional quality and fatty acid composition in preparing the blend (Taşan and Geçgel, 2007). Polyunsaturated fatty acids are vegetable oils used for baking and frying. For example, soybean oil is considered superior to many vegetable oils. However, it exhibits low thermal stability and low oxidative stability. To overcome the problem of low oxidative stability of soybean oil, methods such as modifying the fatty acid composition and increasing natural antioxidants are used. For this purpose, blends of soybean oil with hydrogenated soybean oil and high oleic sunflower oil have been tried (Abdulkarim et al., 2010).

Vegetable Oil Production and Consumption Values in the World

34% of global crude oil production and 61% of exports are made by Indonesia and Malaysia, while 64% of crude oil production comes from palm and soybean oil. In the production of olive oil, the EU countries (mainly Spain, Italy, Greece) are in the top 75% share. Turkey, in the crude vegetable oil from oilseeds production is far behind in the world ranking. But olive oil is a big producer and is ranked 5th in the world with 4.7% share (Öztürk, 2016) .

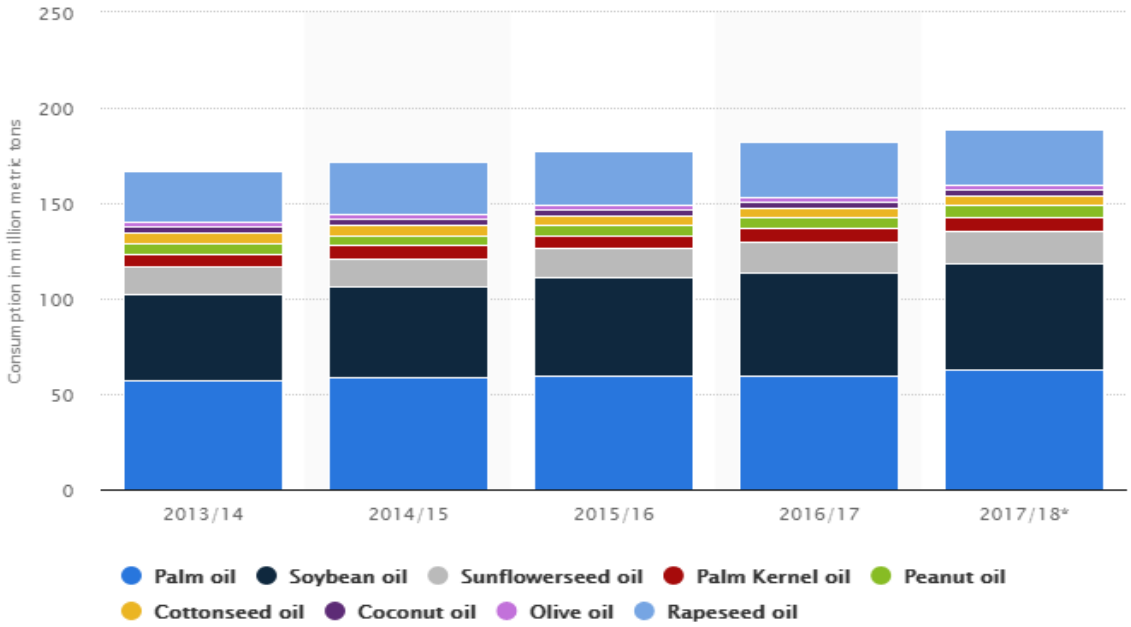

Figure 1. Consumption of vegetable oils worldwide from 2013/14 to 2017/2018, by oil type (in million metric tons)

In 2016/17, sunflower oil consumption amounted to 16.52 million metric tons worldwide. The global vegetable oil production amounted to around 182 million metric tons in 2016/2017. When the global oil consumption is examined, most consumption has been in the palm oil with 64%. This was followed by soybean oil with 53%, rapeseed oil with 27% and sunflower oil with 15%. Olive oil consumption in the EU countries which ranks first, followed by the United States and Turkey (Anonymous, 2018).

Literature On Issue

Food products is based on the frying process throughout the world. For this reason, oxidative stability in fats is an important point. Synthetic antioxidants are used to minimize rancidness and extend shelf life. Adding natural antioxidants rich oils to edible frying oils is an easy process to increase stability. For example, the stability of sunflower oil has been strengthened with the addition of black cumin oil, rich in tocoferol and thymoquinone (Kıralan et al., 2017). Linseed oil is one of the most important oils because of the high amount of α-linolenic acid (ALA, omega-3), which is an essential fatty acid. At the same time, linseed oil is very prone to oxidation. To improve the oxidation stability, flaxseed oil is blended with other vegetable oils rich in bioactive and antioxidant components. For this process, 80:20 cold pressed *Nigella sativa* oil and sesame oil were added to linseed oil. As a result of the study, the total amount of phenolic components increased in the blended oil samples compared to individual oil. Radical scavenging activity has increased by 10-20%. The results show that the blending of vegetable oils containing strong antioxidant ingredients such as cold pressed *Nigella sativa* seed oil and sesame oil into linseed oil significantly enhances the oxidation stability and that the blended oil technique is a simple and effective alternative to the use of synthetic antioxidants (Hamed and Abo-Elwafa, 2012).

Increase in oleic acid in vegetable oils, an important goal to raise the quality of fat. Because, as the amount of oleic acid in vegetable oils rises, cardiovascular diseases decrease, resistance to oxidation enhances. Sunflower oil with a high oleic acid content can be used alone or blended with soya and canola oils to reduce the linolenic acid content to 2-3%. In addition, sunflower oil is blended with canola and palm oils which are rich in monounsaturated fatty acids in order to extend its shelf life (Duru and Bozdoğan Konuşkan, 2014).

Yalçın et al. (2017) in the study extra virgin olive oil and sesame seed oil were blended in varying proportions and stored for 90 days in room conditions. Oxidation parameters varying during storage have been examined. Extra virgin olive oil is more susceptible to oxidation compared to sesame oil, but the formation of oxidation products is observed to decrease in the oil blend. The results show that extra virgin olive oil can be blended with sesame oil containing strong antioxidants and stored for a long time.

Olive oil and palm olein oil were blended at various ratios to observe the effect of saturated / unsaturated fatty acid ratio on physicochemical properties. The high oil content of palm olein has been reported to show strong chemical stability in frying processes. As a result, the increase of the saturated fatty acid ratio in the blended oil affected the stability of the oil (Naghshineh et al., 2010). A variety of blend oil combinations have been established to strengthen oxidative stability. One of them is the investigation of the effect of grape seed oil addition to sunflower oil on the quality, physicochemical properties and radical scavenging activity. As a result, as grape seed oil ratio in sunflower oil increased, properties such as refractive index, iodine value, peroxide value, saponification value and acid value decreased and radical scavenging activity enhanced. It is also stated that the oxidative stability of the blended oils can be increased by raising the grape seed oil ratio (Codină et al., 2015). In another study where sunflower oil was used, cold pressed tiger nut oil and sunflower oil were formed at 0/100 and 100/0 range. In the prepared samples, degradation in the frying process decreased and oxidative stability increased (El-Anany et al., 2012). It has been found that the blend of olive pomace oil-refined olive pomace oil (50:50) used in frying processes is exposed to lower levels of degradation reactions according to sunflower oil (Yaşdağ and Tekin, 2017) because of the low amount of unsaturated fatty acids. In a study of frying performance, Marco et al. (2007) compared sunflower and palm oil blend to palm oil alone. The free fatty acid value of the sunflower/ palm oil 65/35 (w: w) blend was lower. In the frying process, it has been determined that the oxidative stability is higher than the use of palm oil alone, although it is stated that some oxidation indices appear faster in the blend oil.

Ramadan et al. (2011) investigated the effects of corn oil, black cumin oil and coriander oil additions on functionality, stability and radical scavenging activity; the polyunsaturated fatty acids decreased as the blend ratio increased, while the monounsaturated fatty acids ascended. The oxidative stability of oil blends is higher than that of pure corn oil. The effect of coconut oil addition on vegetable oil blends on fat, fatty acid composition, oxidative stability and radical scavenging activity was investigated in a study using palm, rice bran, sesame, mustard, sunflower, peanut, safflower and soybean oil (Bhatnagar et al., 2009). The resulting seven blends were improved in terms of mono- and polyunsaturated fatty acids, total tocopherols and radical scavenging activity. In addition, reductions in peroxide formation were observed. It has been stated that the oxidative stability of these blends is higher.

Lipidium sativum L. rich in omega-3 ALA (α-linolenic acid) oil and blends of this oil with vegetable oils were used in the feeding of experimental rats to investigate antioxidant enzyme status in their body. Sunflower oil, rice bran oil, sesame oil are used for preparation blended vegetable oils and added to the diets of rats for 60 days. As a result, the percentage of tocopherol was increased in the body of rats fed blend oil compared to the experimental rats fed with the native fat. The activity of antioxidant enzymes (catalase, glutathione, peroxidase) in their body was improved but in some liver enzymes not change (Umesha et al., 2002).

Khan et al. (2010) conducted a serum lipid profile and retinol assay in experimental rats fed a micronutrient rich, edible vegetable oil blend, used mustard oil and palm olein oil. Experimental rats, which fed with fat containing 1% cholesterol before the study, were fed micronutrient-rich fat in the amount of 10% of their diets during the 1 or 6-month period and significant changes were recorded in their serum lipid profiles. It is expressed that the maximum benefit is obtained if the oils are used in blend. It has also been found that when used as a blend, it has a mitigating effect on diseases such as coronary heart diseases, diabetes, obesity, hypertension.

Conclusion

Depending on the previously published studies, the blend oils have superior properties compared to the individual oils. Blended oils contribute to the functional and nutritional properties of vegetable oils consumed in household use. In industrial use, it has advantages such as increasing the commercial potential of the oil, lowering the cost, strengthening the stability. It regulates the fatty acid content of vegetable oils, which are susceptible to oxidation, and prolongs the shelf life. It provides chemical stability of fats in frying processes and storage after frying. Enriched with vegetable oils containing essential fatty acids, provides functional oils to be produced. Blended oils enables the consumer to obtain tasty and healthy oil at a more affordable price. The household use of blended oils is not common because of fraud in vegetable oils. This fraud is most commonly in the form of canola, safflower and soybean oil added olive oil or sunflower oil. Consumers have a negative attitude towards professionally prepared blended oils due to illegally prepared vegetable oil blends.

In blended oil samples; to increase omega-3 ratio sunflower oil is blended with canola oil, and addition of palm super olein oil to sunflower oil to improve frying performance. "A cheap alternative instead of 100% natural extra virgin olive oil" to the market with the slogan of the oil blend is used in varying proportions. There are soya, canola and olive oil blends to ensure omega-3/6 balance. In the same way to increase nutritional value and omega-3 ratio; sunflower oil with high oleic content, natural rapeseed oil, extra virgin olive oil, natural Camelina sativa seed oil, natural sesame oil blends are prepared. In order to benefit from the antioxidant properties, combinations of grape seed, avacado and tiger nut oil with other vegetable oils are produced.

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MICROPROPAGATION OF SOME AMERICAN ROOTSTOCKS USING MERISTEM TIP CULTURE

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Abstract

An in vitro propagation protocol was developed for the production of Teleki 8B, 140 Ruggeri, 110 Richter, Kober 5BB, 99 Richter and SO4 (Selection Oppenheim 4) grapevine rootstocks through meristem tip culture. These plants will be used in nematode virus transmission baits. Meristem tissues were isolated from apical shoot-tips with 5-10 mm in length collected from plants maintained at greenhouse and grown on liquid and solid Murashige Skoog Medium (MS) supplemented with 1 mg/L^{-1} 6-Benzylaminopurine (BAP). The tubes and petri dishes with meristematic tissues were incubated in growth chamber at 24°C during a 16 h light period 8 h dark period. After eight weeks, newly emerged shoots were transferred into MS medium and stored in growth chamber for further growth. At final stage adequate grown shoot were transferred into MS medium with 1 mg/r L^{-1} Indole Butyric Acid (IBA) for root formation and growth. When root formation and growth were completed, all plantlets were transferred to 8 cm pots filled with peat and soil. Total of 153 plants were propagated with this method from 6 grapevine rootstocks.

Keywords: *Meristem tip culture, Grapevine rootstocks, Murashige and Skoog Medium*

Introduction

Grapevine is one of the most widely cultivated fruit crop in Turkey covering an area of 6,9 million hectares. It is grown in varied climatic conditions ranging from temperate to semi tropic and tropics. Turkey is known as one of the mother lands of grapevine and ranks sixth in world grape production. Having favorable ecological conditions and good soils, more than 1250 grape varieties can be grown in almost every part of the country. Approximately 40% of grape product is consumed for raisin, 35% is consumed as fresh and 3% is processed to wine. Various factors are responsible for decrease of agricultural productivity in Turkey. Viruses, viroids, bacteria, fungi, constitute pathogens that some were reported as very problematic in various crop plants in Turkey (Argun et al., 2002; Çığşar et. al., 2002, 2003; Öztemiz et al., 2005; Akbaş et. al., 2007; Uygun et al., 2010; Buzkan et.al., 2010; Yıldırım, 2014; Buzkan et al., 2015; Gazel et al., 2016). Grapevine pathogens can distributed by several ways. Infected plant materials are main reason of higher epidemics. For example once pathogen infected grapevine seedling is planted to non infected vineyard rapid spread to healthy plant is possible due to presence of several nematode and insect vectors. Becides there is no chemical treatment to eradicate virus, bacteria and some fungi infections from infected vine. Establishing vineyards with healthy plants is obvious method for restriction of disease distribution. In vitro meristem tip culture is an efficient method for obtaining disease free material from grapevines. The main reason for use of the meristem culture is most pathogens fail to invade the meristematic region of shoot tips since the multiplication of meristem cells is faster than replication of pathogen. Successful regeneration of healthy plants can be achieved by culturing only disease free apical meristems isolated from infected grapevines.

Meristem culture allows higher genetic stability of regenerated plants with low somaclonal variations (Milosevic et al., 2011).

This method have been efficiently applied to several plant species including dahlia, freesia, geranium, lily, chrysanthemum, citrus to eliminate pathogens (Milosevic et al., 2012). In a laboratory study *Impatiens hawkerii* Bull. was totally eradicated from *Tomato spotted wilt virus* (TSWV) and *Cucumber mosaic virus* free chrysanthemum plants were micro propagated via meristem tip culture (Verma et. al., 2004; Milosevic et.al., 2011). Furthermore *Grapevine Leafroll Associated Virus* 1 and *Grapevine Fanleaf Virus* free grapevines were obtained from the optimum 0.5 mm size of meristem tips (Fayek et al., 2009). On the other hand this method shown to be effective for elimination of *Rhizobium vitis* (Sim and Golino 2010). Meristem tip culture allows multiplication of plant that do not produce seeds and could not be vegetatively propagated. In addition this method is not season and climate dependant. Insitu meristem culture under controlled sterile conditions with healthy tissue from plants allows rapid propagation of a large number of plants in a short time (Khan et al., 2015).

Most of the vineyards in Turkey are infested by phylloxera (*Viteus vitifolii*). Gall formation by feeding of higher population of this pests in grapevine roots results in poor growth and death of the vine. The European grape *Vitis vinifera* is highly susceptible to phylloxera and to overcome damage grafting on resistant American rootstocks is recommended in most parts of the country (Sengel et.al., 2012).

Within existing American rootstocks Teleki 8B, 140 Ruggeri, 110 Richter, Kober 5BB, 99 Richter and SO4 (Selection Oppenheim 4) are mostly preferred in Northwestern Marmara Region due to phylloxera resistance, well adaptation and good growth in local soil and climate conditions. *Grapevne Fanleaf Virus*, *Grapevine Leafroll Associated Virus* and Rhizobium vitis are problematic among production materials in Turkey and were detected in mother plants of Kober 5BB, SO4 (Selection Oppenheim 4) and Teleki 8B rootstocks from which cuttings is collected for new rootstock production. Meristem tip culture is recommended for production of virus free plants of these rootstocks. On this purpose a study was carried out forin vitro propagation of Teleki 8B, 140 Ruggeri, 110 Richter, Kober 5BB, 99 Richter and SO4 (Selection Oppenheim 4) grapevine rootstocks through meristem tip culture.

Material and Method

Meristem cultures of Teleki 8B, 140 Ruggeri, 110 Richter, Kober 5BB, 99 Richter and SO4 (Selection Oppenheim 4) were isolated mid June till the end of August from shoots of actively growing vines grown in greenhouse established in Viticulture Research Institute. Apical shoots 5 to 10 cm long, were collected from vines, larger leaves removed, sterilized by immersing for 10 minutes in 10% sodium hypochlorite and rinsed three times in sterile distilled water (Figure 1). Apical meristem tips no longer than 0.5 mm long were excised under a binocular stereomicroscope in a laminar flow cabinet using flame sterilized needle.

Shoot Proliferation

Meristematic tissues were grown on solid Murashige Skoog Medium (Murashige and Skoog, 1962) (MS) supplemented with 3% sucrose, 1.9 gr/lt KNO_3 and 1 mg/L^{-1} 6-Benzylaminopurine (BAP) and 7 gr Agar- Agar (Table 1). The medium was adjusted with 1N KOH to pH 5.8 sterilized by autoclaving for 15 min at 120 ^{o}C. Culture tubes (30x120 mm) and 60 mm glass petri dishes were used in the culturing process. The tubes and petri dishes with meristematic tissues were incubated in growth chamber at 24°C during a 16 h light period 8 h dark period. After eight weeks, newly emerged shoots were transferred into MS medium and stored in growth chamber for further growth and this process was repeated till obtaining shoots with length suitable for rooting.

Table 1**.** MS media formulation used for meristem culture of grapevine rootstocks

Ingredients	**gr/100 ml**	**Ingredients**	**gr/100 ml**
NH_4NO_3	16.5	$Na_2Mo_4.2H_2O$	0.125
$CaCl_2.2H_2O$	4.4	$CuSO_4.6H_2O$	0.125
$MgSO_4.7H_2O$	3.7	$CoCl_2.6H_2O$	0.125
KH_2PO_4	1.7	$FeSO_4$.7H2O	1.114
$MnSO_4.H_2O$	0.223	Na2EDTA.$2H_2O$	1.49
H_3BO_3	0.062	Thiamine-HCl	0.01
ZnSO4.4H2O	0.086	Pyridoxin-HCl	0.05
KI	0.415	Nicotinic acid	0.05

Figure 1**.** Apical shoots and actively invitro grown meristematic tissues of rootstocks

Root proliferation

At final stage adequate grown non contaminated shoots of 5 cm in length with small leaves were cut from mother plants and transferred into new culture tubes (30x 200mm) containing MS rooting medium with 3% sucrose and 1 mg/L^{-1} Indole Butyric Acid (IBA) which promote root formation and elongation.

When root formation and growth were completed, all plantlets were transferred to viols filled with peat. Before transplanting plantlet roots were soaked to fungicide to prevent fungal soil borne diseases. These viols were covered with nylon to achieve sufficient ex-situ conditions for sustaining plant growth without any loss (Figure 2). This young plants were covered for two weeks and then viols were uncovered everyday for a few hours. After plantlets complete aclimatization period they were transferred to 8 lt pots filled with sterile peat and soil.

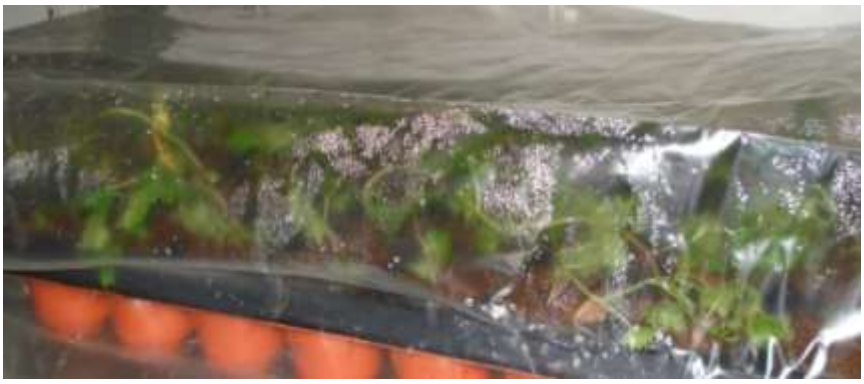

Figure 2. Ex-situ acclimatized new plantlets grown in climate room.

Results and Discussion

Approximatelly 432 meristematic tissues were isolated from Teleki 8B, 140 Ruggeri, 110 Richter, 5BB, 99 Richter and SO4 (Selection Oppenheim 4) rootstocks during two months period. Within these meristem some of them lost due to browning without regeneration. In addition *Aspergillus* and *Penicillium* contaminations were observed in some test tubes and they were discarded. In some cases meristems remained dark brown for a period but later green tissues started to grow and form new shoots. As these shoots reached adequate height they were transferred to new MS medium for further elongation. At the end of rooting process 153 plants were transferred to pots and maintained in climate room at 24ºC 16 h light 8 h dark period (Table 2).

Table 2.Number of plants produced by meristem tip culture

Rootstocks	**Plants transferred to pots**
SO4 (Selection Oppenheim 4)	27
Kober 5BB	43
140 Ruggeri	13
110 Richter	32
99 Richter	21
Teleki 8B	17

The period of axillary shoot collection from mother plant also affect meristem growth. Better meristem isolation and growth was observed when collected at the end of June till mid July. Most of the meristems established at the end of August did not survive.

The effect of culture medium mineral composition and hormones on the micropropagated grapevines has been stated by different authors (Troncoso et.al, 1990;. Balance of hormone amount and medium composition was considered essential to achieve higher meristem regeneration and shoot growth. Experiments of Sarker et. al. (2015) indicated that Citrus micropropagation performance declined parallel to BAP concentration increase or decrease from the optimum dose.

Several researchers also reveal that BAP concentration higher than 1 mg L^{-1} increase the number of vitrified shoots (Subotić et al., 2008; Milosevic, 2011). In addition pale and stunted shoots in some crop plant such as Alocasia higher concentrations observed in higher BAB concentration. In our study meristem tissue growth was promoted by addition of 0.01 gr BAP into 1 liter MS medium. This amount was found sufficient regaring regeneration of new buds and good growth of shoots was achieved. Vitrification, stunting was not observed.

As invitro propagated shoots transferred to rooting medium they produce good roots. The IBA dose was determined adequate for rooting of all rootstock shoots. On the other hand new plantlets of up to 180 cm height was obtained in five months period at rooting medium.

Conclusion

We carried out a study to invitro micropropagate plantlets of Teleki 8B, 140 Ruggeri, 110 Richter, Kober 5BB, 99 Richter and SO4 (Selection Oppenheim 4). Meristem tip culture is effective method to produce plants free of diseases when meristematic tissues less than 0.5 cm excised. Otherwise diseases will be transmitted. In our study we tried to establish culture with meristematic dome and we took small pieces. In miicropropagation process we used standard MS medium with BAP and IBA hormones. After 6 months we achieved to propagate 153 new plantlets.

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ANTIFUNGAL ACTIVITY OF *Origanum onites* L.ESSENTIAL OIL AGAINST SOME PLANT PATHOGENIC FUNGI

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Abstract

Diseases in agricultural areas cause significant losses regarding quality and yield. To reduce these losses, intensive pesticides are used. Extreme pesticides use causes many problems. For this reason, alternative control methods have become necessary. This study was conducted to determine the antifungal effect of *Origanum onites* L. essential oil against *Sclerotinia sclerotiorum* (Lib.) de Bary, *Alternaria solani*, *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *lycopersici*. Essential oils were applied by impregnation with a micropipette on filter paper adhered to the covers of petri dishes.In the antifungal study 0 (control), 0.5, 0.7, 1, 1.5, 2 and 4 µlpetri^{-1} dish doses were used. In the antifungal study, mycelium diameters in the petri dishes were measured by automatic caliper at the end of the 7th day of incubation period. As a result, essential oil of *O. onites* was inhibited mycelial growth in *A. solani*, FOL, *S. sclerotiorum* and *V. dahliae* at a dose of 1.5 µlpetri^{-1} dish by 100%. It was found that 1.5 µlpetri^{-1} dose of *O. onites* essential oil inhibited the mycelium growth of *S. sclerotiorum* by 13.33%. The essential oil of *O. onites* inhibited the mycelium growth in *A. solani, S. sclerotiorum*, FOL and *V. dahliae* at a dose of 4 µlpetri^{-1} dish by 100%. These findings showed that the *O. onites* essential oil has high antifungal activity. This result showed that *O. onites* essential oil had a potential to replace synthetic pesticides.

Keywords: *Origanum onites, antifungal, essential oil.*

Introduction

Origanum genus (Labiatae) is represented by 22 species or 32 taxa in Turkey. Of these species, 21 were endemic, while the endemism rate is 65.2% (Aydin *et al.,* 1998; Dundar *et al.,* 2008). The favorable climatic conditions for the growth of Origanum species are warm-temperate southwestern Eurasia and Mediterranean regions (Kokani, 1997). Among culinary spices, thyme plays a primary role in world trade. It has been produced in particularly in Europe, including France, Greece, Spain and Turkey, as well as in America, including Chile, Mexico and Peru (Olivier, 1996; Barreyro *et al*, 2005). In Turkey, numerous species of thyme have been exported, which are: *Origanum onites, O. minutiflorum, O. majorana, O. syriacum* var. *bevanii, O. vulgare* (Baser *et al*, 1993; Kirimer *et al*., 2003; Toncer *et al*., 2009). Among these species, *O. onites* is the most exported species in the world (Yaldız *et al*., 2005).

Due to the antimicrobial substances they contain, essential oils have an alternative potential against infecting organisms. It is acknowledged by the entire world that herbal products are less toxic to the environment and human health in controlling diseases (Lee *et al*., 2007). In particular, it has been reported that essential oils exhibit favorable antifungal activities against various pathogens, both *in vitro* and *in vivo* conditions (Baruah *et al*., 1996). Origanum essential oil was reported to exhibit anti-bacterial, anti-oxidant, anti-fungal cytotoxic and insecticidal activity (Vagi *et al*., 2005; Tepe *et al*.,2004; Muller *et al*., 1995; Wilson *et al*., 1997; Traboulsi et al., 2002). Once again, in a similar study, the antifungal activity of the Lamiaceae family members on *Verticillium dahliae* pathogens was reported (Rus *et al*., 2015).

In this study, the antifungal activity of *Origanum onites* essential oil on the plant pathogens, including *Verticillium dahliae, Alternaria solani, Sclerotinia sclerotiorum* and *Fusarium oxysporum* f. sp. *lycopersici* was investigated.

Material and Methods

Plant Material and Extraction of Essential Oils: *Origanum onites* plants collected during the flowering phase of Mersin province in 2017 vegetation period. By using Neo-clevenger aparate and hydro-distillation method, the essential oils were obtained from the plants dried in the shade. The essential oils obtained were stored at +4°C until the activity studies.

Fungus Cultures: The fungi of plant pathogen used in the study (*Verticillium dahliae* (Vd), *Alternaria solani* (As), *Sclerotinia sclerotiorum* (Ss) and *Fusarium oxysporum* f. sp. *lycopersici* (Fol) were obtained from the stock cultures in Phytopathology laboratories of Department of Plant protection, Faculty of agriculture, Ahi Evran University.

Antifungal tests: The prepared PDAs, 10 ml per each, were poured into 60 mm plastic petri dish. Sterile drying papers were adhered to the covers of petri dishes and the drying papers were impregnated with essential oils at the determined doses (0.5, 0.7, 1, 1.5, 2, 4) by the help of a micropipette. As a control group, 8 µl sterile distilled water was impregnated onto the drying paper. The surroundings of the petri dishes were covered with parafilm and left to incubate at 25 °C. Seven days after treatment, mycelial growth of the diseases was measured with digital calipers. The treatments were carried out 3 times with 2 repetitions. The inhibition rate of essential oils (%) is calculated according to the following formula.

I:100x(dc-dt)/dc

I: Mycelium growth inhibition rate (%)

dc: Mycelium growth in control

dt: Mycelium growth in treatment (Pandey *et al*., 1982)

Statistical Analysis

The analysis of variance (ANOVA) was used to determine the significance levels of differences between the experimental treatments, and the means were compared by using the DUNCAN test. Statistical analyses were carried out using the SPSS software.

Results and Discussion

The results of the antifungal effects of *Origanum onites* essential oil on *Verticillium dahliae, Alternaria solani, Sclerotinia sclerotiorum* and *Fusarium oxysporum* f.sp. *lycopersici* (Fol) are given in Table 1 and Figure 1.

It was determined that *O. onites* essential oil has statistically significant antifungal activity on the tested plant pathogenic fungi. The negative effect of fungi on mycelium growth has changed due to the pathogen and increased dose. *O. onites* essential oil reduced the mycelial growth of Fol compared to the control and stopped it completely at a dose of 1.5 µl/petri. Similar results were also found in *V. dahliae* and *A. solani* pathogens. It was determined that *S. sclerotiorum* was the most tolerant pathogen to *O. onites* essential oil and it prevented the mycelium growth of the pathogen only at the highest dose (Table 1).

O. onites essential oil inhibited the mycelium growth of *V. dahliae, A. solani* and *Fol* by 100% at a dose of 1.5 µl/petri. However, *O. onites* essential oil inhibited the *S. sclerotiorum* mycelium growth by 100% only at the highest dose (4 µl/petri dish) (Figure 1).

Table 1. The antifungal effects of *Origanum onites* essential oil on plant pathogen fungi

Doses (µl/petridishes	*Fusarium oxysporum* f. sp. *lycopersici*	*Alternaria solani*	*Verticillium dahliae*	*Sclerotinia sclerotiorum*
Control	60.00±0.000^{a*}	60.00±0.000^{a}	60.00±0.000^{a}	60.00±0.000^{a}
0.5	20.87±0.722^{b}	21.09±2.615^{b}	23.68±1.803^{b}	60.00±0.000^{a}
0.7	14.43±1.387^{c}	16.81±0.737^{b}	20.29±2.567^{b}	60.00±0.000^{a}
1	13.55±3.759^{c}	5.38±2.712^{c}	12.09±2.256^{c}	60.00±0.000^{a}
1.5	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{d}	52.00±1.110^{a}
2	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{d}	20.00±1.012^{b}
4	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{b}

* Means in the same column by the same letter are not significantly different to the test of Duncan ($p<0.05$).

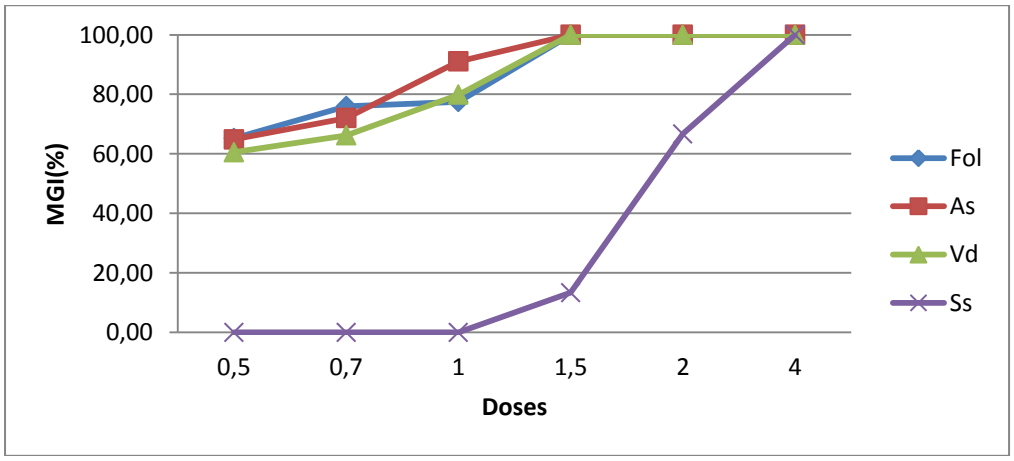

Figure 1. % Mycelium growth inhibition rate(MGI) of *Origanum onites* essential oil on plant pathogen fungi

Similar studies were carried out by different investigators on *Fusarium oxysporum* f. sp. *lycopersici*, *V. dahliae* and other plant pathogenic fungi and antifungal effects of plant essential oils were reported in these studies. It was reported that *Salvia officinalis*, *S. cryptantha* and *S. tomentosa* plant essential oils and extracts have an antifungal activity on the mycelium growth of *Fusarium oxysporum f. sp. radicis-lycopersici* depending on the doses, extracts and essential oils (Yilar and Kadioglu, 2016). Yilar *et al*. (2016) reported that *Vitex agnus-castus* L. and *Myrtus communis* L. essential oils inhibited the mycelium growth of *Fusarium oxysporum f. sp.radicis-lycopersici* and *Verticillium dahliae* by 70.70%-100% and 93.00%-100%, respectively compared to the control. Likewise, in a similar study *Heracleum platytaenium* Boiss. essential oil inhibited the mycelium growth of *V. dahliae* and *Fusarium oxysporum f. sp.radicis-lycopersici* by 73.25% and 100%, respectively at 10 µl dose (Bayan *et al*., 2016). *O. onites* and *T.spicata* essential oils exhibited the strongest antifungal activity against *A. alternata* (Soylu and Kose, 2015). It was stated that *Origanum syriacum L.* essential oil exhibits a high degree of antifungal activity on *Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium* species (Daouk *et a*l., 1995). The antifungal activity of *Origanum vulgare* and *Origanum majorana* essential oils against *Candida albicans* was reported (Lakhrissi *et al*., 2016). Similarly, Arslan and Dervis (2010) reported that *Origanum onites, O. syriacum, O. minutiflorum, O. vulgare, O, marjorana, Thymus vulgaris, T. serpyllum, Rosmarinus officinalis, Salvia officinalis* and *Micromeria fruticosa* essential oils inhibited the mycelium growth of *Verticillium dahliae*. It was also reported that Origanum majorana L. essential oil inhibited the mycelium d growth of *Verticillium dahliae* at 0.5 $mg \cdot L^{-1}$ and 1 $mg \cdot L^{-1}$ doses, and that the MIC concentration for this disease is 5 $mg \cdot L^{-1}$ (Rus *et al*., 2015.

Conclusion

This study showed that *Origanum onites* essential oil has antifungal effect on *Verticillium dahliae, Alternaria solani, Sclerotinia sclerotiorum* and *Fusarium oxysporum* f. sp. *lycopersici* plant pathogens. Today, when the negative effects of the pesticides, used intensively in the control of plant pathogenic fungi in agricultural products, on the environment and people have emerged, these and similar work findings are gaining more importance. However, it is important to improve these and similar study findings and make them available in the agricultural field.

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EVALUATION OF DAMAGE RATES OF LEOPARD MOTH, *ZEUZERA PYRINA* L. (LEPIDOPTERA: COSSIDAE) IN WALNUT ORCHARD IN HATAY PROVINCE OF TURKEY

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Abstract

The leopard moth, *Zeuzera pyrina* L. (Lep. Cossidae), is one of the principal pests of the walnut trees in Turkey. The leopard moth larva is a xylophagous species that infest the trunk, shoots, and branches of numerous fruit-bearing threes. Accumulated damages by larval instars may cause the death of young trees or loss of the branches in older trees. The signs of damage in walnut trees include dark fluid ozzing from bark and also larval orange faeces expulsion from larval tunnels that can reach up to 50 cm. Damage results in tree weakness, the attraction of bark beetles and finally plant death. The study was conducted in 2016-2017 to evaluate damage rates of the leopard moth on walnut CV 'chandler' in a orchard of Yayladağ district of Hatay province of Turkey. The walnut orchard contained 3345 walnut trees (380 da), and evaluation of the damage rates was done tcounting the number of the damaged branches and truck of walnut trees. As a result of the investigation, the damages rates of this pest varied in each of the sampling years. The walnut damages rates by leopard moth were 16 per cent in 2016 and 8.87 per cent in 2017.

Keywords: *Damage, Leopard Moth, Walnut, Turkey.*

Introduction

The walnut, *Juglans regia* L., (Juglandaceae: Fagales) is one of the most significant nut trees in the world. In 2014, worldwide production of shells was 3.46 million tons, China contributed 46% (1,600); other major producers were United States (520), Iran (450), Turkey (180) and Mexico (130) of the world total (Faostat, 2017). The walnut is one of the essential nut trees in Turkey which production is consisting of approximately 868.528 dekar with the total production of 195.000 tons of fruit per annum, and Hatay province's share is 2.361 dekar and 1428 tons [Anonymous, 2016]. The leopard moth, *Zeuzera pyrina* L. (Lepidoptera: Cossidae) is an damaging polyphagous pest infesting over 150 plant species, including fruit trees, such as walnut, apple and quince, and some forest trees, such as elm and chestnut (Balachowsky and Mensil 1935; Carter 1984; Castellari, 1986; Gatwick 1992; Kutinkova et al., 2006; Alford, 2007). The leopard moth, *Zeuzera pyrina* L. is one of the most important pests of walnut trees in Turkey. The larvae of leopard moth feed inside the stems and branches of young trees, causing dieback of shoots, yellowing of leaves and killing of many young trees (Alford, 2007). The purpose of the current study was to evaluate the damage rates of leopard moth in a walnut orchard in Hatay province of Turkey.

Material and Methods

The study was conducted in 2016-2017 to evaluate damage rates of the leopard moth on walnut 'chandler' variety orchard in Yayladağ district of Hatay province of Turkey. The walnut orchard contained 3345 walnut trees (380 da). The walnut trees damage assessment resulted as the percentage of trees with at least one recent larval gallery in November of both years. For this purpose, observations of all the trees were carried out to register the number of infested plant limbs. The percentage of damage was calculated by dividing the number of infested walnut trees by the total number of sampled walnut trees.

Results and Discussion

The damages rates of the leopard moth larva varied each of the sampling years. In the first year, the damage ratios of leopard moth larva was the 16 per cent in 2016, but the 8,87 per cent in 2017 (Figure 1).

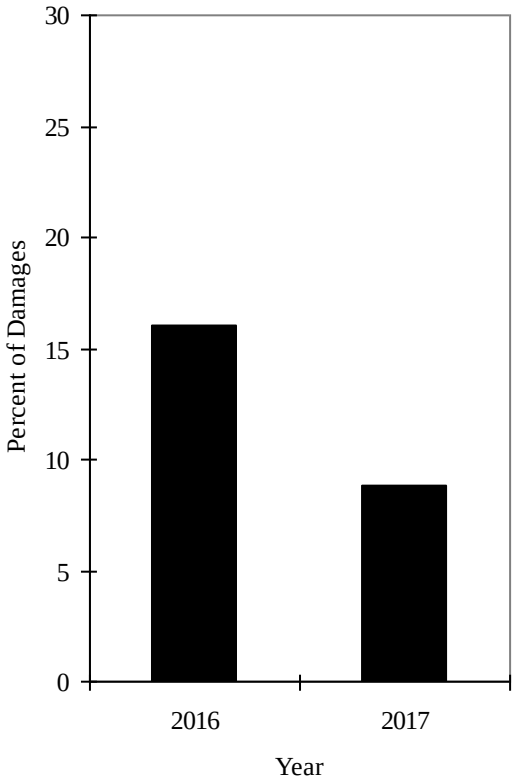

Figure 1. The percentages of damage rates leopard moth in 2016-2017 at the walnut orchard in Hatay province.

Patanita and Vargas-Osuna (2011) reported that the percentage of trees with infested trunks was reduced by 41% in the plots with five traps and by 45% in the plot with eight traps, compared with a 33% increment of the infestation in the control plot. In the following year, the decrease reached 92% in the plot with five traps, 84% in the plot with eight traps and 74% in control.

Conclusions

The present study was conducted to evaluate the damage rates of leopard moth in the walnut orchard in Hatay province of Turkey. As a result of two-year investigations, the damages rates of the leopard moth were observed in 2016 with 16 per cent and in 2017 with 8.87 per cent.

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DETERMINATION OF ANTAGONISTIC AND ANTIBACTERIAL ACTIVITY OF *TRICHODERMA* SPP. AGAINST *RHIZOBIUM VITIS* ISOLATES

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Abstract

Biological plant protection is an important component in the eco-friendly management of plant diseases all over the globe. *Trichoderma* spp. is a strain which is known to produce a number of antibiotics and lytic exoenzymes. The aim of the study is to evaluate the antagonistic and antibacterial activity of *Trichoderma* spp. by agar well method against *Rhizobium vitis*. Seven isolates of *R.vitis* were obtained from Northwestern of Marmara. *Trichoderma spp*. fungi were *isolated* from grass and wood taken from several habitats in Istanbul province. Eleven isolates were chosen among 23 isolates collected for isolation of *Trichoderma* spp. For determination of antagonistic activity, agar well diffusion method was used. In the study, the density of *Trichoderma* was prepared approximately $1.5x10^8$cells/ml and examined in five different doses 5,10,15,25 and 30 µl/ml placed into the wellson potato dextrose agar (PDA).Before placing into the wells, pure colonies of *R.vitis* were transferred to test tube containing *sterile distilled water*to get a final concentration of 1×10^8 CFU/mland uniformly spread on PDA.The experiment was conducted five times. Sterile water was used as negative control and streptomycin was used as positive control. After incubation for 48 h at 28°C, the diameters of the inhibition zones were measured with a millimeter scale and recorded to evaluate antagonistic activity.*Trichoderma* spp. applied at a dose of 30 µl/mlwas the most antagonistic againstfour isolates of *R. vitis*among the other tested doses. Particularly, significant inhibitory effect was observed at 30 µl/ml against one of *R.vitis* isolate with inhibition zone diameter of 56 mm.

Keywords: *Rhizobium vitis, Trichoderma spp., biological control.*

Introduction

Turkey is one of major grape producing country and has genetic resources of *Vitis vinifera* varieties accounting for approximately 90 % of total. Grape production reached approximately 4 million tonnes in Turkey (Anonim, 2015). Total grape production is 130.000 tones in Thrace region (Anonim, 2017). One of the common problems of grape growing are not to produce of quality grafted seedlings free from diseases and pests (Çelik et al., 2005; Çelik et al., 2011). *Agrobacterium vitis* Ophel and Kerr 1990 [= *Rhizobium vitis* Young et al. 2001], grapevine crown gall causing agent is the most important bacterial disease of grapevine throughout the world (Burr et al., 1998; Burr and Otten, 1999; Sawada et al., 1990). We have already known crown gall is common in the United States and the Europe but within past 50 years, the disease were also reported from China, Japan, and South Africa and from several other countries in Europe, the Middle East, and North and South America (Burr et al., 1998). It has world- wide host range, the majority of those are dicotyledonus plants including stone fruits, pome fruits, grapevine and pomegranate (Kenndey and Alcorn, 1980). Scientist work for bacterial diseases found *A. vitis* common in Turkey, so it is important bacterial disease for grape growing areas. *A. vitis* spreads through the plant juice and can survive for many years without any symptom formation. This bacteria genus generally originates of soil-borne. The presence of bacteria have been seen after galls occurred from contaminated wounds injuries (Bouzar and Moore, 1987). Vineyards are under serious threat because of

using disease infected plant production material. For this reason, founding vineyards from certified production material free from disease is very important to at the vineyard. Early gall formations taken place in nurseries can cause plant death (Allewedt et al., 1988; Burr et al., 1998); in the next period, if 50% of the plant body is covered with galls, it will cause a serious decreases in plant growth and yield (Schroth et al., 1988). Chemical management against this plant disease is ineffective (Burr et al., 1998). Biocontrol of plant pets and diseases is very important for increasing the environmental pollutions caused by agrochemicals and pesticides worldwide. Agrocin 84 (K84) produced by *Rhizobium rhizogenes* has important role for effectively biological control of *Agrobacterium vitis*. While K84 is using against tumorigenic strains of *Agrobacterium vitis*, it is not found preventive (Burr et al., 1998). Some scientists tried to find other biological control strategies against crown gall disease using such as nonpathogenic *A. vitis* strain F2/5, agrocin, nonpathogenic *A. vitis* strain VAR03-1 (which is inhibitory to most tumorigenic *A. vitis* strains *in vitro)* (Liang et al. 1990; Staphorst et al. 1985; Webster and Thomson 1986; Xiaoying and Wangnian, 1986; Burr and Reid, 1993; Kawaguchi et al., 2005). *Trichoderma* spp. have been developed into some commercial biological control agents and are used in field and greenhouse conditions (Harman, 2000). These products are known to control numerous soil-borne diseases (Sivan and Chet, 1993; Inbar et al., 1996; Naseby et al., 2000).

The objective of this research was to evaluate the potential of using *Trichoderma viride* as biological control agents to diminish the impact of *Agrobacterium vitis*. The efficacy of *Trichoderma viride* was tested to control *Agrobacterium vitis in vitro*.

Materials and methods

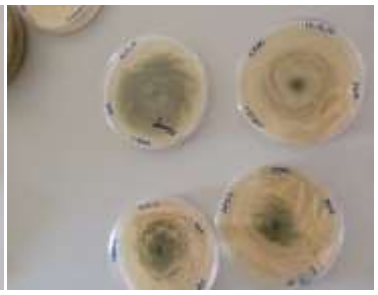
Bacterial isolations and identifications

Field survey were taken place in three provinces (Tekirdağ, Kırklareli and Edirne) in Thrace region in Turkey. 24 ones obtained from grapevine galls were used for this research. Tumors were washed with water, surface disinfected by dipping them into sodium hydrochlorite solution, 1% for 10-20 minutes according to Moore (1988) and Schaad et al. (2001) and were rinsed with sterile distilled water and dried with sterile filter paper. Then the outer layer was removed with sterile scalpel, small pieces were aseptically removed from each tumor, placed into few drops of sterile distilled water, then the resulted suspension was left to stand for 30 minutes and a loopfull of the resulted suspension was streaked on PDA medium. Inoculated plates were incubated at 25± 3◦C till bacterial growth developed dark green olive colonies showing Agrobacterium colony characteristics were selected, purified by preparing a suspension of the colonies in sterile distilled water and restreaked on KB medium, colonies fluorescent under ultraviolet light were eleminated. The isolates proved to be Agrobacterium based on the results of their reactions to these tests, then they were subjected to biochemical and physiological, tests to divide them oxidase, 3- ketolactose production; alkali production from L-tartaric and propionic acids; acid production from: sucrose, melezitose and erythritol, action on litmus milk; 2% sodium chloride tolerance, pigmentation on ferric ammonium citrate; growth on simmons citrate medium as described by Moore et al. (1988) and Schaad et al. (2001). For pathogenicity test, two month old seedlings of tomato (*Lycopersicon esculuntum* cv. Heinz) and Kalanchoe (*Kalanchoe diagremontiana*) were used as indicator plants. The plants were wounded forming a slit in the crown area by a sterile scalpel then 24 hour bacterial culture was applied to the wounded area with a sterile tooth pick. Also, tomato seedlings were sprayed with sterile distilled water, and known pathogenic *Agrobacterium tumefaciens* isolate was used to serve as control. They were kept on a climate chamber at 25± 2 ºC, checked for tumor formation daily (Al-karablieh and Khlaif, 2002).

Fungal Isolations and Identifications

Fungal Trichoderma viride was isolated from grass and wooden plant materials taken from several plantations in İstanbul and inoculated on Potato Dextrose Agar (PDA) medium for growth. Plant samples were collected into sterile polythene bags, transported to the laboratory and stored at 4 °C. For isolating Trichoderma spp, PDA medium was sterilized at 121℃ for 15 min in autoclave, poured to sterilized plates, cooled under sterile conditions.11 plant samples were chosen among 23 isolates collected for isolation of *Trichoderma* spp. Plant materials were cut to the length of 1.5–2 cm with sterile blade cut. Further, they were surface sterilized with 1% sodium hypochlorite for 4–5 min and then washed 3–4 times in sterile distilled water. Following surface sterilization, they were transferred into culture medium and incubated at 26℃ for 7-8 days on potato dextrose agar (PDA). After incubation period, the fungal colonies were picked up using a sterilized scalpel into PDA and purified for individual colonies. Purified fungal colonies were identified at the Plant Pathology Department, Namik Kemal University, based on morphological and culture characters using texts and references described by Barnett and Hunter (1972) and Ramirez (1982). Green conidia forming fungal bodies were selected and microscopic structures as size and shape of conidia and phyalides were observed using AxioVision Imaging System.

The study was carried out with seven diffirent *Agrobacterium vitis* isolates with three replicates and two controls. This trial experiments were made by modifying Bechard et al. (1998)'s study in a laminar flow cabinet. Bacterial suspensions adjusted to a density of 10^8 cfu/ml by using Mc Farland Scale. Seven bacterial isolates 30, 25, 20, 15 and 5 µl volume/petri dish suspensions were spread surface of the petri dish with streile glass rod. Then fungal inoculums were placed in a well positioned at the centre of 94 mm Petri dishes incubated at 26℃ for 7-8 days on PDA. After incubation, the areas of the growth inhibition zones around the central well were measured, and the calculated radius, compared with that of the control, was used to estimate the inhibitory activity. The antagonism between fungal microorganisms and the bacterial pathogen was assigned and calculated according to Wicklow et al. (1980) and modified as follows: the reaction type in which the fungus contacted and stopped the growth of the bacterial colony was assigned a numerical value of growth diameter.


Figures 1, 2, and 3. Applications in petri dishes.

Results and discussion

In petri dishes *Trichoderma* spp. was highly effective in 30 µl volume of bacterial suspensions. 20 and 25 µl volume of suspensions were infected moderately by changing isolates to isolates. Most suitable isolates results are taken NA9-1 taken fromTekirdağ province and NA43-1 taken from Edirne (Table 1).

Table 1. Effect of *Trichoderma* spp. against *Agrobacterium vitis* isolates *in vitro*.

Bacterial isolates	**Isolates location**	**Volume (µl)**	**Average Inhibition zone (mm)**
NA9-1	Tekirdağ	5	2
		15	4.8
		20	3.6
		25	4.9
		30	5.6
NA17-2	Tekirdağ	5	1.8
		15	2.3
		20	2.7
		25	2.1
		30	3.3
NA29-4	Tekirdağ	5	2.8
		15	1.7
		20	3.3
		25	2.7
		30	3.9
NA33-2	Kırklareli	5	1.8
		15	2.2
		20	3.4
		25	3
		30	3.1
NA35-1	Kırklareli	5	5
		15	4.7
		20	3.1
		25	3.4
		30	2

NA41-2	Edirne	5	2
		15	2.7
		20	3.3
		25	3.5
		30	3.1
NA43-1	**Edirne**	**5**	**2.6**
		15	**2.9**
		20	**3.4**
		25	**4.7**
		30	**4.9**

Conclusion

Trichoderma spp. as a rich source of antibacterial compounds can be effective biocontrol agent against agricultural and medical pathogenic bacteria. Trichoderma spp. can produce different compounds under different environmental conditions, hence use of this fungus could be an efficient source of biopesticide. These bioactive compounds produced by Trichoderma spp. can be isolated and might be used in the development of biological control for disease managements. Consequently, next researches are needed to determine structures of these antibacterial compounds for improving agricultural applications that are effective, non toxic and non polluting to control crown gall.

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ORGANIC AGRICULTURE

LAND AND AGRARIAN REFORM IN RWANDA: ORGANIC FARMING PERSPECTIVE

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Abstract

Since 2004, Rwanda is carrying an important land policy and a fundamental program of its farming systems transformation. The pillars of the government plan highlight the changes in land use management and tenure combined with improved inputs utilisation to ensure optimum productivity in the right line of the market-led agriculture. When they mention improved input, someone may understand essentially intensive use of commercial seeds, pesticides and chemical fertilizers. However, bio-fertilizers and natural pesticides are being promoted by the crop and livestock intensification program as well as the necessary techniques mobilised in the high quality of organic fertilizers production and use. This article aims to analyse how organic agriculture can coexist with conventional agriculture and allow agriculture operators to benefit of the market liberalisation. The literature review shows that during the agrarian system evolution, Rwandan peasants are involved in the program of land consolidation and agriculture intensification. Their level of performance has been appreciated throughout their active involvement in the commercial circuit. The main conclusion of this article is that organic farming prospects are inherent to optimal biomass valorisation especially crop residue, agro forestry and livestock dejection incorporated in the production systems. The unstructured interviews revealed that the most performing producers are those who own a minimum of 1ha with a recognized land lease and those who are using a mixture of organic manure and chemical fertilizers in potatoes. It has been demonstrated that the farmers who are enthusiastically participate in cooperatives have also an open mind to consolidate their commercial relationship. Moreover, they have constant ability to take advantage of the market, especially in pyrethrum and potatoes cropping system.

Key words: *Land reform, Organic farming, Rwanda, Pyrethrum, Potatoes*

Introduction

Several authors consent that globally, organic manure are essential for restoring and stabilising agricultural land fertility in Africa and particularly in Rwanda (Kim et al., 2013). However, countries with high density of agricultural population have fewer areas to increase the fodder production for livestock feeding like cattle (Klapwijk et al., 2014). Obviously, cattle still the principle provider of the manure in the Rwandan farming systems. Thus, organic matter from farmyard manure used by most of farmers is not available in sufficient quantity due to the lack of improved composting techniques either the knowledge of the manure management, handling and transporting equipments are deficient (Drechsel and Reck 1998; Kim et al., 2013). The « Umushinga Girinka Munyarwanda»[2], the pro-poor and the most important component of the livestock intensification program in Rwanda cannot allow all farmers in the country to be self-sufficient in organic manure supply (Klapwijk et al.,

[2] Stand for « One cow for one poor family » as a livestock project for milk and manure supplying and increased revenue

2014). This huge program of livestock resources improvement has been implemented by the Rwandan government as a multipurpose development approach. It aims to reduce poverty while it is decreasing malnutrition with milk consumption especially for the children. It intends to increase small-scale farmer's livelihood. This multi-folder program highlights the use of organic manure for soil fertility conservation in a double option of increasing crop productivity jointly with environmental protection. Moreover, it contributes to harmony and reconciliation among Rwandans based on the customary solidarity relationships between the giver and the receiver of the heifer (MINAGRI, 2014)[3]. Besides the different constraints of manure usage and availability, Kim et al. (2013) have found that the key limiting factors of manure expected from the « Girinka program » are lack of manure treatment and transporting tools, distance to crop fields from home and poor construction of cow sheds. Their study has revealed significant differences in manure management as well as in access to information and extension services (Kim et al., 2013).

Previous studies have shown that the organic manure or mineral fertilizers depends on the nature of the soil texture and structure. Among them, Rutunga et al. (1997) find that 10 tons per ha per year, applied on oxisols in Rwanda, is the amount of organic farmyard needed to substitute inorganic fertilizers. Then the core question: can the agrarian system in Rwanda set up entirely on organic or inorganic fertilizers exclusively? Is the rotation of pyrethrum and potato a good farming system to limit excessive use of mineral fertilizers and pesticides? The research assumption is that alternative farming system can contribute to coexistence of organic and conventional agriculture while it is increasing farmers' revenue and connecting them to the market.

Research motivation and objectives

At the era of Rwandan farming system transformation launched by the 2004 land policy reform together with the 2005 legal framework of land tenure and land use, few studies have taken in account of the organic farming prospects beside the promotion of conventional agriculture. It is commonly recognized that in Rwanda, fertilizers both organic and mineral are insufficient in quality and in quantity. But, the main attention is focussed on increasing of subsidized inorganic fertilizers application. This can be demonstrated by the increasing of imported chemical fertilisers the last decades even if we have not yet the authorization to publish those data collected by the National Institute of Statistics of Rwanda. Meanwhile, the use of organic fertilizers is less than the mineral fertilizers use for the big farmers involved in land consolidation and modern agriculture (NISR, 2016). Thus, it is required to search for high quality and affordable alternatives of organic farming focus on the sustainable agriculture. A study has revealed that in Rwanda, the mixture of organic and inorganic fertilizers is the most useful method of the soil fertility restoration in regards to soil configuration and crop category (Bidogeza et al., 2012). Moreover, there are high quality organic fertilizers and bio-industrial techniques already tested to improve the quantity and the quality of organic materials to increase the crop productivity in different agricultural farming systems (Karangwa et al., 2015, Zhang and Sun, 2016).

This article aims to contribute to the organic farming perspectives in Rwanda during the transformation process from subsistence agriculture towards the market-led and knowledge-based agriculture through a long term farming system of pyrethrum and potatoes in Kinigi sector located in Musanze district. About the transformation crossing of subsistence agrarian system in Rwanda, Musabanganji et al. (2016) have illustrated that smallholder farmers are staying in the first three classes according to the theoretical model of smallholders evolution proposed by Zhou in 2010.

[3] http://www.minagri.gov.rw/fileadmin/user_upload/documents/AnnualReports/Annual_Report_FY_2014_2015.pdf accessed on 24 May 2018.

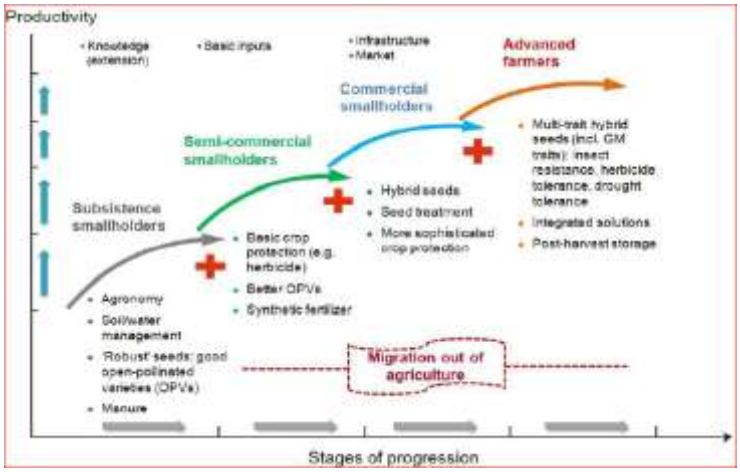


Figure 6 Theoretical model of small scale farmer's progression stages

Source: (Zhou, 2010, p.4 cited in Musabanganji et al. 2016, p.9)

The figure 1 above shows that combined use of manure, recommended agronomic practices (RAP), soil and water management and performing varieties are the basic package adopted by the subsistence smallholder farmers who are involved in a progressive agriculture transformation. They climb the low step towards the high step and former smallholder farmers have adopted improved inputs and gain commercial-minded attitude. The high level of the scale is reached by « advanced farmers » step by step. Those who are taking advantage in the agrarian system evolution are gradually adopting the most improved inputs for increasing productivity. The surplus of production is managed to supplying the market and storage techniques are enhanced for limiting yield loss. In general, most of the smallholder farmers in Rwanda are still yet to the second level of the four steps marking the evolution magnitude described above as « semi-commercial stage» (Musabanganji et al. 2016). The following section presents a comprehensive synthesis of organic and inorganic fertilizers use by both agriculture operators and large scale farmers in Rwanda.

Inorganic users are less than organic one but the first are increasing than the second

Agriculture transformation deal in Rwanda is in the right line of the 2020 vision revised in 2012 with the way forward of a « high-value added and market oriented agriculture » (Republic of Rwanda, Minecofin, 2012)[4]. The agriculture evolution predicted by the 2020 vision is in accordance with the strategic plan of agriculture transformation through intensive use of organic and inorganic fertilizers as well as the modern livestock. The strategic plan of agriculture transformation consider that fertilizers use are the main key to overcome the soil fertility challenge (République du Rwanda, Ministère de l'agriculture et de l'élevage, 2004). Inorganic fertilizers are promoted by the extension services of the Rwanda agriculture board through private sector to boost soil fertility. But, industrial organic manure remains insufficient for yield increasing (Karangwa et al., 2016). The seasonal agriculture survey carried in the « A season » of 2015 reported that in average 21.1% and 51.3% of small scale farmers also called «Agriculture operators » used respectively inorganic and organic fertilizers while 54.8% and 66.8% of Large scale farmers used respectively inorganic and organic fertilizers. The main types of inorganic fertilizers used in Rwandan farming system are DAP, solid and liquid Urea and NPK 17-17-17. The table 1 below revealed that other types of synthetic fertilizers are marginal.

[4] http://www.minecofin.gov.rw/fileadmin/templates/documents/NDPR/Vision_2020_.pdf consulted on 25 May 2018

Table 1. Types of mineral fertilizer used in Rwandan farming system

	Strata	NPK	UREA	UREA (LIQUID)	DAP	OTHER Fertilizers	Total
Agricultural Operators	1.1	19.4	38.0	0.5	42.0	0.1	100
	1.2	79.5	4.5	7.6	8.3	-	100
	2.1	10.1	49.6	1.0	39.3	-	100
	2.2	41.2	45.1	0.5	13.2	-	100
	3.0	11.8	35.3	5.9	47.1	-	100
	All Rwanda	19.8	38.1	0.6	41.4	0.1	100
LSF		27.4	38.8	0.3	28.6	4.9	100

2015 Seasonal Agriculture Survey - Season A

Source : (National Institute of Statistics of Rwanda (NISR), 2015 Seasonal Agriculture Survey Report, January 2016)

Rwanda has an important programme of agricultural systems transformation based on increasing of modern inputs, land consolidation and regional specialisation determined by their agronomic potential. The intensive use of fertilizers is highly recommended to all agricultural investors. « Purchases of agricultural inputs also changed, which is an indicator expected to correlate positively with increases in production. Use of sacks and packing increased from 37% to 48% between surveys for households purchasing any agricultural inputs. This is an indicator of commercialisation of production, since sacks and packaging are purchased primarily if selling is intended. Use of fertilisers also increased drastically, from 18% to 38%. For chemical fertilisers, usage increased from 11% to 29% of households, while for organic fertilisers the increases were smaller (7% to 9% of households). Use of insecticides has also increased: 31% of households used them as compared to 24% in EICV2.» (NISR, 2011). Organic manure stills the main source of fertility restoration in Rwandan rural agriculture. By the way, Niang et al. study (1998) has proposed changes to improve the quality of fodder. « Inclusion of the shrubs increases the overall quality of the fodder and also increases the quality of the grass component. The high production of the pennisetum-based systems is at the expense of adjacent crops. Economic evaluation shows that the optimal grass/shrub combination will depend on both fodder production objectives and on the relative importance of production of fodder and crop. However, it is clear that the leguminous shrubs can be part of the solution.» (Niang et al.,1998). We are in accordance with Yamoah and Grosz for their proposition of Agroforestry, tillage, mulch system, manure and compost as well as inorganic fertilizers as an integrated package to overcome the problem of the soil fertility in a sustainable way of organic farming (Yamoah and Grosz, 1988). The section below describes a long-lasting coexistence of organic and inorganic farming system based on Pyrethrum-Potatoes rotation.

Organic and inorganic farming coexistence: Pyrethrium and potatoes farming model

Pyrethrum is a botanical insecticide produced from Dalmatian Chrysanthemum (*Chrysanthemum cinerariaefolium*) or sometimes from Persian Chrysanthemum (*Chrysanthemum coccineum*), perennials from Eastern Europe and Canada (Duval, 1993). Duval precise that Caucasus Dalmatian chrysanthemum is commercially grown today mainly in the mountainous regions of Kenya, Tanzania and Ecuador. It is also developed in Virunga Mountains of Rwanda and Australia. Its organic nature to grow in volcanic region of the Northern Rwanda has create opportunities to rural people operating in cooperatives to be involved in well organised value chain from the local level to the international business

network according to the Horizon group (2016) in charge of SOPYRWA[5] management. Depending to climate, the plant can be multiplied by division or sowing. It is much easier to start growing chrysanthemums to try to get seedlings from a neighbour or nursery. Otherwise, sowing remains the only option for the first year. For the following years, if you want more plants, you can proceed by vegetative multiplication when the new shoots appear. The nursery for pre-germination is approximately 3 months before the transplantation, either at the end of February or March. The seeds should be placed in a sterilized environment. Otherwise, the seed can lose their capacity of germination in relatively short time. The seeds take 2 to 4 weeks to germinate. The ideal germination temperature is about 20 to 22°C. You can also sow in the ground in August by covering the seeds with a centimetre of fine soil and then dry leaves. The seedlings are transplanted in deep rows spaced 30 to 50 cm apart and 35 to 40 cm in row. It will be 7 to 9 plants per square meter. A loss of 15 to 30% of the transplants can be expected. Chrysanthemums including Pyrethrum can multiply in cold and temperate climates but its optimum potential is reached in the equatorial high altitude zone. Commercial cultivation of Dalmatian chrysanthemum ranges from 1,600 to 3,000 meters above sea level, with altitude pyrethrin concentrations. Semi-arid climatic conditions with a cool winter, 1200 decreasing to less mm rainfall and a 2 to 3 months dry season are ideal. It tolerates temperatures down to -12°C (Duval, 1993). Pyrethrum chrysanthemums prefer dry, gravelly, limestone and sloping soils. The shady places are to avoid the shade of a lot of pyrethrins concentration. In a loamy soil rich in humus and humid climate, the chrysanthemum will grow abundantly but its pyrethrin concentration will be reduced. The chrysanthemums must be kept in well weeded conditions because they are very sensitive to competition. Chrysanthemum is not sensitive to chemical fertilizer and it can resistant to pest. In contrary, harvesting is a critical step in getting maximum pyrethrins in the flowers. It must always be harvested in dry and hot weather. Ideally, flowers should be harvested at full development and even between day 5 and 9 after flowering to achieve the highest level of pyrethrin. The flowers in the center of the plant contain more pyrethrin than the flowers around (Duval, 1993). Every two weeks, flowers can be harvested.

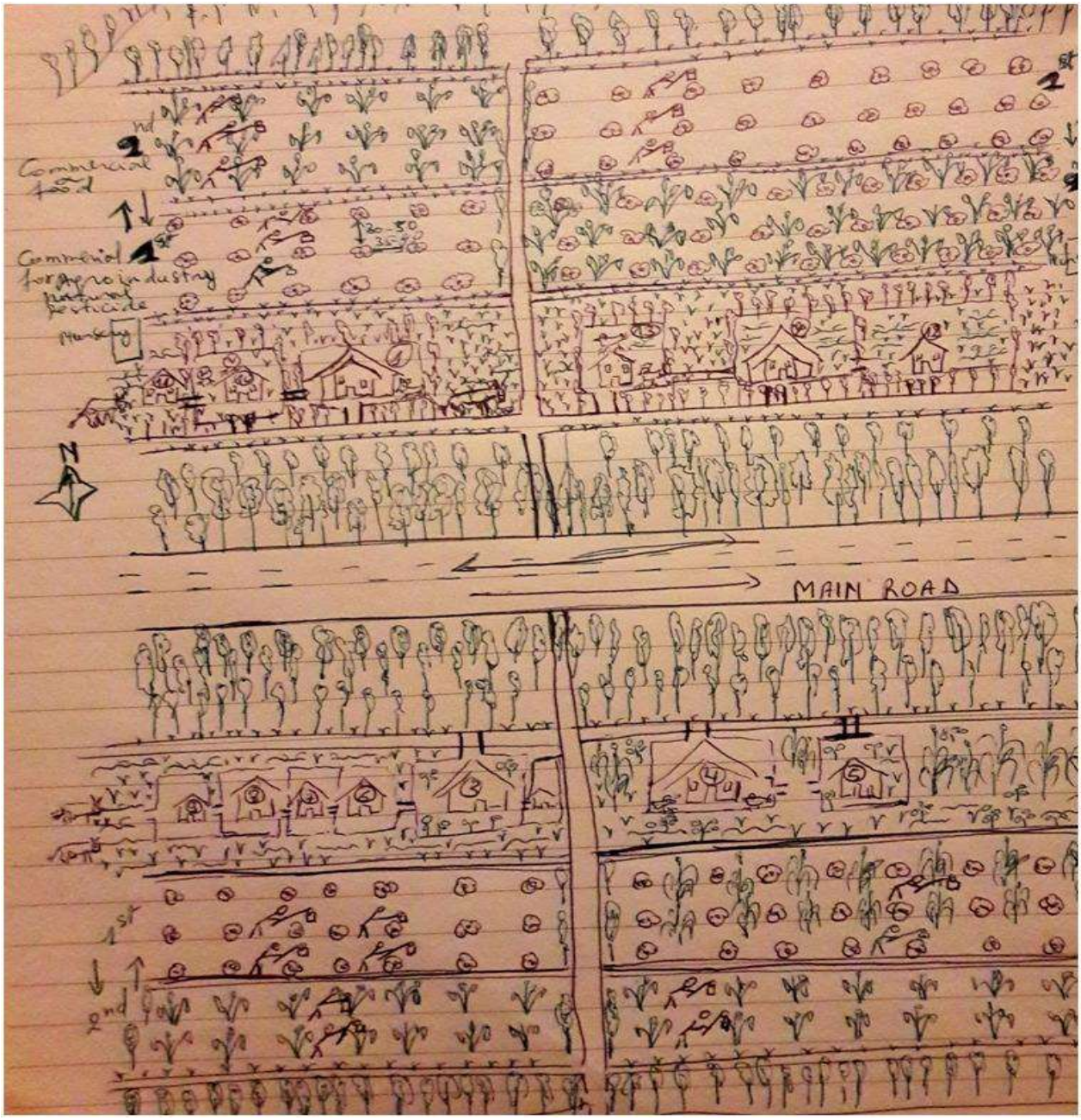
Site of experimentation: volcanic mountains region of the Northern Rwanda

Our site of experimentation is located in a long term coexistence of organic and inorganic farming system in Kinigi sector of the district of Musanze in the Nothern province of Rwanda. The zone of the pyrethrum growing covers 4 districts of high potential like Nyabihu, Musanze, Burera and Rubavu. There are more than 10,000 homes in Rwanda whose livelihoods are based on pyrethrum essentially in the Northern Province. They are all members of seven (7) cooperatives for production and trade. The main buyers of pyrethrum essential oils are the US, Europe and Asia (NAEB, 2012).

Result of field survey in the « Paysannat » of Kinigi sector

Kinigi sector covers a high potential area of potatoes and Pyrethrum production. The growers of pyrethrum and potatoes in the « Paysannat farming system » we have interviewed during our field work revealed us that those who are involved in potatoes production and commercialisation can make a profit of 2,500,000-3,000,000 or 2,500€ of Rwandan Francs per ha for an average of three seasons per year with 15-20 tons of potatoes per ha while the producers of pyrethrum are susceptible to make a profit of 500,000 Rwandan Francs or 500€ per year. While Irish potatoes are budgetivore in pesticides and fertilizers, Pyrethrum is not. The pyrethrum contributes in fertility restoration after a season of potatoes. The total revenue of 3,000€ is equivalent to the annual revenue of middle class in Rwanda.

[5] https://www.horizongroup.rw/share-holder-value/ Consulted on 20th august 2018



Pyrethrum, potatoes, tries farming system in Kinigi Sector

SOPYRWA held by the Horizon Group Ltd since 2008 is the one of the companies involved in the production and processing the pyrethrum together with AgroPY Ltd established in 2011. Those two agro-processing companies are operating in the Musanze district. They make insecticide from pyrethrum flowers that control pests (mosquitoes, Trypanosomiasis bedbugs, lice, ticks) and diseases. « Since 2012, AgroPy Ltd has received attestation by ECOCERT under European Union regulations EC No. 834/2007 & 889/2008 for Pyrethrum 5EW. This product is of huge benefit to coffee farmers in the control of the Antestia Beetle. Organic insecticides allow farmers to benefit from the opportunity to supply organic produce at premium prices» (Horizon group, 2016). « Rwanda intensively promotes pyrethrum growing and has been making quality pyrethrum seeds available for growers. Rwanda teaches farmers on how to increase pyrethrum productivity and advises them on good farming practices. They have also started making pyrethrum seedlings so as to increase its yields. Rwanda is the second world largest producer of pyrethrum and has approximately 15 % of the world market

share... » (NAEB, 2012). Sopyrwa produces in average 3000 tons of dry flowers per annum and processes 10% of pale extract locally. In 2012, almost 26 tons of pyrethrum essential oils have generated 7,517,900$ (NAEB, 2012). The pyrethrum's value of one unit was 81.36 times the value of Coffee and 99.62 times of tea. In the category of horticulture products, it was respectively 111.29, 876.89 and 1,034.48 times the value per unit of natural flowers, vegetables and fruits (NAEB, 2012).

Table 2. Comparative importance of pyrethrum with other agriculture exported product

	Traditional export commodities		Horticulture			
Year 2012	Coffee	Tea	Pyrethrum	Flower	Vegetables	Fruit
Volume in kg	16,989,730	22,453,391	25,785	171	28,699,579	4,040,251
Total revenue in $USD	60,886,260	65,717,926	7,517,900	448	9,542,400	1,138,719
Comparative value/kg in $USD	**3.58**	**2.93**	**291.56**	**2.62**	**0.33**	**0.28**
Ratio Pyrethrum/Other agriculture export products	81.36	99.62	1.00	111.29	876.89	1,034.48

Source : Author from statistics reported by NAEB (National Agricultural Export Development Board, 2012)

About potatoes, Rwanda hosted in 2016 the annual meeting dedicated to the potato week. Since 2011, it has been organized in turn in the 4 member countries of the network of sub-regional producers of potatoes namely the Democratic Republic of Congo, Rwanda, Burundi and Uganda. The Imbaraga organization was organizing the event in the partnership with Agriterra and AgriProFocus, two netherland organization. The FECOPPORWA federation (Federation of Cooperative Producers of Potato in Rwanda), which has 5 unions, 149 cooperatives and 9,796 members, was created in 2009. It mainly aims at strengthening the capacities of cooperatives and representing their interests, as well as the promotion of technology in the value chain. The 4 member countries of the sub-regional network of potato growers draw on the experience of Nedato, a cooperative of Dutch potato producers. Nedato was founded in 1963 and currently has 500 members and remains one of the most dynamic cooperatives on the potato market in Europe. Nedato has specialized in the production and marketing of potatoes, export and processing. Potato producers in Rwanda are already integrated into the formal financing circuit. Although access to finance is still a challenge, the UOB bank (Urwego opportunity bank) supported, in 2016, 995 producers grouped in 27 producer groups and 2 cooperatives, with the outstanding credit which is evaluated at 459,028,392 FRW. Potato producers are integrated in the local, regional and international market as illustrated in the figure below.

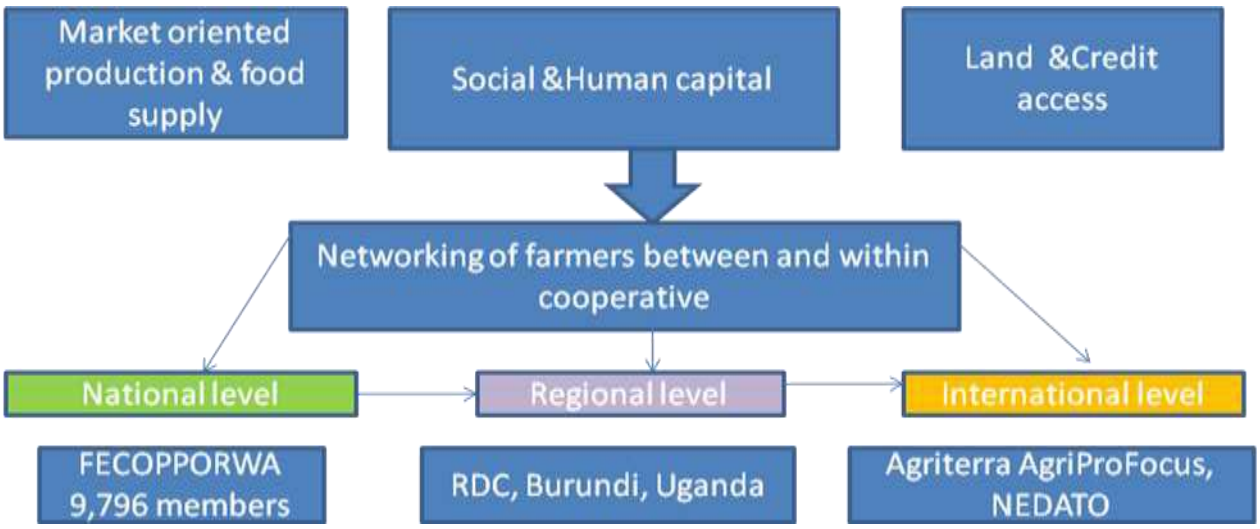


Figure 7 Small scale farmers' integration in the world wide market through cooperatives

During the field work in Musanze District, they revealed us that some of the Potatoes producers are supposed to be Reich compared to other ordinary farmers. They are able to buy cars and are susceptible to invest in diversify trading locally and abroad.

Conclusion

The future of organic farming is depends on optimal biomass valorisation especially crop residue, agro forestry and livestock dejection incorporated in the production systems. The rotation of Pyrethrum and potatoes is the example combination of a crop which is less or no more chemical fertilizers consumer with another one which is highly demanding crop in pesticides and chemical inputs. The most performing producers are those who own a minimum of 1ha of farm land and who are using a mixture of organic manure and chemical fertilizers. Pyrethrum producers and Irish potatoes farmers are actively participate in cooperatives. Moreover, they have an open mind to consolidate their commercial relationship. They have constant ability to take advantage of the market. Their trading strategy consists in individual management of the production but in the collective negotiation of the seller price, grouped command of the inputs and the common use of infrastructures for instance the storage place or dryer areas of the pyrethrum flowers. Those who are involved in Irish potatoes production and commercialisation can make a profit of 2,500,000 or 2500€ of Rwandan Francs per ha for an average of three seasons per year while the producers of pyrethrum are susceptible to make a profit of 500,000 Rwandan Francs or 500€ per year. Pyrethrum production allow to restore fertility when is cultivated in rotation with Irish potatoes. This long term crop system is proof that organic farming can coexist with conventional farming system.

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ORGANIC PRODUCTION OF RASPBERRIES (WILLAMETTE var.)

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Abstract

Raspberries in BiH are grown on an area of 1,628 ha, from which total production of 13.631t is realized. This places raspberries onto the fourth place among fruits. The largest percentage of raspberry production is of conventional type. In recent years, significant activities have been undertaken to introduce the concept of integral production and organic raspberry production. Interest in organic raspberry production is growing in the world, as well as in Republika Srpska, not only in the field of science and profession, but also in farming. This represents a good and safe way for economical production of quality fruits for which there is a safe and stable market. This area is becoming attractive because relatively small areas achieve significant revenues, i.e have positive economic effects that contribute to the sustainability of production. Organic agriculture is based on the minimal use of materials that do not originate from the farm and on the production practice that establishes, maintains and improves the ecological balance. The rules of organic agriculture are defined in the Law on Organic Production of Republika Srpska and the standards and regulations of the EU and other countries.The aim of this paper is to determine the characteristics of Willamette produced according to the concept of organic production, by analyzing the specificity of the technological production process and the basic parameters of fertility of the investigated fruit variety in agro-ecological conditions in the territory of the municipality of Srebrenica.

Key words: *organic production, raspberries, variety Willamette, Srebrenica, Bosnia.*

Introduction

On the territory of Bosnia and Herzegovina, raspberries are grown on an area of 1.682 ha, with a total output of 13,631 tonnes. This places raspberries onto the fourth place among fruits. Raspberry is mostly grown in the area of Podrinje, Upper Carniola and Central Bosnia. In recent years, raspberries have expanded extensively in other parts of Bosnia and Herzegovina (Una-Sana Canton, the region of Krajina, Posavina). Raspberry cultivation in Bosnia and Herzegovina is of small-sized production, whose surface on average ranges from 0.1 to 0.3 ha. Most of the production is located with small individual producers, while in recent years there has been a noticeable appearance of large plantations, whose owners are mostly present in the raspberries business (larger raspberries buyers or cold store owners). Producers are generally affiliated to local associations or are contractually affiliated to cold stores and collective farms operating in the respective regions. Through the associations and collective farms, procurement of fruits and reproductive material during the year is carried out, which in most cases is repaid with raspberries at the end of the season. There is a noticeable increase in storage capacities that does not accompany the rise in production levels, which often leads to a disturbance of the producer-buyer relationship and can endanger production in the long run.

The highest percentage of raspberry production is conventional, although in recent years a significant number of project activities have been implemented to introduce the concept of integral and organic raspberry production. The interest in organic raspberry production is growing in the world, as well as in Republika Srpska, not only in the field of science and profession, but also in farming. This represents a good and safe way for economical production of quality fruits for which there is a safe and stable market. This area is becoming

attractive because relatively small areas achieve significant revenues, i.e have positive economic effects that contribute to the sustainability of production.
The organic production program is very close to the traditional method of production by inputs, but there are also significant differences, primarily in the process control system. Only a product controlled and certified by an authorized control organization can be marketed with the label "organic product". Organic production is very close to traditional, so it is extremely important to choose a suitable area and conscientious producers who understand and want to cooperate in such production. Also, it is very important to build a quality system of internal control that monitors, directs and educates manufacturers.
Climate and soil are external factors that primarily affect the formation of qualitative characteristics of raspberries. However, the soil and climate give the required quality with the appropriate yield when they are in a certain relationship and when they supplement each other. The natural conditions in which the plant grows and develops are decisive for the success of production, since the producer does not have the capability to significantly modify these conditions and adjust them according to the needs of the plant, except for the production of planting material. On the one hand, the production of raspberries depends on natural conditions, such as climate and soil. On the other hand, it depends on the producer`s activity, as he/she can choose the best possibilities in the existing conditions and repair and improve natural conditions in some way, adapting them to some extent to the goals of production.
Raspberry fruits are sensitive and subject to rapid deterioration, so they must be collected carefully and in time. The timing and method of harvest do not only affect the quality of raspberries, but also the advantage and the possibility of their realization. The maturation of raspberries depends on several factors: hereditary traits of species, latitude, altitude, climatic conditions, terrain exposures, soil and applied biological preparations. Fruits of organic raspberries are packaged in packaging made of natural material and are visibly marked, stored with special care and transported in clean uncontaminated vehicles. Raspberry is distinguished as a culture by the fact that its fruits do not mature at the same time, so harvesting takes place on several occasions.
Willamette matures relatively early, at the end of the first decade of June. In the Srebrenica area, the maturation season is delayed for about 10 to 15 days on average. The period of fruit maturation lasts for quite a long time, on average 30 days.
Knowing this, the aim of the paper is to determine the characteristics of Willamette, produced according to organic farming production. This is done by analyzing the specificity of the technological production process and the basic parameters of fertility of the investigated fruit in agro-ecological conditions in the territory of the municipality of Srebrenica.

Material and methods

The research was carried out in the plantation of the company "ZZ Srebrenica" located in Brežani, in the territory of the municipality of Srebrenica. The plantation was established and maintained through an organic production system. Organic raspberry production at this site started in 2007. The plantation was established on a total surface of 4.5 ha at an altitude of 961 m. Raspberries are grown in a spalter system with planting distance of 2.5 m between rows, and 0.25 m between seedlings. In the plantation, agricultural practices common for organic production are used together with the use of authorized preparations for the protection of raspberries from diseases and pests.

Image 1. Details from the plantation in which the research was carried out
Source: author`s photo

Only Willamette is present in the plantation. Previous experience in working with this variety on the field shows its relatively high resistance to the most harmful diseases and pests in conventional production, which is one of the reasons for its introduction into the organic concept.
Soil cultivation is carried out in order to maintain the soil in a loose state, to destroy weeds and to feed the organic fertilizer in the root zone. This contributes to better nutrition of the raspberries, moisture preservation, the presence of air in the soil and the prevention of the creation of a crust on the surface. Regular and proper soil cultivation in raspberry plantation contributes to better income and further development of seedlings, faster planting in full yield, high yields and quality fruits. In the raspberry plantations, the soil should be kept under constant vegetation during the vegetation and without weeds, while in the course of the treatment, mechanical damage to the roots must not occur.
Inter-row space is maintained by grassing and regular mowing. A serious disadvantage in this production process is the use of natural (weed) vegetation for grassing, rather than deliberately sown grass mixtures.
During the research, two segments are included:
a) analysis of the most important measures applied in the process of organic raspberry production
b) the characteristics of the fertility and fruits of Willamette in the organic control system. In the analysis of fertility, sprouts in the regular production system are monitored with loads that represent the usual manufacturing practice. An analysis of fruiting increments developed from the primary and secondary buds, as well as the number of physiologically ripe fruit on them, was made. For the analysis of the characteristics of the fruit, 30 fruits were used, on which the weight of the fruit, length and width of the fruit was measured.
Research was carried out during the harvest in 2015.

Results and discussion

Willamette produces a large number of upright branches with medium long and elastic genital branches, which are rarely fractured under the load of the genus or by the action of stronger winds. Under organic production, there is a significant role to play in the number of sprouts per square meter that are left to proliferate. In a sealed plant, the number of sprouts per square meter ranges from 6 to 8. This yield density per square meter is considered optimal, in order to achieve the required number of buds and thus yield per unit area. After selecting the

optimal number of sprouts, they are transplanted to the required height. In the examined plant, due to the absence of mineral fertilizers, the height of sprouts is usually 150-170 cm, which is slightly less than the optimal values. With this yield height, the desired yields can be realized if the quality of buds found on these sprouts is taken into account. Usually, edges that are too thick or thin do not have a satisfactory quality of buds which should form fetal growths with infructescence. This can affect fruiting.

Raspberry has great demands for moisture in the soil during the vegetation period, especially from the beginning of vegetation until the end of harvesting of fruits. The lack of moisture in raspberry soil is difficult to tolerate, which adversely affects yields, fruit quality, and growth of sprouts. Irrigation is a significant agromera especially in droughty years and in its growing in dry areas. The time and number of irrigation depends on the weather conditions in different arbitrary areas, it is best to be determined according to the specific conditions and possibilities. Raspberries should be irrigated as needed, with irrigation being mandatory before the land drainage occurrs. Rasperries need watering the most during the period of flowering, development and maturantion of the fruit as well as after the harvest so to have normal new sprouts. Irrigation of raspberries is best done more often with smaller amounts of water. Usually it is done superficially by the system of furrows, rain and drops.

On the test site, irrigation is done by "drop by drop" system. Irrigation by the "drop by drop" system is a newer and most suitable way of irrigation in fruit production. The advantages of this irrigation system are that they can be applied on different terrains, there can be no ponding and soil erosion, less water is needed, no fruits and leaves are moistured, thus the possibility of infection and disease is lower. The "drop by drop" irrigation system is easily installed and can also be connected to the water supply system. At the accumulation, a standard water purification filter is placed and then the water is brought into raspberry plantations, most often using a natural fall with plastic tubes.

Table 1. The characteristics of fertility and fruit of the Willamette variety

no.	Better Agronomic performances/ paramters	$\overline{X} \pm S_{\overline{X}}$
1	Number of buds left on a sprout	10,00
2	Number of fruit-bearing increments from the main bud	9.166 ± 0.207
3	Number of fruit-bearing increments from the lateral bud	1.125 ± 0.580
4	Number of fruit from the main bud	98.666 ± 5.244
5	Number of fruit from the lateral bud	8.083 ± 4.668
6	Total number of fruits on a sprout	98.674
	characteristics of fruit	
1	Fruit weight (g)	3.534 ± 0.139
2	Fruit length (mm)	18.996 ± 0.735
3	Fruit width (mm)	18.624 ± 0.606
	calculated characteristics of fertility	
1	Yield per sprout (g)	348,714
2	Yield per length m (kg)	2,097
3	Estimated yield per ha (t)	7,464

Tested of Agronomic parameters testing of the Willamette variety was carried out by leaving ten buds along a sprout and following the basic characteristics of fertility. The number of activated buds is quite high (91.6%) and indicates high fruiting potential in relation to the number of leaves left. In addition to activating the main bud and creating fertile increments on them, activation of the second and second buds (usually less developed) was recorded, and on

average 1.12 buds. An analysis of the number of fruits formed on fruiting increments from the main and secondary buds indicates differences in their fruiting potential. The total number of fruits on fruiting increments, regardless of their character, was 98.67. The average weight of the raspberry fruit was 3.53 g, which is about the standard values (4 g). The minimum raspberry fruit weight is 2.98 g and the maximum is 4.98 g. The average length of the raspberry fruit is 19.90 mm, with a minimum length of 15.09 mm and a maximum of 25.90 mm. From Table 1 it can be seen that the average width of raspberry fruit is 19.44 mm, maximum width of 23.04 mm and minimum of 14.43 mm.

By bringing in the number of fruits per yield and average weight of the fruit, the average weight per sprout (348.7 g) and length meter (2.09 kg) is obtained. The estimated yield per unit area (ha) is 7, 464 t.

The yield obtained is lower than realistically possible. However, in the conditions of reduced application of fertilizers, this yield can be considered acceptable if the price of raspberries produced in this way can compensate for the reduced yield.

Conclusions

- Climate conditions in the Srebrenica area favor the cultivation of raspberries according to organic farming system, especially given the locality and altitude for which it has been established.
- Most of the applied and analyzed technological processes in the production process are in accordance with the principles of organic production.
- The fruiting potential of mixed buds on shoots is quite high, given the way of cultivation.
- The characteristics of the Willamette fruit are consistent with its genotypic characteristics.
- The yield is below the standard for this variety.

Organic raspberry production can be justified in circumstances where the price of a product can compensate for slightly lower yields than conventional production.

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CHEMICAL COMPOSITION AND ANTIOXIDANT FEATURES OF THREE LEAFY CULTURES – BROAD LEAVED DOCK, CURLED DOCK AND LEAF BEET

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Abstract

The paper analyzes the chemical composition and antioxidant features of three leafy cultures: broad leaved dock (*Rumex obtusifolius* L.), curled dock (*Rumex crispus* L.) and leaf beet (*Beta vulgaris* L. ssp. *vulgaris*). Plants were grown in an organic garden with a random distribution and approximately equal cover density. The analysis encompassed following parameters: mineral composition (As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Zn), anthocyanins (total and monomeric), β-carotene, vitamin C, phenols, flavonoids and flavonols. Antioxidant capacity was evaluated through ABTS and DPPH tests.

The researches have shown that all three species have almost equal content of β-carotene, while the broad leaved dock is inferior to the content of anthocyanins. With regards to phenols, flavonoids and flavonols, curled dock is ahead. Next in order is broad lived dock, while leaf beet shows the poorest results with observed features. The same case is with vitamin C and antioxidant properties. Only in the case of macro- and microelements, the results are polycentric. So, leaf beet predominates in the selenium content. Broad lived dock has the advantage in the content of copper and sodium while curled dock is superior in terms of calcium and iron.

Key words: *leaf beet, curled dock, broad leaved dock, chemical composition, antioxidant properties*

Introduction

In the domestic professional community, it has long been known that wild growing species from genera: *Atriplex, Chenopodium*, *Polygonum* and *Rumex* can be used as a substitute for conventional leafy vegetables, whether in form of salads, stewed vegetables, puree or otherwise prepared (Vračarić *et al*. 1968; Grlić 1980, 1986). Particular attention is attracted by representatives of the genus *Rumex*, because in our regions, it is considered that these plants, except for a few species, can be used without any restrictions. This primarily refers to species: *Rumex alpinus*, *R. aquaticus*, *R. crispus*, *R. hydrolapathum*, *R. obtusifolus*, *R. patientia*, *R. pulcher*, *R. sanguineum*. Species *Rumex acetosa* and *R. acetosella* are also edible, but due to the increased oxalic acid content there are limits to the amount of daily portion. Species *R. scutatus* and *R. nivalis* are edible as well, but they are more difficult to reach since they occur at high altitudes (Lakušić, 1982). Apart from the purely nutritional aspect, these species are interesting in terms of their antioxidant properties (Wegiera, 2011; Žabić 2015).

In addition to the basic intention to analyze the chemical composition and antioxidant properties of observed leafy cultures, this paper wants to show wild and cultivated plants can be grown next to each other, and wild species in their chemical composition are not inferior to cultivars (at this point, tastes are not discussed). For that reason we have made certain comparisons with common beet (*Beta vulgaris* subsp. *vulgaris*) variety Swiss chard.

Viewed from a slightly wider angle, applying such approach there is no need for traveling and seeking the wilderness, or purchasing goods in supermarkets, to which they traveled hundreds and even thousands of kilometers (Carlsson-Kanyama, 1997; Pirog and Benjamin, 2003; Welbaum 2015).

Material and method

Material

In an organic garden, which is located in the city of Banja Luka in Bosnia and Herzegovina, three observed species grow: broad leaved dock (*Rumex obtusifolius* L.), curled dock (*Rumex crispus* L.) and leaf beet (*Beta vulgaris* L. ssp. *vulgaris*) var. Swiss chard.

Wild growing curled dock optimum living conditions in Bosnia and Herzegovina is found in the association *Rumici-Alopecuretum geniculati* Tx. (1937) 1950 and is rather numerous in other communities of the alliance *Agropyro-Rumicion crispi* Nordhagen 1940 (Mišić *et* Lakušić, 1990). According to the same source, inhabits ecosystems of classes: *Molinio-Arrhenatheretae, Festuco-Brometea*, *Plantaginetea majoris* Tx. 1950 and *Phragmitetea*. R. Tx. et Prsg. 1942. One ecotype of this species lives like a weed in the ecosystem of stubbles and row crops (classes: *Secalinetea* Br.-Bl. 1951 *et Chenopodietea* Br-Bl. 1951). In vineyards of northern Bosnia curled dock occurs in association *Convolvulo-Agropyretum repentis* Felföldy 1943. A slightly smaller presence in this community has broad leaved dock (Kovačević, 2016). In neighboring Croatia species occur in the *Polygono-Chenopodietum* Lohm. 1950 community, belonging to the vegetation of riverbanks and flooded grassland. In our country curled dock is recorded in the lower course of the river Neretva in Herzegovina (Marković, 1980, referring to the Lohmayer, 1970 *et* Supopp, 1971). Broad leaved dock was registered in the same community, but with a lower abundance. Young leaves of this plant contain vitamin C and other healthy ingredients, thereupon they can be used as a vegetable. In folk medicine they are used as an antiseptic and antiscorbutic. From animal husbandry point of view, this species is considered as a worthless fodder (Mišić *et* Lakušić, 1990). According to this source, it is even considered harmful at a weighting content above 5% because it negatively affects the secretion and quality of milk.

For the time being, there is no evidence that the broad leaved dock was found in natural, anthropogenically uninfluenced plant communities in B&H. Several years ago the authors recorded this species in the virgin forest reserve "Lom", but along the forest path that goes through the protected area, which actually legitimized this species as a ruderal floral element. Čekić *et* Kovačević (2017) found the species in several weed associations of orchards, vineyards and row crops in the Lijevče field, area in the northern part of B&H: *Panico-Galinsogetum parviglore* Tüxen et Becker 1942; *Panico-Portulaceum oleraceae* Lozanovski 1962; *Cynodono-Sorghetum halepense* (Laban 1974) Kojić, 1979; *Chenopodietalia albi* Tx., Lohm. *et* Prsg. 1950. In Croatia, Marković (1984) described the species in a number of ruderal communities: *Urtico-Malvetum neglectae* Lohm. 1950; *Arctio-Artemisietum vulgaris* (R. Tx. 1942) Oberd. apud Oberd. *et al*. 1967; *Chenopodietum boni-henrici* (R. Tx. 1931) Th. Müller apud Oberd. 1970; *Torilidetum japonicae* Lohm. 1967; *Chaerophylletum aurei* Oberd. 1957; *Matricario-Polygonetum avicularis* (Knapp 1946) Th. Müll. in Oberd. 1971; *Junco-Menthetum longifoliae* Lohm. 1953.

Figure 1: Curled dock (left) and broad leaved dock (right)
(photo: S. LJubojević)

Broad leaved dock has a similar usable value as a curled dock. In folk medicine is commonly used against hemorrhoids, severe diarrhea and for general regulation of digestion. It is believed that its use in larger quantities may have negative consequences. According to the some sources, the curled dock belongs to moderate allergens whose period of pollination ranges from June to August, while broad leaved dock has a slightly shorter period of pollination (Anon., 2017).

Beet was first domesticated about 2500 years ago in the Mediterranean area (Lange *et al*.,1999; Welbaum, 2015). There are several approaches to taxonomy and nomenclature of beet (Anon./ITIS, 2018; Kadereit *et al*., 2006; Lange *et al*.,1999; Uotila, 2011; and other). According to Kadereit and Uotila, the beet species (*Beta vulgaris* L.) is divided into three subspecies: *Beta vulgaris* ssp. *adanensis* - Turkish beet, *Beta vulgaris* ssp. *vulgaris* – common beet and *Beta vulgaris* ssp. *maritima* – sea beet. According to Lange *et al*. (1999) and some other sources, biannual common beet encompasses four cultivar groups: leaf beet, garden beet, fodder beet and sugar beet group. Within the leaf beet cultivar group, the following cultivars are differentiated: Swiss chard (silver chard), ruby chard (rhubarb chard) and spinach beet (perpetual spinach). The subject of our interest is the cultivar of Swiss chard. Leaf beet is usually consumed blanched or cooked, although any country or region practices specific ways of its preparation. In folk medicine, leaf beet is being applied to treat a large number of illnesses and disorders (Miraj, 2016).

Method

All three species are grown as a perennials on the no-tillage land belt between vegetable beds on one side, and orchards on the other. Curled dock and broad leaved dock spontaneously settled on the observed surface several years ago (Fig. 1). Since that time there were no interventions on curled dock populations. Their footprint is more or less unchanged. This is not the case with the broad leaved dock. It is trying to spread its footprint in time and space. For this reason in autumn every year some correction needs to be carried. This is achieved by simply digging entire plants from the ground. Such behavior of broad leaved dock perhaps may be explained by its allelopathic affinity (Zaller, 2006). Unlike "dock species", leaf beet was initially grown on beds from seeds, from where it spontaneously settled on the no-tillage land belt (Fig. 2 and 3). In this way, a practically biannual plant is cultivated as a perennial crop.

Figure 2: Swiss chard grown from the commercial seed on the garden soil bed (photo taken in June 2008 by S. Ljubojević)

Figure 3: Swiss chard grown from seed balls(glomerules) spontaneously settled on the garden path (ph. taken in June 2018 by S.LJ.)

Samples for analysis were collected in 2017. From the homogenised material, 5 g of leaves of each species was extracted with 80% ethanol, twice at 25 ml. The solutions were refilled with 80 % ethanol up to 50 mL. Thus, 100 mg/mL concentrations were obtained and further used to determine total phenols, flavonoids, flavonols, neutralization of 2.2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS +) radicals. To determine a total and monomeric anthocyanins, 20 g of samples was extracted with a 20 mL solution (85 ml of 95 % ethanol solution in 15 ml of 1.5 mol/L HCl solution) at 0^{o} C for 24 hours. After being left to stand, resulting mixture was filtered through a filter paper, and the filtrates were used for further analysis. The content of vitamin C was determined by the standard AOAC methods (Anon., 2000). Total phenol content was determined by the modified Folin-Ciocalteu method (Wolfe, 2003). Gallic acid was used as the standard compound and the results were expressed as phenol equivalent to gallic acid (GAE), i.e. μg GAE/g_{FW}. The total flavonoids are determined by the method of Kumaran and Karunakaran (Kumaran, 2007), and the total flavonols according to the method of Ordoñez *et al*. (2006). Quercetin was used as the standard compound, and the results were expressed as μg Quercetine (Qc)/g_{FW}. The antioxidant activity in relation to the DPPH radical was determined by the method of Liyana-Pathirana and Shahidi (Liyana-Pathirana, 2005). The modified method of Re *et al*. (1999) was used for the ABTS radical. The results were presented with the TEAC value (Trolox equivalent of antioxidant activity), *i.e.* as μg Trolox/g_{FW}. Total and monomeric anthocyanins were determined by the spectrophotometrically modified "single" pH and by the pH differential method (Sun, 1998). The overall experiments were performed in three parallel repetitions. Analysis of the collected data was done using Microsoft Excel.

Results and discussion

After all, looking at macro and microelements, the results are quite variable. Leaf beet stands out at a noteworthy selenium content and slightly higher content of nitrogen and phosphorus, but at the same time significantly lags on calcium and iron content. Broad live dock is ahead in copper and sodium, has a slightly bigger potassium content in comparison to both species, but lagged behind them on manganese and latterly curled dock on calcium. The curled dock is superior in terms of calcium and iron, has a slightly higher manganese content, but significantly lags behind broad lived dock on copper (Tab. 1). All three species have almost

equal content of β-carotene, while the broad lived dock is inferior to the content of anthocyanins (Tab. 1). With regards to phenols, flavonoids and flavonols, curled dock is ahead, broad lived dock follows, while leaf beet shows the poorest results with observed features. The same is the case with vitamin C and antioxidant properties (Tab. 1).

Table 1: Synthesis of chemical analyzes

Chemical component	Unit	Broad lived dock	Curled dock	Leaf beet
		Mean value		
Macro- and microelements				
N	%	0.49	0.48	0.51
Na	mg/100 g	285	212.05	215.75
K	mg/100 g	404.75	352.15	379.8
Mg	mg/100 g	52.75	91.95	82.16
Ca	mg/100 g	80.9	189.95	52.11
Fe	mg/100 g	9.2	17.5	1.7
P	mg/100 g	43.95	42.75	46.2
Zn	mg/100 g	0.24	0.30	0.29
Mn	mg/100 g	0.27	0.27	0.28
Cu	mg/100 g	0.75	0.29	0.29
Mo	mg/100 g	0.40	0.36	0.39
Co	mg/100 g	0.35	0.31	0.36
Se	µg/100 g	32.3	31.55	46.55
Pb	µg/100 g	BLD	BLD	BLD
Cd	µg/100 g	BLD	BLD	BLD
Hg	µg/100 g	BLD	BLD	BLD
As	µg/100 g	BLD	BLD	BLD
Ni	µg/100 g	BLD	BLD	BLD
Cr	µg/100 g	BLD	BLD	BLD
Phytochemicals				
Anthocyanins	mg/L	15.23	31.43	32.76
β-carotene	mg/100 g	5.07	5.3	5.25
Flavonoids	µgQcE/g_{FW}	1842.30	3604.70	1255.75
Flavonols	µgQcE/g_{FW}	7901.95	8618.60	6642.75
Phenols	µg GAE./g_{FW}	6453.50	7495.35	5644.15
Vitamin C	mg/100 g	3.76	4.18	1.16
Antioxidant activity				
ABTS	µmol Trolox/g_{FW}	15.61	19.39	4.16
DPPH	µmol Trolox/g_{FW}	5159.55	9200.20	1872.55

Conclusions

Wild growing docks species do not lag behind the domestic leaf beet. More so, in many characteristics they show better results in comparison to discussed cultivated (domesticated) species. Our analysis has shown that dock's leaves have significant amounts of phenolic compounds and respectable antioxidant properties. Phytochemical parameters of leaf beet, except for anthocyanins, are bad, and in terms of the antioxidant properties even worse.

Only in the case of macro- and microelements, the results are polycentric. Accordingly, leaf beet predominates in the selenium content. The broad lived dock has advantage in the content of copper and sodium while the curled dock is superior in terms of calcium and iron.
Mentioned characteristics of docks run green parts of these plants as so-called functional foods - the foods that, in addition to its basic nutritional purpose, may also provide additional health benefit.

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CHROMATOGRAPHIC QIAGEN ISOLATION METHOD OF THE DNA MOLECULES BY USING COLLECTION WITH SILICA MATRIX

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Abstract

Quality separation and purification of DNA molecules from the other cell elements is enabled using chromatographic methods in the analysis of DNA. Chromatographic columns with a silicon dioxide matrix (Qiagen Mini Spin Columns) have wide application in the DNA isolation process. An isolate in which the DNA molecule is purified and ready for further analysis can be obtained washing the DNA molecule from matrix columns using the AE buffer. This work aims to determine the optimal volume of the elution AE buffer needed to wash the maximum quantity of isolated and purified DNA molecule from the membrane column in controversial samples. The thirty samples were taken for the experiment. The Fifteen samples head a small amount of biological material which was putted on the microscope plate by short contacts.

The remaining 15 samples were with a higher amount of biological matrix. The sample concentrations were read by the RealTime PCR method. Ten samples were eluted with 200 µl of eluting AE buffer in the isolation process, the next 10 samples were eluted with 100 µl, while the last 10 samples were eluted with 50 µl. The experiments showed that 100 µl of elution AE buffer is optimal volume needed to wash (from the column) the maximum quantity of isolated and purified DNA molecule from the samples which contained very little biological material.The condition for the release of the molecules from silica matrix is its complete hydration. This can be done using required quantity of AE buffer. The silicon dioxide matrix turned out to be excellent sorbent in solid-liquid chromatography. It also satisfied lysing of the cells and release of DNA in the first steps of Qiagen isolation.

Keywords: *DNA molecule, AE buffer, Qiagen Mini Spin Columns.*

Introduction

One of the most important discoveries in human history is certainly the model and chemical structure of DNA molecule. This discovery has enabled a deeper understanding of all biological mechanisms and processes. It also provided numerous answers about the essence of our molecular biology (Butler, 2005*)*.Each cell contains identical genetic material. In DNA analysis regardless of starting material; blood, tissue, skin, hair or bone from one person, an identical result is obtained.

In the processes of analysis of DNA molecules, the starting material is extremely important, which means that the isolated DNA must be well-purified and of high quality (Tan, Huang, Tie, *et al*., 2013.; Xin, Chen, 2012).

The most suitable methods are chromatographic methods (Chacon-Cortes, Griffiths, 2014). The type of chromatography which will be applied depends on the type of material which will be analyzed, and most often several successive methods of chromatography are applied in order to achieve satisfying purification of the necessary components. In the isolation of DNA molecules from plant material, the most commonly used method is a modified method CTAB (cetyl trimethylammonium bromide) (Doyle and Doyle, 1990.) while a slightly less-used modified method is SDS (sodium dodecyl sulfate) (Schweitzer, *et al*., 1995). Isolation of DNA molecules from cells must have three basic steps regardless of which isolation technique

is used. These steps are: cell lysis, removal of other cellular elements from DNA molecules and isolation of DNA molecules (Đorđević, Marković, 1994.; Vujičić, 2002.).
Lysis of the cell is a common step in almost all techniques for isolating DNA molecule, and for this purpose detergents and enzymes, SDS (sodium dodecyl sulfate) and Triton X-100 are used. Other denaturing reagents such as urea, guanidinium salts and chemical haotrops can be used for lysis of cells and inactivation of cell enzymes, but they can reduce the quality and yield of isolated nucleic acids (Walsh, Metzger, Higuchi, 1991). The cellular residues in the first step are removed by centrifugation where the DNA remains in the supernatant, and the cell residues remain in the precipitate. The mixture of DNA and salt in the presence of ethanol concentration of 70-80% or isopropanol concentration 40-50% leads to precipitation of DNA molecules. The excess salt is removed by washing with 70 % ethanol. Finally, the DNA is resuspended in water or TE buffer (Elkins, Forensic, 2013.).
One of the commercial methods that has wide application in almost all laboratories dealing with DNA molecular analysis is *QIAamp® Mini Kit (QIAGEN, Hilden, Germany)*. Columns in the kit have a matrix of silicon dioxide for which DNA bind under the influence of a centrifugal force. In the kit there are lysing detergents ATL and AL, buffers AW1, AW2 and elution AE buffer (Kurabo, 2011).After the initial lysis of ATL and AL buffers and K proteinase, degraded cells with all the elements are applied to the Qiagen column and centrifuged. High concentration of haotropic salts (guanidine hydrochloride, guanidine thiocyanate, urea, lithium perchlorate) destabilize the intermolecular forces formed by positively charged ions (Figure 1.).

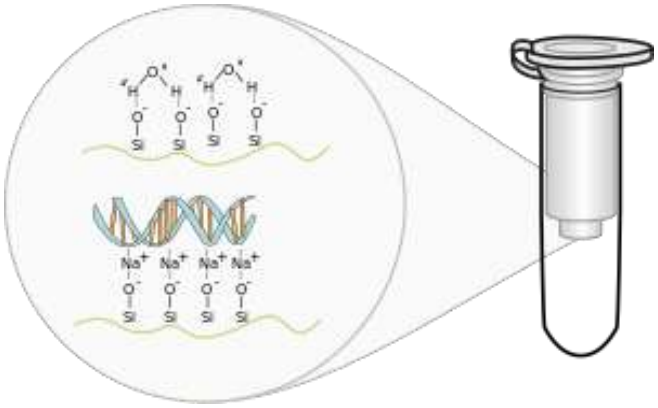

Figure 1. Silicon dioxide on a column with water and with a DNA sample in haotropic buffer

Ions are the connection between the silica matrix of the column (negative charged) and the negatively charged phosphate groups that make up the spine of the DNA molecules. Adding ethanol will further enhance the binding of nucleic acid to the silica membrane (Walsh, Metzger, Higuchi, 1991).
The silica matrix has pores of certain size and density through which all impurities (proteins, divalent cations, positively charged ions including magnesium) and haotropy is passed using centrifugation and rinsing processes of AW1 and AW2 buffers which contain ethanol and different salt concentrations. These solutions will not work on DNA which is attached on the column matrix, but will remove all impurities. The silica membrane DNA molecule is eluted or washed with AE buffer that hydrates the DNA and thus allows the separation from the silica membrane.

Material and Methods

The tests were carried out at the DNA Center for Genetics, in Belgrade. The experiments that have been done aim to establish optimal volume of the elution AE buffer needed to wash the maximum quantity of isolated and purified DNA molecule from samples with Qiagen mini spin colonies. The number of samples which concentrations are read and values obtained using RealTime PCR method were taken as the eligibility criterion.

The thirty samples were taken for the experiment. The Fifteen samples simulated the samples that head a small amount of biological material such as contact marks, where it is assumed that the donor of the biological trail is going to leave a very small amount of biological material. They are prepared in next way:

A biological trace made up of epithelial cells of skin fingers which are excluded as traces on swabs (contact traces) is applied on 15 microscopic plates by direct contact.

- Five samples were (1K2-5K2) were eluted from the 200 µl eluting AE buffer
- The other five samples (1K1-5K1) were eluted from the 100 µl eluting AE buffer
- The last five samples (1K5-5K5) were eluted from the 50 µl eluting AE buffer

The other fifteen samples simulated samples with a little bit more biological material in comparison to the samples described above. This biological material is obtained from fumes, glasses, blood stains, etc.

They are prepared in next way: Traces of saliva were applied on 15 glasses which are excluded as traces on swabs.

- Five samples were (6K2-10K2) eluted from the 200 µl eluting AE buffer
- The other five samples (6K1-10K1) eluted from the 100 µl eluting AE buffer
- The last five samples (6K5-10K5) eluted from the 50 µl eluting AE buffer

The aim of this experiment was to determine the optimal volume of the elution AE buffer needed to wash the DNA molecule from the membrane column in disputable samples.

Results and Discussion

The experiment results are shown in Table 1. From the results in Table 1. it can be seen that the samples that were eluted with 100 µl and 50 µl of AE buffers give similar results.

Minimal volume of the eluting AE buffer needed to wash (from the column) DNA molecules from the samples which contain above the average quantity of biological material such as fumes, blood stains, is 50 µl. Volume of the buffer needed to wash (from the column) the maximum quantity of isolated and purified DNA molecule from the samples which contain very little biological material is 100 µl.

Table 1. Concentrations of samples after elution with different amounts of buffer

Concentrations of samples (ng / µl) after elution with 200 µl of AE buffer - samples of 1K2-10K2	Concentrations of samples (ng / µl) after elution with 100 µl of AE buffer - samples of 1K1-10K1	Concentrations of samples (ng / µl) after elution with 50 µl of AE buffer - samples of 1K5-10K5
-	-	$7{,}04 \times 10^{-3}$
$1{,}1 \times 10^{-2}$	$1{,}33 \times 10^{-2}$	$3{,}66 \times 10^{-2}$
$1{,}09 \times 10^{-2}$	$1{,}29 \times 10^{-2}$	-
$2{,}34 \times 10^{-2}$	$9{,}58 \times 10^{-3}$	$6{,}25 \times 10^{-2}$
-	$8{,}24 \times 10^{-3}$	0,107
-	2,43	1,67
0,151	0,679	1,31
$4{,}3 \times 10^{-2}$	1,12	9,79
-	1,28	9,79

$5{,}15 \times 10^{-2}$	0,207	4.66×10^{-2}

Note: The first five samples in the columns are contact traces applied to microscopic plates, while the other five samples are traces of the saliva applied on glass glasses.

DNA molecule is optimally hydrated with this amount of AE buffer. Also, it is enabled washing from the silica membrane with the same amount of AE buffer. Regardless of the amount of biological material a sufficient amount of DNA molecules was eluted for analysis . The buffer volume of 200 µl of elution is too high. The concentration of isolated DNA molecule dilutes but it's more than enough to wash of residues of haotropic salts that impede elution.

Using the Qiagen method for isolating DNA molecules the certain amount of purified DNA isn't necessary obtained. The obtained amount of purified DNA depends on the amount of DNA molecules in the sample itself.

The silica gel matrix proved to be excellent sorbent in solid-liquid chromatography for the isolation and purification of DNA molecules (Tan, Yiap, 2009). The conditions that must be met in the first steps of Qiagen isolation are high level of haotropic salts and high level of pH which destabilize hydrogen bonds, Van Der Vals forces, and hydrophobic interactions. Also they allow the binding of the negatively charged DNA molecule to the silica matrix. DNA molecules selectively bind, while other molecules directly pass through the column and get removed due to high salt concentration. Adding ethanol allows the washing of salts that interfere with the elution. The additional centrifugation at the end of the process dries the column and liberates it from the residual alcohol that prevents the full hydration of the DNA molecule and its separation from the column. Requirements such as high pH and low salt levels allow the DNA molecule to be released from the column and eluate (Kelly, 2013). DNA molecule is eluted with the different quantities which show us that it is essential to find adequate volume that will achieve the maximum hydration of the molecule which is a condition for releasing it from the silica matrix.

The advantage of the chromatographic method is that the DNA molecule eluted in the AE buffer can be directly used for Real-time PCR (Butler, 2001.; Wong, Medrano, 2005). This method is quickest and no toxic and carcinogenic solutions are used. The problem that can arise is bad selection of the column (inadequately capacity). If a concentration of DNA molecules exceeds the capacity of the column, it can happen that the DNA does not bind to the column and practically wash away from it.

Conclusions

Quality separation and purification of DNA molecules from the other cell elements is enabled using chromatographic methods in the analysis of DNA.

Chromatographic columns with a silicon dioxide matrix (Qiagen Mini Spin Columns) have wide application in the DNA isolation process.

An isolate in which the DNA molecule is purified and ready for further analysis can be obtained washing the DNA molecule from matrix columns using the AE buffer.

The experiments showed that 100 µl of elution AE buffer is optimal volume needed to wash (from the column) the maximum quantity of isolated and purified DNA molecule from the samples which contained very little biological material. The condition for the release of the molecules from silica matrix is its complete hydration. This can be done using required quantity of AE buffer.

The silicon dioxide matrix turned out to be excellent sorbent in solid-liquid chromatography. It also satisfied lysing of the cells and release of DNA in the first steps of Qiagen isolation.

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IMPORTANCE OF ORGANIC PRODUCT CHARACTERISTICS FOR CUSTOMERS IN THE REPUBLIC OF CROATIA

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Abstract

The growth trend of the number of organic producers and of the areas with organic farming in the Republic of Croatia has not been accompanied by the increase in consumer awareness when it comes to the characteristics of organic farming or by the strengthening of the market for organic products in the Republic of Croatia. This paper focuses on determining the motivation, limitations and preferences of consumers regarding their intention to buy organic products as well as their choice of distribution channels. The research covers questions about understanding the differences between organic and conventional products, the meaning of the term "organic product", as well as issues related to the attitudes of the respondents trust in organic products, and the understanding of legislation on organic farming. The survey, conducted between January and April 2018, covered 218 social network users from the Republic of Croatia. The sample was gender balanced, consisting of 52% men and 48% women, of whom 57% were employed, 21% were self-employed and the rest were students and senior citizens. Out of the total number of respondents, more than a half grew up in towns and suburban areas. The results of the research indicate that consumers are insufficiently informed about the legal framework for organic farming with less than a half of the respondents giving a correct definition of an organic product, even though 90% of the respondents believe that there is a difference between organic and conventional farming. When deciding on buying organic products, most respondents consider the health aspects, taste, quality and supporting local production to be more important than the certificate, producer/brand and the packaging/design of an organic product. The results of the research identified the basic limitations that influence the decision to buy organic products and underline the need to strengthen consumer involvement in the enforcement of marketing activities.

Keywords: *organic products characteristics, customer knowledge, preferences, willingness to buy, Republic of Croatia*

Introduction

Organic Agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved (IFOAM, 2005)

The organic market makes up 2% of the total retail in the Republic of Croatia and the consumption of organic products per capita is half the European average (Willer and Lernoud, 2017). Although organic farming has been accompanied by an increase in the number of organic producers and areas with organic farming, certain standards in the use of organic products have still not been reached. If we analyse crops in organic farming, 47% of the surfaces are fields, 41% meadows and pastures, 8% orchards, 4% medicinal herbs, while other surfaces include olive groves, vineyards, fallow land, nurseries and other permanent crops. Vegetables are a crop with the lowest share of agricultural land used in organic production (0.3%). Indices 2015-2016 exhibit a growing trend in the organic farming of livestock except

for pigs and bee colonies, as well as a growing trend of organic products of animal origin (Croatian Bureau of Statistics, 2017). Organic producers in the Republic of Croatia are obliged to maintain their production in accordance with the national and EU legal framework. Organic farming is regulated by the Rulebook on organic farming, which ensures the implementation of the European Union directives on the production, labelling, monitoring, control, marketing and import of organic products. Clearly prescribed obligations and responsibilities points to the established chain of supervision, control and sanctioning of the production and sale of organic products from seedlings and seeds to the final product.

The distribution of organic products is realized through direct, indirect and emerging channels of distribution. The same also largely applies to organic products available at markets, in shopping malls and supermarkets as well as in direct sales on the producers' farms. A wider range of organic products is available in specialized stores that are mainly located in major urban centres and within specialized fairs held several times a year in larger cities. In recent years, several new distribution channels have emerged: Internet sales, groups of solidarity exchange, eco-market and placement through tourism. The domestic organic market is not organized, partly due to an insufficient volume of production, limited processing capacities, insufficient consumer awareness and the insufficient promotional and marketing activities, which, depending on the type and the extent of organic farming, the size of the economy and the distribution channel used, can make some organic products practically invisible in the organic market. Gugić et al., (2017) points out that domestic market is not adequately controlled, resulting in the misuse of the terms "eco" or "bio" by retailers. According to the latest research, consumers buy organic products most frequently at city markets and in supermarkets, and somewhat less often in specialized stores or directly from the producers (Anić at al., 2015). In Bosnia and Herzegovina, Croatia and Slovenia, most consumers buy organic products occasionally and it's mostly fresh fruits and vegetables (Cejak et al., 2010). Renko and Bošnjak (2009) identified the lack of marketing skills of producers, weak cooperation and communication and lack of consumer awareness as obstacles in the development of the organic market, and TV, newspapers and magazines as the most important sources of information on organic products. Furthermore, Croatian producers of organic food have not adapted their assortment and choice of locations to the consumers who point out the lack of information on where to buy organic products as the biggest obstacle. Tolušić (2006) points out that consumers are interested in organic products because of their beneficial effects on health but due to unresolved distribution channels in Eastern Croatia, they buy these products less often.

The objective of this paper is to identify the basic limitations which affect the consumer's decision to buy organic products in the Republic of Croatia.

Material and Methods

The research was based on secondary data sources and primary data collected by a survey conducted between January and April 2018 covering 218 (n = 218) social network users in the Republic of Croatia. In addition to the sociodemographic characteristics of customers (gender, work status, place of growing up, economic status of family) questions were asked about understanding the difference between organic and conventional products, the meaning of the term "organic product", the reasons for buying organic products, the characteristics of organic products which affect the decision to buy them, the frequency of buying, the willingness of customers to pay higher prices for organic products, future intentions of buying organic products, satisfaction with the offer of organic products in the Republic of Croatia. Respondents were asked to share their opinions on understanding legislation which regulates organic farming as well as on the labelling of organic products in the Republic of Croatia. The sample was gender balanced, consisting of 52% men and 48% women, of whom 57% were

employed, 21% were self-employed and the rest were students and senior citizens. Out of the total number of respondents, more than a half grew up in towns and suburban areas, 51% have a higher education, and two thirds of respondents consider the economic status of their family to be very good or good.

Results and Discussion

Organic farming is one of the fastest growing agricultural sectors in the world, with a double figure annual growth rate of land plots with organic farming as well as an increase in the production volume and the number of organic producers (Ummyiah et al, 2017). In a ten-year period, the share of surfaces with organic farming in the Republic of Croatia has increased by 5.4%, and was accompanied by a rise in the number of organic producers (Table 1). This growth trend has not been accompanied by the trend of consumer awareness on the characteristics of organic farming nor by the strengthening of the market for organic products in the Republic of Croatia.

Table 1. Organic farming in the Republic of Croatia – 2007 - 2016 / surface, producers

Year	Utilized agricultural area	Surfaces with organic production	Share of surfaces with organic production as part of utilized agricultural areas,%	Number of organic producers
	ha	ha		
2007	1,201,756	7,577	0.63	477
2008	1,289,091	10,010	0.78	632
2009	1,299,582	14,193	1.09	817
2010	1,333,835	23,282	1.75	1,125
2011	1,326,083	32,036	2.42	1,494
2012	1,330,973	31,904	2.40	1,528
2013	1,568,881	40,660	2.59	1,609
2014	1,508,885	50,054	3.32	2,194
2015	1,537,629	75,883	4.94	3,061
2016	1,546,019	93,814	6.07	3,546

Source: Ministry of Agriculture; Data processing: Croatian Bureau of Statistics, 2017

The research results point to a lack of consumer information on the legal framework for organic farming, more than a half point out that they have no understanding of the legal framework for organic farming and only 12% of respondents consider it to be well-defined, although 90% of respondents believe that there is a difference between organic and conventional farming. In the survey, 1.3% of respondents stated that they do not know the difference between organic and conventional products, a quarter of the respondents consider products from small local farms and products with protected designations of origin (PDO) and

protected geographical indications (PGI), one third of respondents defines organic products as healthy food and 40% of respondents gave a correct answer to the question of defining organic products. The same is confirmed by Gugić et al. (2017) who point out that consumers generally prefer traditional homemade products with a certain quality that are produced using organic principles. Nevertheless, a poor understanding of the nature of organic products makes the consumer identify all local products as organic ones. The respondents decided on the importance of the characteristics that influence their decision to buy organic products on a scale from 1 to 5 where 1 signified a completely insignificant characteristic, whereas 5 a very important one. The respondents consider the health aspects, good quality and taste as well as support for the local production as the most significant characteristics, whereas they care less about the packaging and the design, producer, brand and the shelf life of the product. They don't consider the organic product certificate as significant to making the decision to buy these products (Table 2). The respondents provided an average grade on their understanding of the organic market (3.1) and 4% of them expressed their high satisfaction with the organic products on offer in the Republic of Croatia. Martić Kuran and Mihić (2014) also identify subjective norms, attitudes and health awareness as the best when it comes to predicting if consumers will buy organic products. Hamzaoui-Essoussi and Zahaf (2012) explored the needs and preferences of consumers and determined how they affected them: (1) behavioural factors - knowledge and trust, (2) lifestyle - life according to the principles of sustainable development, and (3) support for the local production. They all affect the decision to buy organic products so the researchers made a distinction between three types of organic products buyers: true, occasional and inexperienced. Arvola et al. (2008) identify subjective/moral norms, positive behaviour, and a self-rewarding feeling as predictors for buying.

Table 2. Importance of organic product characteristics for customers in the Republic of Croatia

Characteristic	Average grade*	Standard deviation
Health aspect	4.51	0.81
Good quality	4.46	0.78
Taste	4.06	1.16
Shelf life	2.97	1.12
Certificate	3.67	1.28
Producer/brand	2.94	1.30
Product from local production	4.12	1.11
Design of the packaging	2.59	1.17
High price	3.32	1.16

**1=not important at all, 5=very important; Source: Author's calculation based on the questionnaire survey results*

Judging from the results of the research, subjective norms linked to healthcare, support for local production and producers, sense of support for ecological balance and biodiversity need to be upgraded by informing and educating consumers about the processes of organic farming and the characteristics that differentiate an organic product from a conventional one. Using an eco-label as confirmation of an organic product needs to be more strongly used in promotional activities. Brčić-Stipčević and Petljak (2012) point to the need of educating and informing consumers about the valid legal framework for organic farming and to the activities that would contribute to the development of the organic market in Croatia in order to raise consumer awareness of environmental legislation and consumer trust in organic products. The strategy of eco-marketing should be oriented towards the management of the product and the brand (Babović et al., 2005). The strategy requires the understanding of factors affecting its

applicability, so by taking the survey into account it is important to involve consumers by identifying their needs, possibilities and requirements and also by setting up distributive channels, which will enable a constant availability of organic products at all times. Of the total number of respondents, two-thirds stated that they were prepared to pay 10-30% more for organic products. Research has shown a weak consumer involvement and understanding of the offer and availability on the organic market, and since the organic market, as well as other markets, is influenced by laws of supply and demand, it is important to diversify supply and finalize organic products as well as understand the needs of the consumers in order to strengthen it. In his research, Petljak (2013) concludes that larger organic producers distribute their products through supermarkets and the meat industry, while smaller organic producers mostly offer their products for a lower price in order to prevent product degradation and production losses. Above mentioned indicates the poor positioning on the market of small organic producers, but also the need to adapt them more to market demands with the aim of a common market entry and/or choice of a distribution channel that would ensure their market visibility and make their products available to consumers. To achieve this, the increase in the level of knowledge about organic products with consumers is imperative.

Conclusions

The main research questions seeks to analyse importance of organic product characteristics for consumers in the Republic of Croatia that influence the decision to buy organic products. According to the research that was carried out, the health aspect, the quality and the taste of organic products as well as supporting local production are all identified as important characteristics of an organic product that influence the consumer's decision to buy them. A weaker evaluation of the organic product certification characteristic may indicate a lack of awareness when it comes to organic production and the characteristics of an organic product. An insufficient level of knowledge of the legal framework regulating organic production, weak consumer involvement and understanding of the offer and availability on the organic market can pose a certain limitation that influences the decision to buy organic products. Another limitation may also be posed by the impact of the "local production support" characteristic in correlation with 60% of respondents who did not give the exact definition of an organic product indicating the lack of differentiation when it comes to an organic product from local production and a product with a protected designation of origin and geographical indication. All of this points to the need for conducting more informative and marketing activities to raise the awareness of the characteristics of organic production and organic products. The same should become the keystone in the planning and implementation of organic product management activities by manufacturers, processors and distributors. Furthermore, any institutional activity directed towards the implementation of measures for educating and informing the consumer about organic production would provide additional support to the organic production sector.

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EFFECT OF CHEMICAL FERTILIZERS REPLACEMENT BY COMPOST ON PEANUT PRODUCTIVITY GROWN UNDER WATER DEFICIT IN EAST OF EL-EWINAT (EGYPT)

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Abstract

Crops productivity is limited due to low fertility level, water holding capacity, organic matter of soil and higher evaporation rate when the crops grown in sandy soils. For these reasons application of compost in sand soil can improve crop production and their resistance to abiotic stress such as water deficit, salinity and extreme temperature. Two field experiments were carried out at Agricultural Research Station, East of El-Ewinat (Egypt) during 2015 and 2016 seasons to investigate the effects of the replacement of chemical fertilizers by compost on peanut productivity grown under water deficit conditions. A randomized complete block design (RCBD) using a split-plot arrangement with three replications was applied. Two water treatments (100% and 70% from the amount of water consumption for peanut) were randomly assigned in the main plots. Five treatments of chemical fertilizers, compost and their combinations were randomly allocated in the sub-plots. The results showed that the peanut yield and its attributes were significantly increased in all studied parameters in both seasons with the replacement of chemical fertilizers by compost. This result indicated that compost improved plant resistance to water deficit due to water availability and use, and improved nutrients availability which appeared critical in improving peanut performance.

Key words: *Compost, chemical fertilizers, nutrient, peanut, water deficit.*

Introduction

Safe agriculture is one of the main attitudes in the world. Also, there has been an increasing awareness of the undesirable impact of chemical fertilizers on the environment, as well as the potentially dangerous effects of chemical residues in plant tissues on the health of human and animal consumers. Therefore, organic fertilizers are very important in plant fertilization in many countries including Egypt due to their beneficial effects on the soil, growth and increase the productivity as well as improving the quality of plant production.

Peanut (*Arachis hypogaea* L.) is an important oil crop in the Egypt. The cultivated area of peanut in Egypt during 2013 season was about 42,000 hectares (FAO, 2013). The production of vegetative oils is considered one of the major economic problems in Egypt because the produced oil amounts do not satisfy the increased demands by the population. Additional, the limited cultivated area to grow oil crops as well as their competition with other strategy crops in the agricultural rotation restricts the insufficient produced amounts of this oil. Although oil consumption in Egypt is about 2 million tons' year^{-1}, there is a shortage of 98 %. Thus increasing oil seed crops is an important aim in Egyptian agriculture. It is successfully cultivated in the newly reclaimed soils which commonly suffers from the deficiency of most nutrients due to the low organic matter content, high $CaCO_3$ content and high soil pH. In addition to increase farming Egypt is known as a heavy consumer of chemical fertilizer and intensive agricultural development and environmental change have led to severe land degradation in this soil. To overcome the problems of these soils and improve the fertility levels, such as compost, organic manures and bio-fertilizers as well as chemical fertilizers should be applied to these soils. Application the compost firstly for improving biological,

physical and chemical properties of soil and secondary to get high and clean agricultural yield produced free from undesirable high doses of heavy metals and other pollutants and improves growth and yield of crops and this is attributed to the better utilization of phosphorus by the mycorrhiza, enhanced nodulation by Rhizobium and better N nutrition.

Water stress is the most critical environmental factor that limits crop production (Bowden *et al*. 2010). Peanut is an important subsistence and cash crop in the semi-arid tropics where it often suffers from drought stress. Yield losses due to drought are highly variable in nature depending on timing, intensity, and duration, coupled with other location-specific environmental stress factors such as high irradiance and temperature. Studies have shown that water deficit stress effects morphological parameters in the form of decreased plant height, leaf area, root length, growth and yield, as well as physiological features in the form of decreased net photosynthesis, chlorophyll content, and water-use efficiency. Other important effects of water stress on plants are the decrease in leaf water potential and turgor loss, closure of stomata, as well as decrease in internal CO_2 concentration and CO_2 assimilation. Generally, water deficit stress or drought stress leads to a decrease in growth of susceptible plants. The initial resistance mechanism of plants to water stress is stomatal closure to prevent further water loss. Stomatal closure decreases the internal CO_2 concentration in the leaf and inhibits (Rubisco) enzyme activity, which ultimately decreases net photosynthesis (Rahbarian *et al*. 2011).

Materials and Methods

Two field experiments were carried out at Agricultural Research Station, East of El-Ewinat, New Valley governorate, Egypt, during 2015 and 2016 seasons under sprinkler irrigation system to investigate the effects of the replacement chemical fertilizers by compost on peanut productivity grown under water deficit conditions. Some soil physical and chemical properties of the experimental site that were determined according to the methods described by **Jackson (1967)** before sowing are present in Table 1.

Table 1: Some physical and chemical properties of representative soil samples (0-30 cm depth) of the experimental site before sowing for 2015 and 2016 seasons.

Soil property		2015*	2016*
Particle - size distribution			
Sand	(%)	73.32	72.45
Silt	(%)	18.46	19.13
Clay	(%)	8.22	8.42
Texture grade		Sandy loam	Sandy loam
EC (1:1 extract)	(dS m^{-1})	1.87	1.92
pH (1:1 suspension)		7.62	7.54
Total $CaCO_3$	(%)	8.65	8.72
Organic matter	(%)	0.057	0.063

* Each value represents the mean of three replications.

A randomized complete block design (RCBD) using a split-plot arrangement with three replications. Peanut seeds (cv. Sohag-110) at a rate of 50 kg $fed.^{-1}$ (fed. = 4200 m^2) were inoculated with *Rhizobium spp*. at sowing on May 4^{th} and 6^{th} in the 1^{st} and 2^{nd} seasons, respectively. Peanut seeds (2-3 seeds) were deposited in rows 50 m length, 60 cm width and about 20 cm spacing between plants within rows and then the plants were thinned after complete emergence (one week from planting) to one plant. The treatments were arranged in two variables in a split plot design with three replications. The first variable was represented by the two different water supply (100% and 70% from the amount of water consumption for

peanut) were randomly assigned in the main plots. The second variable five treatments of different amount of chemical fertilizers or compost and their combinations were randomly allocated in the sub-plots are present in Table 2.

Table 2: A mount of chemical fertilizers or compost and their combinations.

Treatments	N (kg N fed.$^{-1}$)	P (kg P fed.$^{-1}$	K (kg K fed.$^{-1}$)	Compost (Ton fed.$^{-1}$)
100% NPK + 0.0% compost F_1 (as control)	33.5	31	48	0.0
75% NPK + 25% compost F_2	25.10	23.2	36	3.0
50% NPK + 50% compost F_3	16.75	15.5	24	6.0
25% NPK + 75% compost F_4	8.35	7.7	12	9.0
100% compost F_5 (no chemical fertilizer)	0.0	0.0	0.0	12.0

The recommended dose of chemical fertilizers NPK (33.5: 31: 48) were used as controller. Calcium super phosphate (15.5 % P_2O_5) at a rate of 200 kg fed^{-1} added before sowing and during soil preparation and potassium sulphate (48% K_2O) at a rate of 100 kg fed^{-1} added with first dose of N. Nitrogen fertilizer was added at rate of 100 kg fed^{-1} ammonium nitrate (33.5% N). All plots received different amount of chemical fertilizers or compost and their combinations at 15 days before sowing during their preparation for planting.

The amount of water consumption for peanut at East of El-Ewinate government used I_1= 100% as a normal irrigation and I_2 = 70% as a moderate water stress in this experiment according to Abdel-Mawgoud *et al*. (2007), El-Koliey *et al.* (2001) and Mohamed (2007). Sprinkler irrigation system used underground water followed by 120 mints of I_1= 4000 m^3 fed.$^{-1}$ and 84 mints of I_2 = 2800 m^3 fed.$^{-1}$ with flow rate of 60 m^3/h at operating pressure of 400 kPa.). Foliar application of micronutrient combinations was carried out on the plants at 30 days and 60 days after planting at a level of 200 and 300 L fed.$^{-1}$, respectively. Wheat was the preceding crop in both seasons. All agricultural practices for peanut production as well as chemical fertilizer quantity were followed as recommended by Agricultural Research Centre for peanut production at the experimental site. Water application of 25 mm was applied over the entire field area after planting to enhance germination and ensure uniform growth. Irrigation management strategies (every 2 days) were initiated at the second irrigation. An additional light irrigation of about 20 mm was applied to the entire field shortly (two weeks) before uprooting to improve the soil turn over and to minimize pods loss. Plants were manually uprooted and trashed with a hand trash after air drying of about 3 days.

At harvest (120 days after sowing), a sample of 10 guarded plants from each sub-plot were randomly taken in four replications and the plant height (cm), number of pods plant^{-1}, pods weight plant^{-1} (g), seed yield plant^{-1} (g) and 100-seed weight (g) were recorded. Shelling (%) was determined as follows:

-Shelling percentage (%) = Seed yield (kg fed.$^{-1}$) /Pods yield (kg fed.$^{-1}$) X 100.

- Oil percentage (%): dried mature seeds were ground into very fine powder to determine oil % using Soxhelt apparatus and diethyl ether according to A.O.A.C. (1980).

- Oil yield (kg fed.$^{-1}$) = Oil % X Seed yield (kg fed.$^{-1}$)

The analysis of variance was carried out according to Gomez and Gomez (1984) using MSTAT computer software. Means of the different treatments were compared using the least significant difference (LSD) test at the 0.05 level of probability.

Results and Discussion

1. Effect of water deficit:

The results in Tables 3 and 4 showed that the moderate water deficit (70% amount of water consumption) had significantly decreased on all studies treaties except oil%, as compared to

normal irrigated (100% amount of water consumption). The highest main values of plant height (51.36 and 52.83 cm), number of pods plant^{-1} (34.53 and 35.80), pods weight plant^{-1} (34.23 and 34.88 g), seeds weight plant^{-1} (23.42 and 24.64 g), 100-seed weight (70.26 and 71.50 g), shelling (68.34 and 70.56%) pods yield (1432 and 1429 kg fed.$^{-1}$), seeds yield (979.01 and 1009.88 kg fed.$^{-1}$) and oil yield (504.21 and 536.56 kg fed.$^{-1}$) since it's obtained by normal irrigated (100% amount of water consumption) in the first and second season respectively. On other hand, the lowest values of above traits were observed by water stress (70% amount of water consumption), while, there was not significant effect on oil percentage. Yield and quality of peanut were decreased when peanut is deprived of water is high during the last 3 to 6 weeks of the pod maturation period. Furthermore, insufficient water during the growing season may reduce plant growth, normal root expansion, pod growth, and fruit development; however, excessive water can cause excessive vegetative growth and a greater leaf area index, but restrict root growth and development, resulting in decreased pod yield. Drought stress has been the major environmental factor contributing to the reduced crops productivity worldwide. Water deficit affects thylakoid electron transport, phosphorylation, carboxylation and photosynthesis. Drought affects membrane lipids and photosynthetic responses and yield in peanuts. Changes in the lipid content and composition are common in water-stressed plants and this increases membrane permeability. This causes damage and membrane disruption as well as reduction in photosynthesis. Maintaining membrane integrity under water deficit conditions will determine the plants resistance towards stress. Plants have several mechanisms for adaptation to water stress including stomatal conductance and osmotic adjustments. Drought stress has adverse influence on water relations, photosynthesis, mineral nutrition, metabolism, growth and yield of peanut. There are some critical points characterized peanut growth stages, flowering and pod filling growth stages are very sensitive to soil water availability compared with early and vegetative and late maturity growth stages. Water deficit imposed during the vegetative stage of peanut achieved greater final yields and increased water use efficiency and dry matter production including economic yield. Mohamed (2007) found that the seasonal actual applied amounts of irrigation water were 980, 1960, 2940 and 3920 m^3 fed.$^{-1}$ while, the seasonal measured depleted water was 960.83, 1718.92, 2113.10 and 2414.88 m^3 fed.$^{-1}$ when irrigated with 15, 30, 45 and 60 min every 2 days, respectively. The results are in agreement with those obtained in chickpea by Abou Kheira, 2009, Kamal *et al*., 2010, Rahbarian *et al*., 2011 and Amiri *et al*., 2017.

2- Effect of replacement chemical fertilizers by compost:

The presented data in Tables 3 and 4 clearly showed that fertilizer treatments significantly increased all the studied parameters. The data also indicated that the effect of F_4 fertilizer treatment (25% NPK + 75% compost) was more pronounced than other fertilizer treatments as well as the control (NPK). The highest main values of plant height (59.34 and 59.73 cm), number of pods plant^{-1} (39.47 and 40.67), pods weight plant^{-1} (38.92 and 39.40 g), seeds weight plant^{-1} (26.25 and 29.42 g), 100-seed weight (77.25 and 78.65 g), pods yield (1366 and 1479 kg fed.$^{-1}$) and oil yield (510.56 and 577.32 kg fed.$^{-1}$) since it's obtained by F_4 fertilizers treatments in the first and second seasons respectively, as well as, the highest main values of shelling (74.76%) and seeds yield (1105.77 kg fed.$^{-1}$) in the second season only. On other hand, the lowest values of above traits were observed by water stress (70% amount of water consumption). The obtained results revealed that, compost associated with chemical fertilizer mixture significantly increased all growth characters in both seasons compared to control treatment (plants received recommended dose of 100% NPK). Concerning this, it was outstanding that chemical fertilizer treatment unaccompanied with compost, also donate results significantly superior to results of control plants in all study traits during both seasons. Also results in Tables 3 and 4 demonstrated that increasing compost from 6 up to 12 ton caused significant increase in the aforementioned characters due to the application of compost

which could be attributed to either its direct effects by increasing the availability and supplying of nutrients or to its indirect effects by modifying soil physical properties that can improve the root environment, increase plant uptake of nutrients and consequently stimulate plant growth. Curtis and Classsen (2005) reported that the application of compost increased the amount of water available to a plant, its leaf water content, and plant biomass under drought stress.

Table 3: Effect of replacement of chemical fertilizers by compost on some studied traits of peanut productivity grown under water deficit conditions in 2015 and 2016 seasons.

Irrigation (%)	Fertilizers treatments	Plant height (cm)		Number of pods		Pods weight (g plant^{-1})		Seeds weight (g plant^{-1})		100-seed weight (g)	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100		51.36	52.83	34.53	35.80	34.23	34.88	23.42	24.64	70.26	71.50
70		45.73	47.01	26.31	26.77	25.23	25.84	15.92	16.35	56.58	57.25
F test		*	*	**	**	**	**	**	**	**	**
100	F_1 (control)	46.60	47.67	32.20	33.47	31.70	31.47	21.38	22.16	67.65	66.12
	F_2	52.13	54.93	34.20	35.73	34.37	36.12	24.65	25.26	69.46	72.37
	F_3	56.27	58.27	36.27	37.67	36.65	37.26	26.15	27.45	73.50	75.67
	F_4	59.34	59.73	39.47	40.67	38.92	39.40	26.25	29.42	77.25	78.65
	F_5	42.47	43.53	30.53	31.47	29.52	30.13	18.67	18.91	63.42	64.71
70	F_1 (control)	40.53	41.13	23.27	24.73	23.55	24.18	14.26	14.85	54.52	55.17
	F_2	45.67	46.87	25.80	26.42	24.78	25.57	15.56	16.23	56.24	56.87
	F_3	47.27	49.60	27.60	27.47	24.68	25.27	17.12	17.27	57.52	57.85
	F_4	50.73	52.47	28.40	28.87	27.98	28.43	17.42	17.75	58.47	59.18
	F_5	44.67	45.00	26.47	26.40	25.15	25.72	15.23	15.67	56.14	57.16
F test		**	*	**	*	*	**	*	**	*	**
LSD 0.05		0.65	0.94	0.28	0.53	0.52	0.72	0.40	0.47	0.32	0.52

*, ** and N.S. indicate significant, highly significant at 0.05, 0.01 and insignificant, respectively.
F_1 = 100% NPK (as a control)
F_2 = 70% NPK + 25% compost
F_3 = 50% NPK + 50% compost
F_4 = 25% NPK + 75% compost
F_5 = 100% compost

Table 4: Effect of replacement of chemical fertilizers by compost on some studied traits of peanut productivity grown under water deficit conditions in 2015 and 2016 seasons.

Irrigation (%)	Fertilizers treatments	Shelling (%)		Pods yield (kg fed.$^{-1}$)		Seeds yield (kg fed.$^{-1}$)		Oil (%)		Oil yield (kg fed.$^{-1}$)	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100		68.34	70.56	1432	1429	979.01	1009.88	51.57	53.17	504.21	536.56
70		63.52	63.51	959.87	973	609.83	618.7	51.77	53.22	315.35	329.13
F test		**	**	**	**	**	**	-	-	**	**
100	F_1 (control)	67.75	71.06	1389.52	1387.67	941.26	985.53	52.65	53.77	495.48	529.75
	F_2	71.99	70.17	1444.61	1452.33	1038.58	1018.90	51.26	53.34	532.39	543.50
	F_3	70.83	73.78	1486.24	1461.67	1043.99	1078.19	50.24	53.15	524.49	573.01
	F_4	67.60	74.76	1366.43	1479.33	1003.88	1105.77	50.86	52.21	510.56	577.32
	F_5	63.52	63.03	1389.35	1366.33	867.33	860.99	52.82	53.36	458.10	459.21
70	F_1 (control)	60.89	61.70	932.67	942.33	567.81	580.95	52.84	53.95	299.98	313.32
	F_2	63.11	63.70	953.67	962.30	601.47	612.98	51.47	53.52	309.61	328.40
	F_3	69.99	68.56	968.33	983.67	677.78	674.60	50.34	52.86	341.28	356.56
	F_4	62.79	62.46	996.33	1024.67	625.46	640.04	51.23	52.36	320.42	335.14
	F_5	60.83	61.13	948.33	956.33	576.62	584.93	52.97	53.43	305.42	312.53
F test		*	*	**	**	**	**	N.S.	N.S.	**	**
LSD 0.05		2.01	2.61	3.71	4.33	19.98	31.51	-	-	10.94	16.72

*, ** and N.S. indicate significant, highly significant at 0.05, 0.01 and insignificant, respectively.
F_1 = 100% NPK (as a control)
F_2 = 70% NPK + 25% compost
F_3 = 50% NPK + 50% compost
F_4 = 25% NPK + 75% compost
F_5 = 100% compost

These results provide a plausible mechanism for how the combination of compost and chemical fertilizer together led to increase growth parameters over control, this raise spring from their beneficial effects on seedlings represented in nutrients availability and improvement of soil physical, chemical and biological properties resulted in more water retention simultaneously with available elements to be absorbed by plants roots and its insightful effect on the physiological processes such as photosynthesis activity as well as the utilization of carbohydrates. In contrast to mineral fertilizer, adding organic matter to soil improve soil structure, nutrient retention, aeration, soil moisture holding capacity and water infiltration (Deksissa *et al*., (2008). The results are in agreement with those obtained by El-Kramany *et al*., 2007, Sujanya and Chandra 2011 and El-Saady *et al*., 2014.

Conclusion

From the previous results, it is concluded that adding 25% the recommended dose of the chemical NPK fertilization with the 75% organic fertilization compost at a rate of 12 ton fed^{-1} gave the highest values of the traits studied under conditions of sandy soil and sprinkler irrigation system used underground water at East of El-Ewinat (Egypt).

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CONSUMER BEHAVIOR TOWARDS ORGANIC PRODUCTS OF ANIMAL ORIGIN-CASE STUDY: CONSUMERS FROM GREEK REGION OF THESSALY

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Abstract

Greece is a country with a great tradition in livestock farming, which contributes decisively to the country's regional rural development. In recent years, consumers' demand for organically grown products of animal origin has increased. An appropriate legislative framework has been set up, both in primary (plant and animal production sectors) and secondary (processing, standardization and distribution units) to ensure compliance with requirements and production of safe and quality organic products. The present study was conducted in April through May 2018, in central Greek region of Thessaly, which covers an area of about 14000 square kilometers. The region was selected because livestock farming was and still is the basis of the regional economy. In order to explore the knowledge and preferences of Thessalian consumers about organic products of animal origin, four hundred questionnaires were completed by randomly selected individuals, in which respondents were asked to answer key questions about their willingness to trust organic products of animal origin. Results indicate that 92.6% of the respondents are aware of organic products of animal origin, 40.1% of the respondents claimed to purchase organic products of animal origin once a month, mainly directly from the producers, while most (25.4%) have been informed about organic products of animal origin from the internet. An inhibiting factor for organic product market is shown to be high prices. The present research provide detailed statistical information in order to develop an integrated picture of the market and availability of organic products of animal origin in the region of Thessaly.

Keywords: *Organic farming, organic products of animal origin, consumers*

Introduction

Greek tradition in livestock farming is extended, and its contribution to regional rural development decisive. A global shift of consumers towards organic livestock products has been noted in recent years. According to Brown (2000), health concerns, which have been arisen by food scandals, is a powerful factor in turning consumers into seeking a more 'ethical food production'. Still, other studies by Sriman and Forman (1993), Davis *et al.* (1995) report the environmental awareness factor as the main incentive to consume organic products.

Consumers buy organic products because organic farming and organic livestock farming are recognized as a safe food production method that responds to consumer concerns about safe and quality food, animal welfare, environmental protection and sustainable development (Woese *et al.*, 1997, Williams and Hammitt, 2000).

Organic farming (farming and livestock farming) is often referred to as the highest food production system as it is the only one that holistically protects and respects the environment, soil and consumers (Lampkin and Measures, 1995; Fotopoulos, 2000).

For organic production, an appropriate legislative framework has been set up both in the primary (plant and animal production sectors) and secondary (processing, standardization and

distribution units) to ensure compliance with requirements and to produce safe and quality organic products. Extensive farming systems implemented in Greece are largely in line with the organic production requirements under Regulation (EC) 834/2007, therefore Greek livestock farming can respond to new food market trends.

Organic livestock farming exhibits dynamic growth rates. According to Greek Ministry of Rural Development and Food data (Ministry of Rural Development and Food, 2018) organic livestock has reached 1.242.466 farm animals in total, in 2016. Most of the organically farmed animals are sheep (47.8%), goats (26.3%), hens (19.5%), cattle (6%) and pigs (0.4%). There is also an increase in organic crops areas, utilized for feeding of farm animals. Pastures primarily used for animal feeding at an organic stage account for 185.363 hectares. The total number of crops under organic farming producing feed amounts to 8.552 hectares. In addition, there is an increase in the number of enterprises processing organically farmed raw materials, namely 44 enterprises are involved in the processing and preservation of organically produced meat products and 65 enterprises in the production of organic dairy products. An important incentive for changing producers' mindset was the increased consumer knowledge over production methods and their efforts towards consumption of healthier food.

The objective of the present paper is to investigate the behavior of consumers in the organic market of animal origin products, given the growing consumer demand for healthier and safer food, and always considering that consumers appreciate the added assurance of these products.

Material and Methods

The survey was based on the collection of data on the behavior of Thessalian consumer with regard to organic products of animal origin, using a questionnaire formulated for the purpose of this work. The present study was conducted in central Greece, in Thessaly region, which covers an area of about 14000 square kilometers, in order to explore the knowledge and preferences of Thessalian consumers about organic products of animal origin. Livestock farming is the basis of the Thessalian economy. To this end, 400 questionnaires were completed, in which respondents were asked to answer key questions about their willingness to pay for organic products of animal origin. The face-to-face survey was conducted from April to May 2018. Data were processed using the SPSS Statistics, ver. 17 statistical program.

Results and Discussion

The largest part of respondents had an average age of 41-50 years, 51% of the respondents were men and 49% women, 3% primary education graduates, 29% secondary education, 7% graduates of technical education, while the remaining 61% graduated higher education. The largest number of respondents, i.e. 60%, are married. Also, 50% of respondents declare annual family earnings of 10,000-20,000 euros, 29% less than 10,000 euros, 17% 20,000-30,000, and only 4% annual earnings over 30,000 euros.

The largest percentage, i.e. 92.6% of respondents, are aware of the existence of organic products of animal origin and have been informed about this: from the internet (25.4%), SMEs (23.5%), friends (22.7%), press, i.e. newspapers, magazines (14.9%) and specialists (13.5%). With regard to the frequency of purchasing organic animal products (Figure 1), it appears that most of the respondents, i.e. 40.1%, buy them rarely (once a month), then 24.1% sometimes buy (1 time / 15 days), 21% never buy, 10.5% often buys (once / week) and 4.3% always buys (> once per week).

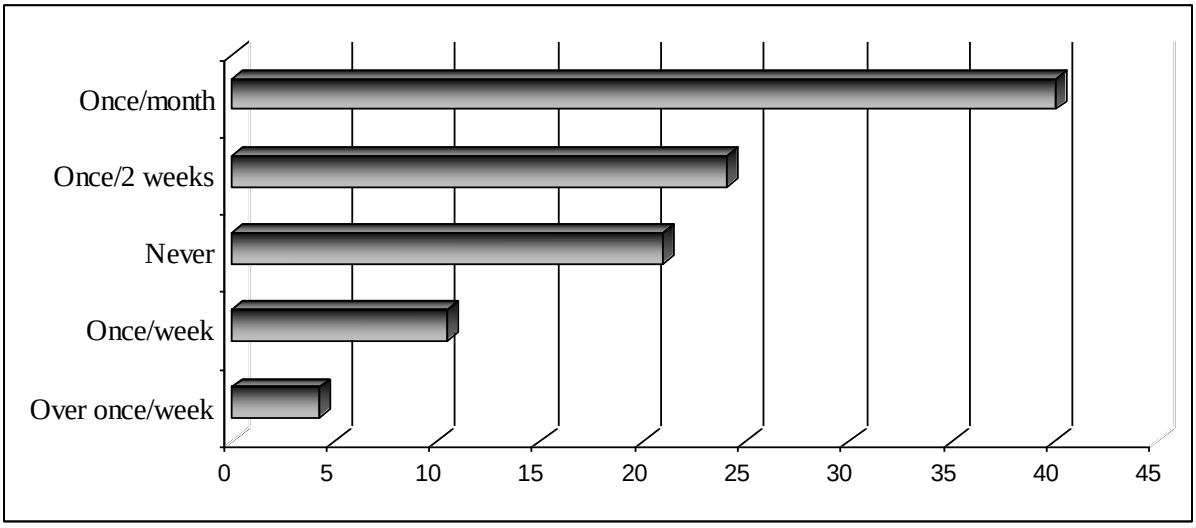

Figure 1. Sample distribution based on market frequency

Most of the respondents (62.6%) are aware of organic products of animal origin in the market. The most frequently purchased organic products by Thessalian consumers are eggs (30.9%), white meats, i.e. chicken, turkey (26.5%), dairy products (21.7%), red meats, i.e. beef, pork, lamb, goat (19.3%) and cured meats (1.6%). A large part of consumers (40.1%) buy organic products of animal origin directly from producers.

With regard to the incentives for consumers to buy organic products of animal origin (Table 1), the highest rates of agreement are observed in the perception of best quality (healthier, safer, higher nutritional value and fewer residues, i.e. pesticides, hormones, antibiotics).

Table 1. Incentives motivating consumers to buy organic products of animal origin

Topics	**Average scoring**
Appearance	2.0
Healthier	3.2
Better quality	3.2
Higher nutritional value	3.1
Better taste	2.9
Safety	3.1
Environment-friendly	2.9
Avoiding genetically modified organisms	2.9
Country of origin	2.7
Less residues (eg. pesticides, hormones, antibiotics)	3.1
More prestige	2.4
Discounted prices or offers	2.0

Average scoring: 1- Totally Disagree, 2- Disagree, 3- Neither Agree / Disagree, 4- Agree, 5- Totally Agree

According to the results of the survey, the reasons that prevent consumers from purchasing organic products of animal origin (Table 2) can be observed in respondents' perceptions of high price, lack of market availability and distrust over the labeling of organic products.

Table 2. Reasons for preventing consumers from purchasing organic products of animal origin.

Topics	**Average scoring**
High Price	3.8
There is no widespread availability in the market	3.7
The same tasting satisfaction as conventional products	2.7
Distrust towards organic products	3.3
Lack of good labeling of organic products	3.3
Lack of trust in the labeling of organic products	3.5
Lack of difference in quality between conventional and organic products	2.4

Average scoring: 1- Totally Disagree, 2- Disagree, 3- Neither Agree / Disagree, 4- Agree, 5- Totally Agree

Conclusions

This research has shown that all consumers are aware of the existence of organic products of animal origin and eight out of ten consumers declare they have purchased those kinds of products. Organic products of animal origin are mainly supplied directly by the producers, probably due to the lack of trust in labeling of organic products.

It seems that the high price prevents consumers from buying organic products. Designing a more favorable pricing policy for consumers is expected to result in an increase in demand.

Consumer perceptions are particularly positive for organic meat, as it is considered to be of better quality, healthier, safer, with a higher nutritional value and fewer residues (eg. pesticides, hormones, antibiotics). High prices are responsible for reduced frequency of purchase, which in a large percentage does not exceed once a month.

We therefore conclude that high price in organic meat is a factor that prevents a portion of consumers interested in organic products and its benefits for health from purchase. In particular, there was found a strong relationship between the organic market, if available at an affordable price and the non-purchase of organic meat.

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ORGANIC FARMERS' MARKETING STRATEGIES IN TUSCANY, ITALY

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Abstract

Organic products reach the Italian consumers through several channels, from on-farm sale to internet, from Solidarity Purchase Groups to supermarkets. Nearly 85% of Italians affirm to have purchased organic food in the last month, with fruits and veggies being the most mentioned, but most consumers admit being very occasional buyers. A survey was performed in the province of Arezzo (southern Tuscany), including 55 producers, randomly extracted from a list of 550 organic farmers belonging to the Italian Confederation of Agricultural producers, a union of small and medium size mostly conventional farmers. With a questionnaire covering 16 variables, data collection was realized from June 2017 through February 2018, via face to face interviews, telephone calls and emails. These farms are mostly family run, with the support of some workers. Most farmers converted only recently, with a minority being organic before 1992. 25 percent did not yet entirely convert. The production systems are diversified, with vineyards, olive and fruit trees, pastures and arable fields, used for several crops. Many have animals: dairy cows, cattle, goats, sheep and small animals. This allows the on-farm production of wines, olive oil, cheeses, jams, breads and cakes, and other foods, frequently used in the on-farm restaurants. That helps the on-farm sale, considered the best modality by 26% of respondents, followed by local markets, specialized organic shops, SPGs and internet. Advertising is done via website, participation in local and national fairs, printed media, small billboards along the roads, and participation in international fairs. 56 percent say that the local market is growing, and 45 percent consider positively the presence of organic foods in the supermarkets.

Keywords: *Direct marketing, Diversification, Agritourism.*

Introduction

This paper explores the behavior and opinions of a group of 55 organic farmers, representative of small and medium size producers in Southern Tuscany and it shows how appropriate strategies allow linking with local consumers, without forgetting international markets. Compared with the situation only a few years ago (Santucci and Pignataro 2002), organic agriculture, with its different approaches, is growing everywhere in Italy (Gamboni and Moscatelli 2015), accompanied and / or stimulated by an ever-increasing market demand (Idda, Madau and Pulina, 2008; Zanoli and Naspetti. 2005). The world market in 2017 has reached €10^9 101, with Europe (39 10^9€) and USA (41) absorbing the largest shares. Very many organic products are nowadays available almost everywhere in Italy. National total consumption was about €10^9 4.7, after Germany (9.6) and France (6.5). Still, the individual expenditure in 2015 was only 38€, much lower than in France (83) or Germany (106) and extremely far from Switzerland (262). This means that there are large margins for expansion, to be explored by farmers, processors and retailers. Organic products reach the consumers through several channels (Santucci 2012), from direct sales at the farm to internet, from Solidarity Purchase Groups (Schifani and Migliore 2011, Maestripieri 2017) to private labels of supermarkets (Santucci and Diotallevi 2015). Large retailers, both Italian and foreign, have entered into this market: 22 supermarket chains propose an average of 130 items each. A specialized, organic only, supermarket chain is fast expanding, from North to South, and it

counts now 256 points of sale. Public procurement, i.e. public purchase for schools, hospitals, and communities is also increasing.
Italian consumers are attracted to buy organic products mainly for "selfish motivations". Italians are scared by residues of fine chemicals, hormones, GMOs, and consider that proper food habits can protect their health and that of their family members (TNS Opinion & Social 2012). Other drivers, like protection of nature, animal welfare and sympathy for farmers, are much less important. A growing number of consumers is looking for locally produced products, also known as "zero miles" or "short food chain" (Scalvedi and Saba, 2018).
85 percent of Italians affirm to have purchased organic products in the last month, with fruits and veggies being the most mentioned. However, only 2 percent of respondents buy only organic, whereas most consumers admit being very occasional buyers. National production, although supported by the EU Rural Development Policies, is not sufficient. While the organic area has grown annually by 6.5 percent, the imports have exploded by 49% annually.

Materials and methods

Tuscany is one of the 20 Italian regions and is located at the center of the country. It includes nine provinces, one of which is Arezzo. The last official figures (December 31st, 2016), indicate there were 4,502 certified organic farms, out of which 1,403 had also some sorts of processing plants (SINAB 2017). This figure positions Tuscany at the 4th place, in terms of number of producers, and at the 6th place for the size of the area grown organically, amongst the 20 Italian regions. Tuscany, and of course its Arezzo province, is famous for the cultural value of its towns (Florence, Siena, Pisa, Lucca, Pistoia, just to mention a few), for the richness of its museums, for the beauty of its landscapes and for the quality of many food products. Many products have received a Geographical Indication and are also exported worldwide (wines, cheeses, olive oil, meats, special cereals and beans, etc.). Thanks to agritourism and to a continuous promotion, rural Tuscany attracts visitors from all over the world and is a main destination for food and wine lovers, who can enjoy 22 routes organized to discover wines, olive oils, and local products.
Within this scenario, a survey was carried out in the province of Arezzo (Southern Tuscany), including 55 producers, randomly extracted from a list of 550 organic farmers belonging to the *Confederazione Italiana degli Agricoltori* (Italian Confederation of Farmers), a major small and medium size farmers' union. The sample size (10%), is large enough to ensure that in the worst possible scenario we have a probability of 0.88 to have an error of estimation smaller than 10% (Iarossi 2006). With a questionnaire covering 16 variables, the data collection was realized from June 2017 through February 2018, via face to face interviews, telephone calls and emails. A total of 88 farmers has been contacted, but 33 did not cooperate, advancing different motivations. In case of denial, the next name in the list was contacted. The 16 variables include:

1. Total farmland (ha)
2. Organic share (%)
3. Year of first organic certification
4. Area of different crops and orchards (ha)
5. Animal productions (n. of heads)
6. On farm processing
7. Human resources
8. Agritourism (rooms and apartments)
9. On farm restaurant
10. Certification body
11. Main point of sale for organic output
12. Methods for promotion and communication

13. Participation in groups and associations
14. Opinion about local consumers
15. Opinion about presence of organic items in supermarkets
16. Likely future changes in the farm

Results and discussion

As expected, the farms are mostly family run, with the support of some permanent and seasonal workers. Most farmers have converted only recently (Table 1), with a minority being organic before 1992, when the first EU direct subsidy to organic areas was introduced by the EU Regulation 2078/92 (the subsidies are still existing as Measure 11 of the Common Agricultural Policy Pillar 2).

Table 1. Year of conversion

Year	%
Before 1992	5.5
1992 - 2000	12.7
2001 - 2010	36.4
After 2010	45.5
Total	100.0

These organic farms show interesting dimensions (Table 2), much larger than the average. This demonstrate that organic management (as well as EU subsidies) is not only attracting smallholders, but also large producers who are looking for alternatives.
About one fourth (23,6%) have not yet entirely converted.

Table 2. Area under organic management

Dimension (ha)	%
< 10	35.0
11 - 20	15.0
21 - 30	25.0
31 - 40	2.5
41 - 50	5.0
> 50	17.5
Total	100.0

As suggested by organic agriculture experts and for increasing technical and economic resilience, the production systems are diversified (Table 3), with pastures, vineyards, olive trees, and arable fields, these latter ones used in rotation for several crops.
Many farms have animals: dairy cows, cattle for meat production, goats and sheep for both milk and meat, and small animals (Table 4).
This allows the on-farm production (Table 5) of wines, olive oil, cheeses, jams, breads and cakes, and other foods, frequently used in the on-farm restaurant.
As written before, agritourism is a very common diversification strategy, that increases job opportunities, adds value to row output, improves agricultural income and in general the livelihood of farmers.

Table 3. Land use (ha, organic area)

Land use	%
Pastures	23.3
Olive trees	20.5
Vineyards	17.0
Orchards	9.9
Cereals	9.7
Fodder grasses	9.2
Industrial crops	6.3
Veggies	4.1
Total	100.0

Table 4. Farms with animals (n=55)

Species	Farms %	Heads (n.)
Dairy cattle	21.8	627
Meat cattle	10.9	185
Sheep	9.1	365
Goats	10.9	480
Pigs	9.1	87
Horses	9.1	19
Small animals	14.6	135

Table 5. Farms with processing activities (n=55)

Products	%
Wines	40.0
Olive oil	38.2
Cheeses	25.5
Jams	18.2
Other products	9.1

25 percent of our respondents declare to have a small restaurant, generally open in winter only on week-ends and every day during the spring-summer months. 38.2 percent also offer some types of hospitality, ranging from a simple room with bathroom to fully furnished apartments, where clients can even cook their own meals.

This helps the on-farm sale, considered the best modality by 26 percent of respondents, followed by internet, local markets, specialized organic shops, Solidarity Purchase Groups (SPGs) (Table 6). The on-farm sale to middlemen and processors is still appreciated by the largest producers, who must market large quantities of output, while smaller producers tend to prefer a direct contact with the final clients. Advertising for the farms and their products and services, and search for new clients (Table 7) are mainly done via the website, nowadays with pages also in foreign languages (English, French, German, and occasionally Russian and Japanese), printed media (also in several languages), participation in local and national fairs, small billboards along the roads near the farm, and participation in international fairs.

Most respondents (70.9%) belong to groups and associations for the promotion of their organic products. As a matter of fact, many are members of the *Associazione Nazionale Agricoltura Biologica (ANABIO)*, established by the *Confederazione Italiana Agricoltori*, and many are also members of the *Associazione Italiana Agricoltura Biologica (AIAB)*, an independent organic association.

Table 6. Best modality for marketing

Modality	%
At the farm, to processors and traders	27.3
At the farm, to consumers	25.5
Through internet, to consumers	16.4
At local open markets	10.9
To organic shops	10.9
To Solidarity Purchase Groups	9.1
Total	100.0

Table 7. Promotion and communication

Modality	%
Website of the farm	90.9
Trade fairs in Italy	81.8
Leaflets and other printed materials	74.6
Billboards along the roads	40.0
Trade fairs abroad	36.4
Sponsoring events	34.6
Registered trademark	16.4
Ads transmitted by local radios	9.1

The general attitude about the evolution of the market is positive: 56.4 percent consider that the local market is growing, while 43.6 percent think that it is constant. Nobody indicates a negative trend. Another interesting point is that the growing presence of organic foods in the supermarkets is not considered a dangerous menace by most respondents, but rather an opportunity (Table 8). 45.5 percent of the respondents believe that the offer of standardized, industrial, organic items will increase the number of consumers and will then push the consumers to look for "more natural", "locally produced" "you know who makes it" products, to be purchased at the local markets, at the farms or via internet.

Table 8. Presence of organic products in supermarkets (n=55)

Opinion	%
Positive, it will expand the market	45.5
Indifferent, the clients of supermarkets are not my clients	36.4
Negative, I am losing clients	18.2
Total	100.0

It comes consequently obvious that almost all respondents are planning investments and reorganizations in their business (Table 9). Many have already applied for grants and subsidies included in the Tuscany's Rural Development Plan 2014-2020, while many others are elaborating farm development plans with the help of technical and financial consultants.

Table 9. Future changes (n=55)

Innovations	%
Capital investments to diversify / to improve my productions	56.4
Reorganize marketing	36.4
Improve / change the organization of work, for family and farm hands	32.7

Conclusion

This study confirms that organic farmers are nowadays a thriving force within the Italian agricultural sector. Supported by the Regional and European policies and thanks to the growing demand, organic producers are dynamic and positive. Organic farmers have increased their resilience, through diversified activities, that include agri-tourism, on farm processing, and smart marketing strategies, that prefer local and direct consumers, but do not neglect middlemen and foreign markets. The increasing number of organic items offered by supermarkets is not generally considered a menace, but an opportunity, because it will expand the number of consumers and their curiosity towards organic food.

This dynamism is confirmed by the structural and organizational innovations planned for the next future, that the Regional Governments implementing the EU Rural Development should favour and support.

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THE INFLUENCE OF LIQUID ORGANIC FERTILIZERS FORMS ON ECOLOGICALLY GROWN POTATOES

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Abstract

Investigation of the effect of liquid organic fertilizers on organically grown potatoes were carried out in light clay loam, shallow gleysol carbonaceous illimerised soils - *Calc(ar) i-Epihypogleyic Luvisol - LVg-p-w-cc* - at the experimental station of Aleksandras Stulginskis University in Lithuania in 2013-2014. The experiments were carried out with liquid organic fertilizers Biokal 1 and Fitokondi, in the manufacture of which herbal extracts were used; fertilizers Fertenat and Ruponis made from biohumus, and fertilizers Humistar rich in humic and fulvic acids. The forms of liquid organic fertilizers had an uneven influence on organic potatoes. The potatoes, which were sprayed with Biokal 1 fertilizer, total yield of potato tubers essentially increased by 0.89-1.47 t ha^{-1} or 5.34-9.14%, and marketable yield increased by 0.89-1.54 t ha^{-1} or 10.24-19.15% compared to spraying with fertilizers Fitokondi, Humistar, Fertenat and Ruponics, and no significant differences in the output of marketable yield were determined. Under the influence of Biokal 1, the content of dry matter in potato tubers compared to spraying with Ruponics and the starch content compared to spraying with Ruponics, Humistar and Fertenat substantially increased, and the content of nitrates in potato tubers significantly decreased compared to potato spraying with fertilizers Ruponics and Humistar. After spraying potatoes with fertilizer Biokal 1, the number of one-stem tubers compared to spraying with Humistar, Fertenat and Ruponics, substantially increased.

Keywords: *potatoes, organic farming, liquid organic fertilizers, yield, chemical composition.*

Introduction

Compared to intensive farming, in organic farming, the products unpolluted with synthetic chemicals are grown; synthetic pesticides are not used and the environment is not polluted; the fertilization with manure, compost and other organic materials increases soil fertility (Rembiałkowska, 2007; Mansour *et al.*, 2009). The cultivation of organic potatoes (*Solanum tuberosum* L.) in the world has considerably widened due to their demand on the market. On Western European organic farms, potato cultivation plays an important role in organic farming. Potatoes are grown not only for food, but also for processing (Finckh *et al.*, 2006; Haase *et al.*, 2007). Organic potatoes accumulate less nitrates in comparison with those grown intensively; the content of dry matter, vitamin C, phenolic compounds, amino acids, total sugars and minerals increases in tubers (Hamouz *et al.*, 2005; Maggio *et al.*, 2008; El-Sayed *et al.*, 2015). The investigation carried out in Lithuania showed that all fertilizers reduced the amount of starch in tubers, but increased the total amount of sugar and nitrates: most content was in potatoes, which received the highest nitrogen content (Baniūnienė and Žėkaitė, 2007). When fertilizing through leaves, the shortage of nutrients in plants was reduced during a critical moment. The plants fertilized additionally through the leaves absorb about 50% of nutrients in the first six hours (Budzynski *et al.*, 2003). Leaf fertilizers did not increase the yield of potato tubers; calcium, boron and amino acids containing fertilizers Boramin Ca, potassium and nitrogen fertilizers Final K reduced the nitrate content in potato tubers, while

the leaf fertilizer Delfan containing amidic nitrogen and amino acids increased it. Leaf fertilizers had no effect on starch content in tubers, and the content of dry matter in tubers was mainly increased by Boramic Ca fertilizers containing calcium, boron and amino acids (Staugaitis and Laurė, 2008). The investigation conducted at the Centre for Agroecology of Alexandras Stulginskis University showed that the yield of organically grown agricultural plants under the influence of liquid organic fertilizers Bijododžio and Biokal 1 was not only increasing, the quality of the production grown was improving, but also the exposure of plants to diseases was reduced (Sliesaravičius *et al.,* 2006; Pekarskas, 2008).

The aim of the paper is to investigate and evaluate the influence of different types of liquid organic fertilizers on the yield of organically grown potatoes, the chemical composition of tubers and elements of yield structure.

Material and Methods

Total nitrogen content found in the soil during the investigation conducted in light clay loam, shallow gleysol carbonaceous illimerised soils - *Calc(ar) i-Epihypogleyic Luvisol - LVg-p-w-cc.* - at the experimental station of Aleksandras Stulginskis University, in Kaunas region, in Lithuania in 2013-2014, containing alkaline (7.1-7.2), low content of humus (1.79-1.83%) (organic carbon found 1.04-1.06%), average content of phosphorus and potassium (146.2-142.0 and 121.0-126.0 mg kg^{-1}), total nitrogen in the soil was (0.123-0.141%).Soil samples were collected prior to experiment setting up from the depths of 0-20 cm, by three replications, and were analysed at the Agrochemistry Research Laboratory of the Lithuanian Centre of Agrarian and Forest Sciences. The pH of the soil was determined by a potentiometric method (ISO 10390:2005), organic carbon by dry combustion method (ISO 10694:1995), humus - organic carbon content was multiplied by a factor of 1.724, mobile phosphorus (P_20_5) and potassium (K_2O) A-L (Egner-Riemann-Domingo method), total nitrogen Kjeldahl (ISO 11261:1995).

Experimental scheme: 1. not sprayed; 2. Sprayed with Biokal 1 10+10+10 l ha^{-1}; 3. Sprayed with Fitokondi 10+10+10 l ha^{-1}; 4. Sprayed with Fertenat 10+10+10 l ha^{-1}; 5. Sprayed with Humistar 10+10+10 l ha^{-1}; 6. Sprayed with Ruponics 10+10+10 l ha^{-1}. Potatoes were sprayed with liquid organic fertilizers on the surface of formation of main stems (BBCH 22-24), during the stages of the growth of main stem (BBCH 35-37) and the end of flowering (BBCH 6N9). The investigations were carried out with early potatoes *Fakse* (Denmark) breed plants. Seed rate was 2.5 t ha^{-1}. The total experimental field size - 14 m^2 (3.5×4) and accountabl area-9 m^2 (3×3). Test variants in repetitions were arranged randomly. Plant protection measures against diseases and Colorado beetles were not used. If was necessary, the Colorado beetles were removed mechanically from potato plants. Potatoes were planted with potato planter. The number of one-bush stem and the length of one-bush stem was determined by measuring 10 potato bushes in all variants of three repetitions. The number of one-stem tubers, the mass of one-stem tubers and the mass of one tuber was determined in all variants of three reperitions after digging all potatoes of 5 stems that were counted and weighed. After digging potatoes of all four repetition variants, potatoes were weighed and sorted. The tubers with diameters of 50-70 mm were classified as marketable potatoes. The potatoes of smaller diameter were assigned to the group of seminal (35-50 mm) and feeding potatoes (25-35 mm). The chemical composition of potato tubers was investigated in the Agrochemical Research Laboratory of the Lithuanian Agrarian and Forest Sciences Centre. The dry matter content was determined by heating to 105 °C until constant weight, starch - by comparative weight, nitrates - by ionometric method, nitrogen content using the Kjeldahl apparatus, phosphorus content by spectrometric method with ammonium molybdate, and potassium content by flame photometric method.

Liquid organic fertilizers Biokal 1 consist of 57% of herbs and 38% of biohumus extract, 5% of essential oils and mineral water. Herbal extract consists of the following herbs: the common nettle (*Urtica dioica* L.), the common horsetail (*Equisetum arvense* L.) and the greater celandine (*Chelidonium majus* L.). Biocal 1 chemical composition: 230.0 mg l^{-1} N; 370.0 mg l^{-1} P_2O_5; 480.0 mg l^{-1} K_2O; 110.0 mg l^{-1} Ca; 30.0 mg l^{-1} Mg; 10.0 mg l^{-1} Fe; 50.0 mg of l^{-1} Co; 100.0 mg l^{-1} Cu; 5.0 mg l^{-1} Se, pH 7.8. Fitokondi contains 80% of aqueous extract of medicinal plants, 13.3% of aqueous extract of biohumus, 6.6% of potassium soap (50.0 ml l^{-1}) and 0.1%. - essential oils (eucalyptus oil - 1.5 ml l^{-1}). In the production of liquid organic fertilizers Fitokondi, extracts of seven plants are used: the common nettle (*Urtica dioicia* L.), the common comfrey (*Symphytum officinale* L.), the shingle oak bark (*Quercus imbricaria*), the common horsetail (*Equisetum arvense* L.), the grand wormwood (*Artemisia absinthium* L.), the tagetes (Tagetes) and the greater celandine (Chelidonium majus L.). Chemical composition of Fitokondi: 0.02 g l^{-1} N; 0.01 g l^{-1} P_2O_5; 0.15 g of l^{-1} K_2O; 0.02 g l^{-1} Ca; 0.01 g l^{-1} Mg; 50.0 mg of l^{-1} Co; 100.0 mg l^{-1} Cu; 5.0 mg l^{-1} Se, pH 7.0, dry matter - 1.0%. Fertenat is manufactured using cavitation technology and biohumus of horse manure. Chemical composition of Fertenat: 1.51% total nitrogen; 104.30 mg kg^{-1} P; 212.40 mg kg^{-1} K; 81.53 mg kg^{-1} Mg; 341.0 mg kg^{-1} Ca; 0.45 mg kg^{-1} Cu; 3.93 mg kg^{-1} Zn and 0.35 mg kg^{-1} Mo. Humistar is made according to a special technology using organic substances and contains 132.0 g l^{-1} off humic acids, 33.0 g l^{-1} of fulvic acids, 55.0 g l^{-1} K_2O with pH of 13.0. Ruponics is made from biohumus of bovine manure using a special technology. Ruponics contains 1.0% of dry and 2.10 g of l^{-1} of humic substances, pH value 8.5-9.5. Chemical composition of Ruponics: 2.00% N; 1.60% P_2O_5; 4.00% K_2O; 0.62 g l^{-1} Ca; 1.04 g of l^{-1} Mg; 150.0 mg of 1^{-1} Fe; 0.06 mg l_{-1} S; 0.44 mg of l^{-1} Cu; 5.10 mg l^{-1} Zn; 2.70 mg l^{-1} Mn; 41.00 mg l^{-1} Mo and 6.20 mg l^{-1} B. Data were statistically evaluated by dispersive analysis method using the ANOVA program (Clewer, Scarisbric, 2001).

Results and Discussion

The investigations revealed that the largest total (17.58 t ha^{-1}), marketable (9.58 t ha^{-1}) yield of potato tubers, seed potatoes (3.28 t ha^{-1}) and the largest output of marketable yield was obtained after spraying the potatoes three times with liquid fertilizer containing herbal plant extract Biokal 1. The minimum total and marketable tuber yield received after spraying with liquid organic fertilizer made from biohumus Ruponics, and the smallest marketable output, but the maximum feed potato yield (5.30 t ha^{-1}) was received after spraying with biohumus fertilizer Fertenat. The total amount of liquid organic fertilizers investigated, as compared to non-sprayed potatoes, significantly increased the total and marketable yield of tubers, and the yield of the total output was substantially increased only after spraying potatoes with fertilizer Biokal 1. After spraying potatoes with fertilizer Biokal 1, total (0.89-1.47 t ha^{-1} or 5.34 to 9.14%) and marketable (0.89-1.54 t ha^{-1} or 10.24 to 19.15%) tuber yield substantially increased comparing to spraying with fertilizers Fitokondi, Humistar, and Fertenat and Ruponics, and no significant differences in the yield of the output was determined. After spraying potatoes with liquid organic biohumus fertilizer Ruponics, the total potato yield compared to spraying with Biokal 1 and Fitokondi substantially decreased, while the marketable tuber yield compared with the spray with Biokal 1, Fitokondi and Humistar. Compared to non-sprayed potatoes, the essential increase in the yield of seed potato tubers was conditioned only by spraying with fertilizers Biokal 1, Fitokondi and Ruponics. When comparing the liquid organic fertilizers among themselves, no significant differences between the yield of seed and feeding potatoes were determined (Table 1).

A number of investigations with liquid organic fertilizers have been carried out in Lithuania. The investigations were carried out in both ecological and intensive farming systems, involving most plant species. They were very unevenly effective, as well as low efficient

(Sliesaravičius *et al.,* 2006; Staugaitis and Laurė, 2008). Previous investigations have shown that liquid organic fertilizers Biokal 1 are very effective for various organically grown agricultural plants. Under their influence, the crop yield increased substantially and had a significant influence on the chemical composition of plants (Sliesaravičius *et al.,* 2006; Pekarskas, 2008). This is confirmed by these investigations. Liquid organic fertilizers were of uneven effectiveness in the cultivation of potatoes. The use of herbal extracts in the production of liquid organic fertilizers increases their effectiveness. The effectiveness of liquid organic fertilizers with herbal extract depends on their chemical composition. Fertilizers Biokal 1 were richer in nutrients and their efficacy was higher than Fitokondi.

Table 1. The effect of liquid organic fertilizers on the productivity of organic potato tubers and the output of marketable yield

Treatment	Total yield t ha^{-1}	Marketable yield (50-70 mm) t ha^{-1}	Output of marketable yield %	Seed potatoes (35-50 mm) t ha^{-1}	Potatoes for feed (25-35 mm) t ha^{-1}
unsprayed	14.40	6.88	47.78	2.42	5.11
Biokal 1	17.56	9.58	54.56	3.28	4.71
Fitokondi	16.67	8.69	52.13	3.11	4.88
Fertenat	16.31	8.14	49.91	2.86	5.30
Humistar	16.47	8.67	52.64	2.85	4.95
Ruponics	16.09	8.04	49.97	3.01	5.05
LSD_{05}	0.50	0.60	5.34	0.51	0.98

After spraying potato with liquid organic fertilizer containing herbal extract Biokal 1, the dry matter content in potato tubers compared to spraying with Ruponics, starch content compared to spraying with Ruponics, Humistar and Fertenat significantly increased, and content of nitrates in potato tubers was significantly reduced compared to spraying potatoes with fertilizers Ruponics and Humistar. Liquid organic fertilizers did not significantly affect the accumulation of nitrogen, phosphorus and potassium in potato tubers (Table 2). Investigations in Lithuania have shown that the influence of liquid organic fertilizers on the chemical composition of plants can be very diverse. They can have little effect on changes in the chemical composition of plants or have a significant effect thereto (Sliesaravičius *et al.,* 2006; Staugaitis and Laurė, 2008, Pekarskas, 2012). Investigations have shown that liquid organic fertilizers according to individual elements of chemical composition under irregular influence as well as their effectiveness can be very different.

Table 2. The effect of liquid organic fertilizers on the chemical composition of organic potatoes

Treatment	Dry matter %	Starch %	Nitrates mg kg^{-1}	N, %	P, %	K, %
unsprayed	20.11	15.35	118.10	0.23	0.051	0.35
Biokal 1 10+10+10 l ha^{-1}	20.17	16.00	109.25	0.24	0.056	0.36
Fitokondi 10+10+10 l ha^{-1}	20.02	15.75	110.60	0.24	0.051	0.35
Fertenat 10+10+10 l ha^{-1}	19.87	15.55	109.45	0.24	0.051	0.34
Humistar 10+10+10 l ha^{-1}	19.87	15.45	114.35	0.24	0.056	0.36
Ruponics 10+10+10 l ha^{-1}	19.72	15.35	113.45	0.23	0.052	0.36
LSD_{05}	0.40	0.22	3.14	0.04	0.012	0.033

The largest number of one-bush stems, their length, the number and the mass of one-stem tubers were obtained after spraying potatoes with liquid organic fertilizers containing herbal extracts Biokal 1 and Fitokondi, and the biggest mass of one tuber was determined in potatoes sprayed with Humistar and Ruponics. Spraying potatoes with fertilizers Biokal 1, Fitokondi and Humistar essentially extended one-bush stems, and when spraying with all the liquid organic fertilizers tested, the number of one-stem tubers and their mass, as compared to non-sprayed potatoes, increased substantially. Liquid organic fertilizers did not have a significant effect on the number of single-leaf stems and the mass of one tuber. Comparing the influence of liquid organic fertilizers on the values of the yield structure indicators, it was found that after spraying potatoes with fertilizer Biokal 1, the number of one-stem tubers compared to spray with Humistar, Fertenat and Ruponics substantially increased. Liquid organic fertilizers did not have a significant impact on the number of one-bush stems, their length, the mass of one-stem tubers and the mass of one tuber (Table 3).

Table 3. The effect of liquid organic fertilizers on structural elements of potato yield

Treatment	Number of stems per plant	Length of stems per plant cm	Number of tubers per plant	Weight of tubers per plant g	Weight of a single tuber g
Unsprayed	3.92	33.82	12.72	441.75	36.45
Biokal 1 10+10+10 l ha^{-1}	4.12	37.88	16.61	554.50	35.16
Fitokondi 10+10+10 l ha^{-1}	4.12	37.54	16.25	543.05	35.50
Fertenat 10+10+10 l ha^{-1}	3.98	35.96	14.90	526.50	36.64
Humistar 10+10+10 l ha^{-1}	4.08	37.06	15.17	536.75	36.75
Ruponics 10+10+10 l ha^{-1}	4.02	35.85	14.36	516.00	37.53
LSD_{05}	0.25	2.71	1.10	48.33	4.06

Conclusion

Spraying potatoes with liquid organic fertilizers containing herbal extracts Biokal 1 resulted in significant increase of total and marketable yield of potato tubers compared to spraying with fertilizers Fitokondi, Humistar, Fertenat and Ruponics, and no significant differences in the output of marketable yield were found. Under the influence of Biokal 1, the content of dry matter in potato tubers compared to spraying with Ruponics, the starch content compared to spraying with Ruponics, Humistar and Fertenat substantially increased, and nitrate content in potato tubers compared to potato spraying with fertilizers Ruponics and Humistar significantly decreased. Liquid organic fertilizers did not significantly affect the accumulation of nitrogen, phosphorus and potassium in potato tubers. After spraying potatoes with fertilizer Biokal 1, the number of one-stem tubers compared to spraying with Humistar, Fertenat and Ruponics substantially increased. Liquid organic fertilizers did not have a significant effect on the number of one-bush stems, their length, the mass of one-stem tubers and the mass of one tuber.

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ORGANIC FARMING IN MOLDOVA - SOLUTION FOR REBALANCING THE SOIL RESOURCES AND ENVIRONMENT

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Abstract

Organic farming is quite developed around the world, but in recent years it has begun to increase in the Republic of Moldova, with relatively modest results compared to EU countries. At present, only 2% of the land is managed as "organic", and organic products exports account more than 11% of agricultural exports. Organic farming recorded a significant increase, from 80 ha in 2003 to 61280 ha in 2012, due to state support (subsidies) and a decrease with the reduction of subsidies. For these reasons, a significant number of agricultural farmers have renounced to organic farming, which has led to a reduction in the number of organic farmers, from 185 in 2009 to 27 in 2016. Another issue of expanding organic farming is irrational use and degradation of soil resources, resulting in lower productivity and profitability of this sector, especially for small farmers, and for rebalancing the soil resources and environment. Organic farming is performing well in this respect, it keeps all technological components, but corrected according to soil requirements, as: conservation tillage, without the physical degradation of soil; organic fertilization based on the use of local organic waste, plant debris and residues from agriculture; biological methods for the control of pests and diseases, as well as agro- and phytotechnical methods for weed control. All actions during a fairly short period have been established and are being used successfully. So the soil is healthier and at the same time the human health, consuming an organic natural product is ensured, and the environment and soils are in an ecological balance.

Keywords: *Organic farming, Subsidies, Soil Degradation, Moldova.*

Introduction

Organic production is a global farming and food production system that combines best environmental practices, a high level of biodiversity, the conservation of natural resources and the application of high standards of animal welfare. It is a method of production that is growing in the European Union states, the Republic of Moldova registering much lower results in this chapter. For Moldova, organic agro-food production and its marketing is a real chance of penetrating foreign markets, as the demand for organic agricultural products is increasing. This can be considered as a new opportunity for agricultural exports.

Organic farming has begun to develop in the world in 1940 and in Moldova since 2003. In 2005, Law No.115 on organic production was approved, and in 2006 - Decision No.149 for implementation of the Law on Production agro-food industry. For comparison, the largest share of organic land (24%) is recorded in Denmark, but in Moldova, organic farming is practiced only on 2% of the total area of agricultural land (Ecologizarea, 2015). In an agrarian country with 3/4 of chernozems and favourable climate conditions, such as the Republic of Moldova, organic farming must have the highest priorities and advantages.

The purpose of the research - to analyze the situation regarding organic farming, the dynamics of areas and number of producers who practice organic farming and to highlight the barriers and factors that lead to the cessation of these direction.

Materials and methods

For the purpose of an in-depth research into the dynamics of the development of organic agriculture in the Republic of Moldova, methods such as: analysis, comparison, analogy, synthesis, observation, grouping and consultation of specialized literature were used. It should be noted that official statistics do not present separate data on organic production. At the same time, there are no statistics on the volume of organic products exported, certified by international / EU bodies, and not by the national accreditation system.

Results and discussion

In 2017, agriculture generated 12.2% of the gross domestic product of the Republic of Moldova and about 21% of the total number of jobs. The given sector offers livelihoods for a significant part of the population, contributing to 50% of total national exports (Indicatori, 2018). However, the performance of the agricultural sector is increasingly affected by factors related to social, economic and environmental dynamics. Internal or external workforce migration, primarily caused by low wages, has reduced the availability of human capital for rural development. Irrational use and degradation of natural resources, including water and soils, results in lower productivity and profitability in this sector, especially for small farmers (Leah, 2010, 2017).

The general trend in the agricultural sector is also reflected in the development of organic farming. Organic farming in Moldova has contributed significantly to global agricultural exports. While only 2% of the land is managed "organic", exports to organic products account for more than 11% of agricultural exports. In the period 2003-2014, organic farming has seen a significant increase in the area used, from 80 ha in 2003 to 55365 ha in 2014 (Agricultura, 2015), thanks to the state's financial support (subsidies) and a decrease of those with the reduction of subsidies starting with 2015 (Figure 1).

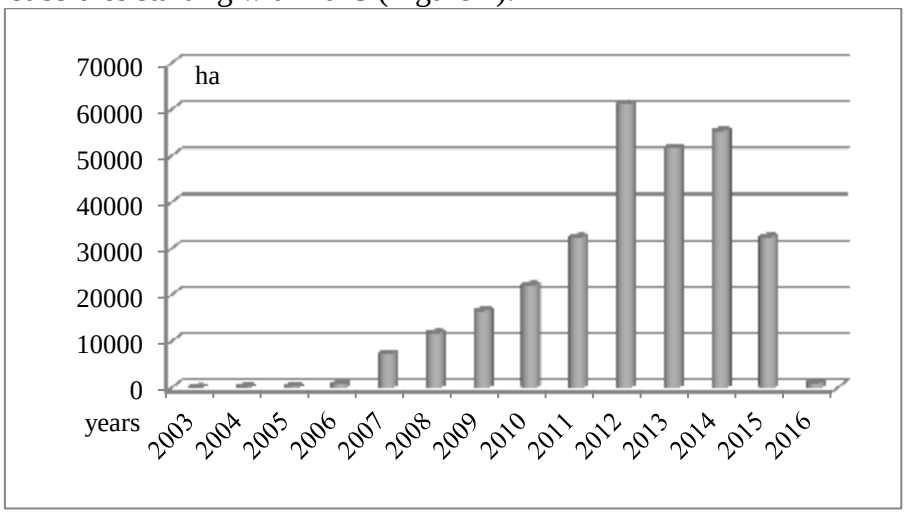

Figure 1. Dynamics of land use for organic farming, ha
(Agricultura ecologică în Republica Moldova - UNCTAG, 2018)

For these reasons (reduction of subsidies), a significant number of agricultural producers have renounced to organic farming, which has led to a reduction in the number of organic farmers and converters from 185 in 2009 to 38 in 2015 (Agricultura, 2015), (Figure 2).

The decrease in the number of economic agents and the areas registered in organic farming is marked by a number of inseparable factors such as (Ecologizarea, 2015):

• From 2014, the export of "ecological" products is carried out without the supervision of the national authority;

• National inspection and certification bodies are not recognized by the European Commission as equivalent;
• On the territory of the Republic of Moldova there are 10 inspection and certification bodies recognized by the European Union Commission to certify organic food products in third countries but are not registered at national level;
• Lack of information on the record of the areas, types of crops cultivated in organic farming, determined by the transfer of the economic agents to the inspection and certification bodies that are not registered at national level;
• Lack of a legal framework to grant exemptions for organic agri-food production;
• Lack of state supervision system and control regarding the traceability of organic products.

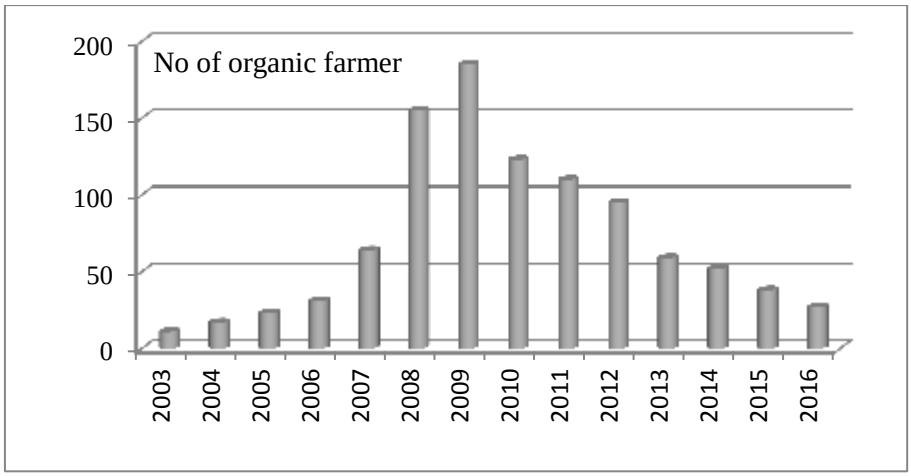

Figure 2. Number of organic farmers registered and undergoing conversion (Agricultura ecologică în Republica Moldova - UNCTAG, 2018)

Under these circumstances, the objective of further expanding the area of agricultural land used for organic farming from 1.9% in 2011 to 5% in 2015 has not been achieved and the 20% target by 2020 will be difficult to achieve without the implementation of comprehensive policies and mechanisms to support farmers (Agricultura, 2017).
One of the main reasons for increase in the dynamics registered during the period 2007-2012 was the favourable policies supported by the Government of the Republic of Moldova, with subsidies granted in 2007 for reimbursement of expenses during the conversion period. Since 2012, subsidies have been granted only for the establishment of multi-annual plantations, which may have had a negative impact on the sector. Since June 2014, the transition period has ended and the system of import authorizations has been replaced by the equivalent control bodies system. Thus, as was mentioned that during the period 2003-2012 in Moldova there were excellent conditions for the development of ecological agriculture, especially the direct neighbouring and association agreements with the European Union (Studiu, 2014).
An identified barrier to the development of organic farming is the impossibility of accessing subsidies by certification bodies that are not registered in the national certification system. Certification of organic products is currently carried out on two parallel platforms. Thus, the vast majority of certification bodies certify organic agro-food production according to the standard that is equivalent to the European Union standard but are not registered at national level. Another part of the certification bodies certifies the products to the National Control Bodies, but they are not in conformity with the standard that is equivalent to the European Union standard. This leads to an imbalance between the interests of the producers. On the one hand, those who can integrate into the national system, which is not in compliance with EU legislation, cannot benefit from subsidies, and on the other hand producers who cannot export agri-food products with "ecological" mention on the EU market (Productie organică, 2015).

Another issue of expanding organic farming is irrational use and degradation of natural resources, including soil and water, which results in lower productivity and profitability of this sector, especially for small farmers (Leah, 2017; Bacean & Țăranu, 2015).

The degradation of the productive capacity of soils due to over-exploitation over the last 50 years has been manifested by the intensification of erosion processes through landslides, humus and nutrient deficiency, salinization, periodic excess of humidity, clogging of depressions with low humus soils deposits, deterioration of soil fertile layers structure.

The small and very small reserve of humus in the soil is an essential issue in the development of organic farming. Soils with humus deficiency account the 60% of the agricultural land. There is a risk that in the coming decades the content of humus in arable land will decrease on average by 10-25%, which will substantially affect the physical qualities and micro-biodiversity of soils. Annual losses for this cause are estimated at 10% of the harvest.

Exhaustion of NPK reserves in soil can only be covered with mineral and organic fertilizers. The lack of fertilizers makes the share of the exhausted land categories and increase the harvest losses (20%).

The soils depletion on the area of 200 thousand ha for vineyards and orchards has led to the disruption of natural stratification and to the removal of low humus layers with high carbonate content. The fertility of these plots, used for field crops, is 10-20% lower compared to similar undisturbed soils (Leah, 2017).

Local soil pollution is maintained, although the application of chemical fertilizers per hectare between 1991 and 2015 has reduced by 5.3 times. At the same time, the degree of biological soil pollution in the localities increased due to the lack of functional systems for the removal and use of domestic and livestock waste (Leah, 2012).

In the last 25 years there has been deformation of field crops rotation, the reduction of the legume in crops rotation by 6 times, the decrease of the mineral and organic fertilizers application by 15-20 times. All these processes have led to the formation of a negative balance of humus and nutrients in the soils. As a result, physical, chemical and biological degradation takes place, soil productivity is decreasing, poverty is increasing (Leah, 2010).

In Moldova, soil protection can be achieved through the development of an environmentally friendly and organic agriculture that does not affect the components of the environment and, at the same time, produces high quality products. In this respect, the chemical control of the pests and the biological one must be gradually replaced, the monoculture practice should be avoided and all the necessary measures should be taken to improve the degraded soils, without omitting the necessity of reforestation and optimization of the storage of the various wastes and industrial residues (Foaie de parcurs, 2017).

In order to move from conventional agriculture to organic farming, it takes a conversion period that, depending on the crop, takes two to three years. The conversion period is a complex process involving the reorganization of the production unit's activity, the change of production processes and the introduction of new technologies in accordance with the legislation in force. So, during this period, soil fertility will be improved by introducing appropriate crop rotation to exclude the use of pesticides, chemical fertilizers and genetically modified organisms. The conversion period to organic farming is two years before sowing, for annual crops and meadows; three years before harvest, for perennial crops and plantations (Leah, 2014; Reglementări tehnice, 2008).

In the period 2015-2016, in order to remove existing gaps in the promotion and expansion of organic agriculture, legal and regulatory provisions have been developed at national level, which require harmonization with international / community standards and regulations. For example, the "Regulation on how to use the Fund's means to subsidize agricultural producers" was complemented by two new measures: support for the promotion and development of organic farming, and consultancy and training services.

The "National Agricultural and Rural Development Strategy for 2014-2020" mentions that the organic farming sector is underdeveloped. Thus, Objective 2 includes specific actions for the sustainable management of natural resources in agriculture. The specific objective 2.2 on supporting environment-friendly production technologies and organic products requires the development and promotion of organic farming through the implementation of clean technologies. Organic production must be subsidized or supported, especially those products that are becoming more and more demanded both on the domestic and on the external market. These measures, together with efforts to adapt to climate change, must be taken into account.
Integrating environmental provisions into agricultural policies will help reduce the risk of environmental degradation and improve the sustainability of agricultural ecosystems. To increase the share of organic farming, the following measures are needed:
(a) promoting the efficient production, processing and marketing of organic products in order to increase the income and well-being of farmers (Ecologizarea, 2015);
b) encouraging the improvement of the processing and marketing of primary organic agricultural products by supporting investments (supporting environmentally friendly agricultural practices, offering quality products and respecting the efficient use of soils;
c) promotion of the renewable energy resources use, development of new technologies and innovations, implementation of compensation systems for landowners whose properties are part of state-protected natural areas;
(d) organizing public education and awareness-raising programs for farmers in the field of the environment and setting up the necessary training infrastructure to further promote farmers' professional education in the field of sustainable agriculture;
e) development of environment-friendly techniques and infrastructure (creation of a mechanism for periodic verification of water quality for irrigation, conducting of pedological and agrochemical analyzes for permanent monitoring of soils, provision of integrated protection of plants against pests and diseases, promotion of production techniques conservative farming, preservation of the landscape / agricultural ecosystems through the use of extensive agricultural practices, development of waste management mechanisms in agriculture, especially in livestock farming);
d) enhancing educational and extension activities to ensure the education and training of staff of different levels engaged in the production and processing of organic products;
e) implementation of climate change adaptation measures aimed at promoting agricultural crops that have the potential for harsher climatic conditions (drought, high temperatures), soil treatment, water conservation and reduction of soil moisture loss through evaporation.
In Moldova there are a number of factors that can contribute to the promotion of organic farming: increasing the level of investments; developing an internal market; developing the culture of management in agriculture; application of no-till and mini-till conservation soil technologies; improvement of irrigation systems (Leah, 2014). These factors may have some immediate effects on organic farming: stimulating land conversion; application of subsidies, increased demand for organic produce, brand promotion in export and rebalancing the soil resources and environment. It is expected that by 2020, the total area of agricultural land occupied with organic production in our country will reach 150 th. hectares (Agricultura, 2017). If implementation of a monitoring and control system adjusted to European standards is not accepted, Moldova risks remaining at this level of organic farming development.

Conclusion

Ecological or organic farming has a great potential for development in Moldova; here are all necessary resources, we must abandon myths and turn potential into reality - fair and sufficient subsidies, educating producers and consumers, competent and corrupt control institutions, creating a modern infrastructure, combat the soil degradation etc.

Promoting and developing organic farming are considered the ways of ensuring sustainable land management in the agricultural sector as it contributes to biodiversity conservation, soil protection and carbon sequestration, helps avoid soil erosion and pesticide contamination, contributes to ecological rebalancing of environmental components.

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ORGANIC FARMS IN POLAND AFTER ACCESSION TO THE EUROPEAN UNION

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Abstract

In the last decade, the Polish agriculture experienced significant changes related to the number of agricultural holdings, production potential, agricultural production organization, as well as production and economic outcomes. These changes were driven by both market and institutional factors. Among the important factors that determine this transformation, technological progress and the growing dependence of agricultural production on industrial factors of agricultural production should be also indicated. In the case of conventional agriculture, this activity often contributes to environmental degradation. Organic production is the alternative system of agricultural production. Organic production provides benefits to society (food safety) and environment (respect for natural resources). In the view of increasing society awareness, consumers more likely choose organic products. This system of agricultural production is also supported within the rural development programme, which improve the difficult economic situation of organic farmers. The aim of paper is the indication of changes in number, production potential and economic efficiency of organic farms. The paper focuses on the presentation of the development direction of organic farms, which has taken place in the last decade. These results were illustrated on the background of all individual farms in Poland allowing identification of the ranges of convergence and diversity in the development of farms in total and organic ones. Central Statistical Office data for 2005 and 2016 were used. These data allowed formulation of conclusions representative for farms` population in Poland. Research indicated, that after the accession of Poland to the EU, there has been the dynamic development of the organic production system, as indicated by the multiple growth of the number of organic farms and their production and economic potential. Changes regarding production simplification and specialization which are taking place in organic farms should be deemed more intense when compared to those in conventional farms. In the case of organic farms, they additionally resign from the livestock production.

Keywords: *Organic farms, Central Statistical Office data, Poland, accession to the EU, production profile, farms' specialization, farms' concentration*

Introduction

Organic farming is the example of the sustainable agriculture form (Zegar, 2012; Wachter, Reganold, 2014). Organic farms represent one of the most interesting and prospective forms of environment-friendly agriculture. Their distinctive feature is the use of organic production methods – in line with the soil, plant and animal requirements. Owning to the fact that cultivation is conducted without agrochemicals and under controlled production methods, organic farming contributes to the conservation of biodiversity and the protection of natural resources, as well as high-quality food production (Kristiansen, Taji, Reganold, 2006).

In accordance with paragraph 1 of the preamble of Council Regulation (EC) No 834/2007, *Organic production is an overall system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes. The organic production method thus plays a dual societal role, where it on the one hand provides for a specific market responding to a consumer demand for*

*organic products, and on the other hand delivers public goods contributing to the protection of the environment and animal welfare, as well as to rural development (*Council Regulation (EC) No 834/2007 of 28 June 2007, L 189/1). The essence of organic farming comes down to a holistic approach to management, which takes into account, on the one hand nature processes, on the other hand ethical values (Stolze, Lampkin, 2009). Organic agriculture shows many potential benefits (i.e. higher biodiversity, improved soil and water quality, higher nutritional food value) as well as many potential costs including lower yields and higher consumer prices (Seufert, Ramankutty, 2017).

The last years point to the dynamic development of organic agriculture in the world, including in Europe. Comparing 1999 and 2015, the number of the world's organic producers has increased by more than 1,000%, and 2.4 million organic producers is currently. Compared with 1999, when 11 million ha were organic, organic Agricultural land has increased almost five-fold. In 2015, Europe took second place in the world in terms of area covered by organic crops (Willer, Lernoud, 2017), while Poland took the sixth place among European countries (Agricultural and Food Quality Inspection, 2017).

Changes in the number and area of agricultural land used in accordance with the rules of organic farming is the result of several components, including use of production technology (promote environmentally friendly production methods), the growing demand (demand for "natural" food), or the sustainable development policy, which strongly accentuates the need for development and support of organic farming (Watson, 2006). Referring to the first question, there is emphasized the need of searching such production solutions that respect nature (including sustainable intensification methods), due to increasing environmental pressure of farming. Parallel with increasing demand for organic food, sales of organic products has been one of the fastest growing market segment within the global food industry in recent years (Willer, Lernoud, 2017). In the case of the latter question, the policies for organic farming developed in Europe since the late 1980s have been developed in the context of production surpluses, loss of biodiversity due to agricultural intensification and a heavy reliance on commodity support for mainstream agriculture. The market for organic products was initially developed as a means to support the financial viability of farmers trying to deliver broader objectives (Stolze, Lampkin, 2009).

Organic farming is growing rapidly, although its range is still niche, as the model of conventional (industrial) farming is dominant in developed countries (Seufert, Ramankutty, Mayerhofer, 2017; Zegar, 2012). According to the definition, organization of agricultural production in organic farms should differ significantly from that encountered in conventional farms. As indicted Meemken and Qaim (2018), smart combinations of organic and conventional methods could contribute toward sustainable productivity increases in global agriculture. The production in organic farms should depart from specialized and simplified technologies and be based on crop rotation, organic fertilisers, natural feedstuffs, natural plant protection products, selection of species and varieties with natural resistance to diseases and local breeds and species. Taking into account organic farms development in recent years, it is important to define the direction of their development, including the scale of occurring organizational changes in relation to those typical of conventional farms.

The aim of paper is the indication of changes in number, production potential and economic efficiency of organic farms. The paper focuses on the presentation of the development direction of organic farms, which has taken place in the last decade. This decade is particularly important because of the Poland accession to the EU, which involved a number of adjustments to the legal implementation of EU policy, as well as strong agriculture subsidies, especially organic farming.

Materials and method

Public statistic of Central Statistical Office – 2005 and 2016 Farm Structure Survey (FSS) data were used[6]. These date were collected on the basis of uniform methodology that allowed to investigate the direction in which tends Polish agriculture. FSS research are carried out in individual EU countries, that results are finally aggregated in EUROSTAT databases. The proposed use of data from FSS to measure economic sustainability of farms and agriculture can be applied to other countries to conduct comparative analyses between them, taking into account the different requirements of sustainability.

The analysis concerns all individual agricultural holdings with at least 1 ha of agricultural land maintained in good agricultural and environmental condition. The research focused on individual farms using organic methods of agricultural production (organic farms) that have a certificate given to them by a certifying authority. The farms` characteristics concerned economic and production potential used in the research were the following: area of agricultural land (ha), labour input (expressed in AWU[7]), animal population (Livestock Units, LU[8]), the value of standard output (EUR thousand)[9] and standard gross margin (European Size Units, ESU)[10]. There was used a set of useful economic indicators, such as: land productivity (the value of the standard output per hectare of agricultural land), labour productivity (value of the standard gross margin per full-time employee), households income sources[11] and farms market activity (in general and local)[12]. Organic farms` results were illustrated on the background of all individual farms in Poland allowing identification of the ranges of convergence and diversity in the development of farms in total and organic ones.

[6]The study is carried out at intervals of several years. In the last decade, FSS was conducted in 2005, 2007 and 2016. Access to more detailed data, which would be representative of the whole agricultural sector was not possible.

[7] 1 AWU (Annual Work Units) is equivalent to full-time, that is 2,120 hours of work a year.

[8] 1 LU is a conventional unit of farm animals with a mass of 500 kg. See tables of conversion coefficient for livestock from physical units to livestock units (Toczyński et al., 2013).

[9] Standard output is the mean of 5 years of the value of production corresponding to the average situation in the region. Total standard production of farms is the sum of the values obtained for each agricultural activity on the farm by multiplying the coefficients of the standard output for a given activity and the number of hectares or number of animals; see (Goraj et al., 2012). It is an economic category that allows for comparing the volume of production, while offsetting the impact of price fluctuations in regional and temporal terms. There were used 2013 standard output indicators (based on the average values for the period 2011-2015).

[10] Sum of standard gross margins (SGM) – the difference between output and specific (direct) costs of all activities occurring on the farm – indicates the economic size of the farm, otherwise the productive potential of the farm. 1 ESU is equivalent to EUR 1,200. Standard gross margin on a particular crop or animal is a standard (average of three years in a particular region) value of production obtained from one hectare or from one animal less the standard direct costs necessary to produce. There were used 2004 standard gross margin indicators - the last SGM calculated indicators, used in Farm Accountancy Data Network (FADN). In subsequent years, there was used FADN farms` typology based on the coefficients of standard output.

[11] The predominant source of farm's income indicates its socio-economic type. This classification distinguishes the following groups: farmers' holdings – with majority income from agricultural activities, employees' households – with majority income from employment, entrepreneurs' households – with majority income from non-agricultural activities, pensioners' households – with majority income from pension, other – with majority income from other sources. Data collected under the FSS 2005 and 2016 allow for the classification of farms according to the predominant source of income of a farming family, but they do not include the absolute level of income, among other thing agricultural income.

[12] FSS data do not include detailed farms' economic account categories. These issues on organic farms economic situation were presented in the publication (Wrzaszcz, Zegar, 2016). These studies were based on Farm Accountancy Data Network data (www.fadn.waw.pl), which enabled the analysis of different production-economic categories, the cost level and subsidy value.

Results and discussion

During the analysed period – after the accession of Polish to the EU – there has been dynamic, more than five-fold increase in the number of organic farms[13] (tab. 1). This was accompanied by changes in the production and economic potential of organic farming – group of organic farms. In 2005-2016, the utilised agricultural area at the disposal of organic farms increased by 10 times, labour input increased by 4 times and livestock population nearly by 6 times, while standard gross margin and output increased appropriately by 11 and 12 times.

In the case of all farms whose results show the situation of conventional farms in Poland, the changes in production potential had a different direction. The number of farms has definitely decreased (by 1/5), which was associated with the outflow of labour force, partially of livestock population and of standard gross margin. Slight progress has been reported in standard output. These statistics indirectly confirmed the outflow of farmers and those employed in agriculture to other sectors of the economy. On the other hand, the results of organic farms show that farmers managing farms in accordance with the principles of the organic system have seen versatile benefits of this economic activity. Growing trends in case of organic farms in Poland were mainly the result of legal regulations – farms' subsidising in the form of agri-environmental programmes. The additional determinant was the changing consumers' and generally society preferences towards "more natural" and nutritious food (Łuczka-Bakuła, 2007; Babicz-Zielińska, 2010). Increasing demand probably will grow in subsequent years, that will be reflected in agricultural producers' choices.

Despite relatively high dynamics of organic farming development it is still a niche system of agricultural production. In 2005 and 2016, respectively, those farms used 0.5% and 4.6% of utilised agricultural area and their standard output was, respectively, 0.3% and 3.7%. There are several reasons for this situation. The demand for conventional products is still dominant in the market, despite the growing demand for organic products. Conventional products are offered at lower prices, that create a significant barrier for organic ones to compete. In addition, organic farming requires high precision from a farmer when performing agricultural practices due to the limited possibilities of using industrial means of production and related logistical challenges (acquisition of outlet markets, also local; efficient organization of transport of organic products, necessity of fast sale and processing). This precision requires the farmer to have multiple skills, which are derived from his extensive knowledge of natural environment and production.

Table 1. Farms' production and economic potential

No.	Specification	2005			2016			2016/05; Δ in %	
		Total	Org	O in T %	Total	Org	O in T %	Total	Org
1	Farms number (thousand)	1,723.9	3.04	0.18	1,398.1	16.15	1.16	-18.90	431.79
2	Agricultural land (thousand ha)	13,060.6	58.60	0.45	13,181.4	608.13	4.61	0.92	937.78
3	Labour input (thousand AWU)	2,035.2	5.61	0.28	1,617.0	22.06	1.36	-20.55	293.14
4	Livestock (thousand LU)	6,430.3	20.02	0.31	5,923.5	116.87	1.97	-7.88	483.73
5	Livestock farms (thousand)	1,247.6	2.42	0.19	712.6	8.85	1.24	-42.88	264.99
6	Standard output (million EUR)	20,824.1	70.28	0.34	21,824.3	817.26	3.74	4.80	1,062.81
7	Standard gross margin (thous. ESU)	9,963.9	33.40	0.34	9,283,4	405.56	4.37	-6.83	1,114.40

[*] *Org., O – organic farms; T – farms in total.*

Source: Prepared on the basis of FSS 2005 and 2016 data.

Organic farms are significantly different from conventional farms (tab. 2). Organic farms, on average, characterised by definitely greater production potential, and next, economics. The

[13] These issues were presented e.g. in: (Wrzaszcz, Zegar, 2014).

greater area of organic farms than of conventional farms is caused by the economic reason – smaller value added per area unit – which preconditions the needs to pursue farming on greater area and to search for external sources of financial support, including those mainly taking on the form of governmental programmes. The differences between these farms' groups have been intensified over time.

Table 2. Farms' production and economic potential – an average farm

No.	Specification	2005			2016			2016/05; Δ in %	
		Total	Org	O/T in %	Total	Org	O/T in %	Total	Org
1	Agricultural land (ha)	7.58	19.30	154.76	9.43	37.67	299.52	24.44	95.15
2	Labour input (AWU)	1.18	1.85	56.54	1.16	1.37	18.13	-2.04	-26.07
3	Livestock* (LU)	5.15	8.26	60.19	8.31	13.20	58.85	61.28	59.93
4	Livestock/agricultural land (LU/ha)	0.49	0.34	-30.61	0.45	0.19	-57.24	-8.73	-43.85
5	Standard output (EUR thousand)	12.08	23.15	91.64	15.61	50.62	224.28	29.22	118.67
6	Standard gross margin (ESU)	5.78	11.00	90.31	6.64	25.12	278.31	14.88	128.36

** ad. 3. Livestock per an average livestock farms.*
Source: Prepared on the basis of FSS 2005 and 2016 data.

Comparing the changes in organic farms to those which have taken place among all farms, it should be noted that in general these two groups of farms followed in the same direction, i.e. increased their production and economic potential, although the scale of these changes was more dynamic in the case of organic farms. In addition to the more intense increase in area and standard results in organic farms, labour inputs have also been more limited. These figures indicate the more efficient use of labour in organic farms. These changes are also a derivative of introduced organizational changes related to, in particular, the decreasing intensity of stocking density in organic farms, which progresses much faster than in conventional farms. This evidences the fact that farms are heading for agricultural production simplification, especially organic ones. These changes are in contradiction to the idea of the functioning of organic farms, which should provide for a closed circulation of macronutrients within the farm as a result of combining the crop and livestock production.
The agrarian structure of organic farms has clearly differed from that for all farms (Chart 1). Organic farms were dominated by farms with more than 5 ha, while very small farms dominated the structure of all farms. In addition, many more organic farms had an area of more than 50 ha when compared to all farms (in 2005, this was, respectively, 8% and approximately 1%). In the analysed period, the differences in this area deepened among the highlighted groups of farms. More and more large and very large farms were managed according to the organic system principles. The dynamic growth of large organic farms indicates their market orientation, adapted to the requirements of processors as well as of retail chains. This is a phenomenon whose justification is purely commercial (Zegar, 2018). Given the data presented, it can even be concluded that this process is progressing more quickly in organic farms rather than in conventional farms.
Agricultural production simplification in organic farms is the effect of market conditions, which by definition, is in contradiction with the desired organization of agricultural production. The result of this process is the fact, that organic farms have started to balance on the brink of legal requirements, adjusting to the market rights. More frequent farms` orientation on the mass market (chain stores) moves to the processes of organic farms` concentration and specialization (Chart 2 i 3). Alarming is the fact that the share of organic farms number with mixed (crop and animal production) drastically decreased, simultaneously in favour of farms oriented at crop production. Organic farms more and more often are targeted only at crop production, both traditional – connected with farming on arable lands, as well as orchard production, while part of them uses only permanent grasslands – meadows

and pastures. Livestock production elimination is determine by economic factors and animal production requirements within the scope of field crop structure, and also – or primarily – the need to narrow specialization of agricultural production forced by the market (uniform and large batches of goods). In addition, the process of agricultural production simplification, typical for conventional farms, took on the rise in recent years in the case of organic farms. Changes that take place in organic farms are not beneficial in environmental terms. The principles of sustainable development indicate the importance of a closed circuit of organic matter and nutrients within a farm. Organic farms, without animal production and natural fertilization (mineral also, because of legal requirements) can have organizational problems with balancing important soil ingredients. Since animal breeding predetermines proper functioning of the agricultural ecosystem, which is the guiding principle of organic farming (Tyburski, Żakowska-Biemans, 2012).The purchase of natural fertilizers is some solution, but it is no popular practice at the market. At the same time, organic farms geared exclusively towards the livestock production resigned from this type of production while different trends took place in conventional farms. An important reason for the resignation from the livestock production in organic farms was the underdeveloped processing sector, in particular, meat processing (Agricultural and Food Quality Inspection, 2017)[14].

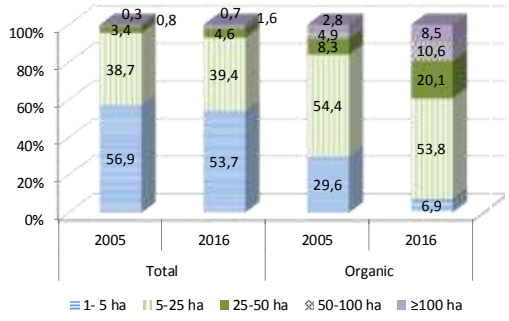

Chart 1. Agrarian structure of farms

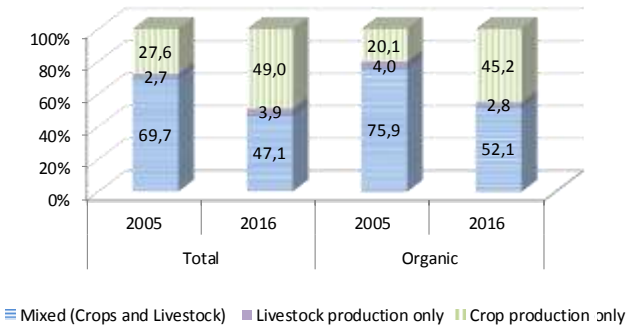

Chart 2. Farms production orientation - structure

Source: Prepared on the basis of FSS 2005 and 2016 data.

In 2016, 50% of organic farms specialized in field crops. The share of this group of farms has increased by more than twice since 2005. The second place was occupied by mixed farms with crop and livestock production, although their share in the structure has decreased by almost the half in the analysed period (from 32% in 2005 to 18% in 2016). On the other hand, specialization in permanent crops and horticulture concerned and continues to concern a small percentage of organic farms, just like rearing of granivores. Noteworthy is the significant share of farms specializing in rearing animals fed in the grazing system, although in this case there has been a significant decrease (from 23% in 2005 to 13%).

When comparing organic farms structure and all farms in terms of their type, it can be concluded that these farms differed significantly and, over time, the differences have deepened. The share of farms specializing in field crops also increased among all farms, although the rate of these changes did not match up to that in the case of organic farms. Specialized horticultural farms, farms rearing ruminants and granivores maintained among all

[14] In 2015, in terms of the number of organic processing operators, the largest share was that of processing of fruit and vegetables – 32.1%, other agri-food products (inter alia, spices, beverages) – 28.2%. The share of grain milling operators was – 20.3%. Definitely smaller was the share of operators in other industries: meat and fish processing – 7.7%, coffee and tea processing – 5.0%, milk processing and cheese making – 3.1%, vegetable and animal fat processing 2.4% and sugar production – 1.2% (Agricultural and Food Quality Inspection, 2017).

farms, with the increase in the share of farms specialized in permanent crops. Over time organic farms become similar to all farms in terms of production simplification and specialization, nevertheless, organic farms are increasingly less specialized in livestock and non-specialized with mixed production. Organic farms progress in field crop specialization is faster. In contrast, in the case of all farms, a relatively stable percentage of those specializing in the livestock production (this applies mainly to cattle, swine, poultry) is maintained.

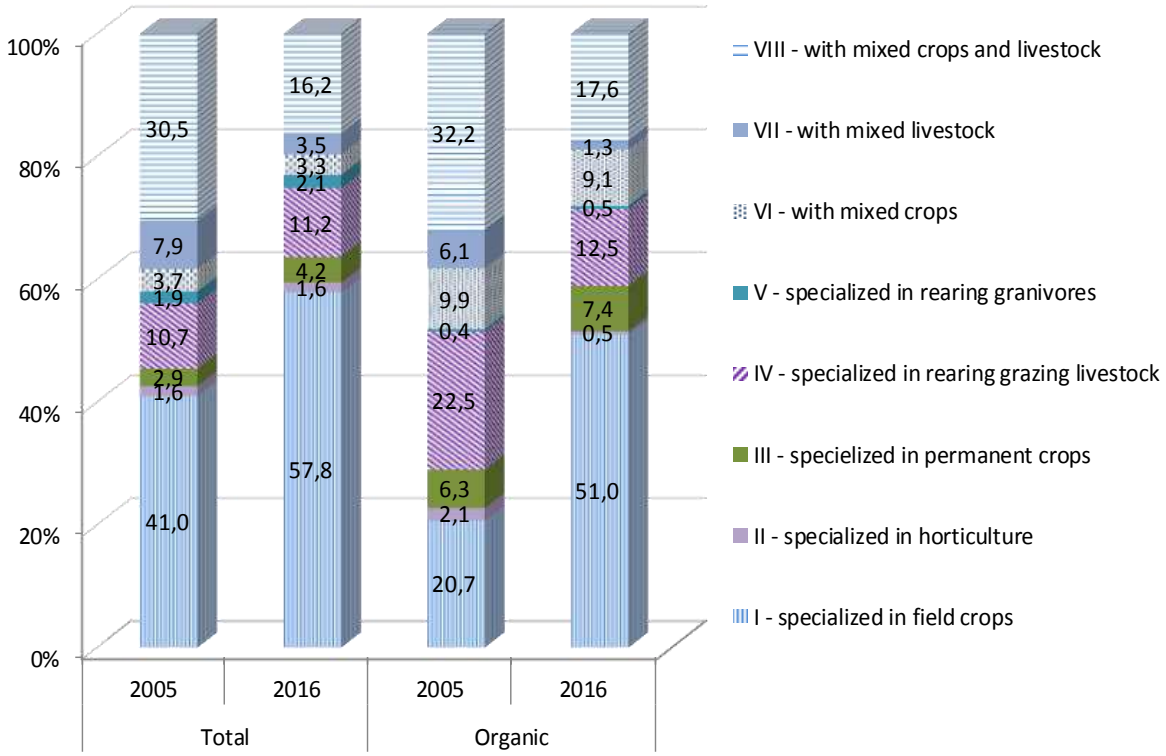

Chart 3. Farms' typology (according to general farms' types classification)
Source: Prepared on the basis of FSS 2005 and 2016 data.

Table 3. Economic indicators of farms` sustainability

No.	Specification	2005			2016			2016/05; Δ in % or p.p.	
		Total	Org	O/T in % or p.p.	Total	Org	O/T in % or p.p.	Total	Org
1	Standard output (thousand EUR/ha)	1.59	1.20	-24.78	1.66	1.34	-18.83	3.84	12.05
2	Standard gross margin (ESU/AWU)	4.90	5.95	21.57	5.74	18.39	220.25	17.27	208.90
3	Local market farms (%)	16.23	27.47	11.24 p.p.	17.50	24.48	6.98 p.p.	1.28 p.p.	-2.99 p.p.
4	Market farms (%)	69.43	76.78	7.35 p.p.	66.86	89.66	22.80 p.p.	-2.56 p.p.	12,88 p.p.
5	Farmers` farms (%)	40.43	60.64	20.22 p.p.	37.47	58.06	20.59 p.p.	-2.96 p.p.	-2.58 p.p.
6	Farms with non-agri. incomes (%)	76.42	69.86	-6.56 p.p.	82.18	72.47	-9.71 p.p.	5,76 p.p.	2.61 p.p.

* *p.p. – percentage points; non-agri. – non-agricultural.*
Source: Prepared on the basis of FSS 2005 and 2016 data.

The last question in the paper is farms' market and economic activity (tab. 3). The value of standard output per hectare indicates land productivity. It is an important indicator of agricultural production volume in the context of food security. In this regard, the organic farms didn't match conventional ones, although their land productivity distance decreased in recent years. However, organic farms had an advantage in terms of labour productivity (ESU/AWU), which strengthen over time. This is the effect of both, standard results

improvement, as well as efficient labour use. Standard gross margin per full-time employee indicates the potential labour charges and the assessment of labour economic efficiency.
Definitely, the greater part of organic farms is focused on local market compared to conventional farms. This is justified because of the "sensitivity" of organic products and social benefits provided to the local communities. Entities operating on local market provide benefits to local communities, which are reflected not only in more convenient ways to buy goods (often in lower price as compared with products produce in longer food chains), but also contribute to the development of neighbourly ties and sustaining of rural areas vitality.
In recent years, organic farms strongly improved linkage with market – in 2016 r about 90% of farms sold at least the half of agricultural production. Taking into consideration analysed farms population in total, percentage of market farms was significantly lower, 67%.
These data have confirmed the growing importance of market organic farms. Simultaneously, among the organic farms, the greater percentage of farms gained the prevailing agricultural revenues (farms oriented on active functioning on market), than in the case for conventional farms. The majority of farms is targeted on agricultural activity, which confirms their market orientation and potential possibilities to ensure an adequate volume of agricultural production. In this light, non-agricultural income have less importance.

Conclusion

1. After the accession of Poland to the EU, there has been the dynamic development of the organic production system, as indicated by the multiple growth of the number of organic farms and their production and economic potential. A major determinant of this process was the subsidising of organic farms from rural development funds.
2. Organic farms are significantly different from conventional farms in terms of their potential and production orientation – they are considerably larger and economically stronger (on average). In the analysed period, the advantage of organic farms was growing.
3. Changes regarding production simplification and specialization which are taking place in organic farms should be deemed more intense when compared to those in conventional farms. In the case of organic farms, they additionally resign from the livestock production.
4. Organizational changes in organic farms are dictated by the market and institutional conditions. Organic farms adapt to market needs while meeting the legal requirements of the organic production system, which determines the receipt of subsidies.
5. The advantage of conventional farms over organic farms is related to the land productivity. Different relationships apply to the labour productivity and local market activity, where organic farms are leaders and increase their advantage over time. The high and increasing over time percentage of market organic farms and those making their living mainly from the agricultural activity indicate their strengthening market position.
6. In view of the promising forecasts of the demand for organic food and the planned subsidising of the organic production in the next EU budget perspective, we can expect the further development of organic farms.
7. The substantive criteria of the organic production system should be subject to discussion. Assuming that the current legislation is maintained, the further simplification of the agricultural production in organic farms is highly probable, which may contradict the need to implement practices consistent with the idea of sustainable development.

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IMPACT OF VERMICOMPOST EXTRACTS ON STRAWBERRY PRODUCTION AND SUSTAINABILITY OF AGROECO SYSTEMS

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Abstract

Quality and balanced fertilization is one of the most important orchard management practices in fruit production. However, fertilization in conventional production systems is mainly focused on obtaining the highest yield. This usually requires increased utilization of synthetic nitrogen fertilizers, which significantly contributes to a series of undesirable effects and results in excessive environmental pollution. Since organic production system is considered as an important factor of the strategy for the development of agricultural sector, it is necessary to increase this kind of production. To find a way to overcome the mentioned problems, the use of vermicompost extracts is appeared as potential solution. Therefore, we conducted a study on the effects of vermicompost extract on yield-related characteristics (yield per plant, yield per unit area), physical (fruit weight, length, breadth, and firmness) and chemical fruits properties (total phenolics and antioxidant capacity) of 'Senga Sengana' strawberry cultivar, as well as microbiological properties of strawberry rhizosphere (total microbial count, numbers of soil fungi, actinomycetes, aminoheterotrophs, oligonitrophilic bacteria and *Azotobacter*). The obtained results indicate that application of vermicompost extracts in organic strawberry production had a positive effect on plant yield and fruit quality. Positive effects on soil biogenicity have also been observed. Therefore, vermicompost extract application can be considered as an appropriate practice in production of healthy and environmentally safe strawberries with satisfying basic postulates of sustainable agriculture.

Keywords: *Vermicompost extracts, Microorganisms, Organic strawberry production, Yield, Fruit Quality.*

Introduction

Ever-increasing studies have warned that the use of nitrogen chemical fertilizers, which is constantly increasing and whose use will be doubled and even tripled by 2050, causes various problems that adversely affect the environment and human health. Despite these, according to the Food and Agriculture Organization of the United Nations, the annual global consumption of chemical fertilizers in the period 1960-1990 increased from 46,000,000 to 130,000,000 tonnes, reaching 190,400,000 tons in 2015 (*http://www.fao.org/3/a-av252e.pdf*).

Introduction of living cells of microorganisms into the soil, the use of vermicompost or compost products - teas, extracts or leachates for improving plant nutrition are great natural resources that can contribute to overcome the mentioned problems.

In many countries such as Germany (Ernst et al., 2008), Spain (Monroy et al., 2009), USA (Arancon et al., 2006) and Vietnam (Yadav et al., 2010), vermicomposting is viewed as one of the key steps in sustainable waste management. Thanks to better physical characteristics, greater microbial and enzymatic activity, as well as larger content of easily accessible nutrients, vermicompost has a number of advantages over chemical fertilizers, and according to Venugopal et al. (2010) and Abul-Soud et al. (2009) vermicompost is significantly more

acceptable by manufacturers compared to compost. Quaik et al. (2012) associate increased interest in vermicomposting with its positive impact on the environment.
Beside the aforementioned methods of plant nutrition, with the aim of sustainable use of natural resources, a considerable attention is paid to the use of compost products - teas, extracts or lichates. Ingham (2002) states that compost teas (fluids extracted from compost) and the benefits of their application were known even by the ancient Romans. However, its role has been neglected due to the increasing use of chemical fertilizers. In recent years, interest in their use has increased along with requirements for health food production. Considering the fact that compost tea contains useful microorganisms and soluble nutrients, both organic and inorganic, its application enables the efficient use of nutrients in the sustainable and economically justified manner and improves waste management from agriculture. Diver (2002) points out that compost and herbal teas are tools that can increase the productivity of crops and inoculate philo- and rhizosphere with soluble nutrients, useful microorganisms and microbial metabolites.
The aim of this study was to determine the effects (advantages and disadvantages) of the application of some alternative fertilization methods (liquid biopreparation based on vermicompost) on yield-related characteristics and fruit quality attributes of strawberries grown in the organic production system.

Material and Methods

An open field trial was conducted on 'Senga Sengana' strawberry plants at the experimental plantation located near Čačak, Republic of Serbia (43^{o} 53' N latitude, 20^{o} 20' E longitude, 225 m altitude) during the two consecutive seasons (2016–2017 and 2017–2018) . The experiment was set up at a randomized block design, in three replications. The frigo strawberry plants were planted in a single row system. The rows were spaced about 80 cm apart, and the plants were set 15 cm apart in the rows. Treatments contained vermicompost-based product (vermicompost extracts) prepared at the Fruit Research Institute Čačak, whereas untreated soil and plants served as control.
Production of biopreparation is based on a specially obtained liquid extract of vermicompost enriched with various strains of useful microorganisms (bacteria strains of the genus *Azotobacter* sp., *Bacillus* sp. and *Pseudomonas* sp. as well as *Trichoderma* sp. fungus). Its application was carried out in three ways: rhizobial (R), foliar+rhizobial (F+R) and foliar (F). Treatments were carried out five times during vegetation period (in accordance with the corresponding phenological development stages of strawberry and 30 days after harvest) in the amount of 400 l ha^{-1} of the respective preparation. The generative potential of the strawberry plants, which included yield per plant (g) and yield per unit area (t/ha), was determined by collecting and measuring the weight of picked fruits in each harvest
To assess the physical fruit properties a sample of 60 fruits in each replication were randomly selected. Samples were colected at full maturity stage. An average fruit weight was measured using the Adventurer Pro AV812M balance (±0.01g accuracy) and the data were expressed in g. The fruit dimensions (mm) including length and breadth, were also determined in the selected samples using the 'Carl Roth GmbH' vernier scales (±0.05 mm accuracy). Firmness of fruit was measured using a hand-held shore-type penetrometer and data were expressed in N. Chemical properties of the fruit were studied as well, such as the content of total phenolics (TPH) and antioxidant capacity (AC). TPH was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Liu et al., 2002) with results expressed as mg gallic acid equivalents (GAE) 100 g^{-1} FW. AC was determined by the ABTS test according to Re et al. (1999) and the results were expressed as Trolox equivalents, mmol 100 g FW.
For the purpose of determining microbiological properties in strawberry rhizosphere, total numbers of microorganisms, fungi, actinomycetes, oligonitrophils, aminoheterotrophs, and

Azotobacter were determined as colony forming units (CFUs) on agar plates by Serial Dilution Plate method (Pochon & Tardieux, 1962).
The mean value from the 2-year investigation were presented in Figures.

Results and Discussion

Effect of the examined preparation (vermicompost extracts) on the generative potential of strawberry is shown in Figure 1.

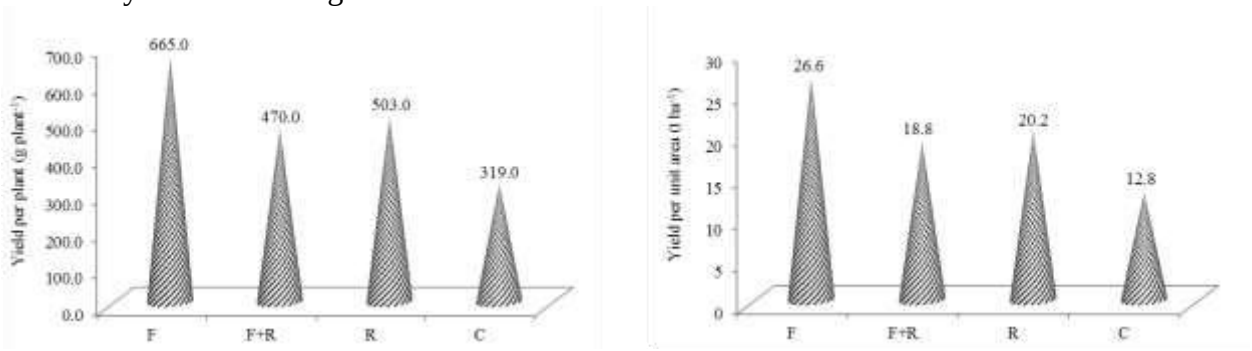

Figure 1. The influence of the vermicompost extracts application on strawberry yield

Higher yield per both plant and unit area was determined in all variants of the vermicompost extracts application. This occurrence can be explained by the fact that the microorganisms contained in biopreparation leave ready food for plants in the soil and / or affect the increase in the content of bioregulators such as indoleacetic acid or gyberilic acid (Arshad and Frankenberger, 1993; Glick, 1995). Diver (2002) points out that compost and herbal teas represent tools that can increase crop productivity and inoculate philo- and rhizosphere with soluble nutrients, useful microorganisms and microbiological metabolites. Singh et al. (2010) having examined the influence of vermicompost lichates found the increase of leaf area index, vegetative growth and yield of strawberry. A positive influence of compost tea on the yield of strawberries was found by Welke (2005) too.
The applied vermicompost extracts positively influenced the weight, firmness, length and width of the fruit (Figure 2). However no statistical significance was observed. Positive effects of biofertilization on the firmness of strawberry 'Senga Sengana' were claimed by Sas-Paszt et al. (2008).

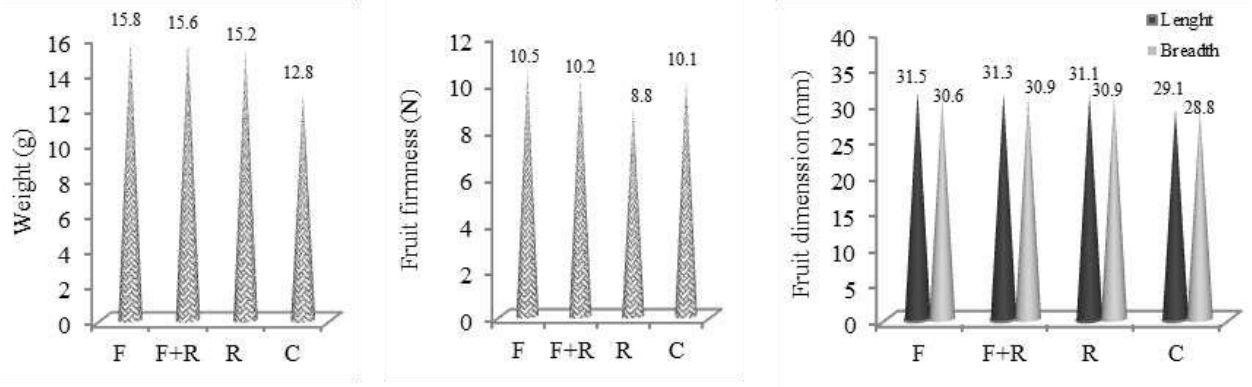

Figure 2. The influence of the vermicompost extracts application on physical attributes of strawberry fruits

The conducted research also points to the positive impact of vermicompost extracts on the content of total phenolic and antioxidant capacity (Figure 3) which is most likely the result of more intensive mineralization processes in soil under that conditions. In such conditions, the activity of the root itself increases, and its physiological functions become more intense. This

is in agreement with the results obtained by Kivijärvi (1999), pointing out that the synthesis of phenolic compounds in berries can be increased if the production technology is implemented without the use of pesticides and mineral nutrients.

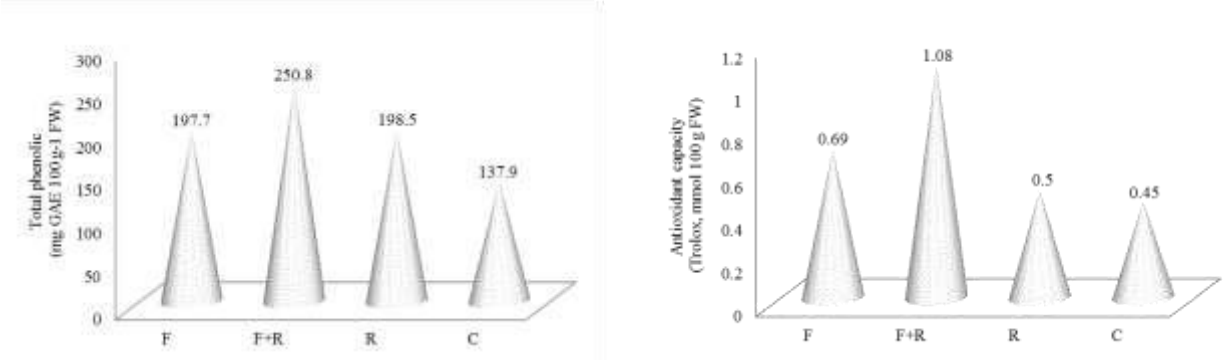

Figure 3. The influence of the vermicompost extracts application on TPH and AC in strawberry fruits

The conducted research has also shown the stimulating influence of vermicompost extracts on the presence of different groups of microorganisms in the strawberry rhizosphere (Figure 4). Our previous research (Pešaković et al., 2013; Pešaković and Milivojević, 2014) relating to the effect of microbial inoculation on soil biological activity also showed a positive influence of inoculation of strawberry rhizosphere, before all, by the diazotrophic *Klebsiella planticola* TSHA-91, but also by a mixture of bacteria of the genus *Azotobacter, Derxia, Pseudomonas* and *Bacillus*. This phenomenon is the result of nitrogenfixation ability of the strains contained in the biofertilizer but also the cumulative effect of a number of effects such as inhibition of phytopathogen development, phytotohormone synthesis (Sukhovitskaja et al., 2004) detoxification of heavy metals and synthesis of exocellular polysaccharides. Statistically significant differences were recorded only in the F+R variant.

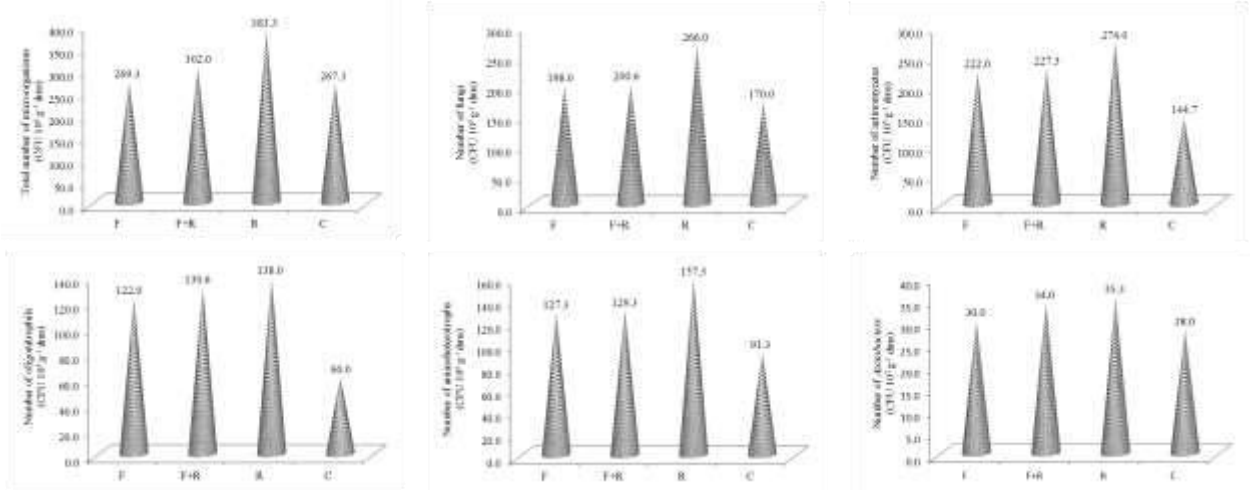

Figure 4. The influence of the vermicompost extracts application on the soil microorganisms

Conclusions

Application of biopreparation based on vermicompost (vermicompost extracts) enriched with various microorganisms strains has positively influenced the generative potential, fruit quality and the microbiological properties of soil in the open field organic strawberry production.

Bio-fertilizers applied in this study can contribute to the improvement of existing technology, allowing substitution of chemical fertilization by biofertilization that satisfies the basic postulates of sustainable agriculture.

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THE YIELD AND CONTENT OF ESSENTIAL TRACE ELEMENTS OF WINTER WHEAT GRAIN IN ORGANIC AND CONVENTIONAL GROWING TECHNOLOGY

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Abstract

The choice of winter wheat cultivation technology affects the quality of the processing. The selected cultivation technology should ensure optimal yield of grain of maximum quality. The paper examines the influence of organic and conventional winter wheat production on yield and content of essential trace elements of winter wheat grain. The trial was set in 2016/17 on the experimental field "Radmilovac" of the Faculty of Agriculture of the University of Belgrade" (Serbia), on chernozem luvic soil type. In conventional technology, the variety of common soft wheat Ilina (*Triticum aestivum* ssp. *vulgare*) was growing in conventional tillage systems. In addition to basic fertilization with NPK fertilizers in the autumn, together with the basic cultivation of soil, in spring added two different amounts of nitrogen (60 and 120 kg/ha N) in top dressing. There was control treatment (without N), also. Standard cultural practices in wheat production were applied, and the harvest was carried out on June 29th. In the organic technology variety Nirvana (*Triticum aestivum* ssp. *spelta*) was the object of investigation. Organic growing technology included conventional soil tillage, fertilization with microbiological fertilizers without chemical protection of crops. In addition to grain yield, the content of the most important trace elements in grain in both technologies was examined. The grain of yield was statistically very higher in conventional than in organic production. The Fe, Mg and Zn contents were higher in wheat grain from organic growing technology and the content of other elements and grain yield were higher in conventional growing technology.

Keywords: *grain of winter wheat, growing technology, elements, yield.*

Introduction

The success in cultivating winter wheat, both in conventional and organic production, begins with the selection of the plot, through the selection of the genotype until the choice of agro-cultural practices in accordance with the meteorological conditions of the area. The choice of winter wheat cultivation technology should ensure optimal yield of grain of maximum quality (Dolijanović *et al*., 2018). One of the most important challenges for agriculture, besides enhancing food production, is to provide almost all the essential minerals and organic nutrients to humans for maintenance of health and proper organ function. Humans need more than 22 mineral elements; some of them are required in large amounts, but others, such as Fe, Zn, Cu, I and Se, are required in trace amounts because higher concentrations can be harmful (Grusak and Cakmak, 2005). Cultivated plants mostly adopt elements in mineral form from the soil, as useful, so harmful and potentially dangerous. If the concentration of a certain element in the plant is in significant positive correlation with the extracted fraction of that element from the soil, it can be assumed that this fraction is the main source of supplying the plant to the given element during the vegetation period, although the plants can absorb the elements from the air and over the surface of the sheet with foliar nutrition (Barać, 2017).

Toxic metals, such as lead and arsenic, reach the soil, and consequently in plants using chemical agents in industrial and agricultural processes. The reason for the contamination

may be the proximity of the road, as well as some mineral fertilizers and pesticides (Žunić Smiljana *et al*., 2017). Anthropogenic inputs are associated with industrialization and agricultural activities such as atmospheric deposition, waste disposal, waste incineration, urban effluent, vehicle exhausts, fertilizer application and long-term application of sewage sludge in agricultural land (Yu-Ying *et al*., 2004). The soil physico-chemical characteristics such as pH, soil organic matter (SOM), texture etc. are very important in determining the retention and mobility of elements in soils.

The aim of these investigations is to determine the concentration of macro- and microelements in the grain of two different genotype of winter wheat in the specific conditions of growing, which results in a good quantity and nutritional value of the wheat grain depending on the production technology.

Material and methods

Investigation of different genotypes alternatives types of small grains in organic and conventional condition was conducted on Radmilovac experimental station of the Faculty of Agriculture in Zemun during vegetation season 2016/17 on chernozem luvic soil type. Winter wheat is cultivated using a conventional soil treatment system - which includes ploughing with a 25-cm drip plow and pre-harvesting with a lawnmower and harrow. In addition to basic fertilization with NPK fertilizers in autumn, along with basic soil treatment, winter wheat with nitrogen (N) (60-N_1 and 120 kg/ha a.m. N-N_2) and control variant (without top dressing) were present. The top dressing was carried out in the phase of drilling with mineral fertilizer KAN (25-27% a.m. N). The surface of the elemental plot was 6 m^2 with the sowing density of 650 kernels per square meter. The seed sowing of the Ilina variety was carried out on October 28th. Standard measures of care in wheat production were applied and the harvest was carried out on June 29th.

Organic cultivation technology included conventional soil cultivation, fertilization with microbiological fertilizer without chemical protection of crops. Just before the seeding of winter wheat (Nirvana variety), the bio fertilizer "Uniker" (nutrient mobilizer) was applied, containing strains of proteolytic and cellulolytic bacteria in the amount of 10 l/ha. For the top dressing in early March, microbiological fertilizer preparation "Slavol" was used in a dose of 5 l/ha. The preparation contains two groups of bacteria: azotofixatores and phosphomineralisators, as well as biostimulators. The essence of the action of the applied preparation consists in the fact that in addition to supplying plants with nitrogen and phosphorus it also enables the production of entomo toxins that protect plants from insects, which together affect the rapid growth of plants in an environmentally acceptable way. Grain yield in both cultivation technologies is determined at harvest time and calculated to 14% moisture. For the preparation of representative samples by using the random square method, 500 grams of wheat grains were taken. The ICP analysis sample was prepared by the method of "wet digestion" according to the literature with small changes (Duran *et al*., 2008). In short, approximately 0.5 g of the sampled sample was weighed and transferred into a 25 ml glass. Then, 7 ml of nitric acid (HNO_3) and 1 ml of hydrogen peroxide (H_2O_2) were added. The mixture is stirred and placed on a water bath in which the water temperature is about 80 ^{0}C. Samples were heated for 5-6 hours at constant temperature. After completion of the destruction of the samples, the contents were quantitatively transferred to normal vessels of 50 ml which were added to the line by ultra-pure (deionized) water. The solutions of normal vessel samples were filtered through quantitative filter paper, pore size 2-4 μm into stand-alone cuvettes (PP).

In order to determine the content of macro- and microelements (Ca, Fe, Mg, Cu, Mn and Zn) in the wheat samples, the analytical technique inductively coupled plasma with optical emission spectrometry, ICP - OES (*Inductively coupled plasma - optical emission*

spectrometry), was used. The analysis was performed on the Spectroblue instrument (SPECTRO Analytical Instruments GmbH, Germany) equipped with Spectro Smart Analyzer data processing software, and US EPA Method 200.7 (1994) was applied.
Statistical analysis of data for all traits was performed using analysis of variance for factorial experiments and for individual comparisons, we used the least significant difference (LSD test). Correlation analysis was performed by Statistica 6.0 package to determine the relationship among the characters according to Pearson method.

Meteorological conditions
The meteorological conditions during the growing season are presented in Table 1.

Table 1. Average air temperatures and precipitation sums from October to June at Radmilovac

Months	Temperature (°C)	Precipitation (mm)
October	11.1	76.8
November	7.7	71.8
December	0.9	2.6
January	-3.3	23.4
February	5.4	23.5
March	11.5	27.0
April	12.7	51.8
May	18.4	86.1
June	24.3	53.0
Average/Sum	9.9	416.0

Meteorological conditions during the study period had a significant influence on the formation of high yields of wheat. The data in Table 1, shows that low rainfall during the winter spring months. Low air temperatures in January, the lack of moisture in the soil and increased rainfall in May, had an influence on the reduction of yield in that year.

Results and discussion

Breeding programs dedicated to organic agriculture would focus on traits including improved nitrogen and nutrient efficiency, adaption to soil microbes, improved competitiveness against weeds and resistance to insects and diseases currently controlled with chemical pesticides with the incorporation of these traits into high yielding cultivars, organic agriculture will be better equipped to realize its full potential as a viable alternative to conventional agriculture (Przystalski *et al*., 2008). Factorial analysis of variance has showed that grain yields in organic technology significantly depend on the chosen fertilizer. Combined application of bio-fertilizer in the fall and microbial in the spring, the highest average yields of alternative wheat were obtained regularly. The yield obtained by this variant, compared to the control variants of fertilization were highly significant (table 2). Roljević Nikolić *et al*. (2018) cited that application of fertilizers in organic production, depending on the variant and varieties, influenced the increase in the total number of microorganisms in the level of 60.8% (Nirvana), which is one of the reasons for increasing grain yield.
The largest content of microelements (Cu, Mn and Zn) was measured in a variant of the combined application of bio-fertilizer and microbial fertilizer, and the smallest in the variant of the independent application of microbial fertilizer in top dressing. The highest content of macroelements (Ca, Fe and Mg) was measured in the control variant, except for Fe, where the variant of top dressing with Slavol gave the best results (Table 2). In the paper Popović Brigita *et al*. (2014), once foliar treatment resulted in a statistically significant increase in the content of all examined microelements (Fe, Zn, Mn and Cu) in wheat grains.

Table 2. The grain yield, content of macro- and micronutrients in grain of winter wheat (genotype Nirvana) in organic technology

Type of fertilizer	Grain yield	Chemical composition (mg/kg)						
	(kg/ha)	Ca	Fe	Mg	Cu	Mn	Zn	Average
Control	2580	108	28	1240	3.01	18.3	30.5	237.9
Uniker + Slavol	3920	107	19	1163	3.80	19.6	35.2	224.6
Slavol	3920	102	42	1018	2.38	17.1	28.7	201.7
Average	3473.3	105.6	29.6	1140.3	3.06	18.3	31.5	221.4

LSD	Yield	Ca	Fe	Mg	Cu	Mn	Zn
0.05	1070.9	3.58	0.42	18.8	0.14	0.34	0.13
0.01	1415.5	4.73	0.55	24.9	0.19	0.45	0.18

In the conventional winter wheat growing technology, the highest yield of grain (9200 kg/ha) was achieved at the most intensive level of top dressing of 120 kg/ha a.m., and the smallest in the control variant (8360 kg/ha). The differences were not statistically significant (Table 3).

Table 3. The grain yield, content of macro- and micronutrients in grain ow winter wheat (genotype Ilina) in conventional technology

Level of top dressing	Grain yield (kg/ha)	Chemical composition (mg/kg)						
		Ca	Fe	Mg	Cu	Mn	Zn	Average
Control	8360	189	24.2	1153	5.25	22.6	28.62	237.11
N_1	8600	160	26.8	1124	1.38	22.8	26.58	226.93
N_2	9200	145	19.5	936	1.31	19.2	23.60	190.77
Average	8720	164.7	23.5	1071	2.65	21.5	26.27	218.27

LSD	Yield	Ca	Fe	Mg	Cu	Mn	Zn
0.05	1695.6	3.38	0.34	16.90	0.08	0.26	0.14
0.01	2241.1	4.46	0.45	22.34	0.11	0.34	0.19

The differences in the content of the macro- and microelements measured in the control and variants with the top dressing of different nitrogen doses are statistically significant. The largest content of the elements in the control variant (except Fe and Mn) was mostly measured, and the smallest in the variant with a higher dose of nitrogen in top dressing. The general fact that the use of fertilizers and pesticides in agricultural production increases the concentration of zinc in the soil has not been confirmed here.

Between conventional and organic production, a very significant difference in the yield was recorded, while the differences in the concentration of all the investigated elements showed a difference, but at a significantly lower level. On average, in organic cultivation technology, a greater content of iron, copper and zinc in wheat grains was found in relation to conventional production, while significantly lower Ca, Mg and Mn content was measured (Table 2 and 3). Several experiments have been performed in order to compare the effects of organic and conventional (mineral) fertilisers on the crop yield and nutritional status of plants, since organic yields are often lower compared with conventional production (Martinez-Ballesta *et al*., 2010).

Conclusion

The investigation different growing technology and their effects on productive characteristics nutritional quality leads to the following conclusions:

- The importance of genotype response to growing conditions on environmental grounds is very large, the choice of the genotype is important as the level of implementation of other cultural practices.
- In the investigated varieties of winter wheat cultivated in both growing systems, all macro- and microelements were detected. The differences in the content of all examined elements in wheat grain from conventional in relation to organic cultivation technology were noticed.
- The content of macro-elements was higher in conventional while the content of microelements was higher in organic growing technology.

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RESEARCH ON ORGANIC IMPORT REGULATION REGIME SHIFTS OF SOME IMPORTANT COUNTRIES (EU AND US) REGARDING TURKEY ORGANIC FOREIGN TRADE

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Abstract

In this paper, the developments in the organic product import regimes of the EU countries, which constitute more than 50%, and of the USA, which constitute about 20% of Turkey's export value of organic agricultural products, are researched in terms of the quantity, price, quality and market access of Turkey's organic product export. For this purpose, a questionnaire study was conducted with all 'foreign trade authorized' control and certification bodies (CBs) and 41% of the companies selling organic products in 2015 in the province of Izmir, which has a significant share in the production and export of organic products in Turkey. The results of this research indicate that there has been a rapid increase in the export of organic agricultural products of Turkey, presumed to be a third country by the EU, with the new Regulation (EU) No 834/2007 abolishing the Regulation (EU) No.2092/91. This regulation has resulted in a positive effect on exporting organic agricultural products because it has paved the way for rapid commercialization, a wide range of suppliers and plenty of opportunities for clients. In recent years, some countries such as the EU, the USA and Japan have made important strides in the development of world organic commodity trade by making mutual recognition agreements that facilitate the trade of organic products. Mutual agreements made in developed countries have facilitated and increased the trade of organic products among these countries. Therefore, it is suggested that either a mutual recognition agreement should be made between Turkey and the EU and the USA, which are important countries for the export of organic products, or the acceptability of Turkey's own standards in these countries should be established.

Keywords: *organic agriculture, organic product trade, organic product import regime, organic agriculture legislation, list of third countries.*

Introduction

Since the establishment of the International Federation of Organic Agriculture Movements (IFOAM) in 1972 for the global development of organic farming practices, the first examples of which were developed in the early 20th century, developed countries have designed their own regulations. In 1991, the Regulation No 2092/91 entitled 'Organic Production of Agricultural Products and Indications Referring Thereto on Agricultural Products and Foodstuffs' entered into force for the first time in the EU. Subsequently, organic production and trade has been reshaped by the Council Regulation No 834/2007 on organic production and the labeling of organic products adopted in 2007 and enacted in 2009, Commission Regulation No 889/2008 laying down detailed rules for the implementation of Regulation No 834/2007, and Regulation No 1235/2008 entitled 'Detailed Rules for the Implementation of Council Regulation (EC) No. 834/2007 as regards the Arrangements for Imports of Organic Products from Third Countries (Abay et al.,2011). The new Commission Implementing Regulation (EC) No 2016/1842 of 14 October 2016 and the Regulation No 1235/2008 on the electronic inspection certificate for imported organic products have taken their final shapes today. In the United States, the access of organic products to the US market and the principles

of production were determined through the enactment of the National Organic Program (NOP) in 2002 by the US Department of Agriculture (USDA).

While the size of the total organic food market in the world was 15 billion dollars in 1999, it reached 89.7 billion dollars in 2016 and increased about six times from 1999 to 2016. According to data from the year 2016, about 90% of the organic food market value in the world is in North American and European countries (Willer & Kilcher, 2009; Sahota, 2018).

In the EU, the organic market is continuing to show more growth tendency than organic agriculture land. The fact that the increase in producers of organic products fall behind the increase of processors and importers in the EU shows that organic production is not able to meet the demand for increased organic products (Willer & Lernoud, 2017). For this reason, a significant part of the organic products consumed by the Member States of the EU is provided by imports from third countries.

The United States first made a mutual recognition agreement with Canada in 2009. Similar agreements with the United States continued with the EU in 2012 (EC, 2016), Japan and South Korea in 2014, and Switzerland in 2015. From the date on which these agreements entered into force, the parties to the treaty agreed on the control systems and standards of each other, so the products certified in one country can be sold in the other country. These agreements have had positive effects on the foreign trade of organic products (Jackie and Demko, 2015). In Turkey, organic agriculture has begun with the production of raisins, dried figs, dried apricots and nuts since the mid-1980s under the leadership of the European organic farming firms through contract farming. There was no national legislation to regulate organic production in Turkey between the years 1984-1993, and the production was carried out based on the rules of the importing country. Between 1994 and 2002, some developments took place at regulatory level. The first regulation, Regulation No 22145 on 'Ecological Production of Vegetable and Animal Products', entered into force on December 18, 1994. In this period, organic farming activities started to develop and spread especially in the Aegean region (Ersun and Arslan, 2011) Organic Farming Law was published on December 3, 2004, and following the 'Regulation on the Principles and Implementation of Organic Farming' which was put into effect on June 10, 2005, 'Regulation on the Principles and Implementation of Organic Farming' was published in the Official Gazette No 27676 in August 2010. In addition, Turkey has applied to take part in the EU Third Countries List (MFAL, 2010)

The number of organic farmers, organic agriculture land and organic production in Turkey has increased over time, and a significant number of organic products have turned towards export. Products such as dried figs, dried apricots, seedless raisins, chickpeas, red lentils, hazelnuts, as well as organic products such as pistachios, pine nuts and fig paste are the exported products. The countries of the European Union, particularly Germany and France, where the largest number of organic products are exported, constitute the largest markets with the USA together (USDA, 2015, 2016). In the world, organic products are exported to 68 countries. The fact that the exports of organic agricultural products are predominantly oriented towards the USA and the EU necessitates the monitoring of regulations and changes of access to these markets.

Despite having its own ecological advantages, being largely in compliance with the EU organic production regulations, expanding organic product range and showing an increase in production quantities of organic products, Turkey is not considered as equivalent by developed countries in terms of import regulations. Taking the growing trade of organic products in the world into account, it can be clearly seen that it is extremely important for Turkey to grow exports by ensuring the equivalence between the organic farming regulations of Turkey and other foreign countries.

Materials and methods

In this study, both primary and secondary data were used. The primary data that constitute the main material of the study were obtained from the questionnaires conducted with companies exporting organic agricultural products and control and certification bodies in Izmir province. Secondary data used in the research were obtained from the related theses, reports, articles and domestic and foreign sources on the internet sites such as IFOAM, FIBL, Ministry of Food, Agriculture and Livestock (MFAL).

Based on the list obtained from MFAL website, a survey was conducted with 22 firms exporting organic agricultural products in Izmir province. There are leading, but few firms among those Turkey-based firms which export organic products. Besides, eight control and certification bodies authorized by the MFAL and the authorities of importing countries to export organic products were included in the study. These control and certification bodies have first begun and are continuing their operations in Izmir province in Turkey, and their independence and competence have been accredited. While conducting the questionnaire with the control and certification bodies, the general manager or another authorized person was interviewed face-to-face in all bodies, except for one.

In this paper, tables and graphs as well as simple statistical methods such as arithmetic average and percentage calculation were used to analyze data.

Results and discussion

All firms surveyed exporting organic agricultural products have been exporting for 12.50 years on average. It has been determined in the study that the main organic products exported by the firms surveyed are dried figs, raisins and dried apricots (over 50.00%) as well as a wide variety of organic products. In addition, 15 out of the 22 firms surveyed carried out more than 50.00% of total exports of organic agricultural products to a single country. These countries are the USA, Germany, France, Holland and Denmark (Table 1).

Table 1 Surveyed Firms Exports of Organic Farming Products to the US and EU Countries

Countries	50% and more	%	Less than 50%	%	Total number of firms (22)	%
France	2	9.09	15	68.18	17	77.27
Germany	4	18.18	12	54.55	16	72.73
Holland	2	9.09	11	50.00	13	59.09
USA	6	27.27	7	31.82	13	59.09
England	-	-	10	45.45	10	45.45
Italy	-	-	8	36.36	8	36.36
Switzerland	-	-	4	18.18	4	18.18
Czech Republic	-	-	3	13.64	3	13.64
Sweden	-	-	3	13.64	3	13.64
Austria	-	-	2	9.09	2	9.09
Spain	-	-	2	9.09	2	9.09
Poland	-	-	2	9.09	2	9.09
Norway	-	-	2	9.09	2	9.09

Denmark	1	4.55	1	4.55	2	9.09
Romania	-	-	1	4.55	1	4.55
Hungary	-	-	1	4.55	1	4.55
Other	-	-	11	50.00	11	50.00

* Since more than one product is exported, the total is more than 100%.

Companies that export organic agricultural products after 2009 cannot formulate an opinion on the EU regulation change because they cannot make a comparison with the previous system. For this reason, as of 2009, 66.66% of the companies exporting organic agricultural products indicated that it is now easier to certify organic products to be exported, and 33.34% indicated that there is not any change. 90% consider that the new arrangement in the EU facilitates quick trade and certification of the products. Since organic production is a controlled production method from the outset, in the previous system companies had to communicate all phases of export including the organic product supplier information to competent authorities in the relevant countries. This process had to be repeated in every export. After the aforementioned process has been removed,

a) the exporting firms should be able to work with the suppliers they wish or work with more suppliers in order not to meet with an obstacle while exporting products in case of changing suppliers,

b) it is now possible to certify more products in order to meet the demands of the EU countries, even if there are a few demands. For these reasons, 50.00% of the companies stated that the new regulation facilitated working with more suppliers, and 40.00% stated that certifying more products got easier.

31.82% of the companies surveyed have and 68.18% have not been certified by a different CB since the year they started organic export. Among the firms that previously worked with a different CB, 42.86% of them changed their CB to receive higher quality certification services, and 57.14% changed their CB due to special reasons such as commercial issues or customer requests. 13.33% of these firms stated that Regulation No 1235/2008 affected the quality of organic products positively, and 86.67% stated that this regulation had no effect on the quality of products. 73.33% of the firms surveyed stated that Regulation No 1235/2008 facilitated the transportation of poultry while exporting organic products, 20.01% said that it did not bring about a change, and one firm said it got more difficult.

Of the firms that think it got easier to access to the market to export organic products, 90.90% stated that the new regulation saves time; 63.63% stated that instead of giving separate import permits to each country desiring to export organic products, the certificates obtained from authorized CBs are valid in the EU countries, which facilitates access to the market. 72.72% of these companies can make sales to meet instant demands, 36.36% can work with more suppliers, and 45.45% can certify more products, so it gets easier to reach the market. As of the year 2009, 13.33% of companies exporting organic agricultural products stated that they had rarely experienced quality loss when they reached foreign countries, while 86.67% stated that there was no quality loss. The two firms stating that quality loss was rarely experienced expressed that removing the EU's import permit process shortened the period, but this did not affect the quality loss because the formalities were completed before the export process. More clearly, these firms are of this opinion because they containerized the goods when they got import permits from the relevant countries before the export took place. The eight surveyed CBs operating in Izmir are all EU-based companies. These CBs have been operating for an average of 14 years domestically and in the EU, and for about 12 years according to US-NOP regulation.

87.50% of the CBs agreed that the concept of 'conformity' is 'the control and certification bodies operate on the basis of the EU directive', while 12.50% participated in this statement partially. Similarly, 87.50% of the CBs agreed that the concept of 'equivalence' is 'the control and certification bodies establish their own standards and operate with the approval of the EU Council', and 12.50% agree with that statement partially. All CBs thinking that the new EU directive facilitates the certification of organic products to be exported have all indicated that this is due to the fact that the amendment to regulation has resulted in less bureaucracy and saved time. 87.50% of the CBs think that the new regulation facilitates the certification of organic products in terms of the companies exporting to our country, and 12.50% think that it does not bring any convenience. 57.14% of the CBs thinking that this regulation brings convenience to the exporting firm reported that the workload of the firms in the certification process decreased because of the decrease of bureaucratic transactions, and 85.71% of the time was saved by shortening the process. They also pointed out that trade is accelerating, giving the exporters a broader supply chain and broader buyer opportunities.

On the other hand; in accordance with the Regulation No 834/2007, in case of a discommodity detected in their bodies operating in other countries, the EU Commission could disauthorize CBs that the organic product firms in Turkey need to work together with to export to the EU. In such a case, the loss of all EU countries' markets is the main problem with the cancellation of the authority of the CB's headquarter with all the organs operating in other countries along. In addition, while the relatively new EU system and the previous system were co-operated, CBs stated that they had gone through a complex and lengthy process in order to have sufficient technical equipment and knowledge in order to become an equivalent institution listed in the Regulation No. 1235/2008. 75% of the CBs participating in the survey did not apply to the EU Commission to be an equivalent company. For this reason, the headquarter of these CBs has to apply to the EU Commission to include the CBs in Turkey. However, applications of all applicants have been accepted. These firms stated that they applied to serve domestically and abroad as a company based in Turkey, to work with more export firms and to increase competitiveness in the sector. These applicants stated that the application process to the EU Council was lengthy and complicated. In addition, 50% of the CBs had some complications / difficulties because the CBs of their suppliers were not be included in the annex to Regulation No 1235/2008, and thus this caused a setback to the export of organic products.

Conclusion

In recent years, some countries such as the EU, the USA and Canada have taken important steps in the development of organic commodity trade in the world, with mutual recognition agreements facilitating the trade of organic products. Yet, the agreements made include the trade of organic agricultural products between developed countries, but have not yet been addressed to developing countries. Mutual agreements made in developed countries ensure that the organic products in these countries can be sold without subjecting them to additional control and certification procedures, which reduces the difficulties in the trade of organic products. As a result, the trade of organic products between these countries is being facilitated and increasing (Jaenicke and Demko, 2015) While legal regulations in Turkey have been implemented effectively in organic agriculture since 1994, it is still necessary to monitor the institutions that control and certify based on foreign standards and collect data, review and fill out the legal gaps in equivalence issues in the existing system in terms of importing organic products and inputs and facilitate the procedures. In addition, considering the EU countries where the organic commodity trade is largely conducted worldwide, it can be said that the Third Country List remains important in the foreign trade of organic agricultural products, despite EU amendments to Law No 834/2007.Turkey, which is now a third country, applied to enter the Third Country List of the EU in 2003 and has completed several phases of

supervision successfully. However, it is not on the list yet. Moreover, Turkey does not have mutual recognition agreements with the US and other countries. For these reasons, Turkey needs to comply with the regulations of its target markets to export organic agriculture products, and the same products are compulsorily subject to multiple certification. Considering the gradual increase in demand for organic products by the USA and the EU, which are important countries for Turkey's organic product export, Turkey holds an important position in terms of a) its compliance with the regulations of these countries, b) its potential of organic agriculture, c) its being a country whose regulations are considered equivalent by these countries based on the growing export especially since 2012. Thus, it is going to be possible for Turkey to increase organic product exports, have an important place in the organic products market in the world, and increase its competitiveness with other countries in terms of varieties of organic agricultural products.

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THE YIELD AND QUALITY CHARACTERISTICS OF WIDELY GROWN GRAPE VARIETIES UNDER LOWLAND AND HIGHLAND CONDITIONS IN MERSIN, TURKEY

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Abstract

This study was carried out in Mersin (Turkey) in 2012-2013. Mersin, a city of Mediterranean Region, has very high potential for viticulture, while the early ripening table grapes are cultivated in the lowlands, late ripening table grapes and raisins grapes cultivation is done widely at highland regions. In this study, it was understood that Yalova İncisi, Tarsus Beyazı, Ergin Çekirdeksizi, Trakya İlkeren and Victoria grape varieties were cultivated at the lowland (≤500 m) and Kişniş, Göğüzüm, Takkara, Dilmit and Tilkikuyruğu grape varieties were cultivated at the highland of the Mersin. Grape growing maturity period in Mersin begins with in the second half of June at lowland (Trakya İlkeren) and ends with at the end of November (Tilkikuyruğu) at highland. The highest yield of grapes per vinestock was determined at Ergin Çekirdeksizi (10.42 kg) at lowland and, on the other hand, in Tilkikuyruğu (16.93 kg) at highland. Varieties' cluster of grapes weights was determined between 318.09 g (Trakya İlkeren) and 410.48 g (Ergin Çekirdeksizi) and a hundred berry weight was found out between 285.0 g (Ergin Çekirdeksizi) and 759.4 g (Victoria) at lowland. However, cluster of grapes weights showed a change between 192.00 (Kişniş) and 446.61g (Tilkikuyruğu) and a hundred berry weight was from 93.6 g (Kişniş) to 651.8 g (Tilkikuyruğu) at highland. TSS contents of varieties differed from 14,8% to 13.1% (except Tarsus Beyazı) at lowland. On the other hand, that showed an alteration between 15.6% and 19.2% at highland.

Keywords: *viticulture, yield, quality, lowland, highland, Mersin.*

Introduction

Turkey ranks the fifth in terms of vineyard areas (435.227 ha) and the sixth in terms of grape production (4.000.000 ton) throughout the world (Anonymous, 2017a). Mersin province, which is located in the Mediterranean Region of Turkey. Summer months are hot and extremely humid, while winter months are warm and rainy. Mersin is one of the exceptional provinces where agricultural production is aimed at three periods; i.e. "early", "mid-season" and "late". Early fruit types and varieties, greenhouse vegetable growing, banana and strawberry production are predominant at the coastal part of the province. Late season fruit and vegetable growing, aimed at exportation, is considerably important in highland areas. Mersin province has suitable climate conditions for viticulture in terms of annual average temperature (19,1°C), effective temperature totals (3150,4 day-degree) and annual amount of precipitation (592,3 mm) (Anonymous, 2017b). Agricultural soil of the province are generally argillaceous and calcareous, with high pH rate.

Mersin province ranks the fourth with 22.148 ha vineyard area and the third with 287.429 tons of grape production throughout Turkey (Anonymous, 2017c). Table (88,8%), dried (6.8%) and wine (4.4%) grapes are grown in the province. Mersin province ranks the second throughout Turkey in table grape growing (255.303 tons). While the amount of table grapes grown per unit area is 800 kg/da in Turkey, this amount is 1400 kg/da for Mersin. It is considered that this increase in production is caused by cultivation techniques such as irrigation, fertilizing, training system and pruning in addition to selection of cultivars. Early

season grape cultivation is prevalent in the lowland parts of the province, while late season cultivation is prevalent in the highlands (Tangolar and Gök Tangolar, 2003).
Grape harvest period of the province is quite long. The length of this period is influenced by selection of cultivars in addition to ecological conditions. Early or late ripening of cultivated variety allows the product to be evaluated more easily and at higher prices.
In this study, cultivars that are widely used in viticulture in vineyard establishments at lowland and highland parts of Mersin province have been determined and yield and quality characteristics of these cultivars have been determined.

Material and Methods

In the study, vineyard establishments in Mersin province were reviewed in two parts; lowland area and highland area. In terms of altitude, 0 to 500 m was accepted as lowland while 501m ≤ was accepted as highland. The number of establishments to be surveyed in Mersin was determined according to simple random sampling method (Yamane, 1967). Accordingly, a total of 297 surveys were conducted throughout Mersin; 125 in lowlands and 172 in highlands.
Top 5 grape cultivars that are most commonly cultivated in lowland and highland viticulture of Mersin province were identified according to the results of the conducted survey. These grape cultivars were used as materials of the study. 10 vineyards with similar characteristics (economical yield age, same type of cultivation) were identified for each cultivar. Each vineyard was accepted to be a repetition. Samples were obtained from a total of 100 vineyards. 10 vine were randomly selected in each vineyard and 2 cluster samples were obtained from each vine. Sampling of each cultivar was made during grape harvest of establishment owners. In order to identify quality characteristics of cultivars; cluster characteristics [cluster weight (g), cluster height (cm), cluster width (cm)], berry characteristics [berry weight (g), berry height (mm), berry width (mm), berry color (L, *a, *b), berry removal force (g)] and must characteristics [TSS (%), pH, acidity (%), TSS/acidity rate] were analyzed (Ergenoğlu, 1985; Lancaster *et al.*, 1997; Tangolar *et al*. 2005). Yield per vine ratio was determined as a result of multiplication of the number of clusters in each vine with average cluster weight for 10 vine in each vineyard.
Average values regarding yield and quality characteristics of cultivars are given in tables together with their standard deviations.

Results and Discussion

Eighteen grape cultivars, which are cultivated in lowland part of Mersin province, were determined according to survey results. It was identified that top five cultivars, which were most commonly cultivated, were; Yalova İncisi (52,56%), Tarsus Beyazı (15,17%), Ergin Çekirdeksizi (8,55%), Trakya İlkeren (6,86%) and Victoria (3,94%).
On the other hand, it was determined that fifty two grape cultivars were cultivated in highland part of Mersin province. It was identified that top five cultivars, which were most commonly cultivated, were Kişniş (16,84%), Göğüzüm (13,61%), Takkara (12,82%), Dilmit (12,56%) and Tilkikuyruğu (10,26%). It was observed that Trakya İlkeren was the earliest ripening cultivar (June26) in Mersin lowland viticulture, and that harvest of Yalova İncisi and Ergin Çekirdeksizi cultivars followed in approximately one week intervals. It was seen that Tarsus Beyazı and Victoria cultivars were harvested in the last week of July. It was determined that the harvest of Takkara and Kişniş cultivars started at the end of August, harvest of Göğüzüm and Dilmit started during the first week of September, while harvest of Tilkikuyruğu started during the first week of November in highland viticulture (Table1)
In our study, our findings regarding ripeness dates of cultivars bore resemblance to the results of Sabır (2008) and Gök Tangolar (2009) regarding Trakya İlkeren and Yalova İncisi; results

of İnal (2000) regarding Takkara; and results of Özdemir and Tangolar (2005) regarding Tarsus Beyazı. However, it was observed that ripeness times of Trakya İlkeren, Yalova İncisi and Ergin Çekirdeksizi cultivars were approximately one week earlier than our findings in the study conducted by İnal (2000) under Alata/Erdemli conditions.

It was identified that yield values of cultivars per vine vary between 6,58 kg (Trakya İlkeren) and 10,42 kg (Ergin Çekirdeksizi) under lowland conditions, and 5,96 kg (Göğüzüm) and 16,93 kg (Tilkikuyruğu) under highland conditions (Table1).

Our findings regarding yield of cultivars per vine were significantly lower than the findings of Sabancı (2009) for Trakya İlkeren and Yalova İncisi cultivars. In our study, yield value determined for Ergin Çekirdeksizi cultivar was similar to the findings of İnal (2000) and Sabancı (2009); while yield values for Tarsus Beyazı, Tilkikuyruğu and Takkara cultivars were higher than the findings of İnal (2000). Tarsus Beyazı and Yalova İncisi cultivars yielded respectively the highest and the lowest values in terms of cluster width in the lowland; while respectively Göğüzüm and Kişniş cultivars yielded the same values in the highland. In terms of cluster weight, the highest and the lowest values were observed on respectively Ergin Çekirdeksizi (410,48 g) and Trakya İlkeren (318,09 g) cultivars in the lowland; in comparison with Tilkikuyruğu (446,61 g) and Kişniş (192,00 g) cultivars in the highland (Table1).

Our findings regarding cluster weights of cultivars bear resemblance to the findings of Sabır (2008) and Gök Tangolar (2009) for Trakya İlkeren, and the findings of İnal (2000) for Ergin Çekirdeksizi and Takkara cultivars. Values identified by Sabır (2008) for Yalova İncisi, Ergin Çekirdeksizi, Tarsus Beyazı and Tilkikuyruğu cultivars; by İnal (2000) as well as Özdemir and Tangolar (2005) for Tarsus Beyazı cultivar; and Kamiloğlu and Polat (2009) for Yalova İncisi and Ergin Çekirdeksizi cultivars were found to be lower than the values that we obtained in our study. It was also seen that cluster weights identified by Gök Tangolar (2009) for Yalova İncisi and İnal (2000) for Trakya İlkeren, Yalova İncisi, Tilkikuyruğu cultivars were higher than our study. While Victoria yielded the highest value and Ergin Çekirdeksizi yielded the lowest value in terms of berry width, berry height and weight of 100 berries among cultivars that are widely cultivated in lowland viticulture; Tilkikuyruğu yielded the highest and Kişniş yielded the lowest values in terms of the same characteristics in highland viticulture (Table 1). Our findings regarding 100 berry weights of cultivars were similar to the findings of Sabır (2008) for Tilkikuyruğu, and Kamiloğlu and Polat (2009) and İnal (2000) for Yalova İncisi and Ergin Çekirdeksizi. Berry weights of Trakya İlkeren and Tarsus Beyazı were found to be higher than the findings of Sabır (2008) and İnal (2000). Furthermore, berry weights of Tilkikuyruğu and Takkara cultivars were also found to be higher than the values determined by İnal (2000). In terms of removal force; while Victoria yielded the highest value (536 g) and Tarsus Beyazı yielded the lowest value (240 g) in the lowland; Tilkikuyruğu and Kişniş cultivars respectively yielded the highest (418 g) and the lowest (121 g) values in the highland (Table1). It was determined that the berry removal force value of Trakya İlkeren cultivar was higher than the findings of Ozer and Kiracı (2002) and similar to the finding of Kamiloglu (2013). However, the value obtained from Yalova Incisi in terms of this characteristic was higher than the findings of Aydın (2009). On the other hand, berry removal force value of Ergin Cekirdeksizi was lower than the findings of Kamiloglu (2013) and the same value of Tarsus Beyazi was similar to the findings of Ergenoglu (1985).

In terms of berry color characteristics of cultivars; L and b* values were found to be the highest in Victoria cultivar and the lowest in Trakya İlkeren cultivar under lowland conditions. A* value was identified to be highest in Trakya İlkeren cultivar and the lowest in Ergin Çekirdeksizi and Victoria cultivars. In terms of color characteristics under highland conditions, L and b* values were found to be the highest in Tilkikuyruğu cultivar, while a value was found to be the highest in Kişniş cultivar. The lowest L, a*, b* values were obtained from respectively Dilmit, Tilkikuyruğu and Takkara cultivars.

Table 1. Harvest date and yield and some quality features for grape cultivars that are widely cultivated in MersinLocation	Cultivar	Harvest date	Yield (kg/vine)	Cluster weight (g)	100 Berry weight (g)	Berry removal force (g)	TSS (%)	pH	Acidity (%)	Maturity index
Lowland	Trakya İlkeren	Jun26	6.58 ± 1.86	318.09 ± 52.01	461.80 ± 37.71	460 ± 52.4	13.4 ± 0.90	3.46 ± 0.11	0.61 ± 0.06	21.76 ± 2.84
	Yalova İncisi	Jul03	7.59 ± 2.27	359.24 ± 52.90	647.80 ± 73.26	363 ± 41.5	13.9 ± 2.02	3.65 ± 0.19	0.36 ± 0.10	38.46 ± 18.72
	Ergin Çekirdeksizi	Jul10	10.42 ± 3.45	410.48 ± 52.70	285.00 ± 40.48	248 ± 45.2	13.1 ± 1.33	3.05 ± 0.13	0.60 ± 0.12	21.96 ± 5.05
	Tarsus Beyazı	Jul26	8.25 ± 1.75	371.08 ± 26.41	428.50 ± 29.09	240 ± 45.4	19.6 ± 0.71	3.57 ± 0.08	0.43 ± 0.04	45.80 ± 3.90
	Victoria	Jul26	7.25 ± 2.23	404.84 ± 44.84	759.40 ± 43.62	536 ± 110.1	14.8 ± 0.92	3.79 ± 0.12	0.29 ± 0.04	51.28 ± 8.48
Highland	Tilkikuyruğu	Nov02	16.93 ± 4.34	446.61 ± 72.69	651.80 ± 51.08	418 ± 48.8	18.7 ± 1.65	3.59 ± 0.20	0.35 ± 0.07	52.86 ± 13.29
	Göğüzüm	Sep03	5.96 ± 2.00	312.27 ± 50.35	539.05 ± 40.39	377 ± 37.2	15.6 ± 1.80	3.52 ± 0.14	0.44 ± 0.12	35.37 ± 22.10
	Takkara	Aug29	7.34 ± 1.51	274.98 ± 23.61	397.90 ± 43.80	281 ± 29.0	18.7 ± 1.05	3.32 ± 0.07	0.51 ± 0.06	36.65 ± 4.90
	Kişniş	Aug29	14.14 ± 2.45	192.00 ± 23.42	93.60 ± 18.94	121 ± 32.2	19.2 ± 1.68	3.30 ± 0.12	0.67 ± 0.10	28.75 ± 4.45
	Dilmit	Sep03	11.05 ± 2.52	235.05 ± 38.34	223.20 ± 31.16	139 ± 11.6	18.6 ± 0.88	3.57 ± 0.09	0.48 ± 0.04	38.71 ± 2.93

It was identified in the study that harvest of early-ripening cultivars other than Tarsus Beyazı (19,6%) were started to be harvested at 13-14% TSS content in the lowland. pH level of cultivars varied between 3,05 and 3,79, while their acidity contents varied between 0,29% and 0,61%. Maturity index value was the lowest for Trakya İlkeren cultivar, which was the earliest cultivar to be harvested, and the highest for Tarsus Beyazı and Victoria cultivars, harvest of which started at the end of July. In cultivation under highland conditions, harvest of cultivars other than Göğüzüm (15,6%) starts at 18-19% TSS content. In addition, pH varied between 3,30 and 3,59, acidity varied between 0,35% and 0,67%, and maturity index varied between 28,75 and 52,86 in cultivars cultivated in the highland (Table1).

The findings regarding TSS contents of cultivars were similar to the findings of İnal (2009) and Sabancı (2009) regarding Yalova İncisi; and Sabır (2008) and Özdemir *et al*. (2006) regarding Tilkikuyruğu. It was seen that this characteristic was lower for Trakya İlkeren and Yalova İncisi cultivars than the values obtained by Sabır (2008) and Aydın (2009) from their studies. This value was found to be higher for Tarsus Beyazı than the findings of Özdemir and Tangolar (2005) as well as Sabır (2008). Maturity index values established during our study were identified to be similar to the findings of Kamiloğlu and Polat (2009) regarding Yalova İncisi and Ergin Çekirdeksizi; similar to the findings of Sabancı (2009) regarding Trakya İlkeren; and higher than the findings of Özdemir *et al.* (2006) regarding Tilkikuyruğu.

Conclusion

Yalova İncisi, Tarsus Beyazı, Ergin Çekirdeksizi, Trakya İlkeren, Victoria grape cultivars are widely cultivated in lowland viticulture establishments, while Kişniş, Göğüzüm, Takkara and Tilkikuyruğu grape cultivars are widely cultivated in highland viticulture establishments in Mersin province. Grape harvest starts in the second half of June (Trakya İlkeren) in the lowland and continues until late November (Tilkikuyruğu) in the highland. Table grape cultivation is prevalent in the lowland, while mid- and late-season table grape and raisins (Kişniş, Dilmit) cultivation is prevalent in the highland in Mersin province. Among these cultivars, Ergin Çekirdeksizi is prevalent in the lowland while Tilkikuyruğu is prevalent in the

highland in terms of yield per vine and cluster weight. In terms of berry weight and removal force, Victoria stands out in the lowland while Tilkikuyruğu stands out in the highland.

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PHYTOREMEDIATION ABILITY OF SOME CROPS AT CULTIVATION ON THE SOILS POLLUTED BY HEAVY METALS

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Abstract

Due to the fact that soils contaminated with heavy metals have features on the intensity and type of contamination (lead, cadmium, mercury, tin, titanium, chromium, etc.) in various regions of Belarus, and plants differ significantly in the ability to accumulate heavy metals, it is necessary to show the effectiveness of sorbents on the basis of calcinated sugar beet production defecate on the example of growing a technical colza. The use of cheap sorbents based on a defecate on contaminated soils can facilitate their return to an agricultural circulation. The method of X-ray fluorescence analysis (XRF) has been used to assess the environmental safety of soils and plant organs, as well as to determine the level of their accumulating capacity. The analysis of the obtained data has shown that the accumulation of cadmium and lead in colza leaves significantly increases on the contaminated soils (by 82%). The levels of the accumulation of manganese, zinc, zirconium, and strontium significantly increase in a phytomass. A decrease in the accumulating capacity (with respect to copper and iron) of colza plants grown on the contaminated soils has been revealed. It has been shown that spreading heat treated defecate to the soil permits reducing the accumulation of arsenic, copper, manganese, and lead by a technical colza phytomass.

Keywords: *environmental safety, X-ray fluorescence analysis, heavy metals, technical colza, sugar beet production defecate*

Introduction

Sugar industry is one of the priority directions of the social and economic development of the Republic of Belarus, providing food security of the country, contributing to the development of agricultural production. More than 450 agricultural enterprises in four regions of the Republic – Brest, Grodno, Minsk, and Mogilev – are engaged in industrial beet growing. There are formed two types of solid wastes in the production of sugar from sugar beet – a pulp and filtrational sediment (defecate). A pulp is used as an additive to livestock feed, so the issue of its utilization is closed. This is not the case with a defecate.

Defecate (the secondary product of sugar beet and raw sugar production) is a product valuable on its chemical and consumer properties. In the earlier period it was removed from sugar factories to filtration fields in the form of a mud suspension and was known as "a filtrational mud". In the conditions of modern advanced technology, it goes out of production in the form of compressed lumps with the mass fraction of dry substances 65% - 70% and has a short, convenient for the producers name "defecate". At the same time according to the TS RB 37602662.630-99, an official name of this waste is "a filtrational sediment". The sediment is destined for liming (neutralization) of acidic soils. The method of application is in accordance with the "Instruction on liming of acid soils of the agricultural lands of the Republic of Belarus", Minsk, 1997.

Because around the world the most recognized scheme for the treatment of sugar solutions from nonsugars and dyes is accepted a scheme through the processes of defecosaturation, the amount of a defecate increases in sugar-producing countries every year. In the Republic of Belarus the volume of a defecate output in all sugar factories has increased significantly and

reached 362 thousand tons per year over the past 4-5 years. At the same time, the use of a defecate in the national economy is limited. 152 thousand tons of a defecate as lime deoxidizing materials were shipped for the agricultural organizations of the Republic; the remaining amount (230 thousand tons, or 60% of the production volume) was exported to the quarries and a small part of it was placed near sugar factories. And the issue of defecate utilization becomes complicated every year with an increase in sugar production. Recently across countries, attempts have been made to use a defecate in various technical sectors: in the production of building materials, fertilizers, in the creation of new types of effective sorbents, etc. Taking into account the uniqueness of defecate composition, the important significance is attached to it as a component of the feed for pets and birds. So it can replace a career fodder chalk. The physical and chemical parameters of a defecate in the form, in which it goes out of production, are suited to the requirements of neither mixed fodder nor agricultural enterprises. High humidity (about 30% - 35 %) and polydisperse composition (from a dust to large clods) prevent organizing the processes of dosing a sediment and its uniform mixing in combined fodder. The same parameters prevent organizing the uniform introduction of a filtrational sediment in deoxidized soils by the method of blowing out from tanks that is now applied everywhere when using a fine dolomite flour.

Currently, only a small proportion of a defecate is used in the agricultural sphere for soil mineralization, however, most of it is stored in industrial sites or filtration fields as unclaimed wastes. According to approximate calculations, more than 120 hectares of land are alienated for the storage of a defecate in industrial sites at each plant. When storing a defecate on landfills, environmental pollution occurs.

As it is known, carbon sorbents are widely used for wastewater treatment. However, they have a number of disadvantages: the need for regeneration and utilization, high cost, the unsustainable use of natural resources (deforestation).

The carbon-containing sorbent obtained from a defecate, of course, may have lower sorption properties compared to generally recognized carbon sorbents. However, despite this, its production and use has a number of advantages: low cost, the conservation of natural resources, no need to regenerate the spent defecate (Gregersen *et al.*, 1999).

Materials and methods

The object of research is a technical colza in the process of growth on heavy metal contaminated soils with the introduction of heat treated sugar beet production defecate as a sorbent. When taken for the analysis, the soil and plants have been brought to an air-dry state. The samples have been crushed and sieved through a sieve with a cell size of 1 mm. The particles, which have not passed through a sieve, have been again ground in a mortar and sieved. The obtained samples have been studied by the method of X-ray fluorescence analysis (XRF). This is one of the many modern physical and chemical methods of measurement. It is widely used for the qualitative, semi-quantitative and quantitative determination of the elemental composition of substances. The XRF method is based on the measurement of energies (wavelengths in wave dispersion spectrometers) and the intensities of spectral lines emitted during secondary X-ray emission. A permissible error is up to 30 %.

The level of the chemical contamination of soil compositions has been found by comparing the content of the gross forms of chemical elements in the contaminated soil with the established in the Republic of Belarus maximum permissible and approximately permissible concentrations (MPC and APC, respectively) (Petukhova and Kuznetsov, 1992).

We have calculated the coefficient of biological accumulation (CBA), which has allowed characterizing the migration ability of heavy metals in the "soil-plant" system at the cultivation of a technical colza on the soils with the introduction of a defecate.

Results and Discussion

Table 1 shows the comparison of the gross concentrations of metals in the colza phytomass, which has been grown during a bench experiment, with their normal content and MPC levels.

Table 1. Concentrations of metals in a colza phytomass in different soil compositions

mg/kg

Element	Soil + solution of cadmium and lead salts	Soil + solution of cadmium and lead salts + heat treated defecate	Normal content (of air and dry mass, mg/kg)	MPC (of air and dry mass, mg/kg)
	Leaf	Leaf		
As	0.44 ± 0.19	trace quantities	0.1-0.5	5.0-20.0
Cd	0.60 ± 0.12	0.62 ± 0.11	0.05-0.2	5.0-10.0
Co	1.84 ± 0.57	18.26 ± 1.62	0.01-0.30	10.0-20.0
Cu	211.57 ± 7.39	74.95 ± 4.13	6.0-15.0	15.0-20.0
Mn	65.72± 5.04	52.02 ± 4.22	15-150	300
Fe	478.41 ± 10.15	557.25 ± 14.78	20-300	750
Pb	18.80 ± 1.88	3.31 ± 0.74	0.1-5.0	10.0-20.0
Sn	1.52 ± 0.31	1.25 ± 0.43	0.2-1.9	1.9
Ti	trace quantities	12.91 ± 4.93	0.15-80	20.0-30.0
Zn	39.95 ± 2.34	31.01 ± 1.94	15-150	150.0-200.0
Zr	7.69 ± 0.57	19.93 ± 0.87	0.06-12	-

The intensity parameter of the biological absorption of elements by vegetation – coefficient of biological accumulation (CBA) – has been calculated, which has allowed characterizing the migration ability of heavy metals in the "soil-plant" system. CBA has been calculated on the basis of data on the gross content of elements in soils and a plant phytomass (Ilyin, 1991)

The coefficient of biological accumulation has been calculated according to the formula

$$CBA = C_{p/} C_{п}$$

where C_p - the concentration of an element in a plant;

$C_п$ - the gross content of an element in the soil.

The value of biological accumulation has been determined according to the gradation (table 2) proposed by A. I. Perelman (Perelman, 1975).

Table 2. Scale of the intensity of element accumulation by the value of CBA (Perelman and Kasimov, 1999)

Value of biological accumulation	Value of CBA
Vigorous accumulation	10–100
Strong accumulation	1–10
Weak accumulation or medium capture	0.1–1.0
Weak capture	0.01–0.1
Very weak capture	0.001–0.01

The CBA of metals for the biomass of a technical colza, when growing in the contaminated soils without sorbents and soils with the application of a defecate, are presented in table 3.

Table 3. Coefficient of biological accumulation of metals in the colza phytomass grown in different soil compositions

Element	Soil + solution of cadmium and lead salts	Soil + solution of cadmium and lead salts + heat treated defecate
	CBA	CBA
As	0.595	0.000
Cd	0.021	0.069
Co	0.034	2.024
Cu	2.813	2.679
Mn	0.165	0.076
Fe	0.049	0.050
Pb	0.523	0.024
Sn	0.025	0.125
Zn	0.889	1.164
Zr	0.037	0.087

According to the data obtained, the series of the biological absorption of metals by a colza phytomass have been constructed.

Soil composition soil + solution of cadmium and lead salts:

Cu (2.813) > Zn (0.889) > As (0.595) > Pb (0.523) > Mn (0.165) > Fe (0.049) > Zr (0.037) > Co (0.034) > Sn (0.025) > Cd (0.021).

Vigorously accumulated metals in the phytomass of colza grown on the contaminated soil without sorbents have not been revealed. Copper accumulates strongly. The elements of a medium capture are as follows: zinc, arsenic, lead, and manganese. The elements of a weak capture are as follows: iron, zirconium, cobalt, tin, and cadmium.

Soil composition soil + solution of cadmium and lead salts + heat treated defecate:

– colza phytomass Cu (2.679) > Co (2.024) >Zn (1.164) > Sn (0.125) > Zr (0.087) > Mn (0.076) > Cd (to 0.069) > Fe (0.050) > Pb (0.024).

Vigorously accumulated metals in the phytomass of colza grown on the contaminated soil with the introduction of a defecate have not been revealed. Copper, cobalt, and zinc accumulate strongly. The element of a medium capture is tin. The elements of a weak capture are as follows: zirconium, manganese, cadmium, iron, and lead.

Thus, when introducing the heat treated defecate of sugar beet production as a sorbent to soil, the intensity of the accumulation of arsenic, copper, manganese, and lead decreases in a technical colza phytomass.

Conclusion

The application of the XRF method is promising in the solution of environmental safety issues. A sorbent on the basis of heat treated sugar beet production defecate permits reducing the intensity of arsenic, copper, manganese, and lead accumulation in a technical colza phytomass.

Consequently, the production of a sorption material from the calcinated defecate is characterized by a number of positive factors:

- decrease in the amount of a large-capacity waste – defecate;
- reduction of the areas of the fertile soil, applied for defecate storage;
- improvement of sanitary and epidemiological indicators of soils in the areas of sugar factories;

- social effect: improvement of the living conditions of the population due to the reduction of an anthropogenic impact on the environment.

Research in this direction is continuing.

Acknowledgement

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ENVIRONMENT PROTECTION AND NATURAL RESOURCES MANAGEMENT

THE AGRO-ECOLOGICAL PRESERVATION OF APRICOT VARIETIES BY THE BIOLOGICAL CURTAINS IN THE BOUKHMISSA AREA, HODNA PLAIN (M'SILA), ALGERIA

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Abstract

The varietal potential of the apricot in the agricultural area of Boukhmissa in the arid Hodna plain north of the town of M'sila has five varieties. This plant genetic resource contributes economically, socially and ecologically to the region. The overall protection of this potential is imperative and the biological curtains as an indirect protection is more than necessary. The biological curtains (windbreezes) installed for agronomic and environmental reasons. It consists of one or more rows of trees. The Boukhmissa (M'sila) perimeter, where the fruit plantation with apricot tree is the most important speculation, illustrates this practice well. A diagnosis of the situation in 29 study stations showed that the biological curtains in this area were installed in a subjective and anarchic way within the apricot orchards, the five most replicated varieties in the study area are: Red Louzi, Bullida, Paviot, Tounsi and Polonais. The results obtained at the end of this study are: -The use of conifers is dominant in more than 62% of study stations. -The biological curtains have a relatively young age (less than 20 years), which shows that this agroforestry protection system in this area is recent. - The low porosity of the system (28%) due to the relatively high planting density. -With respect to the height of the wind breezes of our stations, and given the relatively young age in most stations, the height is less than 10 m. - Most of the stations have small biological curtains, which are to say less than 3 meters, is more than 86% of the study stations, because of their composition of a single row of plant species Protection. In order to better exploit the biological curtains (wind breezes), an optimal height of protection, a porosity of 40 to 50%, A minimum width of 03 meters, an orientation perpendicular to the direction of the winds and finally a choice of fast growing, long-lived and multi-use species.

Keywords: *Biological curtains, Diagnosis, Varietal potential, Apricot, Boukhmissa*.

Introduction

Hedgerows and windbreaks have long demonstrated their effectiveness and are effective tools for improving the quality of the environment. Indeed, these structures reduce erosion and soil loss, increase biodiversity, promote better use of mineral elements and water in the soil. However, producers often perceive the establishment of such agroforestry systems as a loss of space, production and consequently a loss of income (Lebel and Deroy, 2007).

Scientific studies have shown that when windbreak trees are well chosen, well located and well maintained, agricultural and forest production increases, biodiversity, landscape quality and living conditions improve, water pollution, soils and air decreases (Labant, 2009). Due to its complex structure, the hedge offers ecological niches and shelters especially during the winter, it is also a relatively undisturbed environment (INRA, 2009).

In Algeria the protection of crops against the wind is indeed all the more necessary as climatic conditions are less favorable to agriculture, such is the case in coastal regions, plateaus and steppe areas (Greco, 1966). The practice of installing shelterbelts in agriculture is relatively recent in Algeria. The area of M'sila, by its agricultural perimeters, offers good examples, in

this case the perimeter of Boukhmissa which justifies this practice especially that it is a perimeter of fruit arboriculture by excellence.
The general objective of this study is a diagnosis of the importance of windbreaks among small agricultural producers of apricot orchards in the area of Boukhmissa (M'sila in Algeria) on the one hand and to see the prospects of rational use windbreaks by farmers in Boukmissa. In our study on wind breezes in this area, where the average size of the farms is relatively small, we first try to establish a statement on these protective and promising elements and secondly to identify the optimal, real and possible prospects in this place.

Materials and Methods

The study area is located in the region of Boukhmissa, it is an agricultural area located north of the town of M'sila in Algeria, its area is 4500 ha (Brahimi, 1992).
The direct observation aimed to identify and characterize agricultural stations or plots with respect to windbreaks: the type of windbreak, its location and the species that compose it. At the end of this inventory, we were able to identify twenty-nine (29) stations throughout the Boukhmissa perimeter. The research was conducted in 2012, The data on the stations and windbreaks used were collected according to a previously established questionnaire, for the owners of the plots, the respondents were the farmers themselves This survey questionnaire aims to collect quantitative and qualitative data on study stations and windbreaks used. Station data: Geographic position (Altitude, latitude, longitude), Exposure, Plot position in relation to River of K'sob (East or West shore), Plot size (m^2), Culture in place (Perennial or annual).
Data on windbreaks used: Type of windbreak (live or inert), Windbreak used (coniferous, hardwood), exposure (orientation of windbreak against wind), age (Young, adult or old), height (high and low), width (thick and thin), length (long and short) and the porosity of the windbreaks (low, medium and high).

Results and Discussion

1-Area

The study stations have variable areas, their classification according to this parameter is indicated in Table 1.

Table 1. Variation of the surface of the study stations.

Area (m^2)		Less than 5000	5000 and over	Total
Stations	Number	20	09	29
	%	68.96	31.04	100

The majority of the studied stations have surface areas lower than 5000 m^2 that is about 69 % of the total of the stations of study. This state shows that the studied stations are small parcels of land in the form of fruit orchard according to the dominant speculation of the zone and which is the fruit growing, in particular the apricot tree as pointed out (Bahlouli*et al.*, 2009). In all stations, one station has an area of 15,000 m^2 and three other stations have 10,000 m^2 each.

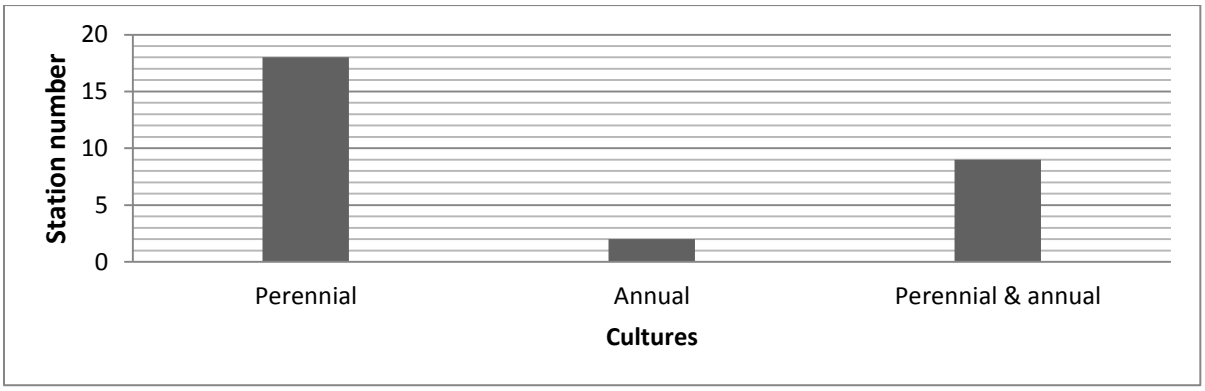
2- Cultures in place

The cultures in place testify to the vocation of the plots (Table 2). Preference is given to perennial crops, with more than 62% of the total number of study stations (Figure 1). These crops adapt to the environment on the one hand and have fairly substantial income for farmers on the other.

Table 2. Cultures in Place of Study Stations

Culture in place		Perennial	Annual	Perennial + annual	Total
Stations	Number	18	02	09	29
	%	62.07	06.89	31.04	100

According to the statistical data of the agricultural services of the area, the most cultivated fruit species are in order of importance: apricot, olive, pomegranate, pear and fig. Apricot has a percentage of 71.75% compared to other perennial species (D.S.A, 2018). For annual crops, there are cereals (wheat, barley, fodder) and market garden crops (onions, garlic, turnips, etc.). As for the other stations, they present mixtures between perennial culture is annual culture.Figure 1.Variation of cultures in place according to the stations.



3- Type of windbreak used

Farmers in the Boukhmissa perimeter use windbreaks to protect their crops. These windbreaks can be either alive or dead (remnants of the size of fruit trees, dead trees, banchages cuts of spontaneous woody species ...). According to this type of windbreak used, the stations surveyed were classified as indicated in Table 3.

Table 3.Number of stations according to the type of windbreak.

Nature of the Windbreak		Alive	Death	Total
Stations	Number	27	2	29
	%	93.10	6.90	100

In the study area, more than 93% of the stations recorded (27 stations), are protected by live windbreaks whether they are woody species such as: Cypress, Aleppo Pine, Casuarina, Atriplex, Tamarix ... or non-woody species such as reed. These types of windbreaks are more effective for crop protection because they create microclimates. For dead windbreaks their use is very low less than 7% of the total windbreaks used. They are used as a barrier for orchards or crop plots against intruders. It is obvious that the use of dead wood causes outbreaks of insects and cryptogams that can cause disease and indirectly disrupt the fall in crop yields.

4- Windbreak species used

The stations were classified according to the species used as windbreaks (Table 4).

Table 4.Number of stations according to windbreak species used.

Windbreak species		Softwoods	Hardwood	Reeds	Total
Stations	Number	17	08	02	27
	%	62,96	29,63	07,41	100

Most stations (17 stations) use softwood species as windbreaks, more than 62% of the total stations. These woody species are evergreen and this is what farmers prefer most for summer and/or winter protection of their plots. The most commonly used species in conifers is the green cypress (Cupressus sempervirens), which is characterized by its longevity, persistent foliage and prominent habit (Photograph 1).

Photo 1. The types of windbreak used in Boukhmissa.

Softwood (Cypress green)-Hardwood (Atriplex)-Reed (Egyptian cane)-Hardwood (Casuarina)

(Zedam A., 2012)

Photo 1. The types of windbreak used in Boukhmissa.

Hardwoods in 08 stations represent 29.63% of the total number. Among the hardwoods used: Atriplex, Myoporum, Acacia cyanophylla, Eucalyptus and Casuarina, which remains the most used. The reed is a grass grass and perennial grass is only present in 02 stations with a percentage of 07.41% of the total recorded.

5- Age of windbreaks used

Depending on the age of windbreak species, a classification could be made only in the live windbreaks (Table 5).

Table 5. Age of windbreaks used

Age (years)		less than 20	20 to 40	40 and over	Total
Stations	Number	14	09	04	27
	%	51.85	32.80	14.81	100

The study stations with the youngest windbreaks are 14 stations or 51.85% of the total, which shows that they are relatively young plantations compared to others and where it has become a practice for the protection of apricot orchards. As for the oldest stations, they are limited to the number of 04 stations or less than 15% of the total.

6- Porosity

6-1- Porosity of the study stations

Table 6. Variation of the porosity of study stations in Boukhmissa.

Porosity class (%)		< 40	40 to 60	>60	Total
Stations	Number	18	09	02	29
	%	62.07	31.03	06.89	100

The majority of low porosity sites dominate the agricultural area of Boukhmissa. Indeed more than 62% of the study stations have this low porosity. According to Vezina (2001), the porosity of a windbreak is effective and is obtained with a porosity close to 40% which corresponds to a moderately dense windbreak. This percentage is present in 31% of the stations surveyed.

According to I.N.R.F (1994), the effectiveness of the windbreak depends mainly on its permeability (porosity).-A windbreak must not be an insurmountable wall; because it

generates swirling phenomena that are very detrimental to crops.- A semi-permeable windbreak (porosity of 40 to 50%) is widely recommended.

6-2- Porosity of the type of species used

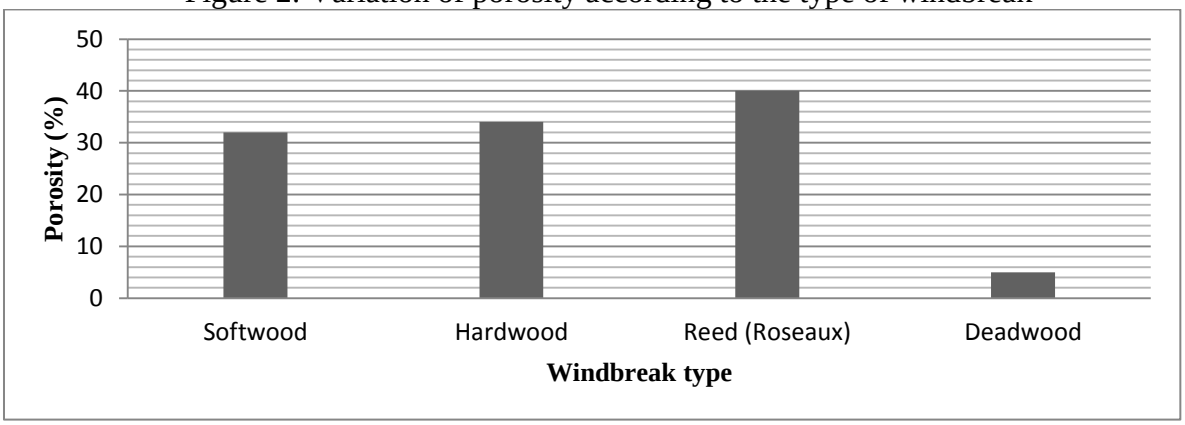
The porosity varies according to the types of windbreaks used where their porosity exceeds 30% whether they are coniferous, deciduous or reeds (Table 7).

Table 7: Porosity of types of windbreak used.

Windbreak used	Softwood	Hardwood	Reed (Roseaux)	Deadwood
Porosity (%)	32	34	40	05

According to Figure 2, the porosity of the planted windbreaks is higher than the dead wood windbreaks which are crammed on the ground, of limited height and sources of disease. For the planted windbreaks, there is a variation according to the species used in a decreasing way "Reed-Hardwood-Softwoods".

Figure 2: Variation of porosity according to the type of windbreak



7- Height

7-1- Height of the study stations

(Parde and Bouchon, 1988) classify trees according to their height in three sizes: first size (> 20m), second size (10 to 20m) and third size (<10m).

Table 8.Number of stations according to the height of windbreaks.

Height class (m)		Less than 10	10-20	Greater than 20	Total
Stations	Number	13	12	04	29
	%	44.83	41.38	13.79	100

The variation in the height of the windbreaks in the study stations shows that a fairly large number of stations have relatively low heights (<10m) where the percentage of these stations is greater than 44%, (Photograph 2).

This can be explained by: - Windbreak plantations are relatively young.

-Non woody species (Reed) and low perennial species such as Atriplex or Myoporrum.

Trees of first magnitude | Trees of second magnitude | Trees of third size

(Zedam A., 2012)

Photo 2.The three types of height of wind breezes observed in Boukhmissa.

7-2- Height of the type of windbreak used

The height of the wind breezes used varies differently (Table 9).

Table 9.Variation of the height according to the types of wind breezes.

Windbreak used		Softwood	Hardwood	Reeds	Deadwood	Total
Stations	Average height (m)	14.37m	5.6m	2.5m	2m	/
	Number	17	08	02	02	29
	%	58.63	27.59	6.89	6.89	100

It is observed that the height of softwoods is the largest with an average of 14.37 m against the other three types where their heights do not exceed 5.60 m. This highly variable difference can be justified by the fact that conifers such as green cypresses are prime trees, unlike deciduous trees such as casuarina, Eucalyptus and acacia or shrubs such as Atriplex, Tamarix and Myoporum. (Figure 3).

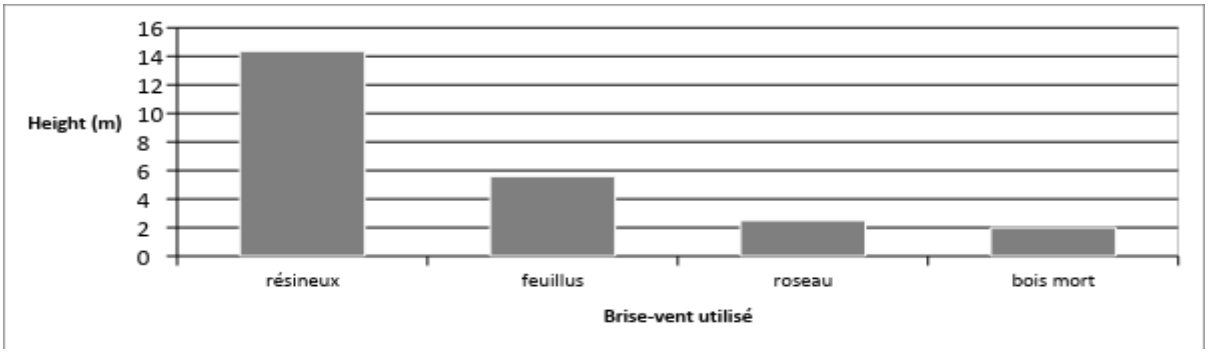


Figure 3.Variation of the height of the windbreaks used.

7-3- Height according to the culture in place

In Table 10 there is a classification of the stations according to the culture in place and the height of the windbreaks.

Table 10.Average height of windbreaks according to crops in place.

Culture in place		Perennial	Annual	Mix	Total
Stations	Average height (m)	11.3	6.5	12.55	/
	Number	18	02	09	29
	%	62.07	06.89	31.04	100

By direct observation on the ground, we notice that most orchards are composed of a single row of windbreaks. According to (Vezina 2001), a row of windbreaks requires less maintenance and takes up less space.

8- Width

(Fortin *et al*., 1999) classify windbreaks by width into: Windbreak at low width (<3m). Windbreak at medium width is effective (3m to 10m). According to this classification, our study stations were classified as follows:

Table 11.Number of stations according to windbreak width.

Windbreak width		< 3m	from 3m to 10m	Total
Stations	Number	25	04	29
	%	86.21	13.79	100

More than 86% of the windbreak width of our stations is not effective. If the thickness of the windbreak increases its permeability decreases but the thickness of the medium width windbreak (3m to 10m) is effective.**9- Length**

The larger the parameter, the more important it is. The ratio of windbreak length to height must be at least 11.5 (Nageli 1953 in Guyot 1989, I.N.R.F 1994 and Vezina 2001). It is a practice in the study area that serves more parcel boundaries or along irrigation canals than for the actual protection of crops. Our stations have been classified according to the length of their shelterbelt (Table 12).

Table 12.Length of the station windbreaks.

Windbreak length	Less than 100m	Greater than or equal to 100m	Total
Number of stations	11	18	29
Percentage (%)	37.93	62.07	100

Stations with a windbreak length greater than or equal to 100m account for more than 62% of the total stations.

10- Orientation

This is the essential factor to consider when setting up a windbreak network. The orientation of the windbreak must be perpendicular to prevailing winds (Nageli 1953 in Guyot 1989, I.N.R.F 1994, Vezina 2001 and Fortin *et al*., 1999). The different orientations of the windbreaks of the study stations are illustrated in groups in Table 13.

Table 13.Number of stations according to the orientation of wind breezes.

Wind Breeze Orientation		North	East	West	North / East	East / South	North / West	South / West	East / West
		A	B	C	D	E	F	G	H
Stations	Number	2	3	4	4	3	4	2	4
	%	6.89	10.34	13.79	13.79	10.34	13.79	6.89	13.79

We noted that 10.34% of stations have windbreaks installed in all four directions. Indeed only two orientations can be justified. It is the directions perpendicular to:
-South -South-East: it is the Siroco, which is a southerly wind or Chehili which causes the drying up of the cultures increases the evaporation and the rise of the temperatures.
- North-North-West: Bahri is a north wind that lowers temperatures and increases the risk of frost. The orientations observed in the study stations of Boukhmissa is concerning these two orientations account respectively 12.92% and 23.26% of the total of the stations. They were installed subjectively and anarchically.

Conclusion

At the end of this work, it is interesting to underline that the objective of our study is first of all on the diagnosis of windbreaks in the agricultural area of Boukhmissa and secondly the release of optimal and real prospects in this environment and this for a good crop protection in place and increased yields. To do this, we conducted a subjective sampling of 29 study stations. The results obtained were analyzed and resulted in the following:
- The majority of the stations (more than 68%) have surface areas of less than 5000m² with a dominance of fruit growing on more than 62% of them and where 27 study stations use breezes live alive.
- The use of coniferous species is dominant in more than 62% of the study stations.
- In general, the windbreaks surveyed are relatively young (less than 20 years old), which proves that the installation of this agro-forestry protection system in this area is recent.
- Overall, the study area has a low porosity with more than 62% because of the relatively high density of planting windbreaks but as for the species used, they have: 32% for conifers, 34% for hardwoods, 40% for reed and only 5% for deadwood.
- Regarding the height of the windbreaks of our stations, and given the relatively young age (20 years old) for most stations, the height is less than 10m where more than 44% of the stations testifies it. For this parameter it is obviously the conifers who hold the most important height. According to the speculation practiced, perennial crops have relatively high windbreaks compared to annual crops, which is justified by the need to protect perennial crops which are the most sensitive to certain types of accidents in the absence of agroforestry systems protection.
- Most of the stations have windbreaks of small width, less than 3 meters, is more than 86% of the study stations, due to their composition of a single row of protective plant species.
- The length of the windbreaks stations seems rather important but this is not sign of plots of large area where this length seems to be justified by its installation much more like boundaries of plots or along the canals and irrigation channels for the actual protection of crops.
- The direction or orientation of the windbreaks observed in Boukhmissa, is concerning these two orientations respectively account for 12.92 % and 23.26 % of the total of the stations. They were installed subjectively and anarchically.
- Choose native species, fast growing, if possible with taproot especially for the case of Boukhmissa which is an irrigated area. A windbreak can only perform its function if it is established according to a judicious plan and is constantly maintained in an optimal state.

As perspectives, for a better exploitation of windbreaks, we have made the following propositions concerning these agroforestry structures:
-A perennial species height of at least 10 m. -A porosity of 40 to 50%.
-Width of 03 meters with spacing between trees from 01 to 02 meters.
-The length must be total to ensure the protection of the parcels.
-The recommended guideline is the installation of tree curtains in the four directions of each plot to ensure better protection and creation of microclimate. If the prevailing winds are decisive, we will proceed with the installation of tree lines perpendicular to their direction.
-Finally, the choice of windbreak species will focus on those adapted to the environment, fast-growing, long-lived and presenting a multi-use.

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CAN WATER QUALITY INFLUENCE THE CHOICE OF BLACK BELLIED SANDGROUSE DRINKING WATER IN ARID REGION?

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Abstract

The black bellied sandgrouse (*Pterocles orientalis*, L. 1758) appears to be one of the most adapted species to extreme conditions of arid regions. However, implementing actions to promote in situ conservation based on understanding the behavioral ecology of the black bellied sandgrouse populations. this study was conducted in the south east of Algeria between 2014 and 2015, we sampled water from 20 watering sites of the black bellied sandgrouse, and we conducted water tests on the following parameters: the hydrogen potential pH, conductivity (EC) and the rate of: potassium (K), sodium (Na), copper (Cu) and zinc (Zn). The results show that the variation in pH between 6.17 and 7.61 with an average of 7.19 ± 0.3 (n = 20), while the EC varies between 0.18 and 24.2 mS / cm with an average of 3.73 ± 6.89 mS / cm. Our results show that the concentrations of potassium varies between 1.27 and 93.92 ppm, with an average of 18.61 ± 24.22 ppm, while the changes in sodium concentrations is between 0.13 and 3799.73 ppm, with an average of 668.72 ± 1023.06 ppm . the concentrations of copper vary between 0.12 and 0.24 ppm, with an average of 0.19 ± 0.03 ppm, while the variations in concentrations of zinc is between 0.86 and 1.68 ppm, with an average of 1.31 ± 0.21 ppm. The black bellied sandgrouse uses has no preference, it drinks readily available water, whether fresh or brackish.

Keywords: *black bellied sandgrouse, drinking behavior, salinity, heavy metals, south east of Algeria.*

Introduction

The black bellied sandgrouse (*Pterocles orientalis*, L. 1758) appears to be one of the most adapted species to extreme conditions of arid regions (Thomas and Maclean, 1981; Thomas, 1984; Maclean, 1984, 1985, 1996; Mourer-chauvire, 1993; De Juana, 1998). According to Hüe and Etchécopar (1957), the black bellied sandgrouse frequent daily the watering site. Hence, understanding sandgrouse drinking regime and regularly used water holes is of great conservation importance (Reuven and Piotr, 2011). In arid region birds, this aspect is particularly important because the fixed elements of the landscape are limited mainly to oases, Dayas, chotts and some water points, which is far from negligible. In the region of Biskra, the diversity of landscapes and water resources are important for the metapopulation of Ziban (Farhi and Bellhamra, 2015). Water consumption is increasing. The potential demand is estimated at 150 liters per capita per day. 97% of the water resources in Biskra are underground. This trend can be explained by the fact that urbanization took a large scale in Biskra (twice as much as the old urban fabric). In addition, the population has more than doubled in 20 years. The amazing enthusiasm for agriculture sometimes pioneering spirit that has more than 1.2 million new palm trees were planted. Gardening in plastic greenhouses, doubled and probably will quadruple by 2030 (Gaouar, 2003). The intensive exploitation of land and water could accelerate fragmentation phenomena. In order to lay the foundations for the sustainability of the farming anthropozoic systems, in competition and to improve the ecological connectivity of these sensitive habitats, frequented by a rich and diversified avifauna, it is more than necessary at first to worry about the quality water in watering sites frequented by arid birds. Therefore, in this work, the questioning is how to implement a

reference database on the abilities of the black bellied sandgrouse to use the available waters of different origins.

Material and Methods

This study was conducted in the region of Biskra (34 ° 48 '00 "North and 5 ° 44' 00" East), it is located about 470 km southeast of the capital Algiers, at an average altitude of 125 m and covers an area of approximately 21.671 Km² (Farhi, 2001).,water from 20 watering sites of the black bellied sandgrouse was tested, between november 2014 and October 2015, on the following parameters: the hydrogen potential pH, conductivity (EC), respectively by using a pH meter and a conductivity meter, the rate of: potassium (K), sodium (Na), copper (Cu) and zinc, by using the flame photometer.

Results and Discussion

1. Hydrogen potential (pH) and Conductivity (EC) of sampled sites

The results of the hydrogen potential and conductivity measurements show a variation of the pH between 6.17 and 7.61 with an average of 7.19 ± 0.3 (n = 20), these results show that the samples studied have a pH that is close to neutral. However, the electrical conductivity EC varies between 0.18 and 24.2 ms / cm with an average of 3.73 ± 6.89 ms / cm (n = 20). (figure1)

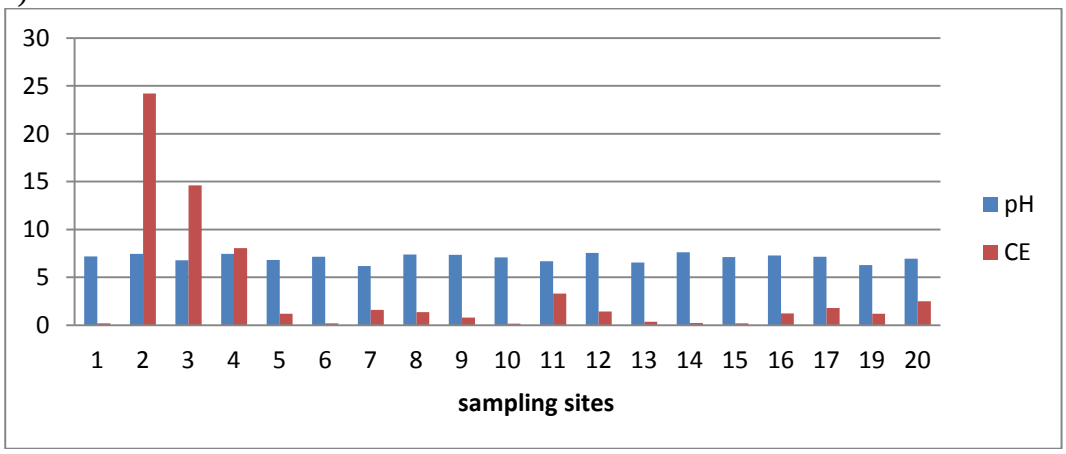

Figure 1: pH and Electrical Conductivity (EC) of Sampled Water

The WHO (2004) standards for pH range from 6.5 to 8.5. Ferns and Hinsley (1995) have shown that the water pH of the watering sites frequented by the black bellied sandgrouse and the pin-tailed sandgrouse in Spain (n = 8) is equal to 7.9 ± 0.1. The pH conditions a large number of physicochemical equilibrium and depends on multiple factors, including the origin of water, agricultural inputs, releases from agglomerations and industries (Tamrabet, 2011). According to Khoul and Touhami (2014) The classification of water as a function of conductivity is as follows: Conductivity equal to 0.05µS / cm: demineralized water; Conductivity from 10 to 80µS / cm: rainwater; Conductivity 80 to 100 µS / cm: low mineralized water Conductivity 300 to 500 µS / cm: moderately mineralized water; Conductivity 1000 to 3000 µS / cm: saline water; Conductivity greater than 3000 µS / cm: sea water. The EC values of our samples are between 1.8us / cm and 242us / cm. This shows that the sampled waters fall into two classes: rainwater and slightly mineralized water (domestic, industrial, and agricultural water). According to Bartholomew and Cade (1963), the role of salt water in the water economy by birds has long intrigued ornithologists, but so far there are only random observations and conclusions drawn from distribution data. However, an examination of the electrolyte metabolism is of particular interest in a survey of land bird water economics. On the one hand, it offers powerful help in understanding the mechanisms

of renal function, and, on the other hand, it can contribute to the understanding of the ecology of desert birds that have access to salt springs and ponds temporary and alkaline. Thomas and Robin (1977) suggest that Moroccan sandgrouse species lack nasal salt glands and appear to be suitable for salt and water conservation. Maclean (1968) noted that the Namaqua Sandgrouse and Burchell's Sandgrouse attend saline springs to drink

2. Concentration of potassium (K) and sodium (Na) at sampled sites

Our results show that potassium (K) concentrations vary between 1.27 and 93.92 ppm, with an average of 18.61 ± 24.22 ppm (n = 20), while the variations of sodium (Na) concentrations are between 0.13 and 3799.73 ppm, with an average of 668.72 ± 1023.06 ppm (n = 20) (figure 2 and 3).

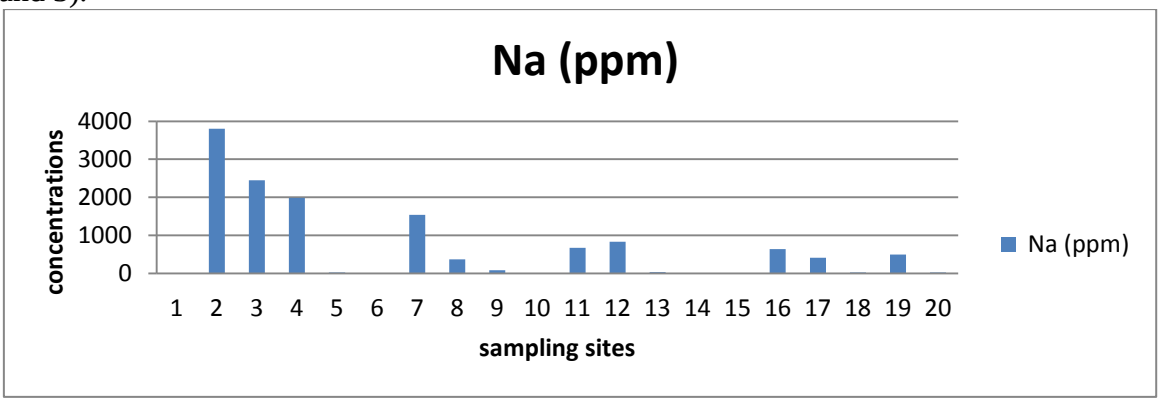

Figure 2: sodium (Na) concentrations of sampled sites

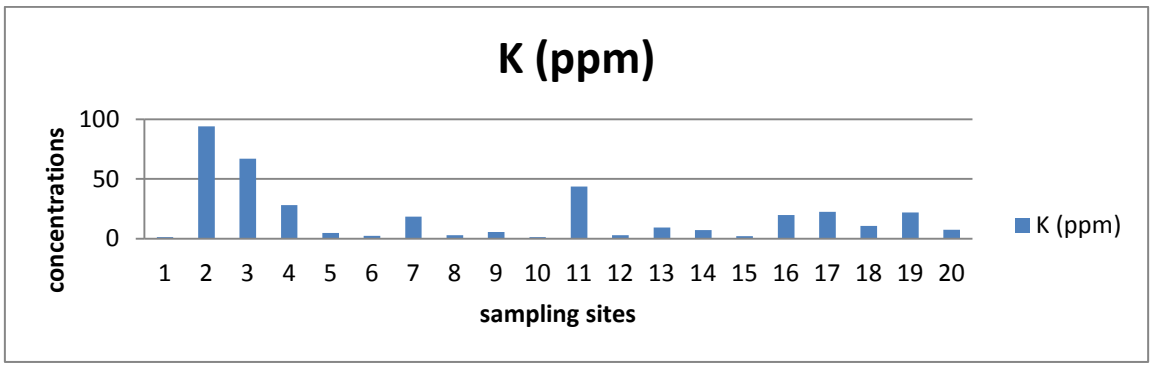

Figure 3: potassium (K) concentrations of sampled sites

In arid regions, not only can water resources be limited, but also saline and / or sodic Thomas and Robin (1977). In the steppes of Morocco, Thomas and Robin (1977) reveal that the concentration of potassium (K) is equal to 10.53 ppm, and the concentration of sodium (Na) is 67.39 ppm in the water of watering sites frequented by the black bellied sandgrouse and the Pin-tailed sandgrouse (n = 1). According to Hinsley (1992) and Ferns and Hinsley (1995), the mean concentration of sodium (Na) in drinking water used by the black bellied sandgrouse and the Pin-tailed sandgrouse in Spain (n = 8) is 120 ± 20 ppm. According to WHO (2003) Sodium ion is ubiquitous in water. Most water supplies contain less than 20 mg of sodium per liter, but in some countries levels may exceed 250 ppm. Saltwater intrusion, mineral deposits, sewage effluents, and salt used in road de-icing can contribute significant amounts of sodium to the water. In addition, water treatment chemicals, such as sodium fluoride, sodium bicarbonate, sodium hypochlorite, together, can cause sodium levels as high as 30 ppm. Domestic water softeners can give levels above 300 ppm. Potassium, much less abundant than sodium, and rarely present in water at levels above 20 mg / l. It does not represent any particular disadvantage although the K one of the possible sources of radioactivity in water (Potelon and Zyman, 1998)

3. Concentration of copper (Cu) and zinc (Zn) at sampled sites

Our results show that copper (Cu) concentrations vary between 0.12 and 0.24 ppm, with an average of 0.19 ± 0.03 ppm (n = 20), while variations in zinc (Zn) concentrations are between 0.86 and 1.68 ppm, with an average of 1.31 ± 0.21 ppm (n = 20)(Figure 4).

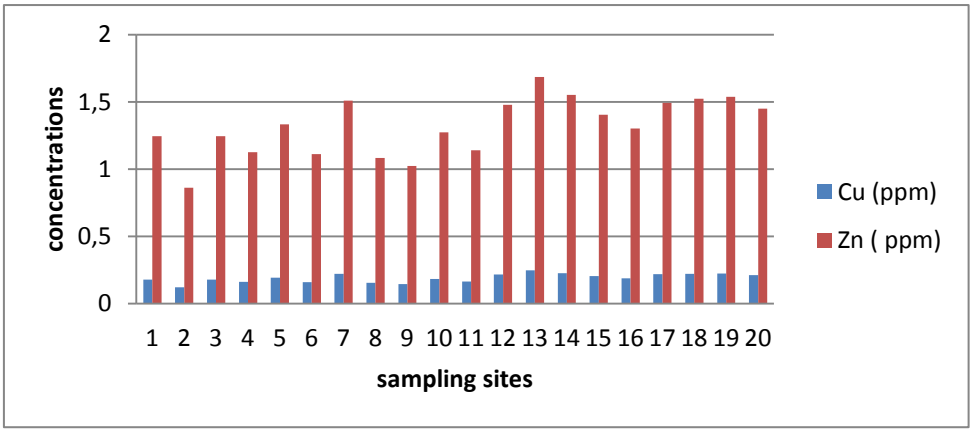

Figure 4: copper (Cu) and zinc (Zn) concentrations of the sampled sites

Loué (1993), suggest that these concentrations are well below the toxicity thresholds recommended for these two heavy metals (2 ppm for each element). However the copper concentration is higher than the norms of WHO (2000) which is of 0.05 ppm, as for the zinc it presents a value lower than norms of WHO which is of 5ppm. According to Nirel and Pasquini (2010), there are two main sources of copper pollution in water: an urban source and an agricultural source. Copper pollution of urban origin is accompanied by pollution in Zn concomitant (roofs, roads ....), which is not the case of copper pollution of agricultural origin. Copper is toxic to animals breeding. The phytotoxicity threshold is reached before that of zootoxicity (Baumont et al, 2004 in Tamrabet, 2011). According to Nys et al (2003), in birds, zinc and copper are physiologically, metabolically and immunologically important, and their toxic levels are much higher than the birds' needs. In captive birds, high doses of Zn may induce a decrease in food consumption that causes a decrease in body weight (Sundaresan et al, 2008)

Conclusion

In the Biskra region, and for several years, agricultural and urban developments as well as climate change have changed the structure of the oasis landscape of the Ziban, which has led to a disequilibrium of anthropozoic pressures, over-exploitation of natural resources and mismanagement of the water resource. The surface waters available for the watering of the birds of the Ziban, including the black bellied sandgrouse, have different origins (rainfall, dams, sewage water (urban, agricultural and industrial) and qualities (physical-chemical parameters). the characterization of the physicochemical quality of this water consumed by the populations of the black bellied sandgrouse, shows that the nature of this water is neutral to slightly acidic and has a wide range of values in terms of salinity and heavy metal content, which makes the black bellied populations, which prove a need for daily watering, subject to numerous physicochemical variations of available surface water.

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A SYSTEM OF SENSORS AND ACTUATORS PREVENTING ANIMALS FROM INJURIES DURING THE GRASSLAND HARVESTING

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Abstract

During the harvesting process-especially in spring time-animals are in danger of being injured or killed by agricultural machines e.g. mowers cutting and collecting grass. Every year a large number of fawns are killed because their instinct does not allow them to flee from the approaching machines. There are some important reasons to protect fawns: not to contaminate the grass, not to blunt the knives, and last but not least not to kill the very young animals. Several systems for detecting animals in a hayfield are available. They differ in usability, comparativeness, sensor technology, and automation capabilities. Our approach consists of a row of optical sensors mounted in front of a grassland mower. The measuring line corresponds to the total working width of the mower. While the measuring line crosses live animals, they are detected within a time period of 20 ms by means of their certain optical features. The detection signal immediately triggers the hydraulic lifting process of the mower mounted at the tractor. This procedure prevents the injury of the animal up to a tractor speed of 13 km/h. The optical sensor detects the live animal due to its surface reflectance in certain wavelengths. The sensor system also includes an artificial light source to be independent from the ambient light (morning/evening light conditions). Several tests have been carried out in the last few seasons to ensure the reliability of the system.

Keywords: *animal protection, grassland harvesting, optical sensor, hydraulic actuator*.

Introduction

In Central Europe the season of the first cutting of grass and the birth of fawns merge. Fawns are led into the high grassland by their does to hide from small predators so they are hard to see. In addition young fawns do not have a scent. Fawns behavior is lay down - see *Fig. 1 a)* - and wait until their doe appears and being feed-and to not show any reaction to any sound and/or approaching objects. This strategy helps them to survive their first weeks after they are born where they are too slow to flee from predators. However, this instinct also keeps the fawns from fleeing from harvesting machines. Every year approximately 200,000 fawns are killed in Germany and 25,000 in Austria during the harvesting process – see *Fig. 1 b) – [Wagner J.]*.

Fig. 1 – a) Fawn hiding in the grass b) killed fawn by rotating knives of the mower

This injuring and killing shall be avoided: A contamination of the silage with protein can lead to botulism (endangering cows), and to several other deceases. The knives of the harvesting machines will get blunt which leads to a higher energy consumption and a lower quality of the grass. In addition there is a legal obligation for farmers to protect animals during the harvest by law. The following systems are state-of-the-art and are in use to protect fawns:
a) Mowing strategy from the inside to the outside of the meadow. This can easily be performed at no extra cost in time and money. Some wild animals will flee to the outer side of the meadow, but most fawns remain due to their instinct *[Poel, Zehm]*.
b) Setting up scarecrows days before harvesting beside meadows–see *Fig 2 a)*–or at the machines. There are optical (flashing lights), mechanical, and olfactory devices beside meadows which prevent does and/or fawns to enter these areas. Machines are equipped with lines of flashing bars. These devices need to be installed and removed regularly and therefore consuming extra time and money. Their impact is low and they work under sever conditions only *[Schmitt S.] [Green C.]*.
c) Checking the concerning area using hounds and removing the fawns by hand just before the harvest. This is tiring and takes a lot of time. The impact is low because of the effective hiding strategy of fawns.
d) Checking the concerning area using electronic devices before the harvesting process and removing the fawns manually. Several sensor systems are in use: infrared sensors, microwave sensors, ultrasonic devices, GPS-localization. One or a series of sensors is mounted on bars (and carried across the meadow manually–see *Fig. 2 b) [Baumgartner M.]*, by tractor, or by drone–see *Fig. 2 c)*). Found fawns are removed immediately or their GPS-position is captured and handed to the operator to remove the fawn. This method is time consuming and costly. Infrared capturing is available at night/morning only while the ground temperature differs from the body temperature of the fawn *[Schmitt P.] [DLR]*.

Fig. 2 – a) Scarecrows optical/mechanical/olfactory b) manual sensor bars c) sensor on drones

e) Checking the area manually (driver of the mower machine) and stop the machine during the harvesting process. This technique requires the driver to monitor the area ahead of the tractor with full attention and to react quickly enough to prevent an injury. Due to the high operational speed and the large working width this system has a low impact.
f) Checking the area whilst harvesting by means of electronic devices and spare cutting locally. This is our approach and will be described in the following chapter. The system is mounted directly on the mover and needs no addition preparation from the operator during harvesting. There is only need to invest once in sensors and actuators at the mower. The reliability is high. *Fig. 3* shows the complete system attached to the mower.

Fig. 3 – Tractor and mower, with sensor bar attached in front of the mower

Materials and methods

Our approach consists of sensors and actuators on the mowing machine. The system operates online during harvesting process and works for speeds up to 13 km/h. The sensors are mounted about 1.5 m ahead of the knifes. Immediately after detection of a fawn a hydraulic system lifts the mower–see *Fig. 4*. While the tractor (and therefore also the mower) moves further the eventual reached height of the knife prevents the fawn to be touched. There is no interrupt of the harvesting process-no direct stoppage is required. In case of a positive detection of a fawn the operator can remove the animal later on.

Fig. 4 – a) Mower with knife during and b) after the lifting process

Each sensor is equipped with an illumination source and a capturing device. A sensor scans an area with a diameter of smaller than 100 mm at the ground. All sensors are integrated into a special sensor bar which is mounted on the mower about 1200 mm above the ground. To allow a simple handling when the machine is switched to street transport position the sensor bar can be folded. Different near infrared wavelengths are used to detect the fawn and segment against other objects as grass and earth. The sensor works with a sampling rate of 500 Hz. The response time of the connected control unit is about 20 ms and the response time of the hydraulic devices is about 350 ms (which is lifting the knife to about 300 mm above the ground). So the overall response time of the system is largely dependent on the hydraulic lifting process. First tests without artificial illumination showed a significant relation to the ambient light conditions. Consequently in the following tests we integrated an artificial illumination source. It operates with low power in the near infrared spectrum and therefore does not harm eyes of humans and animals.

Results and Discussion

The system passed comprehensive tests under laboratory conditions as well as under real harvesting conditions and has been optimized significantly in the last two years. By means of

models within high grass we developed and proofed the correct sensor reaction. We had enough positive true and nearly no negative false reactions of the system so far for going further: Next season an updated sensor system will be tested in an attempt to increase reliability further and to reduce system costs. One of the actual issues is if too much of high grass obstacles the fawn from above. There is need for a minimum amount of fawn signal getting back to the sensor (reliable signal/noise ratio). We tested different measuring areas per sensor but finally got the optimum with respect to the reliability/economic costly ratio. We know of single other systems than ours in use in Austria and Bavaria by local enthusiasts. But due to the fact that the investment costs are dramatically high and/or the operational costs take money (e.g. about EUR 1000.- per day for lending a drone system) and time prior to the harvesting process (e.g. about 1 h per hectare/10,000 m² meadow) until now the systems failed in essential decrease the killing of fawns. Our system has no operational delay and costs at the harvesting process. There is need in one investment for the sensor bar and the lifting hydraulics. We are on to shorten the investment budget by means of approaching a mass product status.

Conclusion

Seasons of fawn birth and harvesting merge in Central Europe, North America, Australia, and New Zealand. Especially in these regions fawns have to be protected against harvesting machines. Due to their natural instinct of hiding in long grass and staying put when enemies are approaching they are likely to be injured or killed by mowers. For preventing these losses measures have to be taken. Systems for fawn protection are already available on the market. They differ in reliability, investment and operational costs. The shown approach comprises a system of sensors and actuators mounted on mowers. Within our studies and recent tests a high number of positive detections have been achieved. Further developments will lead to reduced investment costs and increased reliability. It shall make the market sensitive for the broad usage of such systems.

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A SYSTEM OF OPTICAL/ACOUSTICAL SENSORS/ACTUATORS PREVENTING ACCIDENTS BETWEEN WILD LIFE ANIMALS AND VEHICLES ON ROADS

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Abstract

Roads across free wild landscape pose a permanent danger of accidents in which vehicles collide with wild-life animals. In most cases animals are aware of the sound and visibility of approaching cars. In rare situations, however, either at dawn/sunset or rare traffic, or at specific topologic situations, collisions happen between vehicles and animals. We propose a sensor/actuator system that prevents these collisions by warning animals. This system consists of active electronic components and is operated by solar power. Tests with the previous generation of this device showed a reduction of accidents by 89% and under certain conditions by more than 95%. The system consists of devices that can operate alone or as a group. The device is operated by a microcontroller, it contains optical/acoustic sensors and actuators, a wireless communication facility, and is supported by a power harvesting/storage/control system. One device is 18 cm in height, 9 cm in width and 7 cm in depth, weighs about 400 g and it is mounted on any guide post in the street. One device detects approaching vehicles either by their sound (microphone sensor) or head light (solar cell used as light sensor) and emits a tone of about 4 kHz directing off the street warning the animals. A LED flash light gives an additional optical warning. The tone makes animals observe their environment carefully and keeps them staying several seconds preventing them crossing the street at the same time when a vehicle comes by. A group of nearby devices is connected wireless. They are placed about 30-50 m apart. One can stand in the street and another one 50 m away upwards or downwards remotely from the street for keeping aisles and curved areas safe.

Keywords: *Animal protection, accident prevention, optical sensor, acoustic sensor, energy harvesting*

Introduction

(Nichols A.P. *et al.,* 2014) gives a very broad and fundamental survey of the problem of deer and human behavior at roads, related to the central states of US. There are statistics about size of population with respect to number of accidents, seasonal and hourly occurrences, etc. Their statement corresponds to the observations in Central Europe due to similar landscape and wildlife population (with exceptions). Wild-life animals move for their nutritional support and breeding. They are mostly shy and very sensitive about their surroundings. If they receive an optical or acoustic alarm they stop motion and try to figure out if they are in danger. After a while, which is about 20-30 seconds, they get back from the alarm status and carry on. They know their region and take care about human interaction on places like roads. But they also are aware of timing constraints and make usage of dawn/daylight/night periods. While there is heavy traffic on roads wild-life animals in general avoid these places. But at rare traffic conditions they feel save. If they face a vehicle approaching their instinct allows for an immediate reaction to flee. But this works for vehicle speeds up to approximately 45 mph (70 km/h) on clear areas only. Therefore high speed, rare traffic, winding and/or carved roads or obstacles beside them increases the probability of accidents.

(Meisingset E.I. *et al.*, 2014) checked for deer movement and collisions within a certain area in central Norway 2007-2008. Some of the deer had been equipped with GPS trackers. The 271 detected collisions occurred on major and medium-sized roads only and none at small roads. The identified deer crossings appeared some at main roads, more on medium-sized, but most on small roads. They conclude that speed limits and vegetation clearance had the best impact on avoidance of collisions. (D'Angelo G.J. *et al.*, 2006) evaluated behavior responses of deer to different colors of wildlife warning reflectors. They conclude that these reflectors were ineffective in changing deer behavior such that deer-vehicle collusions might be prevented. Deer reacted accordingly just and short after implementing the reflectors. After theses new situations deer became used to it and ignored the additional light effects. (Riginos C. *et al.*, 2018) tested different road sections in Wyoming, USA, in 2013 and 2014. At each of these sections they compared three different reflector situations: red reflector open, with white and with black canvas. They conclude that the best impact preventing deer crossing ahead a vehicle has white canvas narrow followed by red reflectors. No impact has the black canvas.
Several categories of measures are applied and reduce the amount of accidents by different costs:
a) Inform drivers: warn them at neuralgic sections, aided by speed limits.
b) Control wild-life paths: Erection of secure paths for the animals like tunnels, bridges, corridors, fences, and removal of vegetation from beside road.
c) Warn animals: There are passive (reflective) and active (detection and signaling) devices beside road.
(Huijser M. *et al.*, 2009) developed an in depth cost-benefit analysis of measures for mitigating animal- vehicle collisions. They concentrate on category a) driver warning systems and b) costly road works (bridges, tunnels, fences). Their evaluation relays on the situation in North America and Canada and takes a lot of local statistical data into consideration. (Valitzski S.A. *et al.*, 2009) tested reaction of deer to different sound imissions (frequency, level) originated on approaching vehicles. They conclude, that pure vehicle based whistlers will have nearly no effect in changing the deer behavior. They generally concluded with a weak impact of sound systems, but at the same time they state if the transmitting system is ahead the vehicle and nearby the deer probably there would be a positive reaction.
Within this paper we present a system of advanced sensor/actor modules for warning animals on behalf of appearing vehicles on the road. We designed and built a series of sensors, equipped several desired road sections with them and checked for the accident statistic. These devices are installed on posts besides the road and ahead of shoulders. Regarding the given references they do their signaling nearby the approaching deer with optical (visible light) and acoustical (4 kHz) actuators. This alarm signals are short pulses followed several seconds of pause for not inuring deer too much.

Materials and methods

The system consists of sensors, actuators and communication facilities. It is mounted on guide posts in a series beside the road – see *Fig. 1*. The main objectives of each single device are:

- Detect approaching vehicles by their sound and head light
- Send a single acoustic and optical alarm periodically every 15 seconds while there are vehicles
- Communicate wireless with nearby (30-50 m) devices for handing them the vehicle detection information in advance.

The device is equipped with solar cells and can operate even at low energy input in winter seasons at shadow places. The solar cell additionally works as a brightness sensor detecting the head light of vehicles. A microphone detects the sound. As animals shall be warned a speaker (the microphone works as a speaker as well) sends a 4 kHz tone for 500 ms and a

LED flashes. A transmitter/receiver establishes the wireless communication with nearby devices. A microcontroller operates all sensors and actuators of one device. Low power consumption is achieved by implementing several activation levels/slots depending on daylight/dawn/night conditions. There is a single type only within a housing of 180 x 90 x 70 mm. The weight is 400 g. We concentrate on easy to install and operate, robust, autonomous, and affordable devices. They are now in the third version stage. With respect to the first and second versions improvements in sensitivity, signaling and wireless communication have been carried out. The electronic is completely sealed within a casting resin for being isolated against humid air. By this the system is robust under changing weather conditions like rain during all the year or snow/salt in winter.

Fig. 1 – Devices beside the road and on topological shoulders mounted on guide posts

Results and Discussion

The first version (actuator for optical/acoustical signals but no wireless communication) has been implemented 2003 in Upper Austria. *Tab. 1* shows the reduction of accidents by 89%.

Tab. 1 – Device usage on Upper Austrian roads, accidents without/with first version 2003+; reduction by 89%

Device		*no*			*First version*			
Street \ Year	*2000*	*2001*	*2002*	*2003*	*2004*	*2005*	*2006*	*2007*
B 124	7	5	5	1	6	0	0	0
L 576	1	6	4	1	0	0	0	1
B 38	34	33	33	1	1	0	2	1
L 556	9	10	12	1	0	0	1	0
B 140	4	6	5	0	0	0	0	0
L 564	16	11	33	0	2	2	2	3
Total	**71**	**71**	**92**	**4**	**9**	**2**	**5**	**5**
Total		**234**				**25**		

(Böck C., 2017) states that after this first collection of statistical data by the usage of the first version in 2007 there have been 22,349 devices installed at about 480 km of additional roads

in Upper Austria. In total there has been a reduction from 3364 cases per year (2015/16) to 842 cases per year (2016/17) which is a reduction of 75%.
Due to the communication feature at the third version we have been able to support carved roads by means of placing devices 30-50 m away from the road on topological shoulders. Beginning with spring 2017 tests have been carried out by means of this third version especially on carved roads. *Tab. 2* shows the reduction of accidents by 91%.

Tab. 2 – Accidents without/with third version 2017; reduction by 91%

Street \ Device	*no*	*Third version*	*Remark*
B 124 km 27.8	5	0	*Fig. 2 a)*
L 576 km 3.6	4	0	
L 576 km 1.7	6	0	4 m talus, *Fig. 2 b)*
L 579 km 1.6	10	1	5 m talus
B 124 km 2.8	8	2	8 m talus forested
Total	**33**	**3**	

Fig. 2 – Devices on guide posts a) B 124 km 27.8, b) L 576 km 1.7

The platform ahead the shoulders of carved roads – see *Fig. 2 b)* - cannot be reached by the head light and the sound of approaching vehicles. Therefore wild-life animals in that area are not aware of the traffic at the lower road. If they are in a flee condition towards the road they are eventually too fast for stopping. With the devices at top of the shoulder, which are signaled wireless, sound and light alarms reach wild-life animals ahead the shoulder.

Conclusion

The above given results indicate a dramatic reduction of deer-vehicle collisions by the usage of simple to install/operate and affordable devices. Therefore the local government and insurance companies show high interest in providing essential and dangerous sections of roads in Upper Austria. With the gained experience since the first installations in 2003 we are now on to optimize the system in the sounding alarm (frequency and level). Another field of usage of these devices became visible by the implementation under railway system conditions. Negotiations are on the way.

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WEED SURVEY IN HERZEGOVINA REGION OF BOSNIA AND HERZEGOVINA

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Abstract

This paper has studied invasive weed population of row crops, vineyards, orchards and olive groves in Herzegovina. If the list of weed species is compared to the list of invasive plant species in Bosnia and Herzegovina, it can be seen that 10 species belong to the group of invasive species in BIH. Out of 6 families, 5 families belong to Dicotyledons. The most numerous family is Asteraceae (6 species), and Fabaceae, Rosaceae and Poaceae families are represented by one species each. By analyzing life forms, domination of terophyta and hemicryptophytes can be seen, while phanerophytes and geophytes are represented by one species each. According to the origin, the largest number of species is from America (five types). Analyzing invasive weed species in Herzegovina and their strategies it can be concluded that species with CR strategies and C strategies are dominant. This condition is particularly worrying because C species strategies are more successful in natural and semi-natural vegetation and they negatively effect on native species. The results of this research suggest that it is necessary to conduct further research of C-S-R strategy of invasive species (not only weed species) which will help better understand mechanism and successfulness of their spreading.

Keywords: *weeds, Herzegovina, Grimme CSR plant strategies.*

Introduction

Theory of CSR strategies set by J. P. Grime (Grime, 2002) implies the ability of plant to develop certain strategies of using resources which enable their optimal survival taking into consideration disorder and stress and by adapting to the existing habitat. On the basis of plants adaptability to those two factors, plant can be divided into three basic types: competitors (C), stress tolerators (S) and ruderals (R) and a number of different sub-types.

CSR system is based on the impact of environmental factors on plant life, and the presence of certain strategies can tell us a lot about the condition of the ecosystem. For example, increasing number of C-type species on the habitats can be associated with leaving the anthropogenic habitats maintained, S-type with increased eutrophication and R-type with more common disorders in the habitat (Hodgson et al., 1999). Relating invasiveness to CSR theory, which assumes that plants invest their resources either in the ability to compete, tolerate stress or survive biomass destruction (disturbance), as an adaptive response to the environment (Grime 1977).

Materials and methods

Sampling data

Floristic - phytocoenological research of weed flora and vegetation of vineyards, row crops and orchards were made during three vegetation seasons (2015, 2016, 2017), from spring to late fall. Floristic - phytocoenological surveys were conducted according to the principles and methods of Swiss-French (Zürich-Monpellier) phytocoenological school *Braun Blanquet*'s (1965).

Nomenclature of plant dicots is compliant with the work Flora Europaea (Tutin et al. 1964-1980, 1993) and only partly with Pignatti (1982) and Trinajstić (1975-1986).
Besides the existing one, dicot name recorded by some author earlier can be written in brackets.
Classification of families, species and subspecies in the framework of higher taxonomy categories is mentioned in alphabetical order on the flora list.
Besides the list of species and subspecies, related living forms are also mentioned.
Besides dicot names, flora list contains the living form for every specie in the form of abbreviation according to Horvat (1949) and Pignatti (1982):
P – *Phanerophyta*
Ch – *Chamaephyta*
H – *Hemikryptophyta*
G – *Geophyta*
T – *Terophyta*

The existing data on CSR strategies

The existing data on CSR strategies were taken from internet bases Flora Croatica Database (https://hirc.botanic.hr/fcd/), BioFlor (Klotz et al., 2002) and internal Lookup database for defining CSR strategies (Hodgson et al., 1999). For the species in which CSR strategy was different only in one out of three sources, we took the strategy from the two overlapping sources.

Calculation of the CSR strategy

Method used in this paper for determining CSR strategies was developed by Hodgson et al. (1999). In the period from 1965 to 1987 they conducted a longtime research project, which included field work, laboratory and manipulative experiments. CSR strategies of the bigger number of flora species came out as a result of that research. Using those data as the base, we decided to develop simpler, more transient method for determining CSR strategies. Observing each of three basic categories separately we defined the basic parameters which should be measured for calculating CSR strategy of a certain species. Hodgson et al. (1999) then developed an application in the computer program MS Excel, in which CSR strategy of a certain species is calculated by entering the above mentioned parameters. Table 1 gives an overview of all the mentioned measurable variables, out of which canopy height, flowering start and lateral spread have additional categories such as measurement result or literature data.

Table 1 Description of the variables used in determining of the CSR strategy

Variables Canopy height (mm)	Procedure Plants were measured in the field
Leaf dry matter content (%)	Calculated as the ratio between dry and fresh leaf weight
Flowering period (months in duration)	Data were obtained from literature
Flowering start (six-point classification)	Data were obtained from literature
Lateral spread (six-point classification)	Plants were observed in the field and compared with literature
Leaf dry weight (mg)	Leaves were oven-dried for 48 h/80 °C
Specific leaf area (mm^2 /mg)	Fresh leaves were scanned and scans were processed with ImageJ software to calculate leaf area

The process of assigning CSR strategies to plants (CSR classification) includes measuring a set of traits (Table 1) which can serve as predictors for a specific strategy, and calculating the strategy via previously prepared Excel worksheet (Hodgson et al., 1999).

Results and discussion

Wish for classification of plant species in certain categories regarding their life strategies has existed for long time in the studies of plant species and the reason for that is easier understanding of those organisms. One of such systems was suggested by Grime (1979) in his book *Plant strategies and vegetation processes*. In the book he gave a theory according to which all external factors which limit the amount of the present living and dead plant material in any habitat can be divided into two categories: stress and disorder.

Stress is represented by e.g. lack of water or nutrients which limit the plant production. Disorder is represented by e.g. herbivores, fire, wind, i.e. phenomena which destroy the plant biomass. On the basis of the plant adaptation to those factors Grime made a division into three basic types: competitors (C), stress tolerators (S) and ruderals (R).

Competitiveness implies tendency of the neighboring plants to use the same light quantum, ion of mineral nutrients, water molecule and air volume.

- competitive plants thrive the best in the conditions of low stress and disorder and they are competitive to other species in the habitat, because they are morphologically adapted to use available sources in the best way;
- stress tolerant plants are adjusted to the conditions of high stress, but small disorders and most often they are located in habitats with extreme conditions such as extremely low pH of the soil, small amounts of light, etc.;
- ruderal plants can survive disorder appearance, but not constantly present stress and they are often annual colonizers of the habitat with disorder conditions.

CSR system is based on the influence of the environment factors on flora, thus presence of a certain strategy can tell us many things about the state of ecosystem. For example, increase of C-type species number on the habitat can be connected with leaving the anthropogenically sustainable habitats, of S-type with increased eutrophication and of R-type with more frequent disorders on the habitat (Hodgson et al., 1999). C-type plants are robust perennials with high potential of growth, with fast widening above ground and below ground biomass (Grime, 1979). Taking into consideration those characteristics canopy height, vegetative lateral spread and leaf size were taken for competitiveness variables and roots characteristics were neglected for the purpose of simplicity (Hodgson et al., 1999).

S-type plants are mostly of slow growing, stress tolerant type which vegetate in chronically non-reproductive habitats (Grime, 1979). Taking into consideration that they have long living leaves with high content of nutrients, specific surface, weight and dry mass of the leaf were taken for stress parameters.

R-type species are fast growing and they finish their living cycle fast, they are characterized by the early beginning or extended period of reproduction (Grime, 1979). Beginning and end of flowering were taken for ruderality parameters (Hodgson et al., 1999).

This paper studied invasive weed population of row crops, vineyards, orchards and olive groves in Herzegovina (Municipality of Mostar, Široki Brijeg, Grude, Čitluk, Ljubuški, Stolac, Neum). Comparing the list of weed species to the list of invasive plant species in Bosnia and Herzegovina (Review and conditions of biological and landscape diversity in Bosnia and Herzegovina), it can be seen that 10 species belong to the group of invasive species in BiH. Out of 6 families, 5 families belong to Dicotyledons (Table 2).

Table 2. Invasive species in the weed flora of Herzegovina

Ambrosia artemisiifolia L	*Asteraceae*	T	America	CR
Artemisia vulgare L.	*Asteraceae*	H	America	C/CR
Conyza canadensis (L.) Cronq	*Asteraceae*	T	America	R/CR
Erigeron annuus (L.) Pers.	*Asteraceae*	T	America	CR
Galinsoga parviflora Cav.	*Asteraceae*	T	America	R
Lathyrus tuberosum L.	*Fabaceae*	G	Asia	C
Picris eschioides L.	*Asteraceae*	H	South Europe	CR
Rubus ceasiues L.	*Rosaceae*	P	Europe and Asia	SC
Sorghum halepense (L.) Pers	*Poaceae*	H	Africa and Southeast Asia	C
Urtica dioica L.	*Urticaceae*	H	Europe and Asia	C

The most numerous family is Asteraceae (6 species), and Fabaceae, Rosaceae and Poaceae families are represented by one species each (Figure 1).

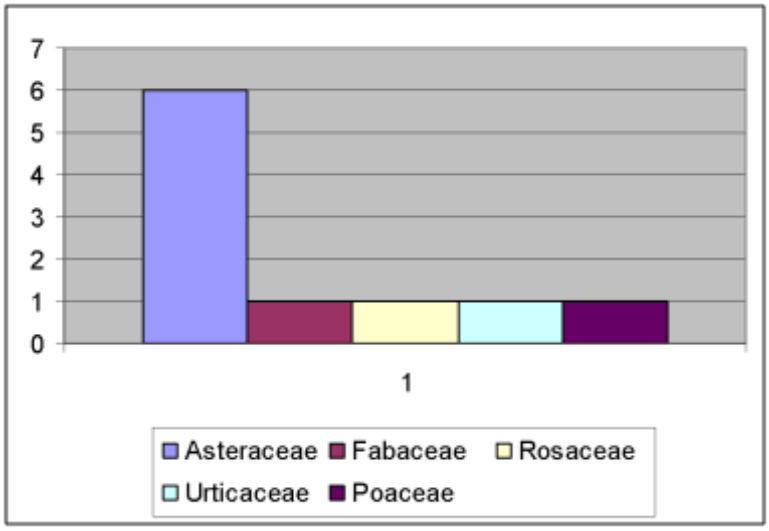

Figure 1. Family representation in the invasive weed flora of Herzegovina

By analyzing life forms (Figure 2) domination of terophita (four types) and hemicryptophytes (four types) can be seen, while phanerophytes and geophytes are represented by one species each.

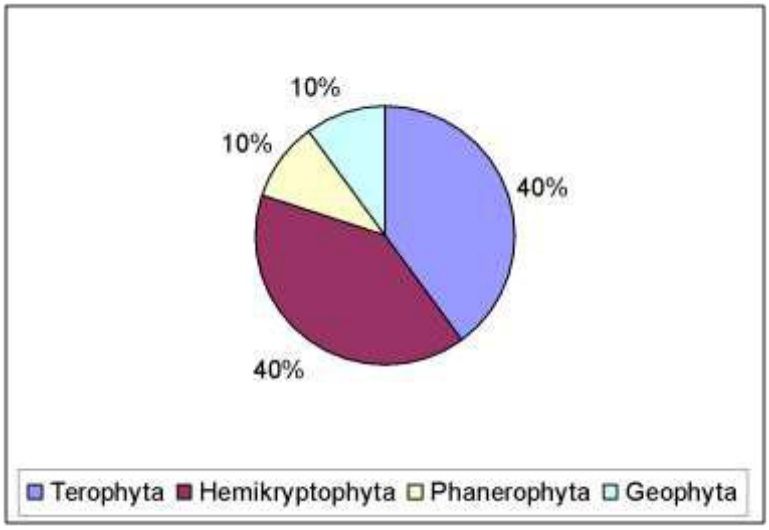

Figure 2. Representation of life forms of the invasive weed flora in Herzegovina

According to the origin the largest number of species is from America (five types), followed by Asia, Europe and Africa. (Figure 3)

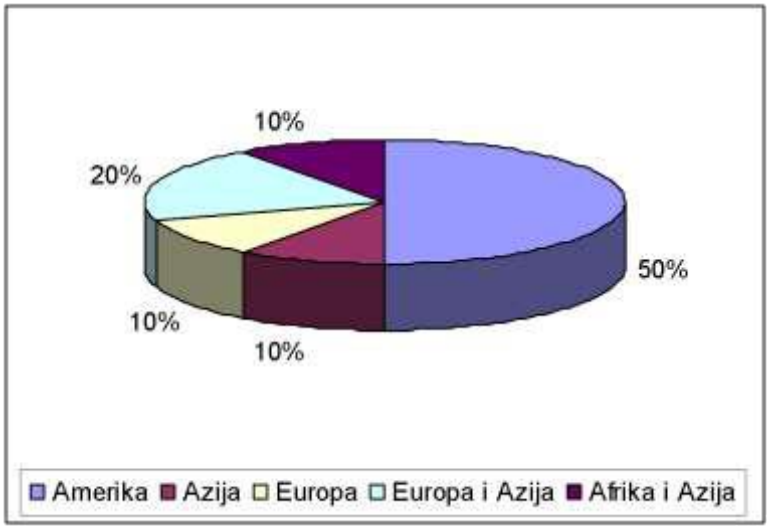

Figure 3. Origin of invasive weed species in Herzegovina

Analyzing invasive weed species in Herzegovina and their strategies (Table 3), it can be concluded that species with CR strategies and C strategies are dominant.

Table 3. Invasive weed species of Herzegovina with their strategies

CR	3	30,00
C/CR	1	10,00
R/CR	1	10,00
R	1	10,00
C	3	30,00
SC	1	10,00
Total	10	100,00

This condition is particularly worrying because C species strategies are more successful in natural and semi-natural vegetation and they negatively effect on native species.

The results of this research suggest that it is necessary to conduct further research of CSR strategy of invasive species (not only weed species) which will help to better understanding of mechanism and successfulness of their spreading. Combining that knowledge with the impact of other factors such as, for instance, the way of their spreading (Vuković et al, 2010.), it will be possible to better understand invasive flora and its adaptation to the environmental conditions which could facilitate the control of its spread.
Taking into consideration that Croatia is very near (border area), the list of weed flora of Herzegovina was compared with the list of invasive species in Croatia (Boršić et al, 2008) (Table 3)

Table 4. Invasive species in feed flora of Herzegovina according to the list from Croatia

Ambrosia artemisiifolia L	*Asteraceae*	T	America
Conyza canadensis (L.) Cronq	*Asteraceae*	T	America
Erigeron annuus (L.) Pers.	*Asteraceae*	T	America
Galinsoga parviflora Cav.	*Asteraceae*	T	America
Sorghum halepense (L.) Pers	*Poaceae*	H	Africa and Southeast Asia
Amaranthus retroflexus L**.**	*Asteraceae*	T	America

Amaranthus retroflexus is the invasive species in Croatia and because of nearness it can be expected it will soon become invasive in Bosnia and Herzegovina too.

Conclusion

Ten invasive weed species with the most numerous family *Asteraceae* were registered on the explored localities in Herzegovina (vineyards, orchards, olive groves and row crops). Regarding life forms of plants domination of terophita can be seen, while regarding origin the biggest number of species are from America. It is important to point out that the most represented strategies in the invasive weed flora of Herzegovina are CR and C strategies and taking into consideration that those C strategies are extremely adaptable, they negatively influence on autochthonous species.
Amaranthus retroflexus L. is especially pointed out on the list of invasive weed species in the Republic of Croatia. It is not invasive in Bosnia and Herzegovina, but it can be expected it will become invasive.

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BIODIVERSITY OF THE BUSKO LAKE FRESHWATER FISH AS A PART OF DIVERSITY OF ICHTHYO FAUNA IN THE KARST FIELDS OF BOSNIA AND HERZEGOVINA

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Abstract

The Busko Lake forms a large karstic, surface freshwater accumulation in the southwest of Bosnia and Herzegovina, at the foothills of Dinaric mountains massif. From the scientific standpoint, especially recognisable is the species diversity of freshwater ichthyofauna, while a significant number of endemic fish species that inhabit the underground karst aquifers, by their status, witness the unexpected changes in composition and diversity of ichthyopopulation. The Cetina River basin encompasses surface and underground waters of Glamocko, Kupresko, Suicko, Livanjsko, Duvanjsko and Sinjsko karst fields. The total number of recorded species is 35, with the greatest diversity in the main waterway of the Cetina River (27 species), followed by the waters of Livanjsko field (18 species). More than half the fish species (18 species; 51%) were introduced and are potentially invasive. Out of the 17 native species, five (29%) are stenoendems. The research task represents continuation of a long-term investigation of qualitative and quantitative traits of the Busko Lake ichthyopopulation. The latest, thoroughly defined ichthyological research was conducted during 2007, 2008, and 2009 on the Busko Lake. The rationale of the current research can be found in a marked extinction of many species, specific for a particular ecosystem, which have either completely disappeared or have had their numbers significantly reduced, while there is a simultaneous, gradual increase in numbers of introduced species. These evident changes in qualitative and quantitative profiles indicate that the observed ecosystem has undergone significant changes, the cause of which has yet to be eradicated. Initially, in this paper, it is essential to conduct a survey and ascertain areal of each individual species, determine distribution of endemic species, and establish protection measures.

Keywords: *Busko Lake, freshwater fish biodiversity, endemic species, Bosnia and Herzegovina.*

Introduction

Biodiversity is a defined phenomenon in the living world, stemming from the fact that the process of speciation still, albeit on a slightly smaller scale, exceeds the process of extinction or disappearance of living species. Speciation is a continuous process of geographical spread of populations and their separation due to fragmentation of habitats and the creation of conditions for propagation of species. Biodiversity is often defined as a number, variety and variability of living organisms, and thus a basis for their survival. Commitments placed before humanity relate to the prediction, prevention and eradication of the causes of biodiversity loss by a model of monitoring and identification of negative changes in ecosystems and habitats themselves, thus ensuring the existence of a small number of endemic and endangered species. Some data indicate that the degree of extinction today is 40% higher than normal, representing a moment of crisis, as the loss of biodiversity unfolds rapidly and uncontrollably. The causes of endangerment of certain species and disappearance of biological diversity are twofold: natural causes (natural disasters) and anthropogenic factors, which directly affect the

changes in natural processes and conditions of the environment (habitat loss, uncontrolled catch and exploitation, introduction of foreign species, pollution and destruction of the environment). The chronology of the previous research in the area of the Busko Lake distinguishes important papers, with their true aims directed at determining the level of ichthyofauna diversity in the southwestern Bosnian karst region. In the earliest period of the study of ichthyofauna in Bosnia and Herzegovina, the first more extensive works were written by Austro-Hungarian researchers, among whom are Heckel & Kner (1858), who in their papers give incomplete, yet the first description of a large number of species from the Cyprinidae family in the Busko Lake and the rivers of Livanjsko, Duvanjsko and Sinjsko fields. Ćurčić (1910, 1913, 1916) in the work *National fisheries in Bosnia and Herzegovina* states that by that time nobody had enough studied *"our"* domestic fish, their way of life and the hunting methods. The author connects the existence of the so-called "cavalry fish", to this area, referring to the genus *Paraphoxinus*, or the gait. It is significant because the species of that genus, *Phoxinellus (Paraphoxinus) alepidotus* (Heckel, 1843), inhabit the karst region of Livanjsko and Duvanjsko fields, and, according to Mučibabić *et al*. (1973), it was present in the Busko Lake until recently. Numerous research papers have different methodological approaches to solving ichthyological research problems in the running waters of Duvanjsko and Livanjsko fields, with a note that there are still insufficient current data on diversity of ichthyofauna of the Busko Lake (Karaman, 1923; Protić, 1926, 1927; Taler, 1951, 1954). In the period that followed, in addition to valorising the state of diversity in the area, analyses of cytophysiological characteristics, as well as serological and immunological tests were conducted within a complex approach to problems of biosystematics, which is of crucial phylogenetic-taxonomic importance. Studies of interspecies and intergeneric hybridization among different fish species were also made, thus following the behaviour of natural and artificially created hybrids. This data made a good basis for solution to a problem of using the applied ichthyology results in practice (Vuković 1963, 1966, 1977; Vuković & Kosorić 1970, 1978; Vuković & Ivanović 1971; Aganović *et al.* 1974, etc.). The continuation of cited papers is related to the period after formation of the Busko Lake accumulation and the somewhat modified ecological conditions of habitats in the investigated area (Mučibabić *et al.* 1973; Habeković, *et al.* 1987; Cvijović & Kosorić 1985; etc.). Kosorić *et al.* (1991) present the real possibilities of using water reservoirs in Bosnia and Herzegovina for production of fish, with the research in support of the familiarisation with endemic ichthyofauna in the Busko Lake area. The last twenty years were marked by the individual papers by authors Guzina, 2000; Dumanić, 2004; Bogut *et al.* 2007; Žujo Zekić, 2009; Škrijelj *et al.* 2015 and others. In this paper, the fundamental ichthyological research was conducted and the aims were set to determine the current state of fish populations diversity of the Busko Lake hydroaccumulation. The results are presented through a qualitative structure analysis of investigated ichthyopopulations and their interrelations within the ichthyofauna of the researched area, with a reference to adaptive reactions of endemic species to the apparent changes in the given ecosystem.

Materials and Methods

Researched area, the Busko Lake, or the Busko Mud as it is still sometimes called today, was once a natural creation and a wetland-marsh ecosystem of the Bosnian karst region. Knowing the Dinaric karst characteristics, (vertical jagged relief, the scarcity of the rugged soil, the scarce flora and the richness of carbonate rocks), there is a clear lack of surface watercourses and underground drainage. Therefore, this significant hydrographical centre is also a "monumental" work of nature. The Busko Lake, the third largest in Europe, is in the southeastern part of Livanjsko field, forming a closed feature with the most imposing morphological characteristics (area 60 km^2) for a karstic area (Figure 5).

Figures 1-4. 1. Site L/1 Prisoje – Tomislavgrad; 2. L/2 Golinjevo-Livno; 3. L/3 Podgradina-Kanal-Livno; 4. L/4 Mukišnica-Grabovica (Tomislavgrad); Figure 5. Cartographic presentation of the geographical position of research sites at the Busko Lake (Boškailo, 2018)

The fieldwork part of the ichthyological research of this aquatic area was conducted within the framework of the project titled "*Survey and monitoring of the life communities of the aquatic ecosystem Busko Lake*", during spring - summer season of 2018, through several field trips. For the assessment and analysis of qualitative and quantitative structure of fish populations within the fishing zone Busko Lake, four sites were marked and studied: L/1 Prisoje - the old bridge; L/2 Golinjevo; L/3 Podgradina-Kanal; L/4 Mukišnica - Grabovica (Figures 1-4). The importance of the proper selection of individual fishing sites that differed in terms of depth, wind direction, geographical position, wealth of aquatic vegetation, percentage of exploitation, etc., reflected on the composition and structure of investigated ichthyopopulations in the area. Analysed representative samples of fish populations were obtained by standard catching methods (the "fill" nets with different diameters, the "barracuda" nets, and fishing by electro aggregate). A general annotated description of research sites is presented in Table 1. Systematic determination of fish was made according to Vuković T. & Ivanović B. (1971) and Kottelat M. & Freyhof J. (2007). Diversity indices Shannon-Wiener index (H) (Shannon-Wiener, 1949) and (Bray-Curtis index) were calculated using the BioDiversity Pro software.

Table 1. General notes on sampling sites at the Busko Lake hydroaccummulation area

Number	Site label	Site name	Coordinates	Altitude (m)	Length of nets (m)	Average depth (m)
1.	L/1	Prisoje – old bridge (Tomislavgrad)	*N 43˚41'117"* *EO 17˚04'896"*	702-712 m	200 -250 m	1,50 m
2.	L/2	Golinjevo (Livno)	*N 43˚41'520"* *EO 17˚01'961"*	702-712 m	200 -250 m	2,50 m
3.	L/3	Podgradina-Kanal (Livno)	*N 43˚42'414"* *EO 16˚50'085"*	702-712 m	200-250 m	3,50 m
4.	L/4	Grabovica-Mukišnica (Tomislavgrad)	*N 43˚38'364"* *EO 17˚04'616"*	702-712 m	200-250 m	2,50 m

Results and Discussion

Qualitative composition of ichthyopopulations at selected sites at the Busko Lake

Investigating the qualitative structure of fish populations in the Busko Lake, and linking the lake's position with its size and ecological environmental factors, it is evident that composition of ichthyocenosis in these karst watercourses is very diverse and specific. A partially interrupted connection of this hydroaccumulation with karst streams in the Livanjsko and Duvanjsko fields, as well as the centuries-old isolation of this karst basin, formed a specific endemic ichthyofauna. It has been previously known that out of the total of five indigenous fish species that exist in the Busko Lake, the four are endemic, with a very narrow areal. Several fish species were subsequently introduced, due to the curiosity and intentions of local fishing associations to increase the commercial value of the lake. All this however depends and relies on biological characteristics of individual fish species, some of which found more favourable ecological niches in a newly formed ecosystem, while others, unable to adapt, moved to less karstic watercourses or completely disappeared from the area.

During current research, we were guided by the very beginnings of the study of ichthyofauna, where special attention was paid to endemic fish species, known spawning periods, the way of life, the influence of certain weather conditions on behaviour of some fish, seasonal oscillations in abundance of communities, appearance of "cavalry fish", conditions at a catchment site (water level, periods of rain, periods of drought), etc.

The current review of a qualitative composition of ichthyopopulations is far from a positive assessment of the state of biodiversity in an artificially controlled ecosystem. According to current results, which differ from those published by Žujo Zekić (2009), at four sites during the spring-summer season 2018, ten species from five families were identified in the Busko Lake:

Family Cyprinidae Bonaparte, 1840, with species: *Chondrostoma phoxinus* (Hackel, 1843), *Cyprinus carpio* (Linnaeus, 1758), *Carassius gibelio* (Bloch, 1782), *Tinca tinca* (Linnaeus, 1758), *Alburnus arborella* (Bonaparte, 1841), *Scardinius dergle* Heckel & Kner, 1858;

Salmonidae Linnaeus, 1758, with species *Salmo farioides* Linnaeus, 1758 (*Salmo trutta m. lacustris* - lake form);

Siluridae Cuvier, 1816, with species *Silurus glanis* (Linnaeus, 1758);

Percidae Cuvier, 1816/7 with species *Sander lucioperca* (Linnaeus, 1758);

Centrarchidae Berg, L.S. 1958 with species *Lepomis gibbosus* (Linnaeus, 1758).

Having obtained data on the state of species diversity, expressed through the qualitative structure of ichthyopopulations at designated sites, obvious is the absence of endemic species from the Cyprinidae family: *Squalius tenellus* Heckel, 1843, *Aulopyge huegelii* (Heckel, 1842), and the species *Hypophthalmichthys molitrix* (Valenciennes, 1844) and *Pseudorasbora parva* (Temminck & Schlegel, 1846), as well as the species *Ameiurus nebulosus* (Le Sueur, 1819) from the Ameiuridae family. Compared to previous studies, the obtained data point to emergence of unnatural competitive relations among investigated ichthyopopulations, at certain habitats, resulting in a poor estimate of the lake's ecosystem bioproductivity.

Degree of ichthyofauna heterogeneity in the Busko Lake (cluster analysis)

The Bray-Curtis index of diversity represents a measure of the degree of similarity and difference in the structure of one community at different locations (communities of ichthyofauna at catchment sites). The cluster diagram (Diagram 1), composed by a series of linear links, shows the two sets of samples, grouped on the basis of similarity of individual sites in relation to biodiversity of the studied ichthyopopulations of the Busko Lake.

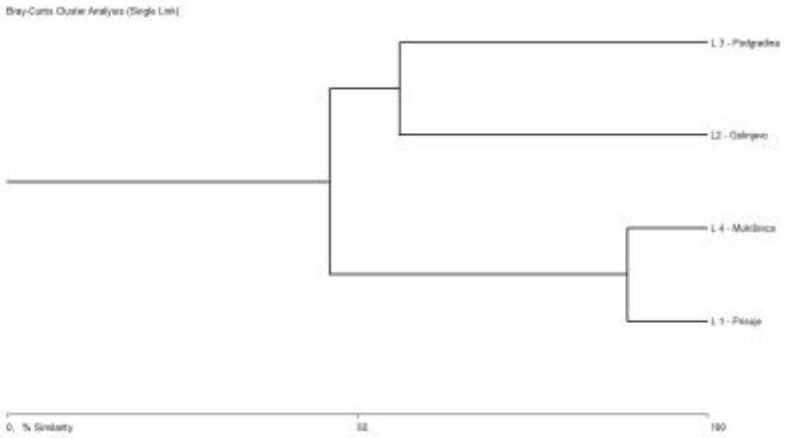

Diagram 1. Bray-Curtis claster analysis of researched sites according to diversity of ichthyopopulations of the Busko Lake

Shannon-Weaver index of diversity

The value of the biodiversity index is a significant indicator of the status and condition of the ecosystem in relation to environmental and anthropogenic impacts, enabling the assessment of the biological diversity of fish communities at the research sites. The Shannon-Weaver diversity index of ichthyofauna is calculated based on determined numerousness and distribution of fish species. It is used in our ichthyological research, and basically measures the order (disorder) within a particular living community. The advantage of this index is that it takes into account the number and equality of species (equal representation). Index values increase with the increase in the number of "special" species, or due to their higher equality, or more even representation in the sample. The results of this test are presented in Diagram 2, with values ranging from 0, for sites containing only one species, up to 1, for sites that have exceptionally high diversity of species.

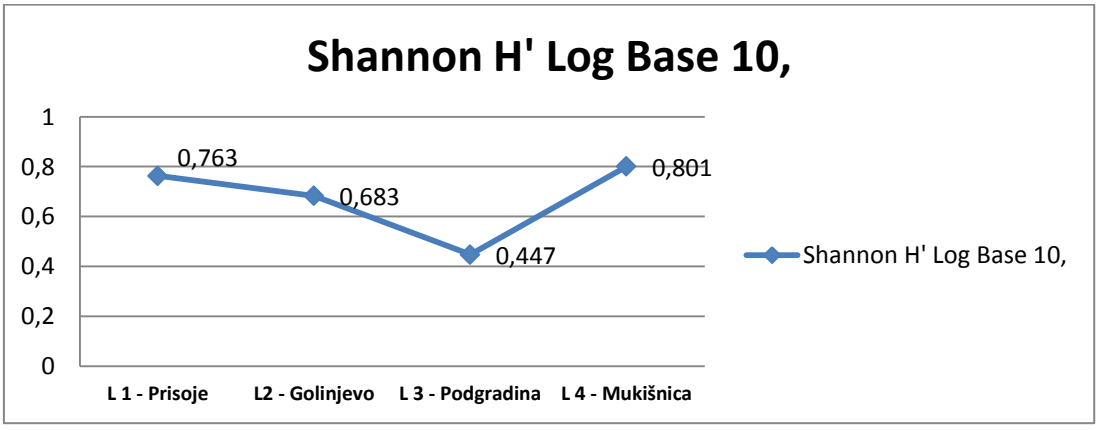

Diagram 2. The Shannon index values for ichthyofauna at researched sites of the Busko Lake

Conclusions

The formation of ichthyofauna of the Busko Lake was especially influenced by a composition of fish communities from the surrounding watercourses, as well as the existing fish fund of the former natural wetland-marsh ecosystem the Busko Mud. Considering that the initial conditions of habitats have been altered, it can be unambiguously concluded that the entire ecosystem has been changed, which is especially noticeable in the composition of ichthyopopulations. This brings into question the survival of certain species. Endemic species of the Busko Lake ichthyofauna appear as indicator organisms, whose absence or health

condition is used to identify a specific type of biocenoses, or as a measuring unit of altered ecological conditions within the hydroaccumulation.
This paper shows a great variation within the observed fish populations as well as individual species, which is conditioned by a series of abiotic environmental factors with an unavoidable anthropogenic effect.

Conclusion

Currently, an interspecific competition is particularly prominent, where in the mutual exploitation of a common natural resource one species has more benefits, thereby reducing its quantity and availability to other species. Hence, the rate of growth, reproduction and survival of the other species are lowered and the species is becoming endangered. Today, this phenomenon has surpassed natural laws. Wherever a human appears as a predominant species, and disrupts the natural relationship of autochthonous species through introduction of "foreign" species, a cascade of events leads to this unbalanced state of the environment. The degree of impact, very seriously undermines the survival of certain endemic species. Considering the basic characteristics that describe the typical cyprinid water, it is clear that the results obtained for the qualitative composition of ichthyopopulations is made up of ten fish species from the five families with the dominance of species from the Cyprinidae family.

Acknowledgement

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STATE OF VASCULAR FLORA IN THE NATURAL PART OF BUSKO LAKE IN BOSNIA AND HERZEGOVINA AND SUSTAINABLE DEVELOPMENT

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Abstract

From the aspect of biodiversity, in the broadest sense of comprehension, Bosnia and Herzegovina is one of the most unique regions of Europe. One such natural resource is the area of the present-day Tomislavgrad municipality. The special attraction, for botanists, is the aquatic complex of the Busko Lake.The research was conducted during vegetation season in spring 2018, in order to determine diversity and distribution of plant species inhabiting coastal parts of the Busko Lake. Using the Braun-Blanquet method (1964), phytocoenological analysis was performed at selected sites. Lowland forests and shrubs have developed in coastal parts of the lake and its tributaries, the most prominent of which are: *Crataegus monogyna, Rhamnus alpinus ssp. fallax,* while less represented species are*: Cornus sanquinea, Salix alba, S. fragilis* and *Populus nigra.*Tertiary vegetation is also present in the area where anthropogenic impact occurs to a greater or lesser extent. This type of vegetation is located around human settlements, rich in habitats and nitrites, edges of roadsand other similar habitats. The species of this vegetation present at researched sites are: *Senecio vulgaris, Lolium strictum, Poa annua, Sonchus oleraceus, Erigeron canadensis, Fumaria officinalis, Lamium purpureum* and others. Macrophytic vegetation is well developed in the coastal belt and shallow waters, represented by species: *Veronica beccabunga, Glyceria fluitan, Veronica anagallis-aquatica* and others. Many human activities lead to the rapid disappearance of rare and ecologically specialized species as well as the fragmentation of their habitats. Environmental protection guarantees the complete preservation of environmental quality, the preservation of natural communities, the rational use of natural resources and energy in the best way for the environment, as a basic condition for healthy and sustainable development.

Key words: *Buško Lake, phytocoenological analysis, floristic composition of vegetation, anthropogenic factor, sustainable development.*

Introduction

From the aspect of biodiversity, in the broadest sense of comprehension, Bosnia and Herzegovina is one of the most unique regions of Europe. One of such natural resources is the region of Tomislavgrad municipality. Particular significance, from the floristic perspective,have the Vran mountain, Dugo field, karstic Duvanjsko field, the Busko Lake and many others. The Busko Lake is a large surface, karstic aquatic complex in south-western part of Bosnia and Herzegovina at the foothills of Dinaric mountain massives. From the scientific standpoint, especially recognisable is the species diversity of freshwater ichthyofauna in the area, and presence of endemic fish and plant species. The Cetina River basin encompasses surface and underground waters of Glamocko, Kupresko, Suicko, Livanjsko, Duvanjsko and Sinjsko karst fields on the territory of Bosnia and Herzegovina and basins of the Jadro and the Zrnovnica rivers in the Republic of Croatia.

The area of today's Tomislavgrad municipality has been a long-standing point of interest for botanists, considering its geomorphological and climatic heterogeny, and hence floristic and

vegetational heterogeny. The influence of biotic factors, especially anthropogenic, was especially significant for flora and vegetation over time, which engrossed attention of botanists. A significant number (70) of plant families have been identified, which is almost one half (43.5%) of the total number of vascular plant families (161) on the entire territory of Bosnia and Herzegovina. Dominant sunflower families Asteraceae and Cichoriaceae are represented with the largest number of species (47) (Abadžić, 2013). Abundant families are also: Caryophylaceaea, Fabaceae, Poaceae, Lamiacece, Rosaceae, Apiaceae. The analysis of the spectrum of floral elements established their numerousness, and therefore divided them into several basic groups of floral elements, the most common of which are: the Eurasian, the Eurosuboceanic, the Supramediterranean, the Mediterranean, the Subatlantic, the Arctic, the Alpine and Pre-Alpine floral element, as well as the Balkanic, Balkan-Apennine and the Dinaric floral element (Abadžić, 2013).

Material and Methods

Field research was conducted in 2018 during the vegetation season - spring (May-June) in the area of the Busko Lake. The survey covered the coastal part of the Busko Lake at sites: Prisoje (old bridge), Golinjevo, Kanal-Podgradina and Mukisnica where phytocoenological analyses were done. Braun-Blanquet (1964) methodology was used, adapted to the research needs. Most plant species were identified on site, during fieldwork. When it was not possible, the samples were identified in the laboratory. Nomenclature of plant taxa was determined in accordance with Tutin *et al*. (1964-1985), Mucina (1997), Rodwell *et al*. (2002), Šilić (1996), and Hayek (1927-1933). The affiliation to the floral element and life form was assigned according to the databy Oberdorfer (2001).

Results and Discussion

The assessment of vegetation was conducted during May and June 2018 at the Busko Lake. These surveys covered four sites in total: Prisoje (old bridge), Golinjevo, Kanal-Podgradina and Mukišnica (Figure 1). Phytocoenological analyses and identification of plant species were conducted at each site.

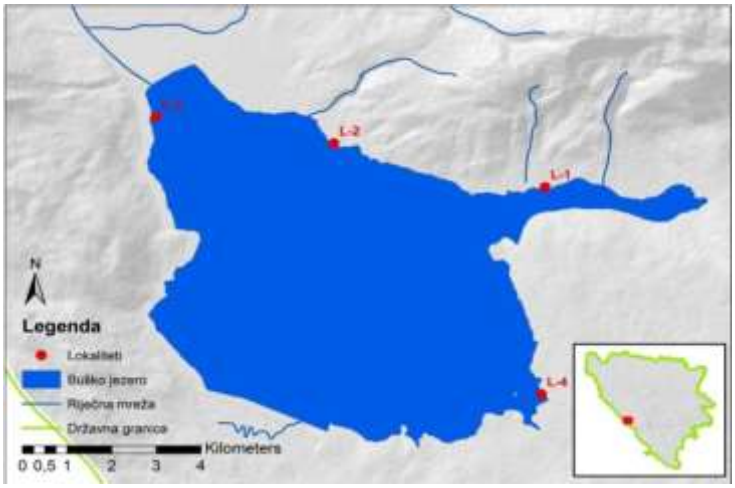

Figure 1. Position of research sites at the Busko Lake

Elements that are directly or indirectly related to determination of the shape and the level of biodiversity are: geological and pedological characteristics, altitude, terrain inclination and the action of anthropogenic factor, manifested through various forms.

Taxonomic analysis

Taxonomic analysis of riparian flora at selected sites of the Busko Lake was performec at the family level. All taxa were sorted into 25 families. Out of 70 taxa identified at selected sites, majority of species were from the family Asteraceae (14), followed by families Poaceae (8), Rosaceae and Fabaceae (7), Ranunculaceae and Scrophulariaceae (3), Polygonaceae and Salicaceae (3), Plantaginaceae and Cyperaceae (2), with all other families represented by one taxon each (Table 1).

Table 1. Taxonomic structure of the most abundant floralfamilies at selected sites

Family	**Number of taxons**	**%**	**Family**	**Number of taxons**	**%**
Asteraceae	14	9,8	Boraginaceae	1	0,7
Poaceae	8	5,6	Campanulaceae	1	0,7
Rosaceae	7	4,9	Clusiaceae	1	0,7
Fabaceae	7	4,9	Convolvulaceae	1	0,7
Ranunculaceae	4	2,8	Cornaceae	1	0,7
Scrophulariaceae	4	2,8	Juncaceae	1	0,7
Polygonaceae	3	2,07	Lamiaceae	1	0,7
Salicaceae	3	2,07	Papaveraceae	1	0,7
Plantaginaceae	2	2.1	Urticaceae	1	0,7
Cyperaceae	2	22,1	Crassulaceae	1	0,7
Apiaceae	2	2,1	Betulaceae	1	0,7
Alismataceae	1	0,7	Potamogetonaceae	1	0,7
Asparagaceae	1	0,7	**Total**	**70**	**100**

Spectar of Life Forms

A range of life forms was determined for the identified plant species at researched sites, amounting to a total of 13, with transitional forms.

Analysis of the structure of hemicryptophytes (H) in the coastal part of the Busko Lake's flora showed that they were represented by a largest number of taxa (39). If we also include hemicryptophytes of transitional forms- H (G), (H) G) i H (Ch), it can be concluded that hemicryptophytes make up a total of 46 species (32,2%). Phanerophyte (P) are the second most numerous life form (10 species), followed by terophya (T) (6) with six plant species and two transitional forms T (H) - (2 species) and T (Ch) - (1 species). A low percentage of hydrophytes (W) (0.16%) can be attributed to a small number of sampling sites, a few field trips and the fact that floral analysis included samples from the coastal part of the lake only. Geophyte (G) were represented by one species and one transitional form G (H), with one species. Similarly, hamephyta (Ch) were represented by two plant species, out of which one was a transitional form Ch (H) (Table 2).

Table 2. Biological spectrum of coastal flora of the Busko Lake

Basic and transitional life forms	**Number of taxa**	**%**	**Total**	**%**
Hemicryptophytes (H)	39	17,94	46	32,2
H (G)	3	1,38		
H (Ch)	3	1,38		
(H) G	1	0,46		
Phanerophyes (P)	10	1	10	7
Terophytes (T)	3	0,18	6	4,2
T (H)	2	0,12		
T (Ch)	1	0,06		
Hydrophytes (W)	4	0,16	4	2,8
Hamephytes (Ch)	1	0,02	2	1,4
Ch (H)	1	0,02		
Geophytes (G)	1	0,02	2	1,4
G (H)	1	0,02		

Spectrum of floral elements

Considering the climatic, geomorphological, pedological and other diversities of the researched area, it was expected that vascular floral species present in the area would show a significant diversity of floral elements, which was found to be 37. The most numerous floral elementsare: nine eurassubozean-smed, four omed-kont, followed by three species each of euras-smed-med, euraskont-smed, euras-smed, smed-euras, no-euras and osmed. Finally, other floral elements were represented by two or one species (Table 3).

Table 3. Spectrum of floral elementsof coastal flora of the Busko Lake(N – number of species)

Floral element	**N**	**Floral element**	**N**
eurassubozean-smed	9	smed-eurassub-ozean	1
euras-smed-med	3	Submed (-subatl)	1
euraskont-smed	3	smed-gemäßkont (pralp)	1
euras-smed	3	no-euras	3
eurassubozean (-smed)	1	(no-) eurossubozea (-smed)	2
euras(subozean)-smed	1	no-euros (subozea)	2
eurassubozean (circ)	1	no-eurossubozea	2
eurassubozean	1	no-euras, circ	1
Euras (subozean)	1	no-euras (-smed)	1
euraskont	1	omed-kont	4
euraskont-(-smed)	1	osmed	3
Euras (kont), circ	1	gemäßkont-osmed	1
Euras (kont) smed	1	med-smed	1

europkont (-osmed)	1	med-smed-euras	1
smed-euras	3	med-euras	1
smed	2	med-smed (-kont)	1
smed-subatl	2	alt-med	1
Smed (-subatl)	1	subatl-med	1
smed-europkont	1		

Vascular flora in Bosnia and Herzegovina is rich in endemic plant species, hence it was no surprise to note the presence of a Dinaric endem *Chouardia litardierei (Breistr.)* Speta (syn. *Scilla protensis* W& K). The macrophytic vegetation in the coastal belt and shallow waters was represented by *Veronica beccabunga, Glyceria fluitans*. In the Busko Lake itself, aquatic plants rooted in muddy substrates were present, such as *Potamogeton perfoliatus, Polygonum amphibium f. natans.*

Influence of pollution on flora and vegetation

The Busko Lake is an area rich in natural beauty, however we also have to take into consideration a significant influence of an anthropogenic factor, which has created various problems on the lake due to human ignorance. Most human activities lead to the rapid disappearance of rare and ecologically specialized species as well as the fragmentation of their habitats. General long-term preventive measures against biotic and non-biotic agents, which could endanger the stability of the lake and its environment, will be based on water management that takes into account natural processes, as well as the maintenance and appropriate improvements of biodiversity conservation. Fundamental biological resources, including the Busko Lake, must be effectively preserved, without further internal fragmentation, significant reduction in size or degradation through development and construction, in order to preserve the natural resources and ecosystem processes that depend on them. Environmental protection guarantees the complete preservation of environmental quality, the preservation of natural communities, the rational use of natural resources and energy in the best way for the environment, as a basic condition for healthy and sustainable development.

Conclusions

The research results that included sampling sites: Prisoje (old bridge), Golinjevo, the channel - Podgradina and Mukišnica on the Busko Lake, indicate that 70 taxa were identified within 25 families. The total spectrum of life forms in the researched area is 13 with transitional forms, while the spectrum of floral elements is 37.

These findings point to the need to organize additional comprehensive field research on parts of the Busko Lake area, which to date have been partially or almost never explored. These activities would certainly lead to new knowledge that would to a great extent update the present understanding of the biodiversity of flora in the Busko Lake area.

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TREATMENT OF FILTERS ON SANITARY LANDFILL

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Abstract

Sanitary landfills are specific places where municipal waste disposal is carried out using sanitary landfilling technology. Biochemical, physico-chemical and geological processes in aerobic and anaerobic conditions take place at sanitary landfills. Anaerobic decomposition of waste matter in the body of the landfill occurs at temperatures of 35-55 ° C and passes through several transformation phases to the final mineralization. In the body of the landfill a very high degree of pollution is formed, which together with the formed gases make the main emission factors of the harmful substances in the environment. The filtrate in the body of the landfill should be collected by the appropriate drainage system, which is lying on a waterproof natural or artificially formed substrate that protects the groundwater. Purification of the landfill filtrate is done by various methods, most often in combination of several types of technological procedures. The aim of the paper is to determine the quality of the filtered filtrate from Bosnia and Herzegovina, at the Ramici landfill that is released into the water stream. The tests were carried out in January, April, September and December 2016. In order to determine the quality of the filtered filter, the determined parameters are: temperature, pH value, electrical conductivity, HPK, BOD, total phosphorus, nitrates, sulfates, chlorides, heavy metals (manganese, iron, lead, etc) and toxicity test Daphnia Magna Straus. The obtained data have been processed and analyzed in accordance with the legal requirements set out in the Regulation on limit values for wastewaters discharged into surface waters (Official Gazette of the Republic of Srpska (Bosnia and Herzegovina) no. 44/01).

Key words: *landfill filtration, treatment, sanitary landfill*

Introduction

Waste materials are substances or objects that arise during the production, service or other activities, objects excluded from use, those which are not for further use and must be discarded. In order to solve the problem of waste materials, there are various functional elements that are grouped into the waste management system, which works in the direction of optimizing such a system, minimizing costs and achieving the maximum effect on environmental protection (Kalambura, Racz, Toth, 2017). Waste management includes the prevention of waste generation, waste reduction and its hazardous characteristics, waste treatment, waste management planning and control, waste transportation, the establishment of a system for operation, closure and maintenance of waste treatment facilities after closure and monitoring. In order to establish a sustainable waste management system, it is important to look at all waste management options. Common technical and technological solutions for the treatment of certain types of waste include procedures for the avoidance and utilization of waste, recycling, treatment by mechanical, biological, thermal and chemical processes and final disposal by landfilling or export. Each of these technologies has its advantages and disadvantages, but everything must meet environmental conditions and must not endanger human health. Only proper treatment of waste materials can create a healthy environment, as a basis for a healthy life. Landfilling of municipal waste is the simplest and cheapest waste management process. The main goal of waste disposal is the long-term sustainable disposal of waste with the aim of protecting the environment and human health (Vujić, 2009). Municipal waste landfilling involves the use of a sanitary technological process that takes place in a

well-preserved location, in accordance with environmental conditions related to the protection of environmental components. The sanitary landfill is a specific construction facility where heterogeneous municipal waste is disposed of in a controlled manner, containing various fermentation and inert components. The deposited waste matter is transformed to the final mineralization by various processes that take place in optional aerobic-anaerobic or anaerobic conditions. As a result of physical-chemical and biochemical processes, carbohydrates, proteins, fats, amino acids, glucose, methanol, methane, carbon dioxide, ammonia, hydrogen and sulfur are formed. In contact with organic matter in the body of the landfill and products of its decay of infiltrated atmospheric precipitation, it builds an extremely high pollution filtrate, which makes the main emissions of harmful substances into the environment together with landfill gas.

The amount of landfill filtrate is difficult to determine. The maximum quantity of landfill filtrates ranges up to 50% of the average rainfall in the landfill area. It is affected by the slope of the site of the landfill site, the quality and type of covering material, the height of the landfill and other. The filtrate in the body of the landfill is collected through a drainage system that lies on a watertight substrate and is discharged into the collection lagoon located near the body of the landfill. The depot filtrate from the collecting lagoon is considered to be the most complex source of pollution in nature, so it is the obligation to be purified before releasing it into a natural recipient (Marković, Milanović, 2016).

Knowing the negative impact of the landfill on natural recipients, the aim of the paper is to determine the quality of the filtered filtrate from the Ramici landfill at Bosnia and Herzegovina that is released into the water stream. The tests were carried out in January, April, September and December 2016. In order to determine the quality of the purified filter, the parameters were determined: temperature, pH value, electrical conductivity, HPK, BPK5, ammonia content, total phosphorus, nitrates, nitrates, sulfates, chlorides, heavy metals (manganese, iron, lead, etc.) and toxicity test Daphnia Magna Straus. The obtained data have been processed and analyzed in accordance with the legal requirements set out in the Regulation on limit values for wastewaters discharged into surface waters (Official Gazette of the Republic of Srpska (Bosnia and Herzegovina) no. 44/01).

The basic hypothesis of the research is that the landfill filtrate after the purification meets the conditions prescribed by the Regulation on the discharge of sewage into surface waters (Official Gazette of the Republic of Srpska (Bosnia and Herzegovina) no. 44/01).

Material and Methods

The waste disposal site in Ramici near Banja Luka is located in the north-western part of the city, at the location of Crkvine (Kalamanda, Vujcic, Delić-Jović, 2015). With all its natural elements, the site of the Ramici landfill is under the influence of anthropogenic degradation. Existing degradation is reflected through the pollution of surface and ground waters, air and soil. Environmental pollution and problems that arise are associated with pollution of surface and groundwater. Treatment of landfill filtrates at sanitary landfills is a very complex task. In order to be released into natural recipients, the criteria for wastewater condition the application of different treatment processes (Jahić, 2006). Since 2014, the landfilled (discharge) water collected at the Ramici landfill has been purified by the reverse osmosis process. When purified, it is discharged into the water stream. In order to determine the quality of treated purified water at the exit of the purification plant, water quality is determined before they are discharged into the watercourse. The quality control of purified landfill water was carried out according to the methodology prescribed in the Regulation on the conditions for discharging wastewaters into surface waters (Official Gazette of the Republic of Srpska (Bosnia and Herzegovina) no. 44/01). For the purpose of the research,

purified landfill water was sampled at the exit from the purification plant, in January, April, September and December 2016, as shown in Image 1.

Image 1. Purification of landfill filtrate at the Ramici landfill
Source: Author`s image

Results and Discussion

Based on the parameters of purified landfill filtrates that are discharged into surface water, the obtained results are shown in Table 1.

Table 1. Results of the parameters of purified landfill water that is discharged into surface waters (January, April, September, December 2016)

Parametar	**Unit**	**Research method**	**Limit value**	**Sample I (January)**	**Sample II (April)**	**Sample III (Septembar)**	**Sample IV (Decembar)**
Water temperature	°C	BAS DIN 38404-4:2010	Up to 30	19.3	19.7	22.0	14.9
pH value	-	BAS ISO 10523:2010	6.5-9.0	7.36	7.39	7.22	6.87
Suspended solids	g/m^3	BAS EN 872:2006	35	1.8	1.6	5.0	2.0
Electrolytic conductivity	µS/cm	BAS EN 27888:2002	-	427	481	435	446
Implanted matter by Imhoff	ml/l	EPA 160.5:1974	0.5	<0.2	<0.2	<0.2	<0.2
Biological Consumption of Oxygen (BOD_5)	gO_2/m^3	BAS ISO 5815-2:2004	25	4.6	4.0	1.0	1.8
Chemical Oxygen Consumption (HPK)	gO_2/m^3	BAS ISO 6060:2000	125	28.6	26.0	9.36	10.9
Ammonia content	g/m^3	BAS ISO 6778:2002	-	2.13	2.96	8.7	8.4
Ammonium nitrogen content	g/m^3		10	1.75	2.43	7.2	9.1
Content of nitrite nitrogen	g/m^3	EPA 354.1:1971	1	0.06	0.10	0.03	0.149
Nitrate		JUS ISO					

nitrogen content	g/m^3		7890-1:1994	10	<0.01	0.31	0.03	5.05
Content uk. nitrogen by Kjeldahl	g/m^3		BAS ISO 5663:2000	15	3.25	5.99	14.3	14.8
Total phosphorus content	g/m^3		BAS ISO 6878:2004	3	<0.01	<0.01	0.01	0.12
Sulfate content	mg/m^3		ASTM D 516:2007	200	2.7	2.7	8.4	6.21
Chloride content	mg/m^3		JUS ISO 9297:1989	250	5.1	5.1	19.7	4.07
Toxicity test Daphnia magna Straus	% wastewater diluted		BAS ISO 6341:2003	>50 %	86.2	86.0	86.0	84.4
Iron content	mg/m^3		BAS ISO 6332:2000	2000	41.3	41.3	61.5	63.68
The content of manganese	mg/m^3		BAS ISO 6333:2003	500	6.4	6.4	170.9	11.9
Lead content	mg/m^3		BAS ISO 8288:2002	10	<0.1	<0.1	<0.1	<0.1
Chromium content	mg/m^3	total	BAS EN 1233:2002	100	8.4	8.0	<5.0	<5.0
		Valence 6		-	4.9	4.4	-	-
Zinc content	mg/m^3		BAS ISO 8288:2002	1000	18.6	18.3	15.0	17.2

By analyzing the data from Table 1. it can be seen that the parameters of purified landfill water, from which the harmful substances have been removed or reduced to the level acceptable for natural recipients, have been determined. The obtained results are compared with the limit values of the parameters according to the Regulation (Official Gazette of the Republic of Srpska (Bosnia and Herzegovina) no. 44/01). Purified water for research purposes was sampled in different periods of 2016 (January, April, September and December). At the sampling point, the following were directly determined: the wastewater temperature was from 14.9 - 22.0°C, which is within the limits of the allowed values, the pH value is from 6.87 - 7.39, the allowed values are up to 9.0. Suspended solids in all tested samples were significantly lower than the maximum allowed concentrations and ranged from 1.6 to 5.0 g/m^3 and allowed 35. The obtained data for electrolytic conductivity showed the lowest value in January 427 μS/cm , and the highest value in April 481 μS/cm.

Imhoff's soluble substances in all tested samples amounted to < 0.2 ml/l, which is lower than the limit values (0.5 ml/l). Biological Consumption of Oxygen (BOD_5) is a basic indicator that serves as an indicator of the presumed impact of wastewater on natural recipients in which the content of dissolved oxygen decreases (Jahić, 2004). The obtained values for BOD_5 in the tested samples ranged from 1.0 g O_2/m^3 in September to 4.6 gO_2/m^3 in January, which is significantly lower than the allowed values (25 gO_2/m^3) compared with the indicators from the Regulation. Analyzing the obtained data for chemical consumption of oxygen (HPK) of the tested samples, it can be concluded that the lowest values were in September 9.36 gO_2/m^3, and the maximum in January was 28.6 gO_2/m^3, which is negligible compared to the indicators from the Regulation 125 gO_2/m^3). The determined values for BOD_5 and HPK in the tested samples were within the limits of the permitted values according to the Regulation, based on which it can be concluded that the content of organic matter in the waters is reduced by the treatment of landfill waters. The content of ammonia in the tested samples ranged from 2.13

g/m^3 in January to 8.7 g/m^3 in September. The values obtained for the content of ammonia, nitrate and nitrite nitrogen in all tested samples are within the permissible limits of the Regulation. Total Kjeldahl nitrogen in tested samples ranged from 3.25 g/m^3 in January to 14.8 g/m^3 in December, which is within the prescribed limits (15 g/m^3). The content of total phosphorus, sulphate and chloride in all tested samples is significantly lower than the prescribed values. The Daphnia magna Straus toxicity test was 84.4% in December, and in January it had the highest value of 86.2% of wastewater diluted, from which it can be seen that the samples were tested within the limits of the allowed (> 50% dilution wastewater). The obtained data for the content of heavy metals (iron, chrome, lead, zinc, manganese) in all tested samples are significantly lower compared to the values prescribed by the Regulation, from which it can be concluded that the content of inorganic as well as organic matter in the tested samples is significantly reduced by the treatment of landfill water.

Conclusion

- At the sampling point, the following were directly determined: the temperature of the waste water was from 14.9 to 22.0° C, which is within the limits of the allowed values, the pH value is from 6.87 to 7.39, the allowed values are up to 9, 0.
- Suspended solids in all tested samples are significantly lower than the maximum allowed concentrations and ranged from 1.6 - 5.0 g/m^3, with 35 allowed.
- Biological consumption of oxygen (BOD_5) in the tested samples ranged from 1.0 gO_2/m^3 in September to 4.6 gO_2/m^3 in January, which is significantly lower than the allowed values (25 gO_2/m^3 in comparison with the indicators from the Regulation)).
- Data for the chemical consumption of oxygen (HPK) of the tested samples of the lowest values in September were 9.36 gO_2/m^3, and the maximum in January was 28.6 gO_2/m^3, which is negligible compared to the indicators from the Regulation (125 gO_2/m^3) . The obtained values for BOD_5 and HPK in the tested samples are within the limits of the allowed values according to the Regulation, based on which it can be concluded that the content of organic matter in the waters is reduced by the treatment of landfill waters.
- The content of ammonia in the tested samples ranged from 2.13 g/m^3 in January to 8.7 g/m^3 in September.
- The values obtained for the content of ammonia, nitrate and nitrite nitrogen in all tested samples are within the permissible limits of the Regulation.
- Total Kjeldahl nitrogen in tested samples ranged from 3.25 g/m^3 in January to 14.8 g/m^3 in December, which is within the prescribed limits (15 g/m^3).
- The total phosphorus, sulphate and chloride content in all tested samples is significantly lower than the prescribed values.
- The Daphnia magna Straus toxicity test was 84.4% in December, and in January it had the highest value of 86.2% of wastewater diluted, from which it can be seen that samples were tested within the limits of the allowed (> 50% dilution wastewater).
- The obtained data for the content of heavy metals (iron, chrome, lead, zinc, manganese) in all tested samples are significantly lower in relation to the values prescribed by the Regulation, from which it can be concluded that the content of inorganic as well as organic matter in the samples tested is significantly reduced by treatment landfill sites at the Ramici landfill.

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REVERSE OSMOSIS, AS MEMBRANE TEHNIQUE FOR CONSUMPTION OF INCIDENTAL DEPONIUM WATER/FILTER

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Abstract

The opening of landfills in our country, as a result of production and consumption, is spontaneous and unprofessional, without taking into account hazardous processes of waste degradation in landfills, or dangerous and harmful matters that are continuously released in form of gases, and in particular of filtrate, causes significant pollution, mostly contaminating river flows and groundwater and other environmental media. One of the most important project tasks in the construction and exploitation of the landfill is the controlled management of the landfill and precipitation waters through the construction of a system that allows separate collection, treatment and discharge of these waters into natural watercourses. Treatment of leachate/leachate filtrate includes purification or non-neutralization of the harmful effect. Raw landfill leachate cannot be discharged into the recipient without prior treatment, due to its exceptional pollution ability. Reverse osmosis, as a membrane technique for filtering leachate filtrate, has been increasingly used to separate and filter organic and inorganic matter from the filtrate. The principle of the process is very simple. The aim of this paper is to examine the fact that the landfill filtrates are among the most problematic types of wastewater, viewed from the aspect of toxicity, and selection of appropriate techniques for their purification.

Keywords: *filters, pollution, reverse osmosis, purification, cleaner technologies*

Introduction

Environmental problems can be individual and local, but their negative impact is manifested and become visible globally. Therefore, in the concept of environmental management and action, the basic motto is: "Think globally, act locally". If we are talking about environmental problems, we can say that they are numerous and objectively difficult to solve. The responsibility for this falls on a man who, in view of his great scientific and technical power and ecological awareness, can protect nature, destroy it or further "create" it in a state of dynamic equilibrium. Naturally, this depends on the character of direction and a new scale of values in the processes of industrialism, post-industrialism, urbanization and adequate design of new technologies. Protection of the natural environment must become an organic part of the overall development projection. Humanity enters the new century and the third millennium with great ecological problems. One of the major problems is large quantities of waste, of various origin and contents in three aggregate states. Disposal and termination of waste is becoming a growing and increasingly general global problem, but also a local problem at the same time. Although located at the very bottom of the waste management hierarchy, landfilling is the most common method of waste management in both European and Pan-European countries. Generally speaking, there is a small degree of separation of different types of municipal waste at the site of production, and the production of waste and landfilling is one of the major problems of our civilization. During life of a landfill, special attention should be paid to the accompanying phenomena occurring on landfills in the form of unpleasant odours, rodents, insects, gases as well as leachate, and their impact on the environment. In particular, attention should be paid to water, both surface and underground, being under the greatest impact of potential pollution from landfills. Sewage waters are

loaded with matters of inorganic, organic and microbiological origin and are even 5 to 10 times more polluted than municipal wastewater. If natural watercourses are directly mixed with landfill waters that have not been previously purified, contamination poses danger for human and animal health. The same happens if soil or groundwater is contaminated by untreated landfill waters. One of the basic problems of waste management at landfills in practice is the problem of collecting and treatment of leachate (filtrate). The intensity of their production, and therefore the quantities, basically depends on a number of factors: age of landfill, types of waste, climatic factors and the like.

Material and Methods

For the purpose of this research paper, intensive research was conducted regarding the most efficient and economically most cost-effective solution for purification of leachate (filtrate), both from the aspect of the quality and quantity of treated leachate, and surface water quality in which the purified filtrates are directly released.

For research to bear results, regular monitoring of the water being purified has been undertaken, including sampling and testing of wastewater for the purpose of quality control, determining total wastewater load expressed in equivalent number of inhabitants, and determining efficiency of the wastewater treatment plant operation.

One of the most important project tasks in the construction and exploitation of the landfill is the controlled management of the landfill and precipitation waters through the construction of a system that allows separate collection, treatment and discharge of these waters into natural watercourses. In order to determine the quality of the leachate, the analytical method examines the following parameters: colour, temperature, electrical conductivity, haze, pH, ammonium nitrate, nitrates, nitrites, chlorides, sulphates, chemical oxygen consumption, biochemical oxygen demand, cadmium, iron, lead, zinc, consumption of $KMnO_4$, suspended matter, washed residue 105°C, dissolved oxygen, ortho-phosphates. Sanitary microbiological parameters examined during the study are: number of colonies of aerobic organotrophs at 22°C, total coliforms, faecal coliforms and faecal streptococci.(6)

As the landfill filtrate is the largest pollutant, its adequate management improves the quality of the environment and its protection. Landfill filtrates are among the most problematic types of wastewater, viewed from the aspect of toxicity and selection of appropriate techniques for their purification. The composition and quantity of leachate depends exclusively on the age and characteristics of the landfill itself.

The amount of landfill gas is unpredictable; it is difficult to determine it. According to some research, the maximum quantity of leachate is between 20-50% of the average amount of precipitation in the area of the landfill. However, science says it is more realistic to go with 5-15% of annual precipitation, due to the loss of a large part of the liquid by evaporation and in the process of anaerobic degradation of organic matter.

Results and Discussion

Treatment of leachate (filtrate) is considered to be purification or neutralization of the adverse effect. Raw landfill leachate cannot be discharged into the recipient without prior treatment, due to its exceptional pollution ability.

The selection of the leachate treatment process depends on the place for final disposal of the contaminated filtrate, which can be:

- Recirculation to the body of the landfill
- Biological purification in natural conditions (irrigation fields, soil filtration, biological lagoons and oxidation channels)
- Purification in artificial conditions in small plants through biological, thermal, physical, chemical and physical-chemical processes

- Discharge to city sewage, with prior treatment.

What kind of processing will be selected depends on the specific location of the landfill, conditions of its substrate, and quantity and quality of the treated leachate.

All of these processes can completely or only partially purify the leachate, most often biological or chemical impurities remain.

The efficacy of water purification using biological, thermal, physical, chemical and physio-chemical processes is presented in Table 1, based on elimination of parameters from the treated leachate: HPK, BPK_5, AOX (aromatic organic halogenate), NH_4 (N) and salts.(10)

Table no. 1. Benefits of applying different methods for filtering

Parameter / **Procedure**	**HPK**	**BPK_5**	**AOX**	**NH_4(N)**	**Salts**
Biological process	suitable	very good	of limited suitability	very good	unsuitable
Physical chemistry					
Flocculation	suitable	suitable	suitable	unsuitable	unsuitable
Membrane	very good	very good	very good	unsuitable	suitable
Adsorption with activated carbon	suitable	suitable	suitable	of limited suitability	unsuitable
Chemical oxidation	suitable	suitable	/	unsuitable	unsuitable
Thermal process					
Drying evaporation	very good	very good	very good	unsuitable	suitable
Striping	unsuitable	unsuitable	unsuitable	very good	unsuitable

*Source: Author's elaboration based on the laboratory analysis

Based on Table 1. it can be noticed that from all analysed leachate purification procedures, membrane technique is distinguished in particular, as part of the physical chemical process for purification of leachate, as the most efficient solution for removing mentioned parameters from the leachate.

Purification of leachate is carried out by using one or combination of several methods. Which method or combination is selected for leachate purification depends on the following criteria:

- Types and concentrations of filtrate matter
- Requirements regarding water purification degree
- Quantity of filtrate
- Conditions for disposal of residues after purification
- Other objective possibilities.

Leachate treatment costs range from 11 to 60 euros per tonne of waste and must be included in the cost of waste treatment.

For the purpose of research, as a support to presentation of leachate membrane filtration, a landfill in Ramici was visited, where leachate is purified through reverse osmosis since 2015.

Remediation and expansion of the Ramici landfill takes into account composition and quantity of the leachate, the landfill location, and it is clearly determined that the leachate is purified through reverse osmosis, all in order to meet requirements of the Ordinance on the conditions for discharging wastewaters into surface waters.

Reverse osmosis, as a membrane technique in leachate purification, has been increasingly used to separate and filter organic and inorganic matter from leachate. The principle of the process is very simple. The basic component in this process is the semipermeable membrane on which surface all changes occur. The product, clean water, passes through the membrane under pressure, while the mixture components or the concentrated solution are retained. Degree of water purification, capacity, economy and purpose of the plant depends on the quality of the membrane and operating pressure of the plant.

Basic problems when designing and constructing a reverse osmosis plant are provision of membranes that need to withstand high pressure differences and retain the proper permeability, and adequate quality of the reverse osmosis module in terms of hermetic seal and durability. The main advantage of this plant over adsorption and ion exchange plants is its

durability, without regeneration or replacement of the membrane module. High operating pressures (up to 16 bar) must be provided for efficient operation of the plant.
Reverse osmotic membranes (RO modules) are made of cellulose acetate, polyamide and other materials. The reverse osmosis block uses semi-permeable membranes, permeators and large pressure difference for moving water through the membrane, in order to achieve improvement in chemical, microbiological and endotoxicological quality of water.(9)
Reverse osmosis includes processes of solution separation where, under external hydrostatic pressure, greater than the equilibrium of osmotic pressure of the solution, the solvent is forced to flow in direction of the solute (A) → pure solvent (B) through the semi-permeable membrane, which separates solute and solvent. The direction of this flow is contrary to the direction of solvent flow which would be achieved with the classical osmosis, under the same conditions, from an area of high chemical potential to an area of low chemical potential, i.e. in direction the solvent (B) → solute (A), and based on this distinctly visible differences comes the name of this separation process - "reverse osmosis".(3)
In practical application of reverse osmotic module, this process is implemented in a way that water enters the membrane under pressure, the water molecules and limited amount of salts (permeat) pass through the semi-permeable membrane while the soluble salts are not allowed to pass and are discharged from the membrane and as flow with increased concentration of salt (concentrate) goes into a central sewage drain.
In the processing of the filtrate, the reverse osmosis found a great application for a variety of reasons:

- Meets requirements for the output concentration of heavy metals and aromatic organic halides (AOX)
- Meets requirements for HPK = 200 mg / l and 75% reduction of this parameter
- In case of direct discharge, no water management fee is charged
- Required area and space for the plant are relatively small compared to other procedures
- Simple shutdown and easy restart of the plant, which gives reverse osmosis great variability in capacity change
- Modular design gives light and quick upgrades
- The return of the concentrate to the landfill is generally granted for economic reasons
- 70% of the device is based on the principle of "disc tubes" or on the principle of a pipe barrier
- The plant can be installed in the container at the place of work, and only requires the foundation and power connection
- The plant can be easily disassembled and moved to another location, without significant investments
- The technology in this plant allows a pressure of 150 bar, and the concentrate management only takes 12% of the total quantity of treated water,
- The reversed osmosis process removes monovalent ions, multi-ion ions, insoluble particles, pyrogenic matter, and 99% organic matter.

In addition to the above-mentioned advantages, it is necessary to list measures to be adhered to when using the reverse osmosis plant, including:

- The filtrate tanks must be properly sized so that the leachate can be released into the surface waters after purification
- Due to the limited capacity of the permeable membrane surface it is not possible to increase hydraulic load short-term, as with conventional devices
- A well-trained and trained workforce is required
- It is necessary to have enough vital spare parts, because of the sensitivity to "work interruption"

- Design should be done specifically according to request for quality and quantity of water for processing with adequate cost estimate
- Problems in the plant can arise due to the development of microbial biomass on membranes, poorly chosen location of waste water suction, wrong selection of membrane material in terms of chemical and biological stability, poor concentration of concentrate and poor maintenance of devices, especially measuring instruments.

Conclusions

Reverse osmosis, as a membrane technique in the treatment of leachate (filtrates), also known as hyper filtration, is one of the more perfect filtration processes that eliminates even the tiniest particles from the filtrate. It is used to purify the filtrate by removing inorganic minerals, salts and other impurities in order to improve the quality of the filtrate through odour, taste, colour, etc., all for the purpose of protecting the recipients in which the filtrates are released.

In the treatment of the filtrate, the reverse osmosis found a wide application, with number of advantages and disadvantages. As the progress of each technology follows the economic aspect of the product, it is to be expected that the improved quality of the membrane and membrane filtration filters will improve already high purification rate, and a greater number of installations will reduce price of membrane models, as the economic aspect is the only limiting factor for the use of reverse osmosis in purification of the filtrate at the landfills.

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LIFE CYCLE ASSESSMENT (LCA) AS A TOOL TO DETERMINE THE IMPACT OF PRODUCTION AND FOOD CONSUMPTION ON ENVIRONMENT

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Abstract

Clean environment is a priority in maintaining a healthy life. Food, as well, is an essential part of our lives and its constant production is highly required. However, the effects of food production and consumption often cause environmental degradation in the three crucial media which include water, air and land. Developing cleaner technology or cleaner production is more and more needed, yet it can still be considered counterproductive for industrial and economic development, as the positive effect of clean technology is not achieved in the short term and its implementation leads to higher costs. The method that is used for evaluation of a particular product and its environmental impact is called *Life Cycle Assessment* (LCA). The LCA technique monitors the entire life cycle of the product, starting from the analysis of the raw material, its transformation to the final product, emissions that are involved in the overall process, to the final disposal scenarios. With this tool it is identified each phase of the product's life cycle, with all the *inputs*, i.e. resources, materials and energy and *outputs*, i.e. emissions into water, air and solid waste that are implemented in all the processes. Given all phases and processes, the LCA tool uses all identified data in performing the *environmental impact assessment.* The aim of this paper is to look at the possibilities of setting up the LCA technique in order to reduce the impact of food production and consumption on the environment.

Key words: *Clean technology, clean production, LCA (Life Cycle Assessment)*

Introduction

A clean and healthy environment is a precondition for quality living and the survival of living beings. Food, as well, is an essential part of our lives and its constant production is necessary. Starting from this fact, we are facing one of the major problems of nowadays - the impact of food production and consumption on the environment, through the amount of waste, as the by product represents incremental harm at both the local and global level. Tremendous growth in waste coming from the food industry sector represents the ecological, economic and social problem of all of us. Increasing concern of society about the impacts that the food production and consumption has on the environment have contributed to the ever growing increase in environmental awareness in the last decade. However, the lack of knowledge is still prevailing in many parts of the world, especially in developing countries, where the environmental awareness is still not at a satisfactory level. Waste from food production and consumption is a product that no longer has usable value. However, waste is generated daily in all industries. Increasing amounts of waste lead to degradation of the environment and disruption of human health, both in developed and developing countries. Determining the characteristics of waste from the food industry is a prerequisite for deciding on its management and disposal. In our region, landfills continue to be the most common form of the final destination of waste. Integrated waste management from the food industry should be based on a concept that aims at reducing waste generation. In particular, integrated waste management focuses on waste minimization through the use of biodegradable raw materials and reduction of unneeded packagings, but also on the establishment of a system in a way that is safe for the environment, while taking into account the economic and social aspects.

The paper evaluates a method that is used for evaluation of a particular product and its environmental impact, called *Life Cycle Assessment* (LCA). The LCA technique monitors the entire life cycle of the product, starting from the analysis of the raw material, its transformation to the final product, emissions that are involved in the overall process, to the final disposal scenarios. With this tool it is identified each phase of the product's life cycle, with all the *inputs*, e.g. resources, materials and energy and *outputs*, e.g. emissions into water, air and solid waste that are implemented in all the processes (Stevanovic, 2011). Given all phases and processes, the LCA tool uses all identified data in performing the *environmental impact assessment.* The main aim of the paper is to analyze and explore the possibility of establishing LCA to determine the impact of production and consumption of food on the environment. The paper discusses the theoretical frameworks for the establishment of the LCA, taking into account the available literature in this field.

Material and method

Various methods and research techniques are used within this study, particularly the analysis and synthesis of collected data from literature research, as well as their processing. Objectivity and reliability of the information makes the work credible and give it importance in scientific research. By analyzing local and foreign literature and other sources, the analysis and synthesis of collected data is carried out through theoretical part. In addition to the literature, theoretical and expert contributions, studies of international institutions in the field of ecology and environment are used in the paper. As previously established, the main focus is set to the use of LCA method for calculation of environmental impact caused by the food production and consumption.

Life Cycle Assessment involves the analysis and monitoring of the life cycle of a particular product, both through the product's decomposition and through its environmental impact. This cradle-to-grave approach, as it is often called, considers all the life stages of the product from the raw materials to the final disposal, and are characterized as inputs and outputs of the process. The input includes resources, materials and energy utilization, while the output refers to emissions into water, air and solid waste. These processes are used to assess the impact of products on the environment.

According to LCA standard ISO 14040 (ISO, 2006a), the LCA consists of four consecutive phases, which are:

- Definition of goal and scope: Defining study parameters
- Inventory analysis: Evaluation of inventory inputs and outputs from all processes that form part of the life cycle of the product
- Impact assessment: Use of inventory analysis results to define environmental profiles and resource consumption for the production system
- Interpretation: An analysis of the impact and resource utilization profile according to the defined objective and extent of the study, including the sensitivity analysis of the key elements of the assessment.

The production LCA is used in assessing the impact of productive activities and the use of a particular product on the environment, but it neglects the intermediate waste generation and treats this phase as the output of the product system. For this reason, the LCA for waste management, which regulates the impact of a product that no longer has a usable value (waste) on the environment, is herein defined.

The European Union predicts that LCA in the future will become an important tool for supporting decision-making in all aspects of waste management (EU Commission, 2005). The need for finding this type of tool appeared in the 1980s, as consumers began to seek information about the impact of the products to their environment. Life cycle application in the environmental product and process analysis led to a new discipline, Life Cycle

Assessment (LCA). This approach has since been widely applied in industry to reduce the burden from the production, use and disposal of many products on the environment. Over the past decade, LCA has also been applied in waste management, providing a new insight into the environmental aspects of waste management. In addition to monitoring the impact of products and production on the environment and human health, the LCA system can also monitor the consumption of resources.

The importance of this technique is more and more evident due to increasing pollution in the process of production and consumption, increasing quantities of waste, loss of useful components from waste, and ever increasing exploitation and consumption of natural resources.

Results and discussion

Clean (or cleaner) technologies and waste prevention offer some parallels. Both have access to the life cycle and both aim at avoiding pollution at the source. However, while the waste prevention focuses only on waste and the prevention of waste generation, clean technology considers all types of environmental impacts. Thus, waste prevention can be considered as part of clean technology. An example is pure organic solvents technology (Clift and Longley, 1995). Although pure technology and pure production are often used synonymously, these two approaches may vary. Pure technology is an approach that goes beyond clean production. Clean production is focused on the production of goods and services with minimal environmental impact according to current technological and economic borders. Priority in clean technology is reflected in preserving and protecting the environment, which often implies higher costs. That is precisely why the development of clean technology and clean production can be considered counterproductive for industrial and economic development. This is certainly true in the case of capital costs, but large investments in new production methods can result in lower operational costs and total savings in long term. However, incremental changes are often easier to implement and have less associated risks of radical changes (Murphy and Gouldson, 2000). Regulations can help overcome such short-term barriers that prevent movement toward clean technology (Murphy and Gouldson, 2000). According to the United Nations Environment Program (UNEP) of the Clean Production Program: "Clean production is a continuous application of an integrated preventive environmental strategy to processes of products and services in order to increase overall efficiency and reduce the risk to people and the environment. processes used in any industry, products, and services in the society " (UNEP, 2001). World-wide successful companies have recognized the importance of this business segment and are showing an increasing interest in carrying out activities that prevent environmental damage. Activities within companies focusing on "green" products and environmental product orientations are of paramount importance and gain greater competitiveness on the market. The purpose of reducing the impact of food production and consumption on the environment is to recognize, assess and minimize the negative impact on the environment. It is a long-lasting and complex process, and requires a systematic review of the environmental impacts, human health and biodiversity security. Summarizing the LCA technique in everyday production, we conclude that the LCA allows monitoring of the impact on air and water, as well as the transition of pollutants from one phase to another, or between these three environmental media. It also prevents the negative impact of production and consumption on biogeochemical cycles and brings to a greater importance of the environmental protection through various technologies and services, as well as improving economic opportunities through projects. Harmonization of needs and possibilities, seriousness and responsibility in planning, and persistence in realization of projects, strategies and action programs must be systematic and done with high interest. The results and progress in this sphere are weak, slow, unattractive, without high awareness,

solidarity and support of both the population and the authorities. Still, some proposals of potential measures are visible in emerging projects, plans and programs done by various environmental organisations. Such initiatives should contribute to lowering the shortcomings mostly related to subjective weaknesses, human omissions, ignorance and lack of coordination in the governance structure. Mistakes and omissions with harmful, dangerous, poisonous substances can have unimaginable consequences at the moment, in the short term, on the long run, but also on a permanent basis.

Conclusion

The empirical part of the paper opened some questions and problems of the management function in this field, mainly with regard to the interdisciplinary nature of the ecological sphere. In functional terms, it is necessary to engage numerous teams in the fields of natural, technical, medical and social sciences, i.e biology, ecology, chemistry, economics, law, medicine. The organization of the administration, especially the sector of economic or production activity, is specifically rationally structured and rounded up according to the subject of activity. From the theoretical and empirical part of the research it can be concluded that there is a discrepancy between the knowledge about problems, planned activities and normative approaches on one side and the real financial and technical-technological possibilities of the local and wider community to realize these and other ideas of pure production and consumption, on the other side. When it comes to the waste management, it can be noted that there had been a great shift from the industrial society, when the economy was characterized by mass production and greater responsibility of businesses and cities for their waste, to nowadays when most of these are still absent. The favorable circumstance of support of EU funds is in favor of equipping companies to master the technological process of production of a range of new products for the market, which would make them self-sustaining and profitable in the competition. This can primarily refer to the production of disposable packaging in the food, chemical, textile and construction industries.

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PRINCIPAL COMPONENT ANALYSIS (PCA) IN ASSESSMENT OF THE LANDSCAPE IN HERZEGOVINA REGION (BOSNIA AND HERZEGOVINA)

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Abstract

The aim of the research was to reduce a large number of attributes in subjective visual assessment of landscapes. Determination of the significance of the main traits in landscape assessment is important for better understanding of the observer and intensifying of landscape changes. Numerous traits influence landscape perception but some of them can overlap. The question is if they can be grouped into components. We have studied which attributes are important for respondents to value landscapes. Principle component analysis (PCA) was done, and the Kaiser criteria was used to determine how many meaningful components should be retained for the interpretation. The presented methodology was applied to the real data obtained from the questionnaire on a sample of students attending the University of Mostar (Bosnia and Herzegovina). Statistical package SPSS 16.0. was used. The statistical analysis of nine variables showed four relevant main components that should be used for more precise decision-making and improvement of landscape development. The first PC could be named component of environmental and biological value, with traits: environmental value 0.872, biodiversity preservation 0.902 and biogeographic position 0.727 (correlation coefficients). The second PC is spatial and developmental component, and it emphasizes the importance of traits: rural development 0.848, spatial planning 0.838 and technological value 0.752. The third PC component is sociological value with traits of the same name 0.937. The fourth PC cultural tourism with traits: cultural heritage 0.944 and development of tourism 0.677.
Interpretation of data through principal components shows some hidden connections and interrelations of data.

Key words: *PCA analysis, landscape, components, traits.*

Introduction

Landscape means geographical area, as perceived by people, whose character is the result of the action and interaction of natural and/or human factors (UNESCO, 2005). Landscape management means action, from a perspective of sustainable development, to ensure the regular upkeep of a landscape. Landscape planning means strong forward-looking action to enhance, restore or create landscapes. When planning and designing public green areas, landscape architect should also give consideration to social needs of the users concerned. Inadequate landscape maintenance may reduce the natural visual quality (Noralizawati *et al.*, 2012). Over time people attach emotional meaning to "place", which can result in a particular attachment to green areas, and can be manifested at a real and symbolic level (Low and Altman, 1992). The visual and aesthetic characteristics of place are one of the most important environmental perception factors (Pereković *et al.*, 2007). Aesthetics of place is important for its "branding" (Miškić-Domislić *et al.*, 2013). According to the subjective paradigm, the quality of landscape can be estimated only by "the eyes of the beholder" (Lothian, 1999). The paper explores the subjective perception of landscape, emphasizes the traits of an object as well as the meaning attached to it by the subject (Crosgrove, 1984). Numerous traits influence the determinants of landscape identity. Knowledge of these traits may significantly influence the intensification of landscape changes acceptable to population.

The main objectives of the PCA are to reduce and analyze the linear correlations of a larger number of multivariately distributed, mutually correlated variables with the aim of obtaining a smaller number of components, new variables, mutually uncorrelated with minimum loss of information. (Orlić, 2012). The maximum number of new variables that can be formed is equal to the number of original variables, and the new variables are uncorrelated among themselves (Sharma, 1996). Many authors argue that PCA is only a specific form of factor analysis (FA) occurring when the decision on the analysis method is made: from the total variance, or the sum of variances of all variables, as is the case in PCA, or only from the part of total variance that is common to all variables, like in FA (Hair *et al.*, 1995). The reason for this is the fact that PCA is found as an option of FA in the statistical package SP SS and STATISTICA. However, the aim of PCA is to create new variables that explain the maximum amount of total variance in data by forming new components (Pecina, 2006). The need to reduce the number of traits is apparent when assessing the main traits.
The objective of this paper is to show the reduction of traits to a smaller number, through implementation of the statistical method of principal component analysis (PCA) and the necessary steps to be followed. PCA is one of the simplest and most popular methods of multivariate statistics used in almost all scientific disciplines.

Material and Methods

The data were collected by the survey method on a sample of students of the University of Mostar (Bosnia and Herzegovina) in 2016. The survey used a questionnaire consisting of questions related to traits determining the value of landscape. The quality of the questionnaire was tested by a pilot study. Data on landscape characteristics were evaluated on the sample of 100 randomly selected students attending the University of Mostar. After this, the final version of the questionnaire was drawn up and the survey was conducted on a new group of respondents with 30 study groups, for nine traits: KA1 environmental value; KA2 rural development; KA3 spatial planning; KA4 technological value; KA5 cultural heritage; KA6 tourism development; KA7 social value; KA8 biodiversity preservation and KA9 biogeographic position. The results of the conducted survey were analyzed using principal component analysis (PCA) (Hotelling, 1936). PCA was processed on the basis of a correlation matrix in the statistical package SP SS 16. After calculating the correlation matrix for all observed traits, principal components are obtained based on correlation coefficients between the variables were extracted. Examination of the Correlation matrix confirmed the suitability of the data for conducting further analysis. Kaiser-Meyer-Olkin (KMO) test measures the sampling adequacy, or whether the data fit into factors. It is obtained as the ratio of sums of squares of correlation coefficients of all the variables in the analysis (except those between themselves which are all equal to 1) to the same sum of squares plus squares of the partial correlation coefficients of each variable *i* with each variable *j* while excluding other variables:

$$KMO = \frac{\sum\sum_{i \neq j} r^2{}_{ij}}{\sum\sum_{i \neq j} r_{ij}{}^2 + \sum\sum a^2{}_{ij}},$$

where r^2_{ij}, ($i \neq j$) is the square of the off-diagonal element of the correlation matrix (square of the coefficient of correlation between the i^{th} and the j^{th} variable), and a^2_{ij} ($i \neq j$) square of the off-diagonal element of the anti-image correlation matrix (the square of the coefficient of partial correlation between the i^{th} and the j^{th} variable). The KMO ranges in a closed interval from 0 to 1. If the KMO value of the measure is less than 0.5, the correlation matrix is not suitable for factor analysis. Except that the KMO value can be calculated for the entire matrix, it can also be calculated for individual variables. Initial results and given eigenvalues,

percentages and cumulative variance percentages for each individual factor were obtained. Eigenvalues of a certain factor are equal to the sum of squares of factor loads by all variables for that factor. Eigenvalue factors greater than one are selected. The percentage of explained variance of each individual factor is calculated on the basis of its eigenvalue of that factor, or as the ratio of the eigenvalue to the sum of eigenvalues multiplied by one hundred. The sum of eigenvalues is equal to the sum of initial communalities. On the main diagonal of the correlation matrix that is analyzed unities are used for initial communalities, the sum of initial communalities is equal to the number of variables. When the eigenvalue of a given factor is divided by the sum of eigenvalues, or with the number of variables and multiplied by one hundred, the percentage of total variance of that factor will be obtained. Using the criterion of the percentage of explained variance, extraction of factors should continue until the extracted factors explain at least 95% of the total variance. The number of factors can also be determined based on Cattell's scree plot. When making a decision, both criteria are usually combined. After determining the number of factors, it is necessary to determine the matrix of factor structure of the extracted factors that contains factor loads. Factor loads show the importance of each variable for the individual factor. The communality (h_i^2) of a variable shows how many variances of that variable are explained by common factors. The factors are then rotated in order to maximize the connection between variables and some of the factors. An orthogonal rotation is performed, and the number of factors is determined. Varimax rotation is more successful in achieving the principle of simple structure than others. After rotating the factor, it is also necessary to determine the factor matrix on which factor interpretation and identification of attributes that have high loads for the same factor are based. The sum of squares of factor loads of a given variable is equal to the communality of that variable. In orthogonal factor rotation, the sum of squares of factor loads for each individual variable after rotation must be equal to the sum of square factor loads before the factor rotation was conducted:

$$\sum_{j=1}^{m} \lambda_{ij}^{\ 2} = \sum_{j=1}^{m} \lambda^{*}_{ij}^{\ 2}, \quad i=1, 2,\ldots,p,$$

Where λ_{ij} is the factor load of the variable i and the common factor j before the factor rotation, and λ^{*}_{ij} is the factor load of the variable i and the common factor j after the factor rotation. It is usual to name components and show the importance of components in prediction of the original variable. After clarification, reliability of the questionnaire is also analyzed. The questionnaire reliability measurement shows the suitability of the measuring instrument and shows that the same measurement indicators would be obtained by its use in repeated measurements. One of the models is the Cronbach α coefficient, which measures the internal consistency of a particular component and the total value. A higher value indicates greater reliability, or shows that the attributes of the same component measure the same occurrence. A value greater than 0.60 indicates an acceptable level of reliability in research.

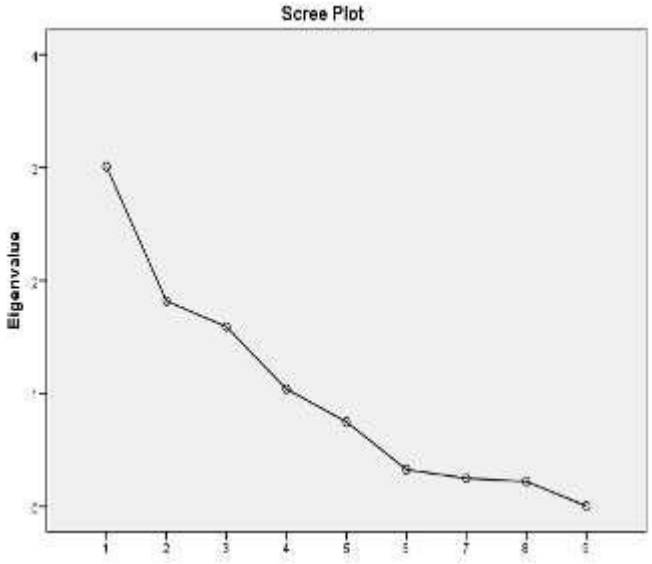
Results and Discussion

The correlation matrix between the variables showed a high degree of correlation, as well as suitability of data for analysis and possible application of PCA. Sample adequacy and test of sphericity were analyzed. KMO was 0.7 and showed that data fit well, and Bartlett's test of sphericity was statistically significant 0.000.

Table 1. Eigenvalue and variability of non-rooted values of principal components

Principal components			
	Eigenvalues	% of Variance	Cumulative %
1	3.008	33.417	33.417
2	1.817	20.187	53.604
3	1.590	17.663	71.266
4	1.039	11.542	82.809
5	0.749	8.320	91.129
6	0.326	3.623	94.752
7	0.248	2.756	97.508
8	0.220	2.450	99.958
9	0.004	0.042	100.000

Table 1 shows that 33.42% of variance is attributed to the first principal component, and even four principal components are needed to cover 83% of variations. Values of Latent roots – Eigenvalues were lower than one after the fourth PC: With those four PC approximately 83% of total variance was explained. Scree plot can also be used to establish the number of components for analysis, or the point at which the curve tends to straighten. When making a decision, both criteria are usually combined. Graph 1 shows that the curve tends to straighten after the component no. 4, for which the eigenvalue is also less than 1.



Graph 1. Component number – Cattell's scree plot

It was not enough to stay at the level of finding PC. Certain traits have a high coefficient of correlation with only one PC. Some have lower correlation level with more PC and next PC have facilitated the need for principal components rotation (Ivanković, 1988). In this way we had clearer distribution of particular traits inside of them, and traits were more closely tied to particular principal components (Table 2).

Table 2 gives the structure matrix data after the Varimax rotation is performed, and shows the coefficients of correlation of individual traits with extracted components.

Table 2. Rotated Component Matrix

	Principal Components			
	1	2	3	4
KA1	0.872	-0.146	-0.097	-0.367
KA2	-0.253	0.848	-0.302	-0.089
KA3	0.183	0.838	-0.301	-0.132
KA4	0.145	0.752	0.003	0.179
KA5	0.047	0.060	-0.064	0.944
KA6	0.366	0.128	0.452	0.677
KA7	0.097	-0.031	0.937	0.017
KA8	0.902	0.055	0.175	0.070
KA9	0.727	-0.018	-0.360	-0.160

Assigning a name to each component shows its importance in the prediction of each original variable. The first PC (KA: 1, 8, 9) could be named component of environmental and biological value, and it contains traits: environmental value = 0.872, biodiversity preservation = 0.902 and biogeographic position = 0.727. Biodiversity preservation has the highest loads in this component. The importance of biodiversity as an important development factor was also adreees by Munić (2010). The second PC (KA: 2, 3, 4) could be named spatial and developmental component and it stresses the importance of traits: rural development = 0.848, spatial planning = 0.838 and technological value = 0.752. The variable rural development has the highest coefficient and load with this component. The third PC (KA: 7) component is sociological value with traits of the same name = 0.937.

The fourth PC (KA: 5, 6) could be named cultural tourism and it is characterized by traits: cultural heritage = 0.944 and development of tourism = 0.677. The importance of these traits was also described by Pavlović (2000) and Bejtović (2008). The Cronbach alpha coefficient measures the reliability of grouping reduced attributes into common components. Traits within the components have satisfactory correlation measures, which confirms the reliability of the survey: PC1 Cronbach $\alpha = 0.85$; PC 2 Cronbach $\alpha = 0.63$; PC3 Cronbach $\alpha = 0.73$; PC4 Cronbach $\alpha = 0.65$. The total value of this coefficient was 0.90 and represents an acceptable level of reliability in the survey, and reliability of the used questionnaire and the possibility of its use as a measuring instrument in a repeated survey.

Conclusion

Based on collected data and application of the principal component analysis (PCA) method, the conducted studies reduced a larger number of traits to a smaller number of components formed on the basis of common traits. The four components were named: environmental and biological value, spatial and developmental, sociological value, and cultural tourism. Interpretation of data through principal components shows some hidden connections and interrelations of data. The obtained results will be used in further studies aimed at measuring the quality and development of landscape. Knowledge of identified traits may significantly influence the intensification of landscape changes acceptable to population.

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WASTEWATER GOVERNANCE IN URBAN TERRITORY – CHALLENGES TO THE CIRCULAR ECONOMY IN BULGARIA

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Abstract

The uncontrolled growth of the cities and urban population has led to need to sustainable governance of natural resources and the waste. One the most important of these resources is water, which becomes a scarce commodity because of its use not only by the households, but also by the industry. In this regard, wastewater recycling is an essential element of the circular economy. Wastewater treatment is a process where extra resources are extracted - remain biogas and sand, sludge and purified water respectively. Generally, the resulting biogas is used for heat and electricity, the sand in the construction, and the purified water is discharged into hydro-basins. In practice, there are several options for utilizing sludge. These are known for improving agricultural or non-agricultural soils. Apart from agriculture, sludge is also used in forestry, reclamation of disturbed terrain - mines and eroded areas, fuel, and construction technologies. The aim of this paper is to analyse the benefits or threats for the society and the economy, as a result of the wastewater governance in urban territory, at the same time, paying attention to the environmental challenges arising from the circular economy. Does the circular economy harm the environment and society?

Keywords: *sustainable governance, circular economy, natural resources, wastewater recycling, Bulgaria*

Introduction

The continued and uncontrolled growth of the cities and urban population has led to the need of sustainable governance of the natural resources and the waste. The goal of the sustainable governance is to reduce consumption and save raw materials, water and energy, thereby contributing to the conservation of resources. During the last decade, economic, social and environmental factors were adopted as key elements of sustainability. In recent years, governance has become the fourth pillar of the sustainability. Linking these concepts and involving the circular economy as the fifth pillar is a challenge of sustainability in the search for new theories and paradigms.

Circular economy models can be divided into two groups. The first one encourages the reuse of resources through recycling, processing, etc. The second one turns old goods into new resources. Knowledge about the essence and objectives of the circular economy is increasingly being applied in the industry. Although the idea of a circular economy is a relatively new concept, it raises fears and scepticism not only in society, but also among the scientific community. The concerns about resource reuse, greenhouse gas emissions, ethics and safety have changed the research approaches. Demonstrating the benefits (or not) of the circular economy will contribute to policy coordination in line with sustainability perceptions and approaches in building business models. When assessing sustainability, a specific historical moment and the specific socio-economic, institutional and natural environment are taken into account (Bashev, 2016). However, this does not automatically guarantee that the same indicators will be reported in future periods. There are contradictions in the business that are related to balancing environmental and economic interests. They involve difficulties in reconciling the objectives of "economic growth" with those of "sustainable governance" and "sustainable development" as indicators for environmental assessment. The main problems

arise from the fact that society and the economy are interrelated. In this case, the question in front of the corporate governance is how to report economic growth that does not damage, but preserves the environment.

The aim of this paper is to analyse the benefits or threats for the society and the economy, as a result of the wastewater governance in urban territory. At the same time, paying attention of the environmental challenges arising from the circular economy. A review is made of the available information on the various aspects of applicability of wastewater sludge in the purification plants. Answers have been searched also for topical issues related to the impact on soil fertility, plant production quality, human and animal health. Opportunities are being explored to exploit this by-product in other systems that do not harm the environment.

The recovery of wastewater sludge is not only a corporate problem. Bearing in mind that wastewater treatment plants and wastewater safety are strategic directions for the benefit of society, the use of sludge should be a national policy with a direct government commitment.

Material and Methods

The paper is based on an extended review of primary and secondary data. The application of wastewater sludge to farmland in EU member-states corresponds Directive 86/278/EU "Environmental Protection Directive". It strongly prohibits the use of sludge from sewage treatment plants in the agriculture, provided that specific requirements are not met. According to European Union laws, sludge from urban wastewater treatment plants is not dangerous when properly managed. Provided that certain requirements are met, they should not pose a risk to the environment or human health, or even to the contrary they can bring benefits. In fact, the EU has a very precise and clear regulation on the requirements for the treatment of sludge, namely: Directive 91/156/EU "Waste Base Directive"; Directive 94/3/ EU according to which sludge from sewage treatment plants is excluded from the category of hazardous waste; Directive 99/31/EU on landfill; Directive 2000/60/EU establishing a framework for Community actions in the field of water policy.

In Bulgaria these issues are regulated in harmony with the European Law on Agricultural Land Protection, Art. 6, paragraph 1, item 3 (SG, No. 35, 1996); Waste Management Act (WAS), (SG, issue 53, 2012); Ordinance on the Procedure and Method for Utilization of Waste Waters by Their Use in Agriculture (Decree of the Council of Ministers No. 330/2004, SG, No. 121, 2014, amended SG No. 20/2011) and others. But strategies and "sustainable practices" that respond to emerging problems in certain countries are not always relevant to others (Bashev, H., 2016).

According to the Ordinance on the Procedure and Method of Utilization of Sludges from Wastewater Treatment by Their Use in Agriculture (promulgated in State Gazette No. 63 of 12 August 2016), it is necessary to take samples from each batch of the following 22 indicators: Dry substance; Total Organic Carbon; Active Reaction - pH (H_2O); Total Kjeldahl Nitrogen; Ammonium and nitrate nitrogen; Phosphorus - extractable and exchange forms, recalculated as P_2O_5; Potassium - extractable and exchange form, recalculated as K_2O; Calcium - exchange forms; Magnesium - exchange forms; Water-soluble sulphates, recalculated as sulphur; Copper; Nickel; Lead; Zinc; Mercury; Chromium; Arsenic; Escherichia coli; Salmonella spp.; Clostridium perfringens; Viable helminth eggs. However, the legislation does not automatically lead to a greater safety.

Results and Discussion

As one of the most important resources, water becomes a scarce commodity because of its use not only by households but also by industry. In this respect, the wastewater recycling is in support of the circular economy. Wastewater treatment is a process in which some extra resources are extracted. Primary, secondary and tertiary purification is expected to separate

biogas and sand, sludge and purified water respectively. Typically, the resulting biogas is used as heat and electricity, sand in the construction, and purified water is discharged into hydro-basins.

The wastewater sludge, also called bio-equipment, is a by-product of wastewater treatment processes. The sludge characteristics depend on the quality of the sewerage network and the type of treatment processes that is used. Practically, there are several options for utilizing (or disposing) sludge. These are the known practices for improving agricultural or non-agricultural soils. Apart from agriculture, sludge is also used in the forestry, re-cultivation of disturbed terrain - mines and eroded areas. Newer options are - power, fuel, construction recovery, new technologies and sludge disposal in special protected landfills.

Wastewater sludge contains organic and nonorganic nutrients that can replace the naturally occurring soil fertilizer, known to the public. The presence of potential toxic metals often limits their use.

The questions are as follows:

• Are the physical, chemical and biological properties of the soil changing, when using sludge in agriculture?

• Is the yield of crops in changed soils higher than in the natural ones?

• What changes occur in soil fertility due to the application of excessive sludge levels over many years?

• Are really sludge contributing to the circular economy or are they an environmental problem?

Will the answers to these questions help to assess the sustainable governance of by-products from wastewater and what are the benefits to the circular economy? It is a fact that plants differ in their ability to absorb from the soil the metals and nutrients, extracted from the sludge. Agriculture is known as one of the most important sectors of economy, which requires the development of a separate model for its sustainable development.

According Milieu Ltd (2010) data, the total sludge in the EU-27 is over 10.1 million tonnes of dry matter, and in 2017 the expectations are to reach over 15 million tonnes (EU-28). About 40% of this amount is used in the agriculture. In different European countries the percentage varies. In Denmark, France, Ireland, Spain and the UK - over half of the sludge is used in agriculture. At three - they are not recycled in the agriculture, and in others less than 5% of the total sludge production is used. Since 1995, the amount of land-filled sludge in the EU has continued to grow. However, in some Member States restrictions have been registered, even stopping the use of sludge in agriculture (such as Switzerland and the Netherlands). Due to the increased public concern about the safety and health of humans and animals in areas such as Flanders in Belgium, Bavaria in Germany and parts of Austria, they have phased out this activity. Some member-states already burn the sludge and the residual ash is deposited. For example, more than 70% of the sludge is burned in Flanders (Belgium), in the Netherlands - 60%, Austria, Denmark and Germany - 40%. This naturally generates side environmental problems. Activities for the recovery of sludge for electricity and heat are increasingly being promoted. In the world's scientific literature, the topic occurs to be quite up-to-date. There are researches on the beneficial impact and possible risk of sludge application in the soil (Singh and Agrawa, 2010). They argue that wastewater sludge in the agriculture provides benefits, as a substitute for chemical fertilizers. The sediment also provides other plant macronutrients, such as potassium and sulphur, copper and zinc. Other researchers point to damages in terms of climate change and greenhouse gas emissions from the sewer. It was found that the carbon from the sludge used in the agriculture would be isolated in the soil. However, this is not entirely scientifically substantiated. As regards to the air pollution, although reducing the use of chemical fertilizer by replacing sewage sludge, the nitrogen oxide emissions associated with this fertilizer of not less than 20% of the nitrogen in the degraded sludge is considered to

be readily available for plants. The condition, structural properties and retention of soil moisture as a result of sludge are also well studied.

In Bulgaria (Marinova and Tsolova, 2005); Baykov, Popova, Zaharinov, Marinova-Garvanska, Kaleva, Kirov, (2013); Popova, Zaharinov, Gentcheva, Pejtchinova, Marinova-Garvanska, Baykov (2017) have extensive research experience and a long list of publications related to the topic. In this area, however, it engenders discussion in the scientific community and practitioners. The publications on the topic are related to the benefits of sludge for agriculture, as a fertilizer for soil fertility and soil structure. Zaharinov (2011) affirms that sludge from "humification products" does not cause "serious" ecosystem damage. It is claimed that "the use of sludge in the agriculture is a cost-effective and environmentally friendly method". Other authors (Dermendzhieva, 2017) have shown that due to the content of "excessive amounts of certain biogenic elements and heavy metals, or pathogenic microorganisms and invasive forms of parasites, they are dangerous to the environment, human health and animal wellbeing". In other words, "there is a risk of microbiological contamination of soils using sludge" (Dermendzhieva, 2017).

The differences of concepts of the benefits and disadvantages of land-use utilization in the agriculture once again suggest that the issue is under-studied and incomplete, raising doubts about the benefits to nature and human health. This reinforces the topicality of the subject, moreover, because (all the more so that) the problem is not only regional or national to the ecological balance but also global. The sludge contains components that can be reused as well as dangerous elements, such as heavy metals.

The sustainable governance of sludge has several basic aspects - legal, techno-economic and informational. They are all interrelated. To account the impact of all factors, they should be analyzed in a relationship, not individually or in isolation.

Some questions arise as follows: Are these indicators sufficient to ensure any safety for humans and animals? Or if the norms of one (or a certain part of it) are above the allowable, what will be the fate of the generated sludge?

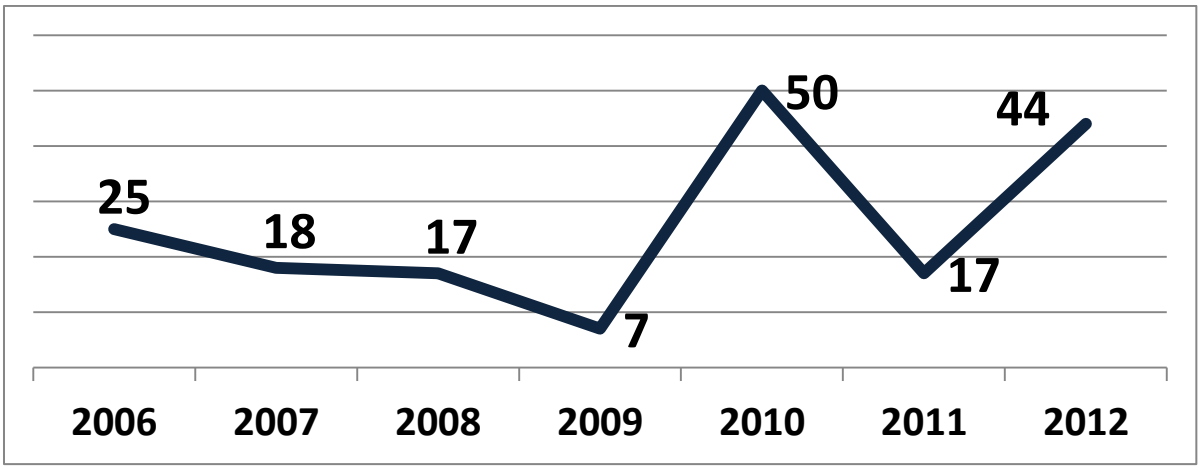
Bulgaria is obliged to implement the statutory provisions of the European Urban Wastewater Treatment Directive (91/271 / EU) on secondary treatment for all urban agglomerations of over 2,000 inhabitants. This means that nearly 90% of the country's population will be covered by the sewerage system. The population will probably continue to move more towards the cities, which will surpass these 90%. As a result, the amount of generated sludge will increase almost twice. Bulgaria has not yet changed the old practices of sludge management (landfilling and recovery in agriculture). They cannot be used in the future, not only because of the risk prevention requirements, but also because of the expected increasing amounts of sludge. The challenges for all stakeholders are growing, and we are all affected by the consequences of "greening" and "green economy" policies. Sludge treatment is the bigger challenge compared to wastewater treatment. Unlike wastewater that after discharge is naturally discharged into water, sewage is collected in a treatment plant, additional costs are generated while being transferred to agriculture, etc. A minimum of 6 months of drying time is required, which reduces the unpleasant odour. However, "sludge remains an environmental problem in terms of their generation, storage and exploitation" (Zaharinov, 2011). According to the legislation, periodic health and environmental expertise and test of stabilized sludge must be carried out.

Are approaches in the circular economy safe for people and nature?

According to the Bulgarian legislation, "sludge is not allowed to be used on meadows, pastures or areas fed with fodder crops if they are used for grazing or the feed is harvested within less than 45 days after the sludge is used; soils on which fruit, vegetable crops and vines are grown, with the exception of fruit trees; soils intended for growing of fruit, vegetables and other crops, which are in direct contact with the soil and consumed in a raw

state for a period of 10 months before and during harvesting; coastal floodplains, riverbeds and protective dykes". The data (Figure 1) show instability / lack of sustainability with regard to the number of permits for the utilization of sludge in agriculture during the period 2006-2012. Naturally, this is an indicator of uncertainty and mistrust among farmers about the quality of sludge, as a humus soil enhancer and about the safety for human and animal health. The practitioners, specialists and researchers interpret differently the conditions and factors for sustainability of agriculture.

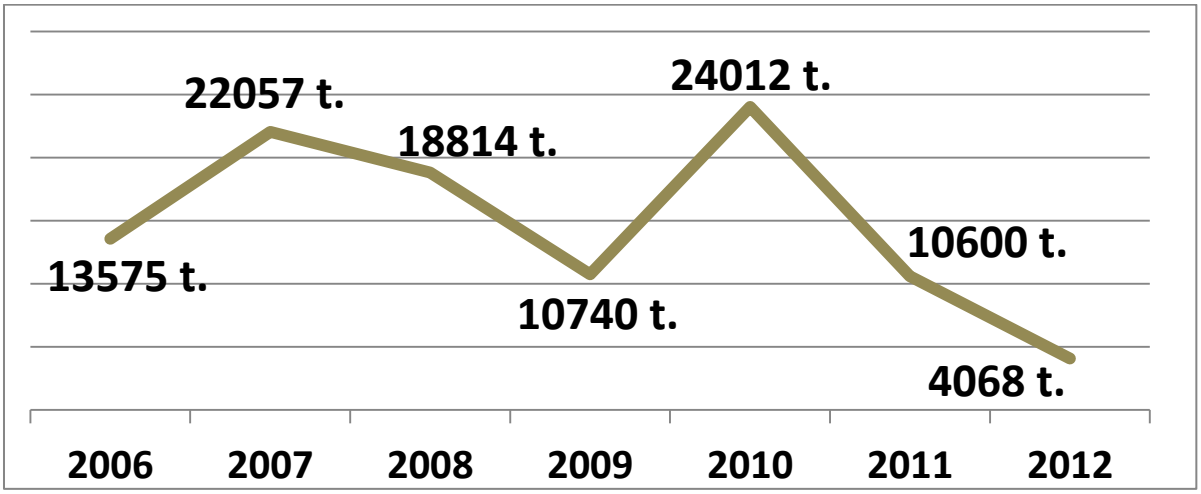
Figure 1. Permits for the utilization of sludge in the agriculture during the period 2006-2012 in Bulgaria



Source: MAF, BSA (2013)

Even more volatile is the emerging picture of the utilization of sludge in agriculture for the period 2006-2012, that has given the constantly increasing amount of sludge from the treatment of waste water. There has been a serious decline in recent years.

Figure 2. Sludge utilization in the agriculture in the period 2006-2012 in Bulgaria, tons



Source: MAF, BSA (2013)

Bulgaria currently operates whit 89 urban wastewater treatment plants, 50 of which are newly built or expanded with European funds from the period 2007 - 2013. The completion of eight treatment plants with funds from the new period and the construction of nine new ones under

the so-called Early Projects under the Operational Program "Environment" will be completed. Thus, sludge will increase simultaneously with the construction of water infrastructure. According to information from the Environment and Water Ministry, 44% of sludge in 2015 is recycled and recovered. This share should grow further in future. By the end of 2016, recycling should reach 55%. 60% by the end of 2018 and 65% by the end of 2020. Energy recovery should reach 10% by the end of 2016. 20% by the end of 2018, and 35% by the end of 2020. Solving a single problem often leads to another one. Sometimes it is more appropriate to rethink and make a reassessment of carefully the opportunities for removing pollution at regional level. Making a hasty decision can cause a global ecosystem crisis and it could have unforeseen consequences.
Another questions arise again:

- Are there any substances contained in sludge that are not monitored but are potentially dangerous for use in agriculture?
- Is the physical-mechanical composition of the soil impaired?
- Is biodiversity in the soil impaired?
- Is the soil poisoned by sludge utilization?

When assessing the sustainable governance of natural resources, it must be known that water, soil and climate are interconnected. This requires that the research should be directed not only to sediments but also to the mixture resulting from the sludge-soil interaction, or how to change the physical-mechanical, chemical and biological properties of agricultural land. In analyzing the physical properties of the soil, the soil-water-climate relationship should be measured. The presence of sludge changes the ability of the soil to retain water, permeability and porosity, which are indicative of its good status. The reducing of organisms, especially earthworms, is an indicator of changes and imbalances. They can be an indicator of sustainability, because their population is heavily dependent on soil contamination. The assessment of the impact of sludge is not only associated with changes in the physical-mechanical, chemical and biological properties of the soil. An economic analysis of plant productivity with purely economic indicators is needed. This means a comparative analysis of the yields obtained with and without sludge in agricultural land.
For these reasons, which are as unexplored factors, farmers are still sceptical about the opportunities about sludge recovery. However, they can prove to be very beneficial for improving the biological properties of the soil.
With regard to chemical changes, the soil must be neither acidic nor alkaline. The most important thing for plants is to have enough nutrients due to the presence of organic matter

Conclusions

The concept of sustainable resource governance includes regulating of consumption, saving raw materials, water and energy to help the protection the environmental. However, following these policies often provokes business contradictions, which are related to the balancing of environmental and economic interests. They involve difficulties in reconciling objectives. Challenges for all stakeholders are growing; we are all affected by the consequences of "economic growth" and "green economy" policies. Therefore, the recovery of sewage sludge with regard to sustainable consumption and production patterns is a challenge in any of science and practice. In support of these opportunities, the EC envisages the signing of two papers for innovations. Legislative barriers to electronic mobility and recycling of batteries will be examined; the other will address regulatory barriers to sustainable wastewater treatment using innovative bio-reactor technology for anaerobic membranes. The Horizon 2020 program envisages investing EUR 650 million in the Industry 2020 in the circular economy. It provides an opportunity to demonstrate the economic and environmental

compatibility of a circular economy approach and at the same time gives impetus to EU re-industrialization. There is a need for a networking concept, which is also supported by funding opportunities for various circular economy projects. The benefits to society will be related to a greener urban environment, conservation of natural resources, economic efficiency, reduction of carbon footprint, Business to Business solutions.

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LANDSCAPE ECOLOGICAL PRECONDITIONS FOR RICE PRODUCING IN BULGARIA

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Abstract

The current research is focused on the landscape ecological preconditions and the restricting role of the rice-growing environment in Bulgaria. The analysis of landscape ecological conditions is a fundamental step in characterizing of specific features of rice production as agro-economic activity. The specific research focuses on the relationship between the agro-environmental requirements of rice crops and the specific landscape ecological conditions in the Pazardzhik-Plovdiv field. The specific combination of the main natural components in this part of Bulgaria is a major factor for the development of rice production since the second half of the 14th century. The favorable climatic, hydrological and soil conditions in the Pazardzhik-Plovdiv field determine the process of growing rice crops as the main one in the agrarian profile of this part of Bulgaria. The natural combination of landscape forming factors, interrelationships and interactions between them, predetermines the existence and functioning of specific hydromorphic landscapes, typical of the lowland territories located around the Maritsa River and its tributaries. On the other hand, the specificities of the anthropogenic workload of these landscapes in historical and geographic aspect are a major factor in the development of anthropogenised rice field agricultural landscape. These agricultural landscapes are characterized by a qualitatively new spatial-temporal structure, functioning and development, which are determined by the restricting role of the natural environment. From landscape ecological point of view, the main focus of rice growing in the Pazardzhik-Plovdiv field is the existence of such specific agricultural landscapes, typical for this part of Bulgaria only.

Keywords: *agriculture, anthropogenic activity, landscape components, landscapes, rice producing.*

Introduction

Rice culture is introduced in the Bulgarian lands in the middle of the 14th century. "In the second half of the 19th century around Plovdiv and Pazardzhik large farms for the cultivation of rice on the rivers have formed" (Gramatikov, 2006). The rice becomes a major culture in the agrarian profile of the Bulgarian economy. The rice is one of the main food sources for realization the population's living on these lands from the middle Ages to the present day. At the various stages of development of the Bulgarian economy and social relations, the rice produced on the Bulgarian lands has occupied an essential place in the product export, either of the Ottoman Empire, or of Bulgaria after the Liberation. Pazardzhik-Plovdiv field categorically stands out as the main rice-producing region in the country. Although in the past attempts have been done to grow rice and in other parts of Bulgaria, Pazardzhik-Plovdiv field, both historically and nowadays, is the only region in the country available with appropriate natural conditions for the development of this sub-branch of the crop breeding. The agro-ecological resource potential of the landscapes is the main factor for the development of one or another agricultural production. The landscape concept allows the differentiation of the natural environment of interconnected and interacting natural systems. In turn, "the formation of the system integrity of the landscapes is conditioned by the interaction and the functional

dependencies between the constituent geocomponents – rocks, air, water, plants, animals, soils " (Borisova, 2013). That is why it is necessary to analyze the individual landscape-forming components, involved in landscape differentiation and their complex specific expression defining their role as a suitable recipient of the rice crop. From point of view of the rice production and the requirements of the rice crops, as well as in the meaning of the differentiation of rice paddy anthropogenic landscapes (hydromorphous), the main focus of the research is on the soil presence and the hydro-climatic preconditions for the landscape genesis. The main purpose of the publication is to argue and justify the suitability of the landscapes, occupying certain areas in Pazardzhik-Plovdiv field, for cultivation of rice, in the light of the landscape-ecological approach. The present study analyzes the landscape-ecological conditions for the cultivation of rice within the scope of the investigated area. A comparison is done between the agro-ecological requirements of the rice as an agricultural crop and the natural factors determining the landscape features. The role of the anthropogenic factor in the differentiation of the rice agro-landscapes is analyzed. The study is based on the complex landscape approach, which find expression in the complex analysis of the regularly interconnected and interacting natural components.

Object of research is the field appearance of the naturally occurring landscape-forming components in combination with the anthropogenic activity within the scope of the studied region.

Subject of research is the processes of correlation between the limiting role of the environment, the vegetation requirements of the rice and the activity of the anthropogenic factor in the range of the rice production in Bulgaria

The main tasks, that this study puts forward, are the following:

- Clarification of the vegetation requirements of the rice crop.
- Analysis of the natural factors and the anthropogenic activity regarding their role in differentiating the landscapes formed under rice crop within the scope of the investigated territory.
- Comparison between the vegetation requirements of the rice crop and the suitability of the landscapes in Pazardzhik-Plovdiv field for rice cultivation.

Materials and Methods

The present research is based on the landscape-ecological approach, which in combination with the comparative and statistical method provides opportunity for a complex analysis of the natural environment. In the present study, we maintain the view that the landscape is "objective existence of a dynamic system limited in the space, evolving over time, but at the same time possessing the dialectical unity of its constituent material components: rocks, air, waters, soils, plants and animals, along with the anthropogenic impact on them " (Petrov, 1990).

„The implementation of landscape-ecological research is a complicated complex of predefined and planned activities, grouped in stages. The aim is to get a maximum accurate notion of the relationships and interactions existing in landscape diversity and using the results for solving scientific and practical-applied tasks (Nam, 2013).

Results and Discussion

Agro-ecological requirements of the rice crop

The agro-ecological requirements of the rice crop, in general, demonstrate a high degree of pretense that is established in the first attempts to cultivate it several millennia before the New Age in the limits of Southeast Asia. According to Milev and others (1973) „favorable conditions for obtaining constant and sustainable high average yields of rice are: high temperature with no sudden fluctuations, dry, warm and sunny weather without winds, rains

and cold waves, especially after sowing, during blooming and ripening, without frequent and significant differences in daytime temperature, flat terrain, soils with impermeable under-fallow layer, and presence of water source, providing sufficient irrigation water in time".

Of course, the different varieties of rice react differently to the conditions of the environment in which they grow. For the cultivation of rice a certain permanent water layer is needed, in which the plant to develop. This aqueous layer regulates the soil moisture and maintains it in a certain state, whereby appropriate conditions for the development of the rice are created. Sowing the rice seeds is done on dry soil - without the presence of water layer, and in other cases seedlings are being planted in the already prepared area. In the specific area of research, the rice field is poured into water layer, which has a certain depth. According to Kitanov (1986) this layer is usually 10-15 cm thick. The presence of such a layer prevents the rice plantations from weeds. In addition, the water layer in rice paddy has a great thermal effect in mitigating the abrupt 24-hour temperature fluctuations to which the rice is quite sensitive, especially in adverse weather conditions (Katsarov and Milev, 1966). At the same time, the height of this water layer should not be too great, providing the opportunity to establish suitable conditions for plant development. „The amount of water supplied greatly affects the duration of the vegetation period" (Tenova and others, 1999). The water layer in the rice paddy also influences the humidity of the ground air layer. This indicator, especially in the flowering period, should be 60% -70%. At lower air humidity levels, the leaves of the rice wither. Of particular importance are the mechanical and physical composition of the soils on which the rice grows. To maintain a constant quantity and level of the water layer, it is necessary that the soil, in which the rice is planted, to be medium sandy-loam. Alluvial soils in the river valleys are also very suitable for rice growing. The water layer is difficult to infiltrate in soils when they have relatively higher clay content. In this way a balance of the height of the water layer of the rice fields is ensured and there is no need for constant and intensive supply of new water quantities.

Precipitations are of great importance for the cultivation of the rice. It can also be grown in areas with a drier climate. Of essential importance in this case is to ensure the water layer in the rice paddy and to maintain a certain level and quantity of the water for the proper development of the plants. The provision and maintenance of the water layer in the rice fields is achieved through a system of irrigation channels which accumulate river waters or groundwaters extracted to the surface. The rice absorbs negligible amounts of water directly from precipitations. The main source of hydro masses in the biogeocycle of rice landscapes is the water infiltrated into the soil, permeated directly from the upper water layer. It is this water that is absorbed by plants and plays a vital role in their development.

From the point of view of the irrigation conditions, the relief is very important. The lowland territories with slightly inclined terrain and the low degree of fragmentation provide the most suitable conditions for rice growing.

From the climatic elements, the water temperature for irrigation, air temperature, soil temperature, solar radiation values and the relative humidity of the air are of greatest importance for rice growing. It has already been specified that rainfalls does not have a direct importance on rice cultivation. However, the amount of rainfalls, even if indirectly, has an impact on the feeding of the water sources necessary for the irrigation of the rice. „The most favorable temperature for rapid and intensive germination is 25-30 ° C. The temperature of the soil and the temperature of the ground air layer depend on the temperature of the irrigation water. The most suitable water temperature for all phases of the vegetation is 22-25ºC." (Katsarov and Milev, 1966). Similar air and water temperatures are characteristic for lowland territories located in the lowland hypsometric belt from 0 m to 200 m.

Landscape-ecological conditions

The conditions of the natural environment are the main factor for rice cultivation in the Pazardzhik-Plovdiv field. It is the only well-established and proven in time rice-growing region in Bulgaria. The contemporary landscapes of Pazardzhik-Plovdiv field are regular combination of interconnected and interacting, specific for this region of the country, natural components. In this sense the analysis of the landscape-forming factors is fundamental in clarifying the specifics of the rice landscapes. The favorable hydro-climatic and soil conditions in the Pazardzhik-Plovdiv field are a logical prerequisite for the development of rice production in this part of the country. From the point of view of the landscape concept and the system approach, the interrelations and interactions of the main natural components in the Pazardzhik-Plovdiv area determine the existence on large areas of the specific hydromorphic landscapes, systemically functioning under the conditions of the Transitional Climate in combination with lowland relief and low drainage module. After certain anthropogenic interference, the landscapes in question undergo transformations in their horizontal and vertical structure and functioning and become hydromorphic agro-landscapes of rice fields. The new system state of landscapes enables the anthropogenic factor to simplify, to homogenize the horizontal landscape structure on a significant area.

Regarding the relief features of the territory, Pazardzhik-Plovdiv area is characterized by a small slope and a very slight fragmentation of the relief. This favors gravitational transport from water basins within the southern foothills of the mountain Sushtinska Sredna gora of great quantities of water at significant distances through canal systems without complicating the technological process. The insignificant incline of the lowland (near Maritsa River) stimulates the naturally accumulation and retention of large quantities of water in the range of the hydromorphic agro-landscapes. On the other hand, the incline of the relief proves to be sufficient to carry out measures for planned or emergency drilling of the mentioned agro-landscapes.

One of the main requirements for rice growing is the temperature of the air during the vegetation period, which is required to be in the range of 25°C - 30°C. Table 1 presents the data on the average monthly maximum air temperatures for three meteorological stations located in Pazardzhik-Plovdiv field.

Table 1. Average monthly maximum of the air temperature (in °C)_station Plovdiv, st. Pazardzhik and st. Ivaylo(according to the Climatic guidebook of P.R. Bulgaria. (1983)

Station	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**	**per year**
Plovdiv	3,6	7,0	11,8	18,5	23,6	27,6	30,3	30,2	26,0	19,4	12,1	6,1	18,0
Pazardzhik	4,1	7,0	11,8	18,8	23,5	27,3	30,3	30,2	25,9	18,8	12,1	6,5	18,0
Ivaylo	4,0	7,2	11,7	18,8	23,2	27,1	29,9	29,7	25,6	19,2	12,2	6,5	17,9

It is clear from the table 1 information that during the vegetation period starting in May-June and ending in September, air temperatures above 25°C are registered, which fact complements and confirms the landscape-ecological prerequisites for the suitability of the hydromorphic landscapes in the concerned rice growing area.

In addition, the 24-hour temperature amplitudes during the rice vegetation period are characterized by low values. For Plovdiv station, they range from 12.8°C in May to 14.3°C in September. For Pazardjik station they range from 13.3°C in May to 14.9°C in September. For Ivaylo station they range from 13.4°C in May to 14.7°C in September. The relatively small value of the 24-hour temperature amplitudes favors the development of rice crop.
The data in regard to the other important indicator - air humidity, is presented in Table 2.

Table 2. The monthly and annual average relative humidity (in %)_station Plovdiv, st. Pazardzhik and st. Ivaylo (according to the Climatic guidebook of P.R. Bulgaria. (1979)

Station	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**	**per year**
Plovdiv	84	81	74	68	69	67	62	62	69	77	84	86	73
Pazardzhik	81	77	73	67	69	67	62	62	68	77	82	83	72
Ivaylo	83	78	75	68	66	58	60	60	65	75	81	82	71

The registered air humidity values during the growing season of the rice crop, namely in the range above 60% -70%, satisfy its requirements regarding this element of the climatic component. Main source of water for rice production in the Pazardzhik-Plovdiv area is Maritsa River and its tributaries Topolnitsa river, Luda Yana river, Potoka river, Pyasuchnik river and Stryama river. The average annual water quantity of Maritsa river at Pazardzhik amounts to about 17.5 m³ / sec, which makes an annual average value of the river runoff of about 550 million m³ (Hydrological guidebook of the rivers in P.R. Bulgaria, 1957). The runoff of Luda Yana River is 3.60 m³ / s at village of Sbor, of Topolnitza River - 10 m³ / sec at village of Lesichovo, of Pyasuchnik River - 2.3 m³ / sec at village of Lyuben, of Stryama river - 8,56 m³/sec at Manole village. The large water reserves of surface water in the field create a prerequisite for securing the necessary quantities of water for the rice production. Through a well-structured system of irrigation channels, water is transported from the rivers directly to the rice field. In the period after the Second World War, hundreds of kilometers of irrigation canals were built in Pazardzhik-Plovdiv field, incorporated in irrigation systems. The main irrigation systems used for irrigation of the rice crops are the irrigation systems „Karabunar", „Topolnitsa", „Aleko-Potoka", "Aleko-Pazardzhik", „Plovdiv" and „Stryama-Chirpan". All of these irrigation systems use the surface waters of Maritza River and its tributaries, but also collect waters, using special technologies, from Belmeken-Sestrimo Cascade, from Topolnitsa dam, Pyasuchnik dam and from some smaller local dams. Accumulated and transported water masses provide direct access to irrigation water for the rice crops almost all year round. The construction of such irrigation facilities allows the accumulation of water that can be used for the purpose even in periods when small amounts of rainfall are observed and consequently reduced the river flow, especially during the summer months.
An important factor for the development of the rice production in the country is the supply of large quantities of groundwater through a network of drilling wells. In pliocene sediments significant amounts of ground water are accumulated, and the powerful alluvial deposits over them prove to be a major source of groundwater. The physico-mechanical composition in the vertical structure of the sediments facilitates their ability to infiltrate and accumulate water masses both from surface water runoff and from atmospheric precipitation. In the

Hydrological guidebook of the rivers in P.R. Bulgaria (1957) it is stated that the depth of the level of the ground water between Topolnitsa river and Pyasuchnik river, for example, ranges from 1 m by the terrace of Maritsa River (low flooded river terrace) and of its tributaries - up to 20 m depth in the water catchment areas between rivers within the lowland. Shallow groundwater reserves create a prerequisite for more intense water exchange between the surface waters and the groundwaters. Thus, the soil and sediment water abundance is characterized by high indices, which is undoubtedly a positive factor for the cultivation of rice. „Among all the structures and basins in our country the alluvial deposits in Pazardzhik-Plovdiv field are characterized by the highest water abundance" (Antonov and Danchev, 1980). The state of the hydrocomponent is the consecutive beneficial landscape-ecological prerequisite for the existence of the rice crops in this part of the country.

The soils on which the rice is grown in Pazardzhik-Plovdiv field are mostly mantle (alluvial), but in some places it enters in the area where the Vertisols are distributed. The alluvial soils (Fluvisols) are distributed in the river terraces of Maritza River and its tributaries Topolnitsa river, Luda Yana river, Potoka river, Pyasuchnik river and Stryama river.

The alluvial soils (Fluvisols) are formed upon alluvial deposits and in the conditions of constant humidification. Their humus horizon has a width of 10-70 cm. Under it, usually with a sharply transition, follow river deposits with different mechanical composition. Their physical clay content fluctuates from 10% to 60%. They are loose with good aeration, high water permeability and average moisture capacity (according to Penin, 2007). These basic characteristics of the alluvial soils (Fluvisols) satisfy the agro-soil requirements of the rice. They favor the intensive flow of moisture turnover in the landscapes, the regulation of the water balance between surface and groundwaters. The alluvial soils (Fluvisols) retain the water quantities required for plants and are a major generator of mineral substances and water necessary for the growth and development of the rice.

The Vertisols occupy the low parts of Pazardzhik-Plovdiv field. They extend over young Pliocene and Quaternary deposits. Contain significant amounts of clay - from 50% to 75%. The relatively higher clay content creates conditions for formation of an original barrier which makes to be difficult for the water masses to infiltrate intensively through the soil profile and to fall into the underground runoff. In this way the soil profile saturates with water. This favors the soil moisture and allows the rice plants to absorb the required water quantity from the waters accumulated in the soil.

Conclusion

Pazardzhik-Plovdiv field is a major rice-producing region not only in Bulgaria, but also on the Balkan Peninsula. The suitability of the contemporary landscapes in the rice growing area is confirmed by the analysis of the landscape-forming factors, the specific landscape-ecological conditions and the limiting landscape-ecological requirements of the rice crops. The large-scale presence of hydromorphic landscapes and the evolutionary assumed interrelations and interactions at system level in vertical and lateral plan, predetermine the establishment of large areas with rice fields in this part of Bulgaria. Despite the crisis in the rice producing sector over the past 29 years, there is now an increase in the areas, planted with rice. During the period 2011-2017 the yield of rice in Republic of Bulgaria is slightly more than 70,000 tons from 12,000 ha. It is necessary to bear in mind, that undesirable consequences of the long-term and monocultural use are recorded. A. Velchev (2011) points out, that „the agricultural utilization of the territories leads to a reduction of the pedo-masses, while the meliorations carried out - to decrease the ecological potential" (Velchev and others, 2011). We agree with such an ascertainment and we would add that in terms of rice cultivation, the negative consequences are not limited to depletion of the soil profile and salinisation, but also to long-term changes at microclimatic level, starting of eutrophication processes, formation of

an environment for breeding of various stinging insects - possible carriers of disease-causing organisms, and etc. That is why a strict annual complex and component analysis of the hydromorphic landscapes occupied with rice crops is necessary, to reduce the risk of the occurrence of different in intensity and range environmental problems.

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SOIL AND AIR TEMPERATURE DURING THE SOWING PERIOD OF SPRING CROPS IN THE REGION OF SOUTHERN BULGARIA

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Abstract

The whole ranges of spring crops are traditionally cultivated in Bulgaria. Over the last thirty years, temperature and precipitation have been studied in Eastern Europe and, in particular, in Southern Bulgaria, with changes in both the average values of meteorological elements and their frequency and extreme event. Rainfalls are irregularly distributed and sometimes are heavy. In the same time droughts are intense and prolonged. These changes influence basic agrometeorological indices and are the subject of a study in detail. High temperatures start very early in the spring, they last long in the summer and negatively affect the growth, development and productivity of plants. All these make the cultivation of spring crops in some regions of South Bulgaria risky. To a great extent, the influence of anthropogenic factors is limited or minimized. However, the correct sowing time is a passive method for avoiding adverse meteorological effects. Initially, the plants begin their development in the soil and in this respect the conditions of heat and humidity in the layers of 2 cm to 20 cm during the sowing and germination period are of scientific interest. There is dependence between air temperature and soil, which is particularly strong during the spring period. The aim of the present study is to analyze the relation between air and soil temperature during the spring period paying attention to using and predicting the sowing time of spring crops in the southern Bulgaria area at the beginning of the 21st century.

Keywords: *spring crops, air temperature, emergence, soil temperature, sowing period*

Introduction

In southern Bulgaria, the cultivation of spring crops in recent years is risky. In the last 30 years is registered strengthening of extreme weather events (Alexandrov, V., 2011, Bocheva,L., 2012). Summer precipitations during the last century show a decreasing tendency in the lowlands of Bulgaria (Koleva E., et al., 2008) and a winter increase in southern Bulgaria (Alexandrov, V., 2006, Kazandjiev V., et al., 2008, Moteva, M.,et al., 2009). The average monthly, maximum and minimum temperatures in South Bulgaria were increased during the spring, autumn and summer (Georgieva at al, 2017).The amount of precipitation in the period from October to March is increasing (Georgieva at al, 2017). These trends of temperature change and rainfall lead to a change in agro-climatic resources (Eitzinger J., et al., 2008). Nowadays in few areas of the country, there are favorable conditions for cultivation of crops (Moteva et al, 2016). Summer droughts are a common occurrence and often coincide with the critical humidity periods of spring crop development. In this sense optimally selected period of sowing is essential for obtaining optimum yield and liability method at minimal cost to mitigate the adverse effects of the weather character. On the other hand the period of sowing depends largely on the development and productivity of crops from year to year. In this respect, the object of the study is the relationship between soil and air temperature during the spring season in major agricultural regions of southern Bulgaria in the period between 1986-2015. The results show the decade values of the two parameters and the correlation between them in the spring, during the sowing and start growth of some early, medium early and warm-loving crops? The analysis is valuable for predicting and optimizing the sowing time of early, medium early and late spring crops in the area.

Material and Methods

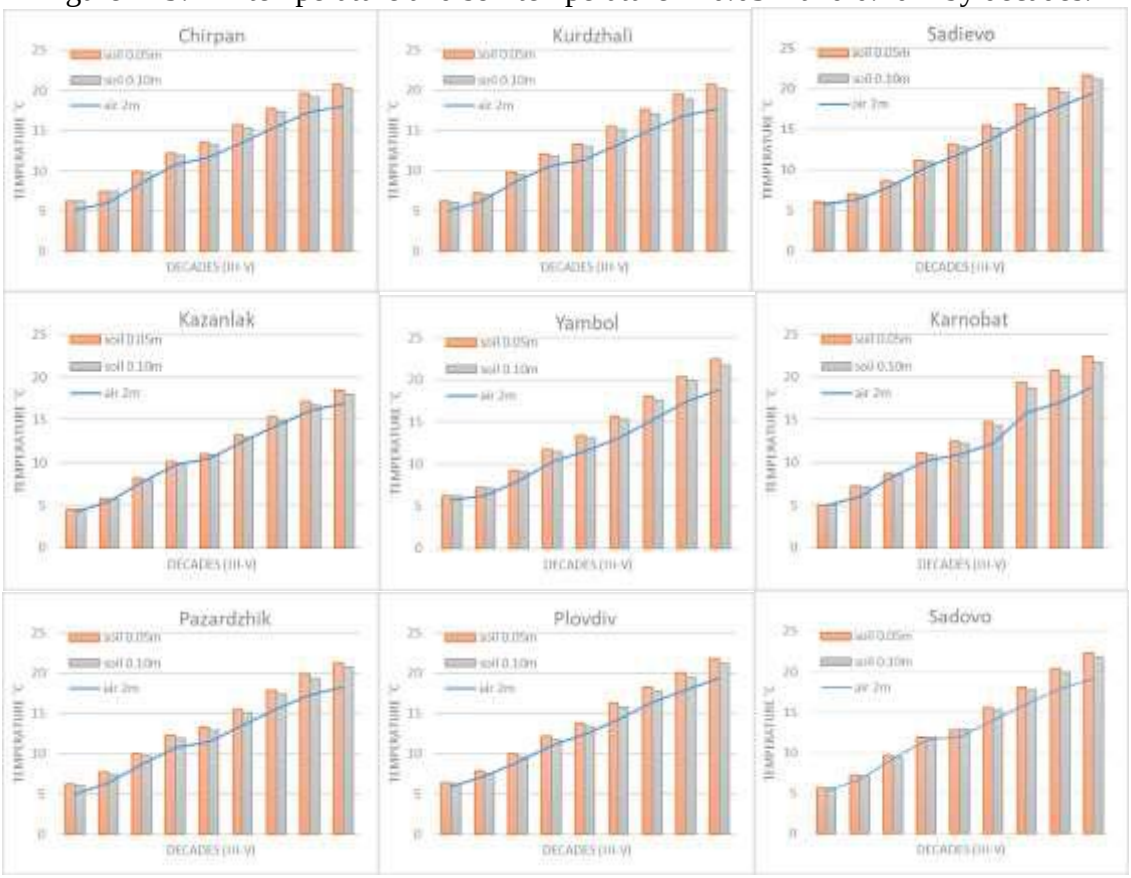
Data on average daily air temperature (˚C) and the soil temperature of 0.05 m; 0.10 m; 0.20 m from weather and agrometeorological stations - Plovdiv, Pazardzhik, Chirpan, Kurdzhali, Kazanluk, Yambol, Sadievo, Sadovo, Karnobat from the network of National Institute of Meteorology and Hydrology (NIMH) in the region of Southern Bulgaria are used. Daily data of soil temperature which is measured at 7, 14 and 21 hours local time in March, April and May are used. The average values are being calculated as the arithmetic mean of the three periods. The decade values of the two indicators have been calculated, analyzed and a correlation has been found between them. The agrometeorological stations are located on the main soil types for the South Bulgaria according to the classifications of FAO (Food and Agriculture Organization of the United Nations***)*** and *Koynov* et al. (1998) - alluvial-deluvial meadow (Plovdiv, Kazanlak); cinnamonic forest soils (Pazardzik, Sadievo) and Vertisols (Yambol, Chirpan and Karnobat). The coefficient of temperature conductivity about the area of the Thracian Lowland are published by Marinova, T., 1993; Doneva et al., 2001. The coefficients for the area are very small in the range between $3.0*10^{-7}m^{2}.s^{-1}$ and $5.6*10^{-7}m^{2}.s^{-1}$. Long term phenological data for the sowing and germination period in the three groups of spring such as sunflower, beans and peanuts from the archive of the Agrometeorology Department to NIMH are used. The dates of sowing (D_1), germination (D_2) and the interphase period ($D_{(2-1)}$) were analyzed in Julian days.The data are collected from the agrometeorological network of the section according to the NIMH methodologies and in accordance with the international standards of the World Meteorological Organization (WMO, 1975 and 1984). Air and soil temperature data are compared with sowing and germination of basic spring crop. The graphic presentation of the figures is visualized with Microsoft Excel. Regression and correlation analysis was used in data processing. Statistical software "Statgraphics" has been used. The study period ranged between 1986 and 2015.

Results and discussion

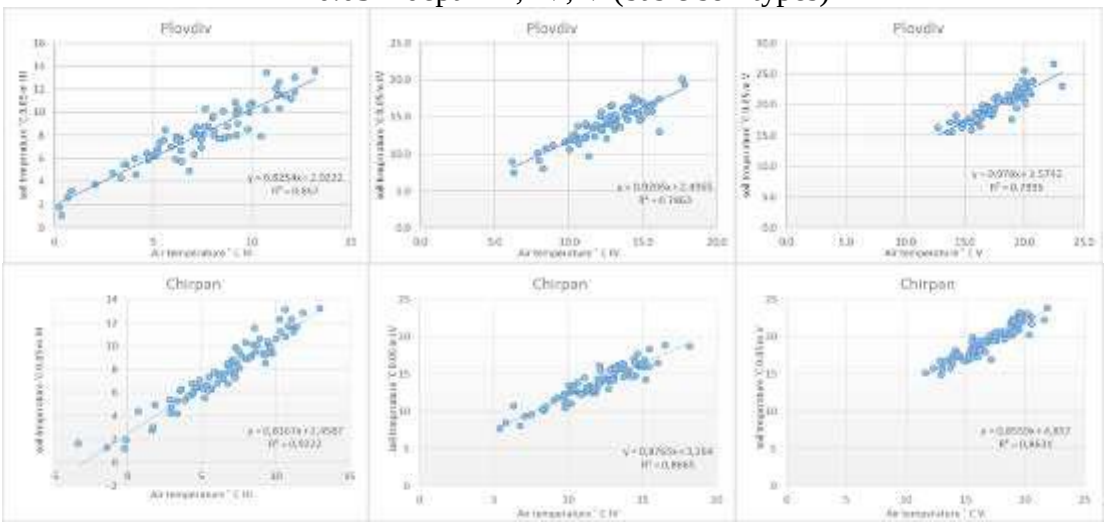
Soil temperature and air temperature are formed by components that are highly variable and depend on the location, seasons and physical characteristics of the soil. Warming and cooling is also a result of the physical properties of the environment. In the spring, soil humidity is the highest, the air temperature is of small amplitude and there is no permanent snow cover in the area. All this smooths the differences. It is therefore logical to seek relationship between both parameters during the months of March, April and May. In the same period is the sowing of the early, middle early and warm-hearted spring cultures. Optimally selected date favors jointly germination Very early seeding and long standing in the soil can lead to seed rot; roots and young plants. Late sowing reduces interphase periods and displaces reproductive phases to hot and dry summer. Since the middle of the last century in our country has begun the search for a connection between the air temperature and that of different depths in the soil (Stanev et all., 1965; Gurova et al., 1975). The relationship between the two temperatures is also discussed by a number of scientists outside of our country (Daolan Zheng 1993; Langholz. H., 1989). The ability to use longer and up-to-date data ranges, and their viewing by months, makes it possible to obtain dependencies with greater accuracy. Decade data is a sustainable, widely used agrometeorological indicator. At the same time the temperature of the soil is measured in climatic points, but in the synoptic stations measurement is only on the air temperature. Good correlation allows determining the soil temperature indirectly, by the measured and predicted air temperature. This helps farmers to start preparing for sowing and also doing it on time. The duration of the vegetation season for the different groups of spring crops is determined by the continuous retention of air temperatures above 2.5°C, 5°C, 7.5°C, 10°C, 12.5°C and 15°C. Given the observed increase in air temperatures and the earlier occurrence of a sustained transition above the biological threshold of spring crops and the

increasing number of droughts of varying intensity, predicting the times of reaching optimal sowing soil temperatures will allow the growing season of crops so as to limit the adverse effects. When sowing the seeds are laid at a depth of up to 0.10 m. The surface soil layer (0.5 m; 0.10 m) begins to warm up in March and its temperature becomes higher than that of deeper soil layers and that of the air (Figure 4-12). In the first ten days of March the temperature of the soil and air rise above 5°C, with the exception of Kazanluk which is the coldest place and Karnobat, where these values are close but not higher than 5°C. The two parameters considered during the second ten days of March are about 7.5°C. The air temperature rises above 10°C in April. There is a slight difference in the average values of the temperature and one-level temperature for the second and third decades of April at all stations. The values are about 12.5°C and for Plovdiv, Sadovo and Pazardzhik about 15°C. The temperatures are rising faster in the first decade of May when the soil temperature reaches 15°C and at the end of the period the temperature of the air in Kazanlak, Chirpan and Karnobat also reaches these values. The data shows that the soil temperature corresponds to the requirements for sowing of early spring crops in the first ten days of March; the middle-aged ones in the last of March and the first of April, and the warm-loving-in the middle of the second ten-day period and the third ten-day period in April. The final results show a positive deviation compared to the period 1961-1990 in both parameters (Fig. 25-26). In search of dependence Information is divided by decades, months and stations. The results show a strong, positive, correlational relationship with all stations and all soil types (St (0.05m-0.20m) = a*AT2m +b). The statistical evaluation of the regression model is shown in Table 1. Since the P-value in the ANOVA table is less than 0.01, there is a statistically significant relationship between the variables at the 99 % confidence level. According to the data, the sunflower produced in southern Bulgaria in recent years is about 25% of the total for the country. There is also an increased interest in growing protein crops. From the early spring crops in the area are cultivated peas, pheasant, and chickpeas as conditions allow (see fig. 4-12) the sowing to be held very early in spring at the end of February, early March. They do not suffer from the negative temperatures that are possible within the period. Early crops are sown after a stable transition of air temperature at 2.5°C to 5°C. As a perspective and drought-resistant culture, we have looked at data on chickpeas. Analysis shows that chickpeas sowing is from the end of February to the beginning of April, the average date in July days is D1 = 79, the earliest D1 = 57, the latest D1 = 96. The duration of the interphase period is on average D2-D1 = 14 days. The average date of germination is D2 = 93 days, the earliest D2 = 71 and later D2 = 107. The length of the vegetation period is average D7-D1 = 100 days and yields about 108 kg / dca. A comparison of the data shows that favorable conditions for sowing chickpeas are formed from 48 to 58 days, around the earliest date, and which is about 30 days earlier than the massive surveys we are watching. At a transition above 5°C and up to 7.5°C the conditions favor sunflower sowing. We pay special attention to it because in recent years it has grown in the region. Good conditions for sowing sunflower are observed during the third ten days of March in Kazanlak and Karnobat and at the end of the second in the other stations. However, sowing occurs on average on D1 = 139 days. Germination starts D2 = 155 and has an average duration of D2-D1 = 16 days. The vegetation period (D7-D1) continues 100 days. From the warm-loving crops we have analyzed the phenological evolution of peanuts, beans. For them, good sowing times are observed at a steady average temperature above 12.5°C or from 97 to 113 days. When sowing at the end of March, the plants will spring up after the average spring frost dates. The peanuts are mainly sown in the Sadovo and Plovdiv regions and on average on D1 = 121 days. The plants germinate at D2 = 136 as D2-D1 = 14; D6-D1 = 133, yield 232 kg /dca.

Figure 1 -9. Air temperature and soil temperature in 0.05m and 0.10m by decades.



Figures 10-23. Trend line of air and soil temperature
in 0.05m depth III, IV, V (basic soil types)



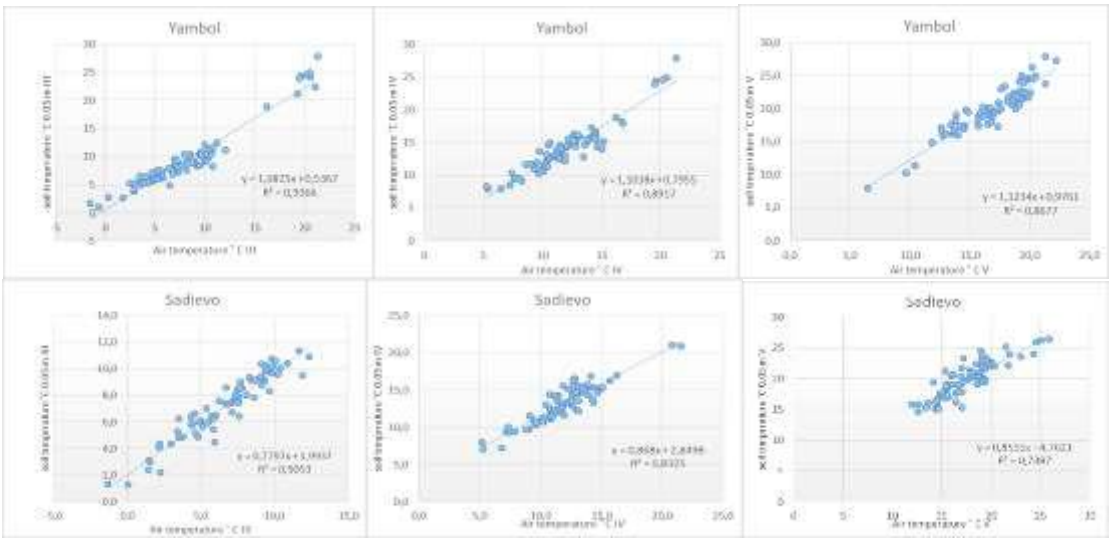


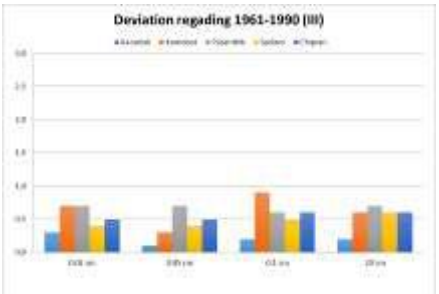
Table 1. Statistical evaluation of the model

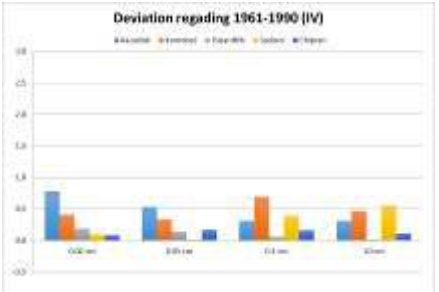
Station	r	St. error of est.	MAE	Station	r	St. error of est.	MAE
PlovdivIII0.05m	0.97	1.2	1.0	YambolIII0.05 m	0.98	1.1	0.9
Plovdiv III0.10 m	0.97	1.2	1.0	YambolIII0.10 m	0.98	1.2	0.9
Plovdiv III0.20 m	0.97	1.2	1.1	YambolIII0.20 m	0.98	1.3	1.0
Plovdiv IV0.05 m	0.95	1.2	0.9	Yambol IV0.05 m	0.97	0.9	0.7
Plovdiv IV0.10 m	0.95	1.2	0.9	Yambol IV0.10 m	0.96	1.1	0.8
Plovdiv IV0.20 m	0.94	1.2	0.9	Yambol IV0.20 m	0.96	1.1	0.9
Plovdiv V0.05 m	0.93	1.0	0.8	Yambol V0.05 m	0.96	1.1	0.8
Plovdiv V0.10 m	0.93	1.1	0.9	Yambol V0.10 m	0.96	1.1	0.9
Plovdiv V0.20 m	0.91	1.2	0.6	Yambol V0.20 m	0.90	1.0	0.8
Chirpan III0.05 m	0.96	0.7	0.7	Sadievo III0.05 m	0.94	0.8	0.6
Chirpan III0.10 m	0.96	0.7	0.6	Sadievo III0.10 m	0.98	0.9	1.0
Chirpan III0.20 m	0.95	0.8	0.8	Sadievo III0.20 m	0.98	0.9	0.9
Chirpan IV0.05 m	0.93	0.7	0.9	Sadievo IV0.05 m	0.95	0.9	1.0
Chirpan IV0.10 m	0.93	0.7	0.9	Sadievo IV0.10 m	0.96	1.2	1.0
Chirpan IV0.20 m	0.92	0.7	0.9	Sadievo IV0.20 m	0.94	0.8	0.9
Chirpan V0.05 m	0.98	0.7	1.2	Sadievo V0.05 m	0.90	1.3	1.1
Chirpan V0.10 m	0.93	0.7	0.8	Sadievo IV0.10 m	0.91	1.2	1.1
Chirpan V0.20 m	0.94	0.7	0.8	Sadievo IV0.20 m	0.90	1.3	1.1

Table 2. Day (Julian days) transition over 5^0 C, 7.5^0 C, 10^0 C, 12.5^0 C, 15^0 C degrees

Area	(Av.T>5^0 C)	Av.T>7.5^0 C	Av.T>10^0 C	Av.T> $12,5^0$ C	Av.T> 15^0 C
South Central Region 1 **(Pazardzhik)**	**53**	**69**	**85**	**103**	**121**
South Central Region 2 **(Sadovo, Plovdiv)**	**49**	**64**	**80**	**97**	**114**
Southeastern 1 (Kazanlak, Chirpan**)**	**58**	**75**	**92**	**112**	**133**
Southeastern 2 (Yambol, Karnobat**)**	**49**	**69**	**88**	**108**	**129**

Fig.25 -26 Deviation of soil temperature regarding 1960-1990 III, IV





Conclusions

Correlations between decade values of soil temperature (0.05m;0.10m;0.20m) and air temperature ($St_{(0.05m-0.20m)} = a*AT_{2m} + b$, $p<0.01$) have been established. These dependencies can be used to assess the temperature conditions during the sowing period only based on the air temperature data. The average decade values of air temperature and soil temperature for the period 1986-2015 were calculated. Both indicators show an increase over the period 1961-1990 in the watched stations. In the same period phenological data show delayed sowings of a vast majority of observed crop. This shows that farmers do not strictly monitor agro-meteorological forecasts and are not ready on time. They omit favorable conditions that affect productivity. The relationship between science and practice needs to be improved.The authors believe that in the early sowing periods, the conditions of plant growth will be improved during the growing season, especially in critical development stages.

Acknowledgements

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POTENTIAL OF CALENDULA OFFICINALIS FOR PHYTOREMEDIATION OF SOILS CONTAMINATED WITH HEAVY METALS

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Abstract

A field study was conducted to evaluate the efficacy of Calendula officinalis for phytoremediation of contaminated soils. The experiment was performed on an agricultural fields contaminated by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. The content of heavy metals in different parts of Calendula officinalis (roots, stems, leaves and flowers) was determined by ICP. The essential oil of the Calendula officinalis was obtained by steam distillation in laboratory conditions and was analyzed for heavy metals and its chemical composition was determined. Calendula officinalis is a plant which is tolerant to heavy metals and can be grown on contaminated soils. Based on the obtained results and using the most common criteria, Calendula officinalis can be classified as Pb hyperaccumulator and Cd, and Zn accumulators, therefore, this plant has suitable potential for the phytoremediation of heavy metal contaminated soils. Favorable is also the fact that heavy metals do not influence the development of the Calendula officinalis, as well as on the quality and quantity of the essential oil. For oil obtained from the processing of Calendula officinalis flowers grown on highly contaminated soils, its key odour-determining ingredients meet the quality requirements of the European Pharmacopoeia Calendula officinalis oil and/or have values that are close to the limits of the standard. The ability to process the calendula flowers in oil and its use in perfumery makes it extremely suitable for phytoremediation of heavy metal contaminated soils.

Keywords: *Calendula officinalis, Heavy metals, Phytoremediation*

Introduction

Environmental pollution with heavy metals is a global problem, and therefore the development of phytoremediation technologies for plant-based clean-up of contaminated soils is therefore a significant interest. Aromatic and medicinal plants have shown to uptake and accumulate toxic heavy metals from polluted areas, and could be used as biomonitors or accumulators of pollutants (Abu Darwish *et al.*, 2009, Angelova, 2012).

Calendula officinalis L. is an aromatic herb that belongs to the Asteraceae family. Also known as pot marigold or marigold. It originates from the Mediterranean (Gazim *et al*., 2008). While the biennial plant is found in a wild state in southern, eastern and central Europe (Van Wyk and Wink, 2004), the annual plant is cultivated. Calendula is grown for decorative and medical purposes (Ramos *et al*., 1988, Muuse *et al*., 1992) and finds application in the pharmaceutical and cosmetic industries. Teas, tinctures and creams made from this herb are used for healing purposes (Re *et al*., 2009). Studies have shown that extracts of C. officinalis have antioxidant (Albulescu *et al*., 2004, Fonseca et al, 2010) and antibacterial (Iauk *et al*., 2003) properties. The compounds found in essential oils of C. Officinalis have biological activity, for example, α-muurolene has antifungal activity, β-caryophyllene has anti-inflammatory, antibiotic and antioxidant activity (Legault *et al*., 2007).

Insufficient is the information available on the potential of *Calendula officinalis L.* for accumulation of heavy metals and its potential for use for phytoremediation. The studies connected with growing the pot marigold on polluted soils are too limited. There is evidence that *Calendula officinalis L.* can grow on soils containing large amounts of Cd and Pb without

suffering from phytotoxicity and is able to tolerate high concentrations of heavy metals (Liu *et al*., 2008). This gave us the grounds to carry out a comparative research, which to allow us to determine the quantities and the centers of accumulation of Pb, Zn and Cd in the vegetative organs of pot marigold, the quality of the oil, as well as the possibilities to use the plant for phytoremediation of heavy metal contaminated soils.

Material and Methods

The experiment was performed on an agricultural fields contaminated by Zn, Pb and Cd, situated at different distances (0.5, and 15.0 km) from the source of pollution, the NFMW (Non-ferrous metal plant) near Plovdiv, Bulgaria.

Characteristics of soils are shown in Table 1. The soils were neutral to slightly calcaric, with moderate content of organic matter and essential nutrients (N, P and K). The psedo-total content of Zn, Pb and Cd is high and exceeds the maximum permissible concentrations (MPC) in 1 (from 0.5 km from the NFMW) (Table 1).

Table 1. Characterization of the soils

Parameter	pH	EC, dS/m	Organic C,%	N Kjeldal,%	P, mg/kg	K, mg/kg	Pb, mg/kg	Zn, mg/kg	Cd, mg/kg
Soil 1 (S1) 0,5 km	7,4	0,15	2,2	0,34	625,6	6960	2509,1	2423,9	64,3
Soil 2 (S2) 15 km	7,5	0,15	1,54	0,12	387,3	6780	49,4	172,7	1

MPC (pH 6.0-7.4) – Pb -100 mg/kg, Cd-2.0 mg.kg, Zn-320 mg/kg
MPC (pH >7.4) – Pb – 120 mg/kg, Cd – 3.0 mg/kg, Zn -400 mg/kg

The test plant was pot marigold. Plant samples were taken immediately after harvesting and the contents of Pb, Zn and Cd in their different parts - roots, stems, leaves and flowers - was determined. The essential oil of the pot marigold was obtained by steam distillation in laboratory conditions which was analyzed for heavy metals and its chemical composition was determined.

The concentrations of contents of heavy metals in different parts of pot marigold (roots, stems, leaves, flowers), and oils were determined by the method of the microwave mineralization.Total content of heavy metals in soils was determined in accordance with ISO 11466. The mobilisable heavy metals contents in soils, considered as a "potentially bioavailable metal fraction", were extracted by a solution of DTPA (ISO 14870). The quantitative measurements were carried out with inductively coupled plasma emission spectrometry (ICP) (Jobin Yvon Emission - JY 38 S, France).

Statistical analyses were conducted with Statistica v. 7.0.

Results and Discussion

The results presented in Tables 1 and 2 show that in the soil samples S1 (taken from the area situated at the distance of 0.5 km from NFMW), the reported values for Pb were exceeding MPC approved for Bulgaria and reached to 2509.1 mg/kg. In the area located at a distance of 15 km, the contents of Pb significantly reduce to 49.4 mg/kg. Similar results were obtained for Cd and Zn. The results for the mobile forms of the metals extracted by DTPA show that the mobile forms of Cd in the contaminated soils are the most significant portion of its total content and reached to 57,2%, followed by Pb with 33,8 % and Zn with 9,8%.

In the soil located at a distance of 15km from NFMW the mobile forms of Cd are the most significant part of its total content and reach up to 70%, followed by Pb - 43.5% and Zn - 22.5%.

Table 2. DTPA-extractable Pb, Zn and Cd (mg/kg) in soils sampled from NFMW

Soils	Pb		Cd		Zn	
	mg/kg	%*	mg/kg	%	mg/kg	%
S1	849.1	33,8	36.8	57,2	236.8	9,8
S2	21.5	43.5	0.7	70	38.9	22.5

*DTPA -extractable / total content

Figure 1 presents the results obtained for the content of heavy metals in the vegetative organs of pot marigold. Most of them (Pb, Cd and Zn) are accumulated in the aboveground parts of the pot marigold (the leaves). By moving the source of contamination away, there is a clear tendency of reduction of the heavy metal content in pot marigold's vegetative organs. The content of Pb in the roots of pot marigold grown at 0.5 km from NFMW reaches up to 228.4 mg/kg, Zn - 126.4 mg/kg, Cd - 36.6 mg/kg. The obtained values obtained for the heavy metals (Cd, Pb and Zn) in the roots are much higher than the values considered by plants by Liphadzi and Kirkham (2005) as toxic for the plants (0.1 mg/kg Cd, 30 mg/kg Pb, 100 mg/kg Zn,).

It is known that calendula forms a bearded root system, 20 cm long tap root and numerous thin, secondary roots. Probably this is the reason why calendula accumulates heavy metals in the roots.

The movement of heavy metals and their accumulation in the vegetative organs of pot marigold is specific to the individual elements. A significant accumulation of Pb is found in the leaves and stems of the pot marigold. The content of this element reaches up to 54.1 mg/kg in stems, and 1506.8 mg/kg in leaves of the pot marigold grown at a distance of 0.5 km from NFMW. The content of Cd in the stems and leaves of the pot marigold reaches up to 39.4 mg/kg and 45.5 mg/kg, respectively, values considered to be toxic to plants (Kabata-Pendias, 2001). The content of Zn in the stems and leaves of the pot marigold reaches up to 214.6 mg/kg, and 604.9 mg/kg, as these values are also higher than the critical values for plants - 100-400 mg/kg.

The results obtained show that significant quantities of Pb are accumulated in the leaves of pot marigold. This is probably due to the anatomical and morphological features of the crop. Its greater accumulation in the leaves is probably due to the fact that they are covered with hairs, which favour the retention of heavy metal aerosols.

The content of heavy metals in the stems and leaves of the pot marigold grown at 15 km from NFMW are significant lower. Increased concentrations of heavy metals in leaves of pot marigold grown at 0.5 km from NFMW are largely due to aerosol contamination.The heavy metal content in the above-ground mass of pot marigold is higher than that in the root system, which is consistent with the results of other authors. Dwivedi and Dey (2002) found that the Pb content in medical plants ranged from 2.624 mg/kg to 32.757 mg/kg, and cadmium from 0.056 mg/kg to 0.419 mg/kg. They found that the content of heavy metals (Pb and Cd) in medical plants was higher in the leaves than in the stems or roots, and the lowest values were found in the seeds. It has been found that with the increase of the concentration of metals in the soil the ability of accumulation of metals in calendula increases.

The heavy metal content in flowers is significantly lower compared to the root system and the above-ground mass of the plants. The Pb and Zn content in the flowers of pot marigold grown at a distance of 0.5 km from the NFMW reaches 419.4 mg/kg and 308.9 mg/kg, respectively, and Cd - to 33.3 mg/kg. Probably a portion of the accumulated heavy metals in the flower of the pot marigold is due to the aerosol pollution.

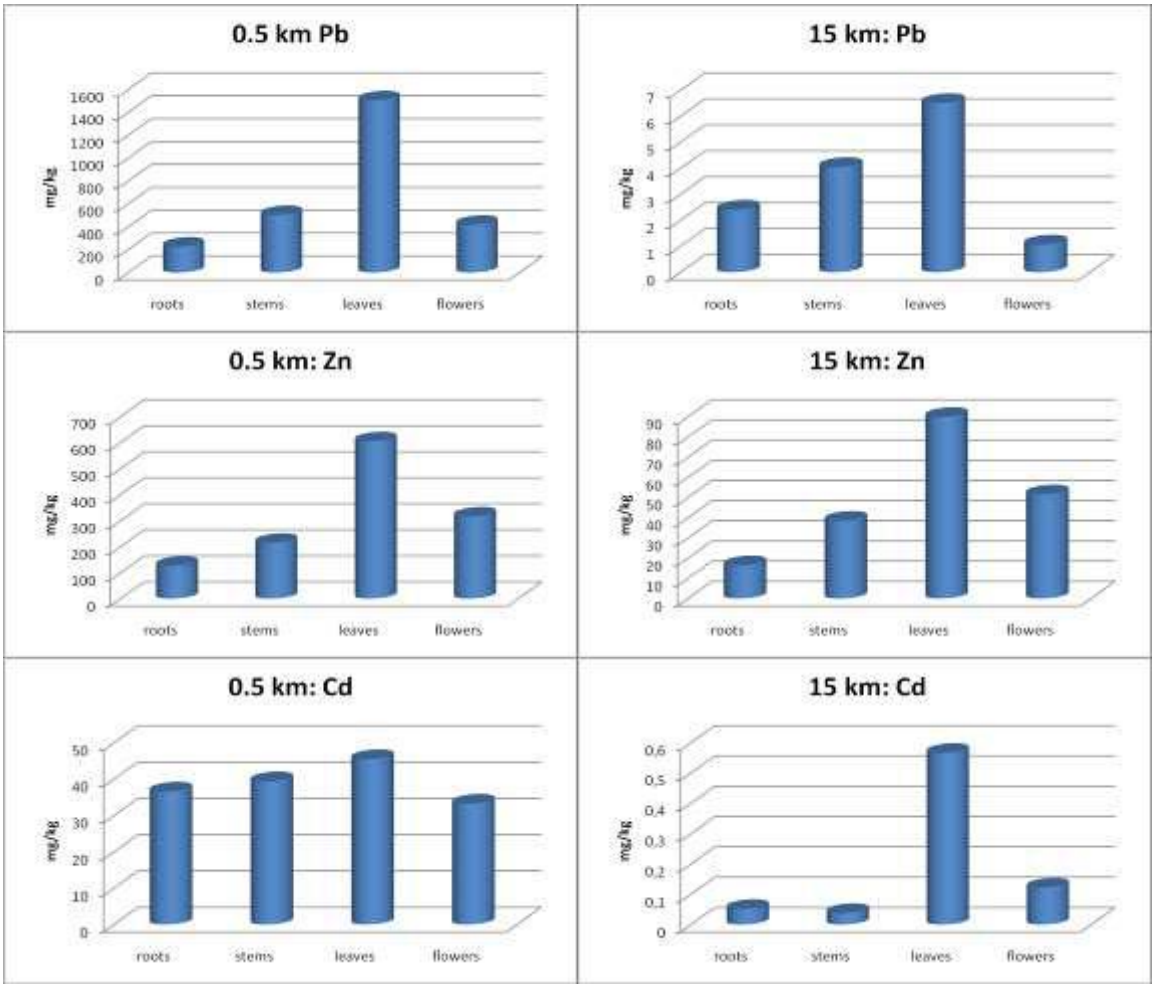

Fig.1. Content of heavy metals (mg/kg) in pot marigold

The content of Pb in flowers of pot marigold grown at a distance of 15 km from NFMW reaches 1.1 mg/kg, Zn – 51.6 mg/kg, Cd - 0.13 mg/kg

The heavy metal content in the essential oil from pot marigold was also determined. The results obtained show that the majority of the heavy metals contained in the flowers of the pot marigold do not pass into the oil during the distillation, therefore their content in the oil are much lower. Pb content in the essential oil of pot marigold reaches up to 0.22 mg kg, Zn up to 0.57 mg/kg, while the content of Cd is below the limits of the quantitative measurement of the method used. Significantly lower are the content of heavy metals in the essential oil of pot marigold grown at a distance of 15 km from NFMW - 0,14 mg/kg Pb and 0,10 mg/kg Zn. The results obtained show that the content of heavy metals in the essential oils is much lower compared to the flowers of pot marigold, and the amounts of Pb, Zn and Cd in the oil of pot marigold are lower than the accepted maximum values and meet the requirements of an environmentally friendly product. The results are confirm the ones established by Angelova et al. (2015), who found that the heavy metal content in the essential oil of lavender is very low and is not affected by the level of soil contamination with heavy metals.

To be able to give a categorical answer to the question what are the abilities of the pot marigold to extract heavy metals from the soil and to assess the potential of pot marigold for phytoextraction, the translocation factor (TF) and bioconcentration factors (BCFroots and BCF shoots) were calculated.

The BCFroots is the ratio of heavy metal content in plant roots to soil content. BCF values can be used as an indicator to assess the ability of plants to accumulate heavy metals

depending on the content of metals in the soil. They vary depending on the concentration and type of heavy metals, the ability of the plants to accumulate, the physiological characteristics of the plants and the environmental factors. The values from 1 to 10 are considered to be high, 0.1-1 are low and 0.01-0.1 are very low (Li et al., 2007). The results we obtained show that this coefficient reaches 0.27 at Pb, 0.53 at Zn and 0.99 at Cd (Table 3).

The bioconcentration coefficient BCFshoots is defined as the ratio between the metal concentration in the above-ground mass of the plant and in the soil and is a measure of the plant's ability to digest and move the metals to the above-ground mass that can easily be collected. In hyperaccumulators, the enrichment factor is higher than 1 and in some cases may reach 50-100 (McGrath and Zhao, 2003). The results obtained by us show that, with respect to Pb, the bioconcentration factor of above-ground mass for calendula grown at 0.5 km reaches 2.4, for Zn up to 3.5 and for cadmium up to 2.3 (Table 3).

Translocation factor (TF) gives information on the ability of plants to uptake heavy metals through the roots and to translocate them to the above-ground mass (leaves) (Liu et al., 2010). Hyperaccumulators usually contain less heavy metals in the roots than in the above-ground mass. (Baker et al., 1994). This specific criterion for hyperaccumulators can reach values > 1, indicating that the content of heavy metals in the above-ground mass is higher than that in the underground parts (roots). The results we obtained show that the TF values for Pb reach 8.8 and 4.4, indicating that a significant amount of Pb is moving to the above-ground parts of calendula. Similar results are obtained for Cd. TF values for Zn reach up to 6.5 (0.5 km), up to 7.8 (1 km) (Table 3). Similar results are obtained for Cd. TF values for Cd range from 2.3 to 11.7.

Table 3. Translocation (TF) and bioconcentration factor (BCF) factors in pot marigold

Element	TF		BCF root		BCF shoot	
	0.5 km	15 km	0.5 km	15 km	0.5 km	15 km
Pb	8.8	4.4	0.27	0.11	2.4	0.49
Cd	2.3	11.7	0.99	0.07	2.3	0.86
Zn	6.5	7.8	0.53	0.42	3.5	3.3

BCFroots=[Metal]roots/[Available metal]soil, TF=[Metal]shoots/[Metal]roots), BCFshoots=[Metal]shoots /[Available metal]soils

The distribution of heavy metals in the organs of the pot marigold has a selective character, it is specific for the individual elements (Fig.2).

There is a distinct feature in the accumulation of heavy metals in the vegetative organs of the pot marigold. The pot marigold accumulates heavy metals through its root system, but a very small portion of the heavy metals are retained by the roots, and most of them translocate and accumulate in the above-ground parts (stems, leaves).

Our results strongly suggest that pot marigold is a crop which is tolerant to heavy metals and can be grown in contaminated soil. The pot marigold may be referred to the group of hyperaccumulators of Pb, and to the accumulators of Zn and Cd, and therefore, it can be successfully used for phytoremediation of soils contaminated with Pb, Cd and Zn.

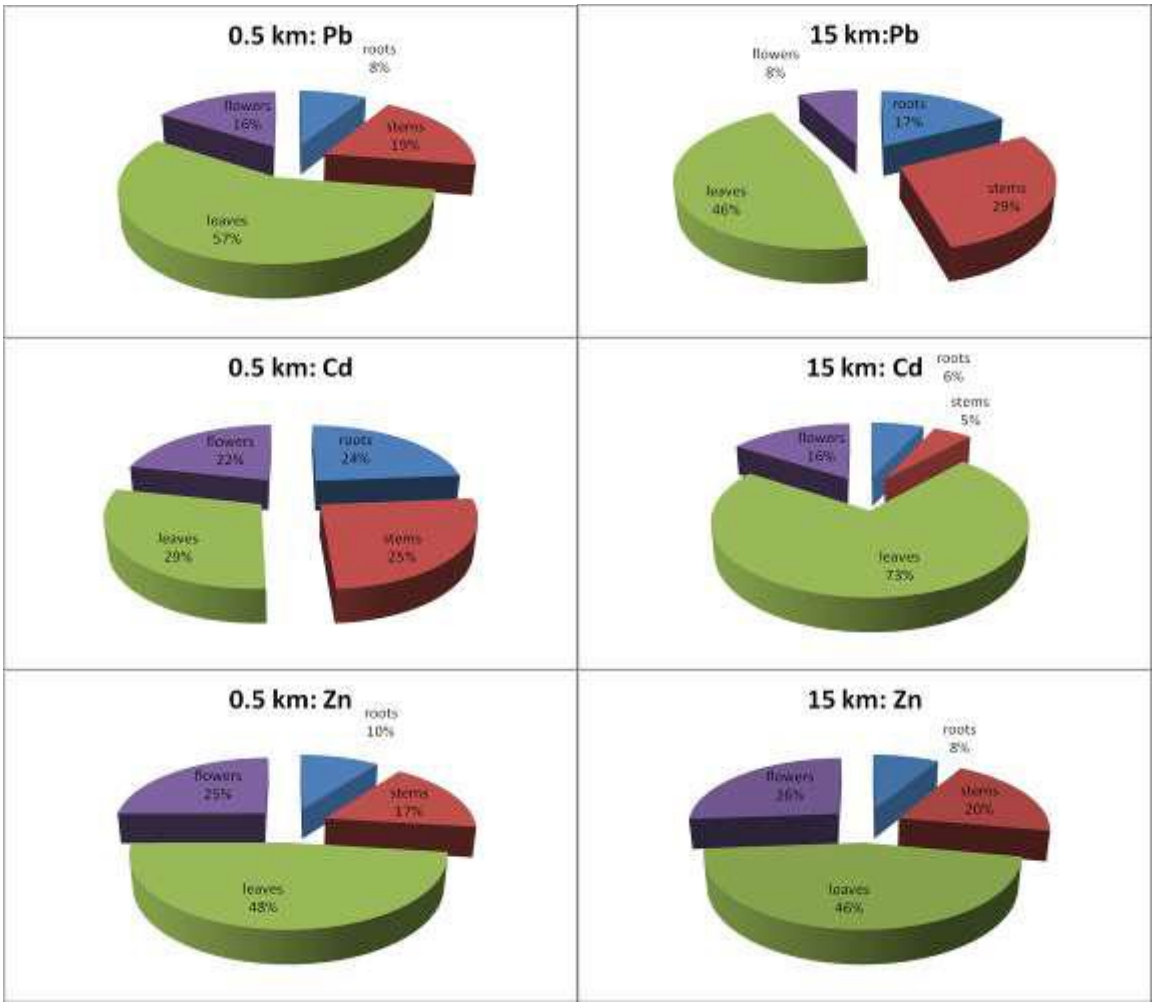

Fig. 2. Distribution of heavy metals in pot marigold

Calendula essential oil was obtained by steam distillation in laboratory conditions. Essential oils samples in hexane (1:1000) were analyzed on Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C mass spectrometer. The compounds were identified on the basis of literature data and estimated Kovat′s (retention) indices that were determined using a mixture of homologous series of normal alkanes (C8-C40) analyzed under Automated Mass Spectral Deconvolution and Identification System (AMDIS) conditions. Confirmation was made by comparing the mass spectra of the components present in the EOs with the reference spectra obtained from NIST'08 and Adams mass spectra libraries. Quantification of the essential oils components was performed using the normalization method of the GC/FID peak areas without any correction factors.

According to the literature, various monoterpenes and sesquiterpenes are contained in the oil. The major components (> 1.0%) identified in the oil from comtaminated soils (S1) are δ-Cadinene (22,088,) epi-α-Muurolol (18,187), α-Cadinol (14,519), γ-Cadinene (8,943). α-Muurolene (4,739), α-Cadinene (3,912), β-Ionone (3,286), Ledene (2,475), γ-Muurolene (2,392), β-Oplopenone (1,811), 1-epi-Cubenol (1,675), Geranylacetone (1,628), Geranylacetone (1,628), α-Calacorene (1,407), Viridiflorol (1,393), Ledol (1,324), α-Humulene (1,241). Traces of minor components (in the range <1.0 and> 0.10%) 1,10-di-epi-Cubenol (1,002), Cadalene (0,923), α-pinene (0,892), α-Copaene (0,877), β-caryophyllene (0,755), α-Copaen-4-ol (0,735), Caryophyllene oxide (0,616), α-thujene (0,319) have been identified in oils from comtaminated soils (S1).

There are no significant composition differences between oils obtained from areas located at different distances from NFMW - Plovdiv, which have varying degrees of pollution.
According to Chalchat et al. (1991) the volatile fraction obtained from Calendula officinalis flowers were included α-cubebene, α-copaene, β-cubebene, α-gurjunene, β-cariophyllene, α-ionone, α-humulene, γ-muurolene, β-ionone, α-muuronele, γ-cadinene, δ-cadinene and α-cadinene. The oil is rich in α-cadinene, α-cadinol, t-muurolol, limonene, and 1,8-cineol and p-cymene (Okoh et al., 2007).
Calendula essential oils vary in composition (Miguel et al., 2004, Danielski et al., 2006) for the different countries. The variation between the individual results for the chemical composition of the oil may be due to the conditions of cultivation, genetic factors, varietal peculiarities and other factors. The composition of the soil, temperature and atmospheric conditions during the growth and development of plants, which influence the composition of secondary metabolites in plants, determines the different plant haemotypes. As α-Cadinol has the highest content of alcohols, this indicates that the oil we studied belongs to α-cadinol chemotype.
An important point in the study is to trace whether heavy metals affect the quality of essential oil. Pot marigold oil is used in a wide range of perfumery, pharmacy and medical products and must have guaranteed indicators of cleanliness and harmlessness, i.e. to meet pharmacopoeial requirements. This means that it must not contain heavy metals and/or contains traces of metals and there must not be any change in its composition.
The data show that the cultivation of pot marigold on heavy metal contaminated soils does not negatively affect the composition of the oil. Heavy metals in the soil have a negligible impact on yield (data not provided) and do not affect the quality of the oil.
The results we obtained show that heavy metals do not affect the development of pot marigold as well as the quality and quantity of the oils it produces. The ability to process the calendula flowers in oil and its use in perfumery makes it extremely suitable for phytoremediation of heavy metal contaminated soils.

Conclusions

Based on the obtained results the following conclusions can be made:

1. There is a clear distinction in the accumulation of heavy metals in vegetative organs of pot marigold. Pot marigold accumulates heavy metals through its root system, but a very small part of the heavy metals are retained by the roots and most of them move and are accumulated in the above-ground parts (stems, leaves and flowers).
2. The quantities of Pb, Cd and Zn in the oil of pot marigold grown 0.5 km from the NFMW, were lower than the accepted Maximum permissible concentrations, and meet the requirements of an environmentally friendly product.
3. Pot marigold is a crop that is tolerant to heavy metals, it can be attributed to hyperaccumulators of Pb and accumulators of Cd and Zn and can be successfully used in phytoremediation of heavy metal polluted soils. Processing of flowers to oil and the use of the obtained oil in perfumery will significantly reduce the cost of phytoremediation.
4. Heavy metal soil contamination has no significant effect on the essential oil composition.

Acknowledgements

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SUSTAINABILITY OF THE CONSTRUCTED WETLANDS FOR WASTEWATER TREATMENT USING RENEWABLE ENERGY SOURCES

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Abstract

Constructed wetlands for the wastewater treatment represent not only effective facility for the removing of contamination, but they also fit good into the environment. Natural and artificial swamps, lakes or accumulations are usually used for these purposes. Despite of their positive characteristics for purification of the wastewater, there is a need for the electric energy, which is in the most cases used for the re-pumping of the wastewater, cleaning of the grid on the entrance in constructed wetland, re-pumping of the purified wastewater, heating during the winter period, illumination and safety reasons. There are many renewable energy sources, which may be used for such purposes. Availability, with accent on intensity and duration of the renewable energy sources depends mostly on the climate characteristics of the observed location in the world. This research will explore which of the renewable energy sources can be used for the production of the electric energy for the constructed wetlands for wastewater treatment, where the primary orientation would be achieving of the systematic sustainability of such constructed wetlands from the energy and hydraulic aspects. For the purpose of the research, there is an intention to present model, which will take into the account all situations, which could happen during procedure of the wastewater treatment. During the operational work of the constructed wetlands, there is a possibility for a surplus of a produced electric energy, which can be used for different purposes or to be sold into existing electric energy grid.

Keywords: S*ustainability, Wetlands, Wastewater treatment, Renewable energy sources.*

Introduction

Constructed wetlands for wastewater treatment (WWT) are well known technology, which is widely used in rural as well as in urban areas. During past decades, technology of wastewater treatment, by using of the constructed wetlands, was continuously improving (Badhe et. al, 2014, Chen et. al, 2014, Harrington and Scholz, 2010, Wu et. al, 2013). As a matter of fact, constructed wetlands for WWT, in their basic form do not require any energy for their operational work, but despite of this, just minor improvement of existing wastewater treatment process should include at least a small portion of the energy. For example, this is re-pumping of the wastewater, cleaning of the grid on the entrance of wastewater in wetland (pre-treatment procedures), illumination and safety reasons.

Nowadays, sustainability of every hydro-technical system is not persisted only from economical view; i.e. by cost-benefit analysis. Providing of such a task should be accomplished by taking into the account technological, social and maybe the most important one-ecological criteria. On the other hand, especially from the engineering point of view, hydraulic and energy balance of the system must be rounded into integral whole by satisfying both mentioned aspects. Biggest accent of this paper is using and production of the renewable energy sources (RES) with regards on the constructed wetlands for the WWT. Most important reason for this is possible neatness of particular RES and their applicability for including them as energy sources. Not of all RES are suitable for this usage. Next reason is the fact that RES

are the only possible energy source for the location outside of the cities, on the islands or allocated areas. Both of this will be examined within this paper.

Theoretical background

Constructed wetlands are engineered wetlands that have been designed and constructed to mimic the natural processes involving wetland vegetation, soils and the associated microbial assemblages to assist in treating wastewaters involving physical, chemical and biological mechanisms to remove pollution (Vymazal et. al, 2006). Constructed wetlands at the beginning were used mainly for treating domestic and municipal wastewater but now their application expanded for treating industrial, agricultural wastewater, polluted rivers and lakes as well for treating collected precipitation from highways and city roads. Within the constructed wetlands, biological processes such as microbial metabolic activity and plant uptake as well as physic-chemical processes such as sedimentation, adsorption and precipitation at the water-sediment, root-sediment and plant-water interfaces are always taking place.

Constructed wetland systems are classified into two general types (Wetlands International-Malaysia Office, 2003): the Horizontal Flow Systems and the Vertical Flow Systems, which are fed intermittently and drains vertically through the bed via a network of drainage pipes, Figure 1.

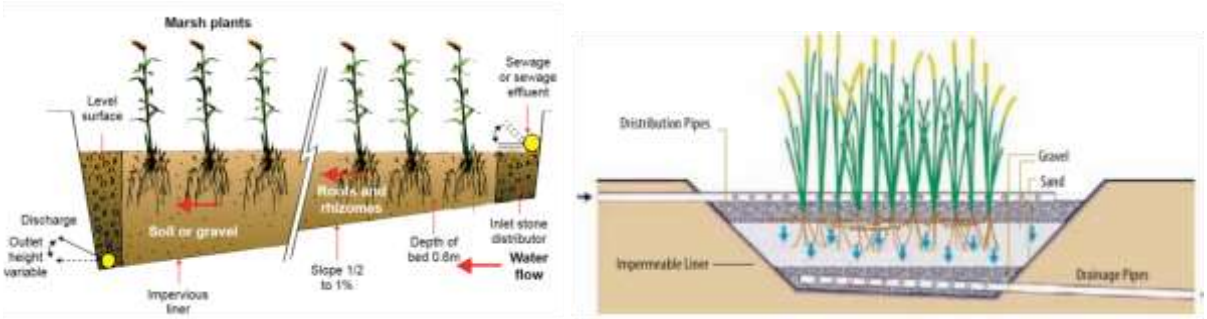

Figure 1. Horizontal and Vertical Flow Systems ((UN-HABITTAT, 2008; Wetlands International-Malaysia Office, 2003)

In Horizontal Flow System wastewater is fed at the inlet and flows horizontally through the bed to the outlet and has two general types: Surface Flow and Sub-surface Flow systems (Wetlands International-Malaysia Office, 2003). In Europe, Horizontal Flow Systems are widely used concept of constructed wetlands (Vymazal et. al, 2006).

Usually, the Common Reed (lat. Phragmites karka) and Cattail (lat. Typha angustifolia) are used for WWT because they have a large biomass both above (leaves) and below (underground stem and roots) the surface of the substrate (Wetland International-Malaysia Office, 2003). Plants in constructed wetlands stabilise the surface of the beds, slow down the water flow and increases water transparency, but most important is that they provide a huge surface area for attachment and growth of microbes. Microbes have the most important function in wastewater treatment. They use the organic substance in wastewater as a source of food and transform it into energy and biomass. Also, the choice of substrate is important and determined in terms of their hydraulic permeability and their capacity to absorb nutrients and pollutants. A mixture of organic clay soils, sand, gravels and crushed stones could be used to provide support for plant growth (Wetland International-Malaysia Office, 2003).

Sustainable model of the constructed wetlands for wastewater treatment

Not only hydro-technical, but also every observed system has so called ''black-box'' with input and output. Same situation is for the constructed wetlands for the WWT, Figure 2.

Figure 2. Main concept of the sustainable model of the constructed wetlands for wastewater treatment

Obvious intension is to minimize energy input E_{Input} and to maximize produced energy, E_{Output}. Wastewater input, W_{Input}, should be in prescribed range of the flow, which is defined by the designers of the constructed wastewater plant. Purified wastewater output, W_{Output}, usually does not have prescribed flow range, because this amount is connected with the W_{Input}. Also, energy and water losses exist in constructed wetlands, but for the practical cases they are usually neglected.

Wastewater (hydraulic) input

Depending on the type of the wastewater, there are many pre-treatment facilities which should be provided before wastewater enters the wetland for WWT. Rainwater, industrial wastewater and settlement wastewater define pre-treatment facilities. Due to the scope and purpose of this paper, further discussion and researching will be focused on the precipitation wastewater. For the precipitation wastewater, there is only need for the protection grid because of large floating and scattered particle, eventually a sedimentary basin due to the grease collected from the highways, roads, sidewalks and pavements. Regarding character of the rainwater/precipitation, inflow regime into the wetland is of the stochastic nature and depends on the climate characteristics of the observed area, Figure 3.

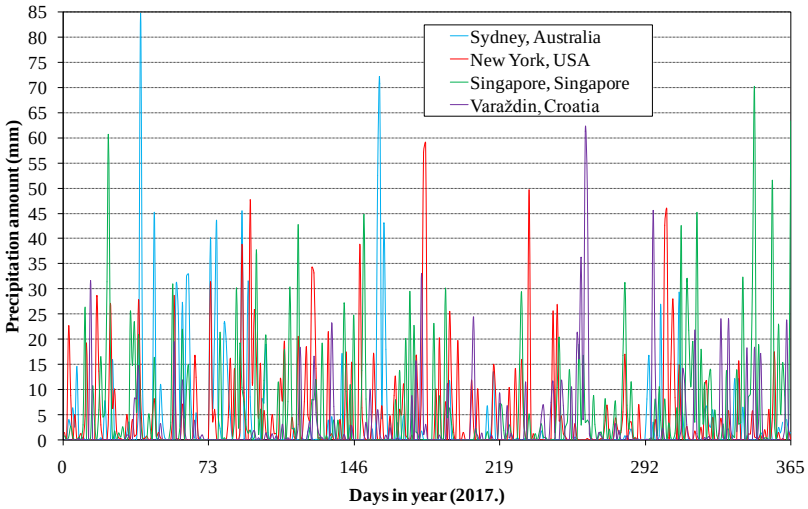

Figure 3. Daily precipitation regimes (modified from Australian Government, 2018.; CNYWeather, 2017; Meteorological Service Singapore, 2017.; MHSC, 2017)

Energy input

Renewable energy would be used as a primary energy source, which will be converted into the electric energy (mostly) and into the heat energy. Electric energy would be used for the re-pumping of the wastewater, cleaning of the grid on the entrance of wastewater in wetland (pre-treatment procedures), re-pumping of the purified wastewater, illumination and safety reasons. Heat energy can be used for heating up during the cold/winter period of the year, especially in the case of snow precipitation.

Solar energy is widely used energy source all around the world in form of the solar photovoltaic (PV) energy and solar thermal (ST) energy. Figure 4. presents daily and yearly insolation regimes for some locations in the world (PVGIS, 2018).

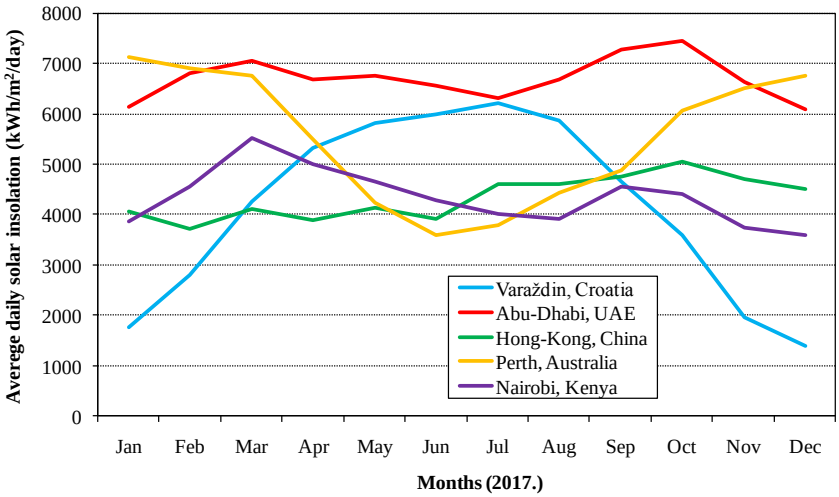

Figure 4. Daily and yearly insolation regimes for some locations in the world

Wind energy also can be used for the producing of electric energy for the already mentioned purposes in constructed wetland for WWT. Wind is a global and local energy source, available at numerous locations with different energy potential (Margeta & Đurin, 2017). Figure 5. presents potential for the production of energy from wind turbines.

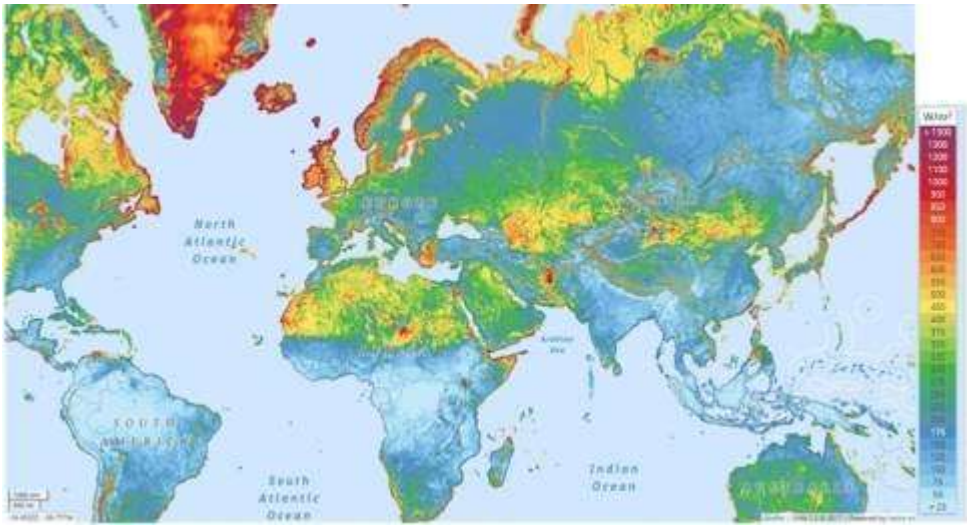

Figure 5. World wind power density (modified from Global Wind Atlas, 2018)

Hydro-electric energy is a classic and economically advantageous source of green energy, but is not often available locally or in the cities (Margeta & Đurin, 2017). Figure 6. shows world map of potential for hydro-generated electricity (Zhou et al., 2015).

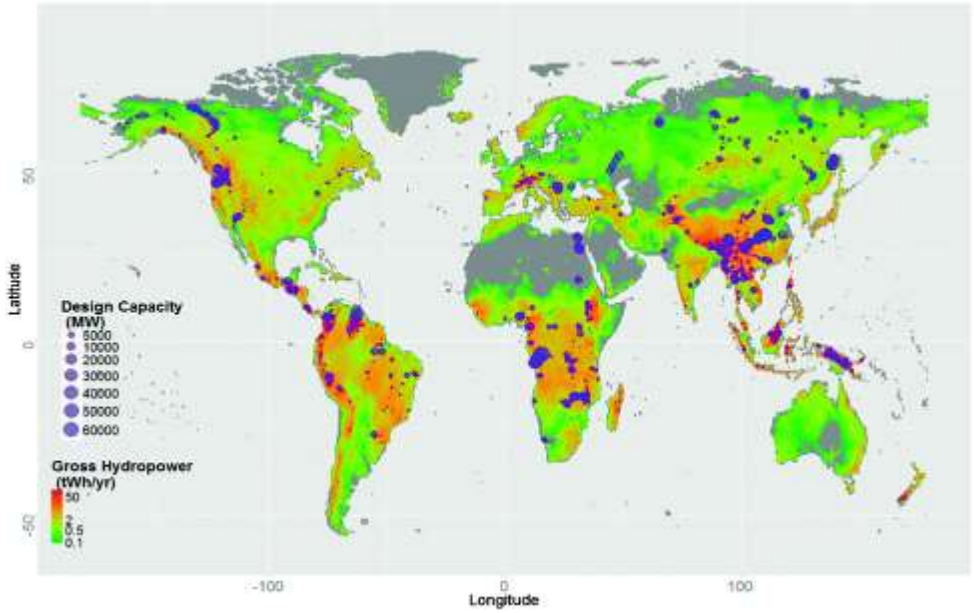

Figure 6. Map of global potential for hydro-generated electricity

Biomass includes any organic matter that is available on renewable or recurring basis; it can be derived from gaseous, solid or liquid biomass. Biomass includes energy crops and trees, agriculture crop residuals, animal manures and other organic waste materials, as well as sludge from wastewater treatment plants (Margeta & Đurin, 2017).

Geothermal energy is obtained from the Earth's internal heat and can be used for generating steam to run steam turbines and electricity generators. A prerequisite for the installation of this energy source is the existence of favourable geothermal conditions near or in the cities (Margeta & Đurin, 2017).

Results and Discussion

Solar photovoltaic (PV) energy for constructed wetlands could be used when the insolation is appropriate for usage. During the intensive precipitation or cloudiness, PV energy cannot be used. But, after ending of the precipitation period, especially after cloudburst, when the rain wastewater inflows are the biggest, using of this kind of energy has full justification. Solar thermal (ST) energy can be used for the purpose of heating during the winter period and snow melting in closer area of the constructed wetlands for the providing of the extended duration of the wastewater treatment, especially when temperatures are under 0 °C. Interruptions of the wind energy production are therefore possible and their characteristics are high unpredictability of occurrence and duration (Margeta & Đurin, 2017). Hydro-energy is very useful energy source for the constructed wetlands for the wastewater treatment plants, because most of such plants are located near rivers and watercourses because of the purified wastewater discharge. Despite seasonal water flow and level changes, purified water in most of the cases generates constant flow at a lesser or greater extent. Biomass is a proven technology as well as its economy. Unfortunately, there are many of locations where this energy is not available in required quantity, i.e. it is not sufficient to satisfy all energy needs of constructed wetlands (Margeta & Đurin, 2017). Particular amount of biomass could be produced by constructed wetland itself (mud, sludge, surplus of the plants, etc.) in a form of an internal energy. Unfortunately, this amount is not satisfying required need for energy within wetland. Geothermal energy is not available at most of the locations near constructed wetlands. Thereby, this energy is not appropriate for such usage.

Conclusions

The course of the conducted discussion show that solar photovoltaic (PV) energy and hydro-energy are most suitable renewable energy sources for the constructed wetlands. As a matter of fact, mutual combination of these two energy sources is very promising for the most locations in the world. In most of the cases, i.e. locations in the world, during summer period, river and watercourses has the smallest flow. Parallel with this period, insolation has the biggest intensity and duration. Therefore, these two sources are covering each other and produce almost constant and continued flow of energy. This paper presents justified and reasoned itemization for the providing sustainability of the constructed wetlands. Further researching will be continued in form of the real case study for the several different locations in the world, considering climate, energy and hydraulic factors.

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MITIGATION OF GREENHOUSE GAS EMISSIONS BY REPLACEMENT OF WHEAT CULTIVATION BY SAFFRON IN THE AGROECOSYSTEMS OF NORTH-EAST IRAN

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Abstract

The aim of this study was to evaluate the greenhouse gas emissions based on energy consumption in the saffron and wheat fields in Khorasan Razavi Province, Iran. For this purpose, the information needed was collected from the saffron fields (43 growers) during the 8 years of growing this crop and irrigated wheat production (65 growers) within 1 year by designing and completing a questionnaire through face-to-face conversations. The results revealed that the total and average annual energy consumptions in the saffron fields were 304.60 and 38.08 GJ/ha, respectively, while the total energy consumed in the wheat fields was 43.10 GJ/ha. The total and annual energy efficiencies based on saffron stigma yields were 0.002 and 0.003, respectively, while energy use efficiency and productivity of the wheat fields were calculated to be 1.95 and 0.16, respectively. The total emissions of greenhouse gases over 8 years of saffron growing season were 33099.1 kg of equivalent carbon dioxide per hectare (kg CO_2-eq ha^{-1}) and 1125.4 (kg CO_2-eq) per kilogram of the stigma. Also, the total emissions of greenhouse gases produced in the irrigated wheat fields 3408.2 (kg CO_2-eq ha^{-1}) and 0.81 (kg CO_2-eq) per kilogram of seed. From among the inputs of saffron fields, electricity (68.20%) and manure (28.80%) accounted for the highest shares in greenhouse gas emissions, respectively, so that the amounts of greenhouse gas emissions were 22574.4 and 9531.4 (kg CO_2-eq ha^{-1}), respectively, whereas in the fields of irrigated wheat production, electricity (88.5%) and nitrogen (4.2) had the largest shares in their emissions. Although organic fertilizers were from renewable energy sources, the greenhouse gas emitted from them allocated a high amount because of their immense consumption in the saffron fields.

Keywords: *Climate change; Greenhouse gases; Direct energy; Renewable energy; Energy efficiency.*

Introduction

The increased and intensive use of energy resources causes environmental problems (Alluvione et al., 2011). It was estimated that agriculture contributes 52–84% of world's combined anthropogenic methane and nitrous oxide emissions in the world. Due to its large area and increasingly intensive management, agricultural lands have a significant negative impact on the Earth's carbon and nitrogen cycles. From among agricultural crops, saffron (*Crocus sativus*) is a plant that has been able to withstand the water scarcity problems of the mentioned areas with its own special characteristics, these areas and encourage farmers with a sustainable benefit since having a very high economic efficiency. With regard to saffron being as an export product and saffron market competition in the global level, the need for more research in this area is becoming increasingly tangible (Kafi et al., 2002).

Saffron as a renewable source plays an important role in the sustainable economic development of the country and is considered as a strategic product for regions lacking talent and agricultural facilities like Khorasan Razavi and South Khorasan Provinces. Little water requirement, possibility of exploitation of 5-7 years at one planting time, a long shelf life, ease of transport, lack of need for heavy and sophisticated machinery, ability to employ the

labor force during the harvest season, irrigation during non-critical times for water needs of other plants, and increases of food, industrial, and pharmaceutical consumptions have led to the enhancement and rapid development of saffron cultivation (Dadkhah et al., 2003).
Currently, world annual production of saffron is about 300 tons and Iran has the first rank in the world by producing 282 tons of saffron per year, which is more than 94% of global production (FAO, 2014; Ministry of Agriculture-Jahad. 2016). Through better management practices, such as selection the suitable crop in rotation, organic and sustainable farming, and efficient use of inputs, agriculture has tremendous potential to reduce the atmospheric concentration of GHGs and sequester carbon in the soil (Fares, et al. 2017).
Therefore, increasing and extension of saffron cultivation are in the region and given the fact that Iran has the most important role in the production of saffron in the world and since this product can be in good standing in rotation with wheat, this study aimed to accurately assess the amount and type of consumptive inputs in the fields of saffron and irrigated wheat productions, as well as determine the amount of greenhouse gas emissions and the share of each of the inputs in the production of these gases during various saffron and irrigated wheat production processes in Khorasan Razavi province as one of the most important producing provinces of most crops in the country.

Materials and methods

General information of the study place:

In this study, the information about saffron and irrigated wheat in Khorasan Razavi Province was collected from the Ministry of Agriculture (Ministry of Agriculture, 2006-2015). Khorasan Razavi province are located in the east of Iran, within 56° 19′ and 61° 16′ north latitude and 33° 52′ and 37° 42′ east longitude. After initial data collection, all the agricultural activities such as cultivation, yield and production of saffron and wheat, land preparation, seeding levels, the amount of irrigation water, fertilizers and pesticides, and manpower required during nearly 8 years of growth and development stages of wheat and saffron were identified. Then, the information on all the inputs and outputs was separately extracted through face-to-face discussions with farmers in the area and according to the designed questionnaires.
To complete the questionnaires, a simple random sampling method was used among saffron and wheat farmers. This method is in fact the simplest sampling approach and the results are reliable and generalizable to the entire community with the observance of sampling principles. The number of samples was calculated according to the equation 1 (Yamane, 1967).

$$n=\frac{n\times s^2\times t^2}{(N-1)d^2+(s^2\times t^2)} \qquad (1)$$

In this formula, n is the number of samples required and N is the total number of the target population. S^2 is the estimation variance of the study traits in the community. Assuming the normality of the desired trait, t was obtained from Student's t table at a confidence level of 95% and d was the acceptable error whose value at the confidence level of 95% was 5%. Based on the above equation, the numbers of samples required to complete the questionnaire for saffron and irrigated wheat were obtained to be 43 and 65 farmers, respectively.

Calculation of the amounts of greenhouse gas emissions:

Greenhouse gas emissions resulting from energy consumption in the production of inputs and different operations were calculated by multiplying at carbon dioxide equivalents at the amount of energy used and the amounts of greenhouse gas emissions resulting from different activities in the fields of saffron and irrigated wheat were computed in the form of carbon dioxide equivalent per surface unit (kg of equivalent carbon dioxide per hectare)
By dividing the amount of greenhouse gases in kilograms of carbon dioxide equivalent per hectare on the production amounts of saffron stigma and irrigated wheat seeds in kilograms

per hectare, greenhouse gas emissions for producing a kilogram of saffron stigma and irrigated wheat seeds or in other words, the equivalent weight (kilogram of carbon dioxide per kilogram of saffron and irrigated wheat grain) were obtained. Similarly, by dividing the amounts of greenhouse gases in kilograms of carbon dioxide per hectare on the amount of energy input and dividing the amounts of greenhouse gases on energy output in GJ, the equivalent global warming in kilogram of carbon dioxide/GJ used energy and the energy produced (kilogram of equivalent carbon dioxide/GJ) were achieved.

Results and Discussion

Energy indices:

The total energy input in the saffron fields during the 8 years of its growing season was 287.82 GJ ha^{-1} and the average annual amount of energy input was 35.98 GJ ha^{-1}. It was while in the fields of irrigated wheat production, the total energy input of 43.1 GJ ha^{-1} was calculated (Table 4). In the fields of saffron production, the highest and the lowest energy inputs were 104.09 and 23.27 GJ ha^{-1} related to the first and third years of production, respectively. Of the main reason for the higher energy consumption in the first year of saffron production compared to the other years was the high amount of used seeds (about 4130 kg ha^{-1} with the energy equivalent of 61.83 GJ ha^{-1}) (Table 1). Bakhtiari1 et al. (2015) reported that in five years of saffron production cycle in Khorasan Razavi and south Khorasan region in Iran, the overall input and output energy use were to be 163,912.09 and 184,868.28 MJ ha^{-1}, respectively. But Mohammadzadeh et al. (2017) demonstrated that overall input and output energy use for five years cycle of saffron was 25,144 GJ ha MJ ha^{-1} and 154,755 MJ ha^{-1} in Azerbayjan province in Iran.

The total energy input during the 8 years of saffron growing season was reckoned 175.23 GJ ha^{-1} with an average annual of 21.90 GJ ha^{-1} (Table 1). The amounts of energy available in the saffron stigma, straw and stubble, and bulb were 0.57, 149.03, and 25.63 GJ/ha during the 8 years of its growing season, respectively, and the average annual energies for the stigma and straw and stubble were calculated 0.07 and 18.63 GJ ha^{-1}, respectively (Table 4). The variation trend of the amounts of energy outputs of the saffron fields during the 8 years of growing season was proportionate to the changes in its production rate. Also, the total energy output of the irrigated wheat during the growing season was 88.44 GJ ha^{-1} (Table 1). In the irrigated wheat fields, the energy amounts of grain and straw and stubble were 62.48 and 21.97 GJ ha^{-1}, respectively. Also, Ghorbani et al. (2011) estimated the total energy output of irrigated wheat fields to be 65336.3 MJ ha^{-1}.

The results of this research demonstrated that saffron energy use efficiencies during the 8 years of its growing season based on the yields of stigma and stigma plus straw and stubble were 0.02 and 14.3, respectively (Table 1). The average annual energy use efficiencies of the saffron fields based on the yields of stigma and stigma plus straw and stubble and corm were 0.003 and 1.8, respectively, while the energy use efficiency in the irrigated wheat fields was 1.95.

Also, during the years of saffron production, the maximum and minimum energy use efficiencies based on stigma yield were related to the 5^{th} and 1^{st} years, respectively, and highest and lowest energy use efficiencies based on the yields of stigma plus straw and stubble and corm were observed in the 8^{th} and 1^{st} years of production, respectively. The higher energy use efficiency in the 8^{th} year was due to the addition of corm yield in calculating the energy use efficiency (Table 1). By examining the energy consumed in the production of irrigated and rain-fed wheat in the city of Saveh, Iran, Safa and Tabatabaeefar (2002) reported energy use efficiencies in the irrigated and rain-fed wheat fields to be 1.17 and 0.99, respectively.

Table 1. Total energy inputs and energy indicators for Saffron and irrigated wheat farms.

	Year (Saffron)										Wheat
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**	**Average**	
Total energy input (GJ ha^{-1})	105.7	25.7	25.4	34.6	26.6	30.5	28.6	27.4	304.6	38.1	43.10
Total energy output (GJ ha^{-1})	11.7	15.1	19.6	23.2	24.4	22.3	17.0	272.6	405.9	50.7	84.44
Net energy (GJ ha^{-1})	9.1	1.7	1.3	1.5	1.1	1.4	1.7	0.1	17.8	2.2	41.34
Energy Use Efficiency $_{(Total)}$	0.11	0.59	0.77	0.67	0.92	0.73	0.60	9.94	14.3	1.8	1.95
EUE$_{(based\ on\ stigma\ yield)}$	0.00006	0.002	0.003	0.003	0.005	0.003	0.002	0.001	0.02	0.003	-

Additionally, the total renewable and non-renewable energies for the saffron fields were 131.9 and 172.7 GJ ha^{-1}, respectively, and the average annual energies were 16.5 and 21.6 GJ ha^{-1}, respectively (Table 5). The share of renewable energies in the saffron fields was lower compared to non-renewable energies (Figure 1). The amounts of renewable and non-renewable energies were 43.3% and 56.7% of the total used energy, respectively. Also, in the irrigated wheat fields, the total renewable and non-renewable energies were 8.4 and 34.71 GJ ha^{-1}, respectively (Table 1).

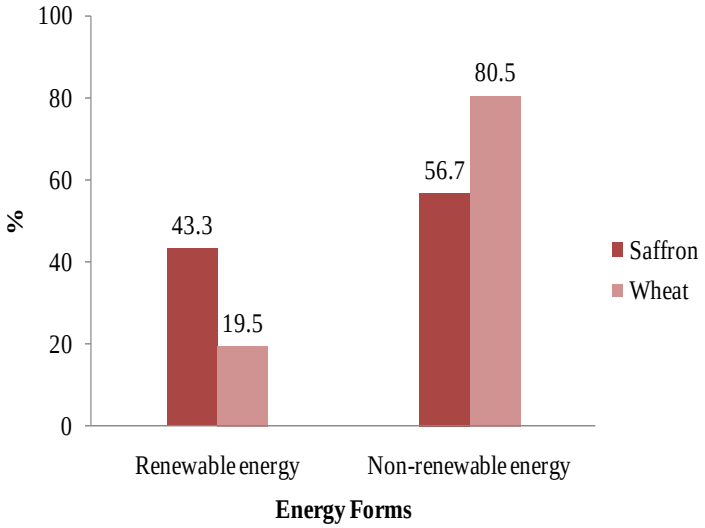

Figure 1. The share of renewable and non-renewable energies in saffron and wheat farms during 8 years production.

Greenhouse gas emissions:

The results of this study showed that during the 8 years of saffron growing season, greenhouse gas emission was 33099.1 kg of equivalent carbon dioxide per hectare and the average annual greenhouse gas emission from the fields of saffron production was 4137.4 kg of equivalent carbon dioxide per hectare (Table 2). During the years of saffron production, the highest and lowest greenhouse gas emissions were observed in the 1st year (70160 kg CO_2-eq ha^{-1}) and 3rd year (2728.9 kg CO_2-eq ha^{-1}), respectively. It seemed that more uses of inputs, especially chemical fertilizers and fossil fuels for soil preparation were of the main reasons for higher levels of greenhouse gas emissions in the first year compared to the other years. The results also revealed that during 1 year of the irrigated wheat production, about 3408.2 kg CO_2-eq ha^{-1} (Table 2). Rajabi et al. (2012) showed that the global warming potential value of

wheat production varied between 268 and 923 kg of equivalent carbon dioxide per hectare. In another study,. Despite the fact that organic fertilizers were from renewable energy sources.
The results of this investigation also revealed that during the 8 years of saffron growing season, the amount of greenhouse gas emissions was 1125.4 kg of equivalent carbon dioxide per a kilogram of stigma, while for producing every kilogram of wheat seeds, 0.81 kg of carbon dioxide was emitted into the atmosphere (Table 2). During the years of saffron production, the highest and lowest amounts of greenhouse gas emissions per stigma production related respectively to the 1st year (22632.4 kg of equivalent carbon dioxide per a kilogram of stigma) and 5th year (401.5 kg of carbon dioxide per a kilogram of stigma), respectively (Table 2). These differences were due to the low production of saffron stigmas in the first year on the one hand and high energy consumption in the same year on the other hand. It was the completely opposite for the fifth year. Rajabi et al. (2012) also reported the global warming potential value of wheat production ranged between 103.8 and 271.5 kg of equivalent carbon dioxide per ton of grain.
Furthermore, during the 8 years of saffron growing season, 108.7 and 188.9 kg of equivalent carbon dioxide were produced per a GJ energy input and output, respectively, while in the irrigated wheat fields, these amounts were 79.1 and 38.5 kg of equivalent carbon dioxide, respectively (Table 2). During the years of saffron production, the highest and lowest amounts of greenhouse gas emissions related to the 4th year (157.9 kg of equivalent carbon dioxide per a GJ of energy input) and 1st year (66.4 kg of equivalent carbon dioxide per a GJ of energy input), respectively (Table 2).
In addition, within the different years, the highest and lowest amounts of greenhouse gas emissions related to the 1st year (601.5 kg of equivalent carbon dioxide per a GJ of energy output) and 8th year (83.8 kg of equivalent carbon dioxide per a GJ of energy output), respectively (Table 2).

Table 2. The greenhouse gases emission in different bases for Saffron and irrigated wheat farms.

	Year (Saffron)									
Parameters	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**	**Wheat**
per unit area (kg CO_2-eq ha^{-1})	7016.0	2869.4	2728.9	5468.7	2850.5	4586.4	4066.4	3512.6	33099.1	3408.2
per unit weight (kg CO_2-eq kg^{-1})	22632.4	1147.8	649.7	994.3	401.5	899.3	1270.8	2341.8	1125.4	0.81
per unit energy input (kg CO_2-eq GJ^{-1})	66.4	111.6	107.4	157.9	107.1	150.2	142.2	128.1	108.7	79.1
per unit energy output (kg CO_2-eq GJ^{-1})	601.5	190.0	139.3	235.9	116.6	205.7	238.6	83.8	188.9	38.5

Conclusion

On basis of the studied results, because of saffron growers in this region are cultivated and manage saffron as modern (conventional) agriculture therefore in view of CO_2 emission saffron production is not reasonable case for cultivation in this region (4137.4 kgCO_2-eq ha^{-1}) compared to irrigated wheat (3408.2 kgCO_2-eq ha^{-1}) per year. But in another study in west of Iran has shown that saffron production with 646.24 kgCO2-eq ha^{-1} had the lowest GHG emissions amongst the studied crops (Mohammadzadeh et al. 2017). In fact saffron

cultivation in Khorasan Razavi is a high input production system, because farmers use all kind of chemical inputs and fossil fuel in the agricultural practices, so it is not an efficient system in term of energy use and CO_2 emission. But in other part of Iran, saffron is a new crop in their crop rotation, so farmers manage their farms as subsistence (traditional) agriculture. In these fields farmers apply renewable energies and reduce application of chemical inputs. Thus type of farm management severely affect on greenhouse gas emission in saffron and irrigated wheat production systems.

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DYNAMIC STUDY OF A LANDSCAPE THROUGHOUT THE LANDSCAPE OBSERVATORY- THE CASE OF CASA NORTH METN, LEBANON

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Abstract

The European Landscape Convention 2000 has given new inputs to landscape research and action especially by inviting the parties in its article 6-C to identify its own landscapes throughout its territory, to analyze their characteristics, dynamics and the pressures that modify them to eventually study their transformations. Therefore, it enhances the importance of the Landscape Observatory as a tool in each country. Despite its small area (10452 km²), Lebanon has a large variety of landscapes. However, it faces several natural and anthropogenic pressures stressed by the nonexistence of a national landscape law. Beirut Northern Suburb, *North Metn* is one of the most affected districts. Its 51 localities are distributed from the coastline to the highest mountains representing different Lebanese geographical characteristics in only 2.5% of the national surface. The current research aimed to explore the landscape transformations within the case study area. The research theoretical base included a comparative study of the landscape Observatory's methodologies around the world. By taking into consideration the national context, the Landscape Observatory was applied by using the Landscape Indicators, the visual monitoring system, field visits, public participatory, road books containing social, cultural and natural information of each locality occurred with aerial maps study (2005 till 2017). The results showed that 45% of the landscape mutated from natural landscapes to urban/industrial/rural landscapes, while 55% of the urban landscape became even denser specially by losing most of the green areas. Hence, the Landscape Observatory is important as an analyzing tool to manage, restore and protect the landscapes.

Keywords: *Landscape, Landscape Observatory, North Metn, Lebanon.*

Introduction

The Landscape Observatory, initiated in the face of rapid and profound changes in the landscape, aims to monitor the evolution of landscapes and territories as stated in the articles of the European landscape convention. This convention defines the landscape as it "refers to a part of territory as perceived by the people, whose character is the result of the action of natural and / or human factors and their interrelations." (Europeen convention 2000). Mankind has shown throughout the decades a lot of effects on the landscape whether positive or negative. Effected landscapes by anthropogenic factors are called: "cultural landscapes" that differs by their social, ecological and spiritual dimension; some are related to specific land-use techniques that maintain biodiversity, others associated in the minds of people with beliefs, artistic and customary practices, witness an exceptional spiritual relationship between man and nature. (Unesco New Delhi, 2012) In Lebanon (10452km²), 70% of the territory is mountainous and its wide seaboard over the Mediterranean presents a wide variety of landscape. The casa of North Metn has a surface of 265 km². Its geographical characteristics represent a smaller scale of the same geographical characteristics of Lebanon. It is one of the busiest most effected casas in Lebanon, where it contains 51 region. These features made the minorities of the region over the centuries, to find refuge which enriched its cultural

landscape. Unfortunately, in addition to the global stress caused by the climate change these landscapes are facing all kind of natural and human threats stressed by the nonexistence of a national landscape law, which leads us to the following problematic: "How will the landscape observatory be a baseline and tool for studying these developments, especially based on landscape indicators? According to which methodology can we master the evolution of these landscapes?" (Ireland Heritage Council, 2010)

In order to apply the landscape observatory methodology in Lebanon, were there is no landscape law, two specific comparisons were made between 10 different landscape observatories: First comparison was a study of identification of the landscape in different Landscape Observatories while the second one was related to methodologies followed or created in Landscape Observatories around the world. It shows that every landscape is defined by its role and objective to the country and the methodology used based on the objective they want to achieve. After this analysis, strength and weakness points helped create a new methodology to follow in our site of study, based on two large systems: Visual monitoring Landscape System and the Landscape Indicators. The methodology followed is applied in Lebanon, in the casa of North Metn. A list of factors of changing the landscape of North Metn were studied in details such as: the effect of rapid urbanization, self-reliance and centralization, the increase of random quarries, the effect of fast road construction, the destruction of the seafront, the decrease of green spaces, rivers and wet landmarks disappearance and dumps. These factors were taken into consideration to study the evolution of the region especially while going through the history books and pictures in the municipalities. Moreover, what helped the most was an essential factor in every Landscape Observatory study which is: the public participatory. Surveys and dialogues with the public added to the analysis important issues and factors on social dimensions to study further more. Following these basics led to the qualification and quantification of the landscape of the casa of North Metn. The objective of this paper, therefore, was to study the transformation of North Metn and assure a future scenario for these changes if actions of protection and restoration were not made.

Materials and methods

Visual Monitoring system (VMS) and landscape indicators as said before were the basic of this methodology. The VMS helps comparing new pictures of a landscape to old pictures of the same landscape from the same angle taking into consideration the monitoring of physico-spatial and visual component besides the sociocultural component of the landscape. In addition to understanding the difference between the three different kinds of transformation: disappearance, mutation and persistence. (CPEUM, 2007)

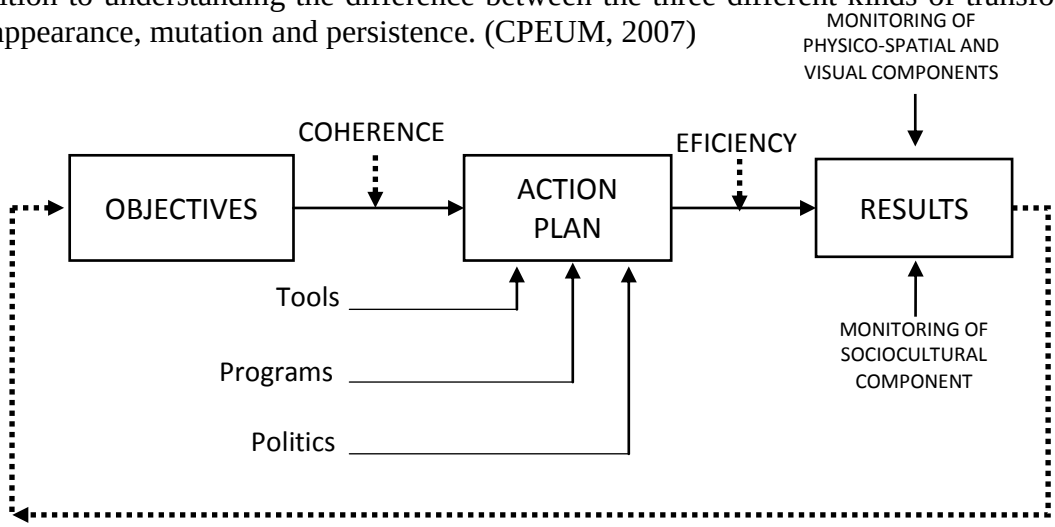

Figure 8: Model for Monitoring Landscape
Source: (CPEUM, 2007)

The "Landscape Indicators", (Joan Nogué, 2005) which present the largest parts of studies in the LO of Catalonia, are key factors for a good monitoring of the state of the landscape in Catalonia and during the application of landscape policies, at all levels. The Landscape Observatory proposes a list of landscape indicators based on economic, social and environmental points of view that will help in the measurement and analysis of the evolution of the landscape of Catalonia following the principles of sustainable development. The indicators began by being simple parameters that represent all the complexity of the landscape. These parameters indicate the current landscape situation and are used as data to define the use of sustainable soil. Due to their importance especially in the field of agriculture by studying the effects of changes, these are defined as "Landscape Indicators." "An indicator is a way to reduce a large amount of data to its simplest form, retaining an essential meaning for the questions that are asked about this data."(OttWascher, 1978)"Indicators show changes over time for each criterion and demonstrate progress towards its specific goal. " (MCPFE 1998). Landscape indicators are either qualitative or quantitative. The indicators are: Landscape Transformation, Landscape Diversity, Landscape Fragmentation, Economic Value of Landscape, Landscape Knowledge, Landscape Sociability, Landscape and Communication, Landscape Satisfaction, Application of Protective Law Instruments, planning and landscape management, public and private action in the field of conservation. The range of studies made was 20 years: 2005-2017: throughout these years, effects were noticeable quickly specially after the war, where the effects of urbanization and mass destruction of green areas has finally occurred. In order to understand further more these transformation, field visits were a must: circuits were chosen based on the existing types of landscape along the way. The Belgaum-Hainaut landscape observatory committee has created and followed a "road book" "contains all the technical information necessary for this: the roadmap (geographical coordinates, technical information of the aircraft, etc.), a location diagram of the point of view, a photograph locating the foot of the camera in its context, a location map." (OP, 2008) And in parallel, questions for the survey has occurred to ask while the field visit. And of course, the table of comparison between the old pictures and new ones with the municipalities' outcome were taken into consideration as well. Each landscape has its own road book due to their showing difference. During these field visits, it was noticeable that some of the old compositions of the landscape are no longer available which adds more value to the study. Programs were used at the end to analyze the survey results, in order to have a better perspective of people's thoughts and satisfaction of landscape. And other programs were used to summarize the results of the study on the maps such as AutoCAD and GIS.

Results and discussion

After applying this methodology, North Metn landscape was classified accordingly: the Littoral and the hills of the littoral, the middle mountains and the high mountains. And each type of landscape transformed quickly according to the comparison between old and new maps: the littoral landscape is known utterly for its high and quick urbanization where the difference in the map of 2001 and the map of 2010 shows a disappearance and a decrease in the green areas while the urbanized areas has grown much more, on the other hand comparing this to the 2016 map shows how urbanization is becoming saturated and the green areas are being demolished. As for the middle mountains landscape transformation, the comparison between maps from 2005 and 2017 shows a big disappearance of green spaces where industries and urban landscape started showing. On the other hand, even the mountainous landscape shows a 60% of green areas while only 35% has concrete and other urban forms and 5% of it are arid as the map of 2005 shows but the green areas decrease by 10% in 2016. In North Metn casa, studies and research show that it handled all kind of transformations

differently with 55% persistence, 40% mutation and 5% disappearance in the past 10 -15 years.

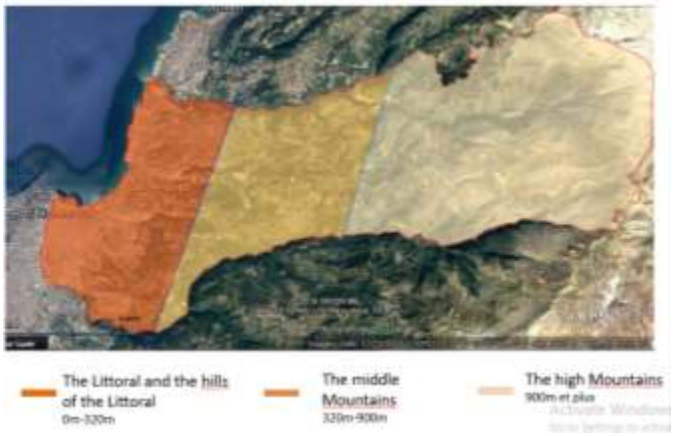

Figure 2: Qualification of the landscape of North Metn

Mainly what persisted are all the urban areas that are saturated with no place left for change or modification, 40% mutated from green areas to urban landscapes, or from a certain urban landscape to an industrial one … However, the 5% that disappeared are mainly the green landscapes that could never become reforested again because they are destroyed by concrete invasion and industries and rural houses. A map showing the frequent transformation in the landscape of North Metn during the last 10 years was a result of these researches as well. The map shows as well future transformations, where buildings and compounds are being constructed throughout these areas. This study helped in creating a future scenario of the future transformations as seen in the figure 4 below.

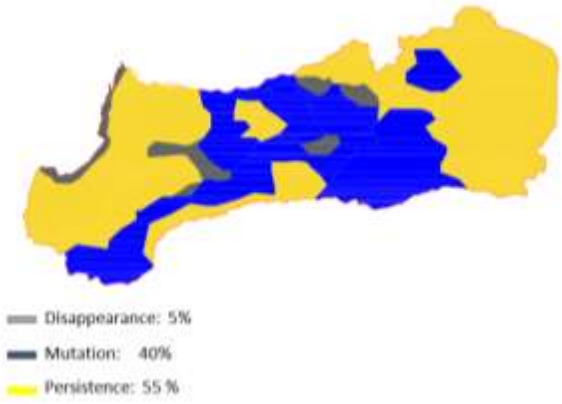

Figure 3: Map showing different types of landscape transformations in North Metn

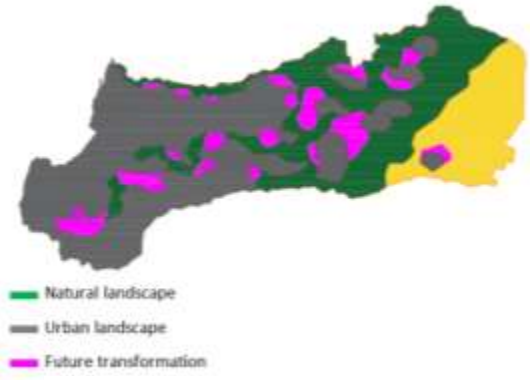

Figure 4: Map showing a scenario of future transformation (next 10 years)

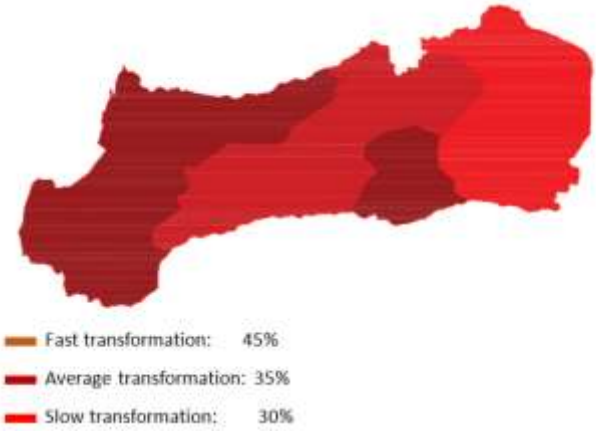

Figure 5: Map showing the frequency of landscape transformation in North Metn during the last 10 years

These transformations were studied on a smaller scale on every field visit, results of mutation and fast transformations throughout the last 10 years are shocking. Some patrimonial features has disappeared, greenery is decreasing, forests are becoming smaller and urbanization is finding its way through the mountains. These results were filed in the road books and helped summarizing the vision of transformation.

Conclusion

Concerns about the setting and the quality of life, as well as the awareness of the environmental impasse in which we find ourselves, have brought the landscape to the fore. The landscape was considered here as an object of observation, but also as an observation tool: the landscape as entrance to the territory. This article's objective was to develop a landscape observatory at the Metn Nord housing estate to finally raise awareness of the importance of landscape, interaction between the landscape and the observer, as well as accomplishing a goal of following the evolution of the landscape and making it a tool for territorial and landscape action, integrating all the populations, the leaders, the elected officials, associations following an organized participatory approach. In their participatory version, landscape observatories appear to be a good compromise between the analysis of the material nature of landscapes: diagnosis, photography, field studies and their subjective dimension: interviews, inquiry, social demand; and contribute to the emergence of a prospective reflection of the management of territories and landscapes. As far as photography is concerned, this research made it possible to become aware of both the interest and the danger it represented. It seems relatively easy to let photography "guide" an observatory of the landscape, to photograph landscapes perceived as representative and to be limited to follow their evolutions. However, photography should be seen as a tool and should not be the only framework for analysis.

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VARIETY AND STATE RESEARCH OF ENERGY PLANTS IN LITHUANIA

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Abstract

Article presents the review of introduction studies on energy plants (variety and state of plants) cultivated at the collection at Kaunas Botanical Garden of Vytautas Magnus University. Collection was formed of 72 samples of plants: 40 genus, 53 species, 33 cultivars, 1 varietes, 3 hybrids. Plants are grown separately depending on their life form – woody plants, perennial herbaceous plants, annual (biennial) plants and introduced plants. Also non-infectious diseases (necrosis, dry branches), fungal disease agents (7 genus, 10 species), pests (2 genus and species) are described.

Keywords: *energy plants, state, variety, Kaunas Botanical Garden of Vytautas Magnus University*

Introduction

Lithuania is in a cool temperate climate zone with moderate summer temperatures and moderate cold winters. The average temperature in July reaches about 17 °C, in winter – apie -5 °C. Therefore, the country needs a lot of energy resourses.

The decreasing imported non-renewable natural resources (coal, oil, gas, ect.) should be replaced with indigenous, renewable ones. One of the most important prerequisites for sustainable development is the rational use of natural resources. Plants can be used for energy purposes: woody plants (*Salix* L., *Populus* L. ir kt.) or tall in hight large-stem non-woody herbaceous plants (*Miscanthus* Andersson, *Silphium* L., *Napaea hermaphrodita* L., *Solanaceae*, *Helianthus annuus* L., *Cannabis sativa* L.) (Jakienė *et al*., 2013; Kryževičienė *et al*., 2005; Makarevičienė and Sendžikienė, 2013). For energy purposes could also be used straw, sodder grain plants, also specially grown *Brassica napus* L. ssp. *napus*, x *Triticosecale* spp. Wittm (Jasinskas and Liubarskis, 2005). Most of these plants are easily propagated, easy to maintain, non-sensitive to soil, fast growing, resistant to adverse biotic and abiotic factors (Jeviė, Lunaèek *et al*., 2000; Jasinskas *et al*., 2008; Jasinskas, 2009; Lewandowski *et al*, 2000, 2003). There are 80 species of energy plants calculated in the world that can be used to generate energy (Bassam, 2012). The biomass of woody plants produces heat, electric power, biofuels; sugar (sugar beet), starch (*Solanom tuberosum* L., grain) – bioethanol; vegetable oil (from *Brassica napus*, *Linum* L., *Glycine* Willd, *Solanum tuberosum*, *etc*.) – biodiesel; *Zea mays* L. – biogass (Jakienė *et al*., 2013; Makarevičienė and Sendžikienė, 2013).

Plantation of energy plants are raised in Europe and studies are carried out on most popular, grown for energy purposes plants (*Miscanthus*, *Panicum* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Typhoides arundinacea* (L.) Moench, etc.), biogas stations generating biomass are being built (Lewandowski *et al*., 2003. Wright, 1990). For the energy needs 35 species of herbaceous plants were investigated in the United States.

Researches of energy plants in Lithuania were started in 2000 in Lithuanian University of Agriculture. Investigations were carried out on the possibilities of the cultivation and usage perennial crops as biofuel, also explored options of herbaceous plants for energy efficiency (Kryževičienė *et. al.,* 2005). During 2007–2009 at the testing base there was investigated *Salix viminalis* L. for the technology purposes of fuel preparation. From 2007 in Lithuanian Research Centre for Agriculture and Forestry scientific project '*The development of new renewable energy sources by formating plantations of short-growing forest plants and*

herbaceous (imprted and native) plants'. The cultivation and use of energy plants for energy purposes in Europe has a tendency to grow. It is an opportunity to use unused, less fertile, rehabilitated areas or territories located on roads and other contaminated air objects.
During 2012–2014 at the Kaunas Botanical Garden of Vytautas Magnus University in the framework of the Public Information and Environmental Education means project "School of green ideas" (No VP3-1.4-AM-09-K-02-001) a collection of energy plants was created.
Aim of work: evaluate the variety and state of energy plants cultivated in Kaunas Botanical garden of Vytautas Magnus Uviversity (Lithuania).

Material and Methods

Research object – energy plants, grown at the area of 60 ares at Kaunas Botanical Garden of Vytautas Magnus Uviversity (Lithuania). The plants were planted in 2012–2014. These plants are grown at separate areas according to their life forms: woody plants, perennial herbaous plants, annual (biennial) plants and introducated plants.
The variety of plants is described on the basis of the taxonomic-analytical method, placing them according to the dependence on the higher taxonomic rank according to A. Takhtajan phylogenetic system (1987). *Pathogens* were identified visually (according to disease symptoms and morphological signs of pathogens – fungi, using lupus) and using descriptors (Grigaliūnaitė, 1997; Minkevičius and Ignatavičiūtė, 1993; Sinclair and Lyon, 2005). Pests are described by (Pileckis *et al*., 1968). The intensity of non-infetious and infectious diseases and pests abundance asssessed on a scale of 0–4 grades, in July – August (0 grades – up to 10 % of assimilated area is damaged, 1 grade – 11–30 %, 2 grades – 31–60 %, 3 grades – 61–80 %, 4 grades – 81–100 %). Plant Latin names are used accroding to *www.Tropicos.org*. Fungi teleomorphic taxons of fungi disease agents are described by the database „*Index fungorum*' (http://www.indexfungorum); pests – according to „*Fauna Europeae*" (http://www.fauna-eu.org/).

Results and Discussion

In 2012–2014 at the collection of energy plants were planted 72 plant samples. Plants belong 1 division, 2 classes, 5 subclasses, 15 families, 40 genus, 53 species, 3 subspecies, 33 cultivars, 1 variety and 3 hybrids (Table 1). The collection is the richest in Lithuania. Plants in the collection are planted in separate areas according to the form of life: woody (short rotation), herbaceous perennials, introduced herbaceous, annual (biennial) plants.
Plants of different life forms are grown at a separate areas: woody (of short rotation) – 15 samples belongint to 6 genus, 11 species, 7 cultivars, 3 hybrids, perennial herbaceous plants (14 samples – 13 genus, 12 species, 4 cultivars), introduced herbaceous plants (18 samples – 10 genus, 15 species, 3 cultivars, 1 hybrid), annual (biennial) – 22 samples: 16 genus, 12 species, 2 subscpecies, 12 cultivars, 1 variety. Poacea family plants compose a larger amount in numbers among the collection plants (14 genus, 16 species, 12 cultivars) (Table 1).

Table 1. The variety of energy plants grown at the collection of Kaunas Botanical Garden of Vytautas Magnus University (Lithuania) ([W] – woody plants, [H]– herbaceous perennials plants, [I] – introducated herbaceous plants, [A] – annual (biennial) plants), 2018

Division, Class, Subclasses, Family, Genus, species	
Magnoliophyta – Magnoliopsida	
	Caryophyllidae
Amaranthaceae [H, A]	*Amaranthus cruentus* L. 'Raudonukai', *Beta vulgaris* subsp. *vulgaris* var. *altissima* Doll 'Ernestina')
Polyganaceae [H, A]	*Fagopyrum esculentum* Moench.: cultivars: 'VB Nojai', 'VB Vokiai'; *Reynoutria sachalinensis* (F.Schmidt) Nak.)[I], *R. japonica* Houtt.[I]
Dilleniidae	
Salicaceae[W]	*Populus deltoides* W. Bartram ex Marshall; *Populus* x *euramericana* L.; *Salix integra* Thunb., cultivars: 'Hakuro Nishiki', 'Pendula'; *S. purpurea* L. 'Gracilis'; *S. matsudana* Koidz. 'Tortuosa'; *S. viminalis* L.
Brassicaceae [A]	*Brassica napus* L. var. *napus* 'Fenja'; *B. napus* L. var. *napus* 'Cult'; *Camelina sativa* (L.) Crantz; *Sinapis alba* L.
Malvaceae[H]	*Althea officinalis, Napaea hermaphrodita* L.[I]
Cannabaceae [A]	*Cannabis sativa* L. cultivars: 'USO-31', 'Finola'
Urticaceae[H]	*Urtica dioica* L.
Rosidae	
Rosaceae[W, I]	*Physocarpus opulifolius* (L.) Raf. cultivars: 'Dart's Gold', 'Diabolo'
Fabaceae	*Galega orientalis* Lam. 'Gale'[H]; *Glycine max* (L.) Merr.)[A]; *Lupinus angustifolius* L.[H] *L. polyphyllus* Lindl.[H]; *Melilotus officinalis* L. Lam.[H]; *Robinia pseudoacacia* L.[W, I]
Linaceae [H]	*Linum perenne* L.; *L. usitatissimum* L. cultivars: 'Kastyčiai'[A], 'Rasa'[A]
Cornaceae [W]	*Cornus alba* L. 'Spaethii'
Lamiidae	
Lamiaceae [H, I]	*Leonurus cardiaca* L.
Solanaceae [H, A]	*Solanum tuberosum* L. cultivars: 'Talentas' , 'VB Aista', 'VB Goda', 'VB Liepa', 'VB Venta'
Asteridae	
Asteraceae[H]	*Artemisia dubia* L.; *A. vulgaris* L., *Helianthus annnus* L.[A] and cultivars: 'Big Smile', 'Dwarf Sungold', 'Floristan, 'Henry Wilde', 'Prado Red', 'Red Sun'; *H. tuberosus* L., *Silphium asteriscus* L.[I]; *S. integrifolium* Michx.[I]; *S. laciniatum* L.[I]; *S. perfoliatum* L.[I]; *Solidago canadensis* L.[I]
Liliopsida – Liliidae	
Poaceae [H]	*Avena sativa* L. 'Jaugila' [A]; *Bromopsis inermis* (Leyss.) Holub; *Dactylis glomerata* L. 'Regenta'; *Festuca arundinacea* Schreb. 'Kingi'; *Lolium perenne* L.; *Miscanthus giganteus* J.M. Greef & Deuter[I]; *M. sacchariflorus* (Maxim.) Benth.[I]; *M. sinensis* Andersson[I] and cultivars: 'Malaperdus', 'Silber'; *Molinia caerulea* (L.) Moench 'Variegata'[I]; *Panicum virgatum* L. [I]; *Phalaris arundinaceae* (L.) Moench 'Pievys'; *Secale cereale* L. 'Matador'[A]; x *Triticosecale* spp. Wittm. [A] cultivars: 'Ada', 'Nilex', 'SW Talentro'; *Spartina pectinata* Link.[I]; *Spodiopogon sibiricus* Trin.[I]; *Zea mays* L. 'Ixxes FAO 190'[A]

Plant state investigation is relevant to plant introduction. During the research period 18% of plants were injured by 2 species of non-infectious diseases (necrosis, dry branches); 8 genus, 11 species of fungal disease agents and 2 genus and species of pests (Table 2).
The greatest variety of violations was on *Populus* x *euramericana*. They were injured by necrosi by 1 grade, rust – (*Melampsora larini-populina*) – 3 grades and by pests of two species: moth (*Lirhocolletis populifoliella*) and leaf beetle (*Phyllodecta vulgatissima*) by 1 grade each. Most strongly injured were *Salix matsudana* 'Tortuosa' and *Salix purpurea* 'Gracilis'. 'Tortuosa' genus rees were injured by mildew (*Erysiphe adunca*) and scab (*Venturia saliciperda*) by 3 grades of each and noticed single injuries of rust (*Melampsora caprearum*). 'Gracilis' genus trees were noticed to have large amount of dry branches (3 grades), rust (*Melampsora caprearum*) injuries of 3 grades and scab (*Venturia saliciperda*) by 1 grade. Another 7 plant species were damaged by one pathogen and plants of two species had injuries of physiological origin – necrosis. Mildew was noticed to be of the stongest violation: mildew *Helianthus tuberosus* (*Plasmopara halstedii*) (4 grades), *Lupinus angustifolius – Erysiphe trifolii* (3 grades) and *Lupinus polyphyllus – Erysiphe trifolii* (2 grades), *Helianthus annus* – (*Plasmopara halstedii*) and *Fagopyrum esculentum* – (*Erysiphe communis* f. *fagopyri*) – by 1 grade each.

Table 2. State of plant grown at the collection of energy plants at Kaunas botanical Garden of Vytautas Magnus university, 2018.

Hosts plants	Disease, pest	*Agents – injury grade*
Althea officinalis	Rust	*Puccinia* malvacearum Bertero ex Mont. – 0
Beta vulgaris subsp. *vulgaris* var. *altissima* Doll 'Ernestina'	Leaf spot	*Ramularia betae* Rostr. – 1
Fagopyrum esculentum Moench.	Mildew	*Erysiphe communis* f. *fagopyri* Jacz. – 1
Helianthus annus		*Plasmopara halstedii* (Farl.) Berl. & De Toni – 1
Helianthus tuberosus		*P. halstedii* (Farl.) Berl. & De Toni – 4
Lupinus angustifolius		*Erysiphe trifolii* Grev. – 3
Lupinus polyphyllus		*E. trifolii* Grev. – 2
Galega orientalis Gale	Physiological injuries	Necrosis – 1
Reynoutria sachalinensis		Necrosis – 1
Populus x *euramericana*		Necrosis: – 1
	Rust	*Melampsora larini-populina* Kleb. in Zeitsch.) – 3
	Pest, moth	*Lirhocolletis populifoliella* Tr.). – 1
	Pest, leaf beetle	*Phyllodecta vulgatissima* (L.) – 1
Rosa chinensis	Physiological injures	Necrosis – 1
	Rose black spot disease	*Diplocarpon rosae* F.A. Wolf – 0
Salix purpurea L. 'Gracilis'	Physiological injures	Dry branches – 3
	Rust	*Melampsora caprearum* Thüm – 3
	Scab	*Venturia saliciperda* Nüesch – 1
Salix matsudana 'Tortuosa'	Mildew	*Erysiphe adunca* (Wallr.) Fr. – 3
	Scab	*Venturia saliciperda* Nüesch – 3
	Rust	*Melampsora caprearum* Thüm – 0

During the research there were detected two species and genus of pests that injured *Populus* x *euramericana* by intensity of 1 grade: poplar moth (*Lirhocolletis populifoliella*) and leath beetle (*Phyllodecta vulgatissima*). The detected injuries are common on mentioned plants in Lithuania. Injuries has no significant influence on plant state, exluding hawks, they have freezed out.

Conclusions

In Kaunas Botanical Garden of Vytautas Magnus University (Lithuania) the energy plant exposition was established. Exposition consists of 72 plant samples, they taxonomically belong to: 1 divisio, 2 classes, 5 subclasses, 15 families, 40 genus, 53 species, 3 subspecies, 33 cultivars, 1 variete and 3 hybrids.

Assessing the state of the plant was found 18% of plants to be injured by disease of non-infectious origin: necrosis – 1 grades, dry branches – 3 grades; 0–3 grades – 8 genus, 11 species of fungal disease agents and 1 grade – 2 genus and species of pests.

The largest variety of injuries were detected on *Populus* x *euramericana*: necrosis (1 grade), rust – (*Melampsora larini-populina*) – 3 grades and by 1 grade of pests of 2 species and genus (*Lirhocolletis populifoliella*, *Phyllodecta vulgatissima*).

Mildew was of the strongest violation: *Helianthus tuberosus* – *Plasmopara halstedii* (4 grades), *Lupinus angustifolius* – *Erysiphe trifolii* (3 grades), *Lupinus polyphyllus* – *Erysiphe trifolii* (2 grades), *Helianthus annus* – (*Plasmopara halstedii*) ir *Fagopyrum esculentum* – (*Erysiphe communis* f. *fagopyri*) – 1 grade each.

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POTENTIAL OF OPPORTUNITIES FOR REUSING SLUDGE, PRODUCED IN RECIRCULATING AQUACULTURE SYSTEMS

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Abstract

Aquaculture, just like any other economic activity, has effects on environment. This can be seen through the eutrophication and quality decrease of surrounding surface waters, due to being polluted by waste. One of the main advantages of recirculating aquaculture systems (RAS) is the absence of dispersed waste, i.e. the possibility to produce concentrated waste and thus simplify the decontamination process. This paper presents a review of RAS waste characteristics, global practices and tendencies of waste decontamination and their potential reuse, as well as recommendations, based on waste characteristics gathered through an analysis in an aquaculture farm. Sludge, produced in RAS, as well as the generated pollution, depends on the volume of feed. The main parameters, determining waste pollution – organic pollution index and dissolved nitrogen and phosphorus compounds - were measured in the analysed farm. The following results were obtained: biologic oxygen usage, showing the pollution of waste with organic material, was 106 mg/l, ammonium nitrogen concentration was 29 mg/l. This was significantly higher than 12 mg/l, found in literature. However, because the water pH index in the system oscillated between 6.27 and 6.7, in 23^0C water temperature; non-dissolved ammonium part was only 0.2%. Nitrite values were from 0.38 to 0.69 mg/, measured RAS phosphate values oscillated between 12 and 14 mg/l, which would correspond to phosphorus concentration of 3.9 – 4.6 mg/l. After determining the volume of sludge, produced in the RAS, relevant techniques for sludge reuse were suggested. The main features of these techniques are that they are eco-friendly, prevent pollution of surface water, are able to create added value and increase the profit of the company.

Keywords: *aquaculture sludge, environmental protection, recirculating aquaculture system, pollution, sludge reuse.*

Introduction

Water saving and concentrated and thus better manageable effluents are primary reasons talking in favour of recirculating aquaculture systems (RAS). Though aquaculture effluents have economic potential which is expressed primarily through nutrients and energetic capacity it contains, their reuse is rarely considered due to lack of site specific research on investment and absence of well-established and viable appropriate technologies. RAS gives possibility to obtain reasonably concentrated effluent flow compared to other production systems in terms of relatively small flow and higher amount of solids, which could be concentrated for further treatment and reuse. Significance of the reuse options is not only for seeking economic benefits but also important for aquaculture sector in regards of EU water protection and green circular economy policy. There are number of big international collaboration projects funded by EC which were called to tackle various aspects of aquaculture sustainability issues as well as aquaculture waste management and reuse: SUSTAINAQUA (Integrated approach for a sustainable and healthy freshwater aquaculture) https://cordis.europa.eu/project/rcn/84683_en.html; AQUAETREAT (Improvement and innovation of aquaculture effluent treatment Technology)

https://cordis.europa.eu/result/rcn/47777_en.html; INAPRO (Sustainable food production through aquaponics) https://cordis.europa.eu/result/rcn/203873_en.html; BIFFIO (Cooperation between the aquaculture and agriculture sectors with the intent to use animal manure and fish faeces for sustainable production and utilization of renewable energy and recovered nutrients) https://cordis.europa.eu/result/rcn/204311_en.html; BIOALGAESORB (Enabling European SMEs to remediate wastes, reduce GHG emissions and produce biofuels via microalgae cultivation) https://cordis.europa.eu/result/rcn/153864_en.html.
In spite of findings and new knowledge generation, these collaborative research point out that there is still room for increase knowledge on various aspects of reuse and bioconversion of aquaculture effluents.

Materials and Methods

The primary aim of the work was the help for decision making procedure on expanding the African catfish RAS owned by rural energy generating and distributing company JSC "Baisogalos bioenergija" in central Lithuania. We have performed extended review of literature on possibility of beneficial reuse of RAS effluents and sludge and evaluated RAS sludge characteristics on site. The company's aquaculture business was launched there as additional income source taking benefits of cheaper energy for heating water for warm water species like African catfish *(Clarias gariepinus)*. The capacity of the farm production is 20 tons/year appeared not to be sufficient to allow establishment on the market, so now feasibility study is carried on for to increase the production tenfold. For this reason, company is looking for possibility to reuse farm effluents and sludge in viable way. The farm consists of 112 m^3 fish rearing space and water treatment unit – mechanical drum filter, trickling biological filter and UV water sterilization unit. Water retention time in the system – 1 hour, water refreshment rate 200 l/kg feed, fish rearing density – up to 400 kg/m^3. The farm effluent analysis was performed and research papers review was made which suggests closer look on various options of beneficial reuse of aquaculture effluents. Possible options of reuse according to literature review include land application as nutrient rich irrigation for various crops; aquaponics; composting and biogas production.

Results and Discussion

Research on JSC 'Baisogalos bioenergija' farm was performed between October 2017 and March 2018. Main wastewater parameters of interest, namely, biochemical oxygen demand (BOD) and nutrients (nitrogen and phosphorus) were measured. Mechanical drum filter operated more than half of time (51%) thus suggesting that there was many suspended solids in RAS water. Literature states for normally one-third total time of operation but it refers to RAS with lower production densities. African catfish tolerates lower water quality so this is the option to use less sophisticated and zero-energy suspended solids filtration system like lamella sedimentation which according to reviewed data can produce equally good effluent if viable design is available for RAS flow rate. This was suggested when biotechnology for raising African catfish was being developed in the Netherlands somewhat forty years ago.
Measured BOD mean rate was found to be 106 mg/l. Effluent settling for two hours removed more than half (53%) BOD leaving its value at 50 mg/l. This point out that half of organic pollution could be removed from effluents with sludge. Nitrogen compounds ammonia nitrogen, nitrite nitrogen and nitrate nitrogen that are crucial for RAS functioning were monitored. Mean value of ammonia nitrogen was equal to 29 mg/l and was higher than can be found in literature (12 mg/l), but the RAS water was slightly acidic with pH value ranging from 6.27 to 6.7 and this helped to fix undissociated part of ammonia which is extremely toxic to fish on safe level. Water temperature being 23^0C, calculated undissociated ammonia ranged from 0.0576 mg/l to 0,144 mg/l and was well below reported in literature value of 0.34

mg/l which hinders growth of the African catfish. Water pH values found on farm are more suitable for plant growth in aquaponics, but biofiltration process is impeded under such conditions. Another toxic to fish nitrogen compound – nitrites – value was found to be 0.38 mg/l and was below the level of few mg/l which reported in literature as safe level for warmwater fish growth. Nitrates measured were between 33 mg/l to 45 mg. Nitrates value depends on water refreshment rate and they are not of great in RAS per se, because they are toxic to fish in high concentrations when reach several hundred mg/l. Nitrogen compounds concentrations in settled water did not differ thus suggesting most of nitrogen being dissolved in RAS water. Phosphorus and its compounds were found do not affect fish growth even at concentrations as high as 52 mg/l (Van Bussel et al., 2013), but it is the major component promoting fresh water eutrophication. Measured phosphate values ranged from 12 mg/l to 14 mg/l which give phosphorus concentration 3.9–4.6 mg/l. Settled water phosphate concentration slightly diminished to 11 mg/l. Further we discuss only reuse of aquaculture effluents with possibility to obtain added value. These options were already mentioned, so let's have closer look at them.

Land application/irrigation

Dry aquaculture sludge nutrient content is comparable to livestock or poultry manure with nitrogen content of 3-9 % and phosphorus 1-4% with lower content of potassium (up to 0.3%). Plants can't use nutrients from organic compounds that are why sludge must be stabilized. It must be kept in mind that when sludge is being disinfected by adding lime for raise pH value up to 12 and kill pathogens it becomes alkaline with high amount of calcium (up to 15%). Normally, aquaculture effluents and sludge is not suspected to contain harmful substances like heavy metals, hazardous materials etc. so it can be used for edible crops fertilization by irrigation for cost effective and sustainable cultivation. There are some reports on research of RAS effluent usage for irrigation. Grassland irrigation in Chile with brackish water RAS effluents with various application rates (30/60/90 t/ha) demonstrated positive effect, but showed the need of potassium addition (Teuber, 2005), but same done with potatoes (50/100/200 t/ha) did not give any additional value (Teuber, 2007). Another research (Brod et all, 2017) revealed that aquaculture sludge application will have the effect only if there are deficiencies of the nutrients in the soil and we need it to raise to optimal level. One more research (Danaher, 2015) demonstrated aquaculture sludge with nutrient content N:P:K = 11:3:1 was beneficial when using as addition to growing substrate up to 25%, because higher content hinder plant roof oxygenation and raises salts content.

Aquaponics

Effective aquaponics system functioning is a challenge because fish and plant need for water chemical parameters are not exactly the same with do not to mention in such system present microorganisms with their own requirements. According to research reports for every m^2 of planting there is need for nutrients from 100 g of fodder with over all plant to fish growing surface being 8:1 and recalculating to volumes this relationship should be 2:1. Fish growing in RAS generates carbon dioxide which must be striped and on the other hand is very beneficial for plant growth – when it doubles the crops increase is 30%. (Rakocsy et al., 2006). Most critical water parameter is pH value, when it drops below 7.5 biofiltration efficiency diminishes this follows with ammonia removal decrease, but on the other hand when pH > 7.0 some nutrients and microelements of interest (PO_4, Mg, Fe, Mn, Ca) form non-soluble compounds which are not accessible for plants. Calcium is main deficient element for plants in RAS water which need supplementation, the other two, namely, potassium and iron can be deficient under certain conditions. Effective aquaponics system states for fish as only additional not commercially important production and source of nutrients. Now on research

agenda is decoupled aquaponics systems (for example, EU funded project INAPRO) which shows promising results.

Composting/vermicomposting

Though RAS produces relatively concentrated effluents compared to other aquaculture production methods, but they are still too diluted (up to 0,5% dry content) to be economically viable to transport to remote sites. To obtain more concentrated sludge few methods are in (primarily demo) use. These are (1) additional concentration in secondary belt filters with further settling and (2) concentration in geotextile bags. These methods allow to obtain sludge concentration in range of 8-12 % dry content. This allows to more versatile use of the sludge or even transport it on the distant sites. Sludge, stabilized in mesophilic conditions is suitable for worm growing (vermicomposting). Warmwater RAS has optimal temperature of 25^0C all year round, worms also tolerate wet substrate with 83-91% humidity (Mishra, 2003). Research revealed worms can process 0.16 – 0.63 kg/m^2 RAS sludge (dry matter) during 2-5 days when continuously provided sludge dry content is 3.5%. After four weeks worm mass grew up by 489%, survival rate was 96% (Yeo and Binkowsky, 2010). Laboratory trials gave excellent results when aquaculture sludge was mixed with cardboard in relation 92:8 with optimal mixture carbon to nitrogen ratio being 25:1 and sludge being properly dewatered.

Biogas production

Energetic capacity of the fodder used on the farm is 20.3 MJ/kg which theoretically equals to 80% of coal and 45% of petrol energy amount. When feed conversion ratio of 1.2, 5% of feed wastage and 75% of feed assimilation we have that farm producing 100 t/year will loss with effluents energy equal to 640 GJ or 177 kW, which is sufficient for yearly supply of 10 households. Anaerobic sludge stabilization is quite complex biochemical process with reduces sludge volume by tenfold and generates biogas consisting mainly of methane and carbon dioxide (Mirzoyan and Gross, 2010). Aquaculture sludge is suitable for biogas generation which was proved by research carried out by different researchers during their laboratory trials: trout RAS sludge with 2% dry matter which is 80% biodegradable at 25^0C yielded 0,4 m^3/kg biogas with 80% of methane (Lanari and Franci, 1998); salmon RAS concentrated sludge with dry matter 6-12%, temperature 35^0C gave 0,28 m^3/kg dry matter biogas with 44-54% methane, stabilized sludge yielded 3.4 – 6.8 kg/t nitrogen and 1.2 – 2.4 kg/t phosphorus. The need for additional stabilization was founded due to high rate of biodegradable fatty acids (Gebauer ir Eikebrokk, 2006). Biogas generation from RAS sludge is a new domain primarily because of lack of sufficiently big production based on RAS. Good example is EU funded BiFFiO project trying to incorporate RAS sludge together with livestock manure to generate biogas and produce fertilizer. Experiments were carried in RAS with production capacity of 1000 tonnes with the yield of methane of 194 l/kg sludge dry matter which is comparable with results obtained from household effluents. RAS under consideration produces 200 tonnes of sludge (dry matter) with potential to produce of 400.000 m^3 methane which is equivalent of 40 000 litres of diesel fuel and which supposed to satisfy 8% of RAS energetic requirements. Besides process produces stabilised sludge with nitrogen content of 4 g/kg sludge and phosphorus content of 0.8 g/kg. One more interesting example comes from Germany, where local 34 kW cogeneration power plants was based on biogas from aquaponics system sludge and plant residuals with addition of food residuals with relationship 1:1:1. For the purpose of such power plant which need 81 m^3 was estimated to have 47 t/year RAS, 860 m^2 aquaponics grow field and 252 kg/daily dry mass food residuals. Overall conclusion states that RAS sludge solely is not viable for viable biogas production due to the need of tremendous production volume. Industrial biogas generator suppliers specify to have 50 000 t/year

substrate feed to the reactor. For this reason now only small minority of livestock waste is involved in biogas generation. Norway practice states for 2.4% only (Del Campo et al., 2010).

Estimations for hypothetical 100 t/year production African catfish farm

One more RAS advantage – intensive, stable, season independent production. Effectively working RAS must always be loaded to near its maximum capacity and this state for stable predictable effluent flow. For Hypothetical 100 t/year African catfish farm some estimations can be calculated:

- at maximum load with selling every four weeks of 7.7 tonnes with standing crop of 30 tonnes in basins. Basins volume 160 m^3;
- with assumed no fluctuations in system loading and feed conversion ratio of 1.2 we can calculate 120 tonnes of fodder per yearly production cycle or 330 kg/day;
- assuming 0.3 kg sludge dry weight per kg feed we have 100 kg sludge daily or 36 tonnes yearly;
- nutrients based on fodder used on farm with 42% protein, 0.9% phosphorus and assumed 40% assimilation, excretion can be calculated as nitrogen 13.3 kg daily or 4856 kg per year, phosphorus 1.8 kg and 650 kg;
- if we assume that sludge concentration is done up to 8%, we have sludge amount of 1.25 m^3 daily or 456 m^3 yearly with supernatant amounts daily 14.4 m^3 and yearly 5250 m^3;
- is to be noted that according to situation actual situation on farm with refreshing rate 200 l/kg feed the effluent amount will quadruple to 20 000 m^3 yearly;
- according our estimations 3300 m^2 area for aquaponics crop will be supported by the system;
- biogas production according to various reports in literature will be from 18 to 40 m^3 daily;
- vermicomposting volume will be 40 m^3 – say, 100 m^2 bed with 40 cm deep;
- composting area needed will be 125 m^2.

Conclusions

We have gathered data from African catfish RAS farm. This species is known and actually appeared to be extremely sturdy so we have large degree of freedom in approach to any integrated effluent treating system stability while doing research. We are convinced that this is good basis for some near market research or demo projects to refine possibilities for coupling RAS with some chosen innovative wastewater reuse system. All these system couplings still lack either reliable real life or even literature based reports. This is obvious because there is no consistency in raising either marine or freshwater, cold or warmwater species, different farm management and different farm environmental setting.

Actually there are two challenges – one to seek for best available technology, which do not solve the problem because after comes the other, namely, one has to make it flexible and adapt it to local situation. This is the real challenge for creative specialists. This is not boring and one has always space for individual judgements and can always withstand industrial "turn-key" solutions.

All interested in reuse aquaculture effluents are warmly welcomed for collaboration in the projects we can set on some particular topic and investigating possible suitable technologies.

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CHANGES IN NUTRIENTS IN THE DOTNUVELE AND SMILGA STREAMS

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Abstract

Due to the intensifying agricultural activity, the leaching of nitrogen and phosphorus compounds from the soil to water has been increasing each year. According to the data of Helsinki Commission, the largest river in Lithuania – Nemunas, which Nevezis flows into, is considered to be one of the nutrient suppliers to the Baltic Sea. The tributaries of the Nevezis River - Smilga and Dotnuvele, collect the water saturated with nutrients from agricultural land, thus degrading the quality of the water of Nevezis, Nemunas and the Baltic Sea. Research data from 2013-2017 has shown that the average N_{total} and NO_3-N concentrations in Dotnuvele and Smilga streams' water in March (12.6 mg l^{-1}, 11 mg l^{-1} as well as 8.5 mg l^{-1} and 8.7 mg l^{-1}, respectively), May (11 mg l^{-1}, 9.1 mg l^{-1} as well as 5.4 mg l^{-1} and 4.6 mg l^{-1}, respectively), and November (4.5 mg l^{-1}, 8.7 mg l^{-1} as well as 3.5 mg l^{-1} and 7.4 mg l^{-1}, respectively) mostly corresponded to a very bad and poor ecological status, and only in August they corresponded to a good and very good water ecological status (2.4 mg l^{-1}, 3.2 mg l^{-1} as well as 1.1 mg l^{-1} and 1.3 mg l^{-1}, respectively). P_{total} and PO_4-P concentrations in the researched streams' water were usually low (0.04 – 0.08 mg l^{-1} and 0.02 – 0.05 mg l^{-1} in Dotnuvele, 0.03 – 0.08 mg l^{-1} and 0.01 – 0.03 mg l^{-1} in Smilga, respectively) and corresponded to a very good ecological status of water bodies, except in August, when the water quality in Dotnuvele was found to be average (0.19 and 0.14 mg l^{-1}).

Keywords: *Concentration, Ecological status, Nitrogen, Phosphorus.*

Introduction

The ecological status of rivers' water usually deteriorates due to the diffuse pollution. caused by intensive agriculture; the concentrated pollution, which is the urban rain as well as the industrial and production wastewater pollution; due to international pollution, which includes the pollution load coming from neighbouring countries; as well as the historical pollution, caused by long-term pollution. In recent years, a declining trend in rivers' water pollution has been observed. The surface water bodies' monitoring data, which is carried out in Lithuania according to the 2011-2017 State Environmental Monitoring Programme, has shown that 49% of Lithuanian rivers meet good or very good ecological status. In the eastern, south-eastern and western regions of Lithuania, where human activity is less intense, 53-65% of all water bodies correspond to very good and good ecological status. Bigger issues in surface water bodies have been identified in intensive agricultural areas, i.e. northern, central and southwestern Lithuania, where 50 to 79% of water bodies do not meet the requirements of good ecological status (National, 2016).

Nevezis is a contaminated river, because 74% of surface water bodies in its sub-basin do not meet good ecological status (National, 2016). The pollution of the Nevezis River is greatly influenced by its tributaries, which flow through the fertile soils of the Middle Lithuanian Lowland collecting nutrients; thus, degrading water quality. Studies in the Nordic and Baltic countries have shown that the trends of nitrogen concentrations have been decreasing mostly in Denmark and Sweden, while they have been increasing in the Baltic States (Stalnacke et al., 2014). The largest of the four Nevezis tributaries in Kedainiai town is the Smilga and Dotnuvele streams. The sources of concentrated pollution in their basins are the urban wastewater treatment plants, rainwater cleaning equipment and JSC Kedainiai Canning

Factory. Not all inhabited houses are connected to the central sewage system, and part of the surface water flows from the town straight into the stream. Sewage flows into the Dotnuvele and Smilga streams from various settlements which are situated in their basins.

Dotnuvele and Smilga streams, flowing in the Kedainiai town territory, were very polluted from the Soviet military airfield and two fuel bases. However, in 2009 – 2012, by implementing the environmental measure, "The management of historically polluted territories", the water and banks of the streams were cleaned from harmful substances. It was hypothesised that after cleaning up these contaminated sections of streams, the water quality would improve. After that, the maintenance of cleaned water bodies and streams' water monitoring was carried out for five years. The purpose of the research is to investigate the changes of nitrogen and its compounds in the Dotnuvele and Smilga streams, depending on meteorological factors.

Materials and Methods

Dotnuvele and Smilga streams, flowing through Kedainiai town, are the right tributaries of Nevezis river. Even though Smilga stream is almost twice shorter (32 km) than Dotnuvele (60.9 km), its basin is almost the same (208.8 km^2) to Dotnuvele's (192.7 km^2).

The cleaning of 1.95 km length of Dotnuvele stream (measured from the mouth of the stream) as well as restoration of 10 m width of coastal strip was completed in 2012. In 2013, 1.98 km length of Smilga stream and 2 ha of coastal land was cleaned, and 5157 m^3 of sludge removed.

Water samples were taken from the mouths of Dotnuvele and Smilga streams four times a year (2013 – 2017) – in March, May, August, and November months. Chemical analysis, according to the methodology described in literature (Unified, 1994), was done by Aleksandras Stulginskis University Chemical Analytical Laboratory of Water Resources Engineering Institute.

In the samples total nitrogen (N_{total}) was determined by applying the spectrometric method, by mineralizing with potassium persulphate, P_{total} was determined by spectrometric method after mineralization with potassium persulphate, nitrate nitrogen (NO_3-N) – by spectrometric with phenol sulfonic acid, phosphorus residue (PO_4-P) – by spectrometric with ammonium molibdate and ascorbic acid. The colorimetric analysis was performed using 'FIA star 5012 system' analyser.

Surface water bodies quality currently is assessed according to 'The Surface Water Bodies State Evaluation Methodology' approved by the Minister of Environment of the Republic of Lithuania (Surface, 2010). According to this methodology, the ecological state of rivers is divided into 5 classes from a very good state, when N_{total}<2, NO_3-N<1.30, P_{total}<0.1. PO_4-P<0.05 to a poor state, when N_{total}>12, NO_3-N>10.0, P_{total}>0.47, PO_4-P>0.4 mg l^{-1}.

Mathematical and statistical analysis of the data was performed using the computer program MS Excel 2010 and Statistica v.5.

Results and Discussion

The highest amount of nitrogen and its compounds is found in the rivers of central Lithuania, where the most intensive agricultural activities are being developed. However, nitrogen compounds are almost always present in the surface water bodies, even those that are not affected by anthropogenic pollution. This means, that their formation is influenced not only by human activity but also by natural factors (Tumas, 2003; Šileika, 2012).

The research has shown that in summer, nitrate concentrations in both Dotnuvele and Smilga streams, are lower compared to the spring and autumn. In summer – the third quarter of the year, the water quality in both rivers was very good: in Dotnuvele 1.1 mg l^{-1}, in Smilga – 1.3 mg l^{-1}, which means, that these concentrations were 7.7 times in Dotnuvele and 6.7 times in

Smilga lower than in the I quarter of the year (Figure 1). This was influenced by an intensive assimilation of aquatic vegetation.

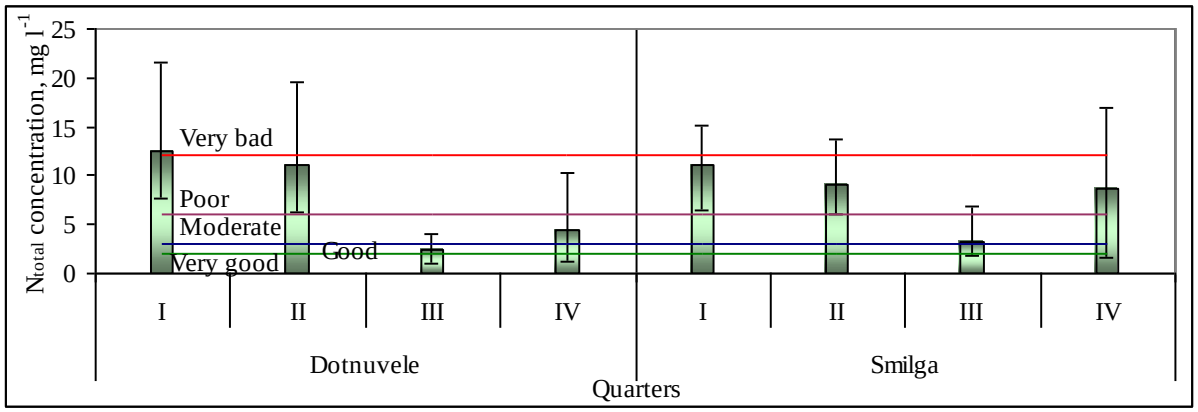

Figure 1. NO_3-N concentrations change in streams' water

During the autumn period (November), intensive autumn rain washes large amount of organic and inorganic fertilizers out into streams and rivers. In addition, plants and algae begin to decompose in the rivers, which increases the nitrate concentration in the water. The average concentration of NO_3-N in Dotnuvele in the fourth quarter was 3.5 mg l^{-1}, while in Smilga - 7.4 mg l^{-1} and they corresponded to the average and poor water ecological status. At the end of the cold season (March), the nitrate concentration in the ecosystems is the highest and corresponds to the poor ecological status of the streams: in Dotnuvele - 8.5 mg l^{-1}, in Smilga - 8.7 mg l^{-1}. Thus, the season of the year affects the nitrate inflow into the water bodies (Kutra and Berankienė, 2006; Stankevičienė, 2012). Over time, there are periods in which nitrate concentrations may depend not only on river runoff but also on other factors: plant vegetation, winter conditions, soil freezing depth, snow cover (Povilaitis, 2003; Lintern et al., 2018). According to Šileika (2012), "comparison of N and P concentrations in the Nevezis River and in the Graisupis Stream revealed that in the Nevezis River N concentrations were lower by 15.4% and P concentrations were higher by 52.2% than those in the Graisupis where non-point pollution prevails".

The highest nitrogen concentrations were observed in the Graisupis stream in the middle Lithuanian lowland, where more intensive agricultural activity occurs (Povilaitis et al., 2014). "The highest monthly N concentration in the Graisupis Stream was registered in December and November – 9.4 and 12.7 mg l^{-1} respectively" (Šileika et al., 2010). Higher N_{total} concentrations were determined in areas with intensive agricultural activity or in soils having a small number of clay particles. Also, water quality of the streams is affected by high fertilisation rates both of mineral fertilizer and manure (Stalnacke et al., 2014).

To determine the trends in eutrophication. it is very important to analyse the change in total nitrogen in rivers' water. In Figure 2, it can be seen, that the higher concentrations of this indicator as well as nitrate nitrogen, were determined during the cold season or at the beginning of vegetation – at that time the status of water of both streams was in a very bad or poor ecological condition: in Dotnuvele – 12.6 mg l^{-1} and 11.0 mg l^{-1}, and in Smilga – 11.0 mg l^{-1} and 9.1 mg l^{-1}.

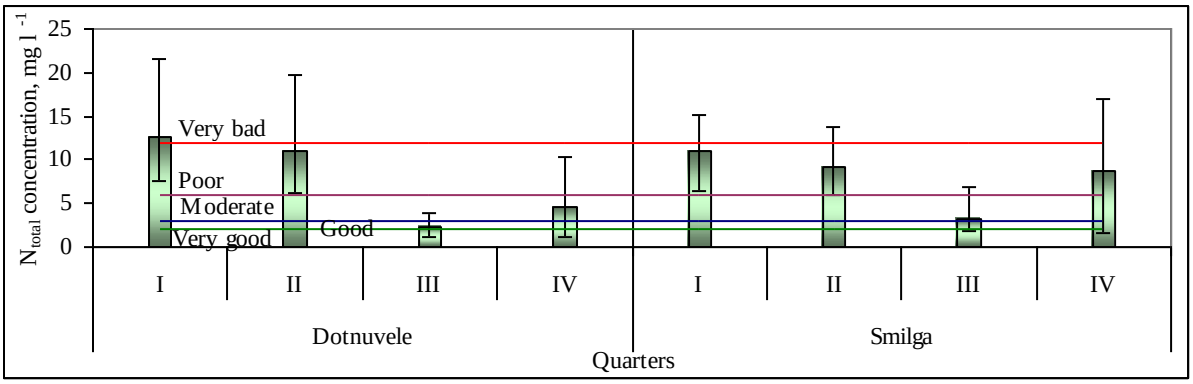

Figure 2. N_{total} concentrations change in streams' water

Dotnuvele and Smilga streams' water receives biogenic matters from agricultural lands, as increased concentrations of these elements were observed mostly in spring, when, during the snow melt, water with dissolved nutrients reached these streams by drainage.

In the spring, agricultural fields still have a very poor vegetation cover, which is able to absorb only a part of the dissolved nitrogen and the other part of it flows into the streams via drainage. The smallest N_{total} as well as NO_3-N concentrations were found in the August samples; as nitrogen is used to mature crop yields, the amount of it flowing into the streams is very small.

In the fourth quarter of the year, N_{total} concentrations are rising again due to the autumn rain, which dissolves nitrogen; as the plant cover is often scarce in this period, it is therefore unable to absorb all of the nitrogen.

The changes in concentrations of PO_4-P and P_{total} in the Dotnuvele and Smilga streams are completely opposite to the changes in nitrogen concentrations. The lowest concentrations of these indicators were observed in the I, II and III quarters of the year and corresponded to the very good ecological status of these streams. They were the highest in the third quarter of the year. The average concentrations of PO_4-P and P_{total} were found to be 0.14 mg $l^{-1}$and 0.19 mg l^{-1}, respectively in Dotnuvele, and corresponded to a poor condition of the water bodies. In the Smilga Stream, water status, according to both of the indicators, was found to be very good (0.04 mg l^{-1} and 0.06 mg l^{-1}), but the deviations from the average showed that in some years the water status in this stream deteriorated to an average ecological status (Figure 3, 4).

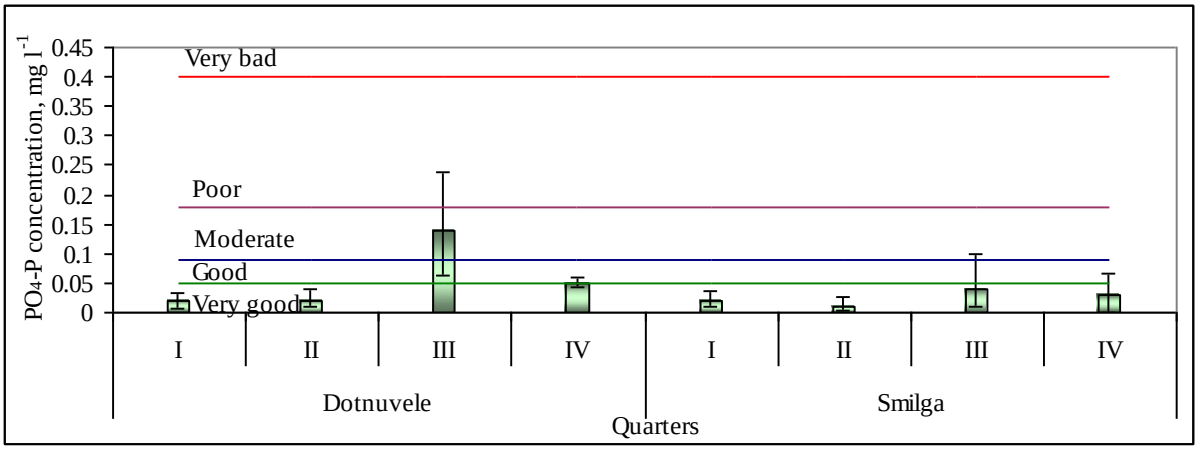

Figure 3. PO_4 – P concentrations change in streams' water

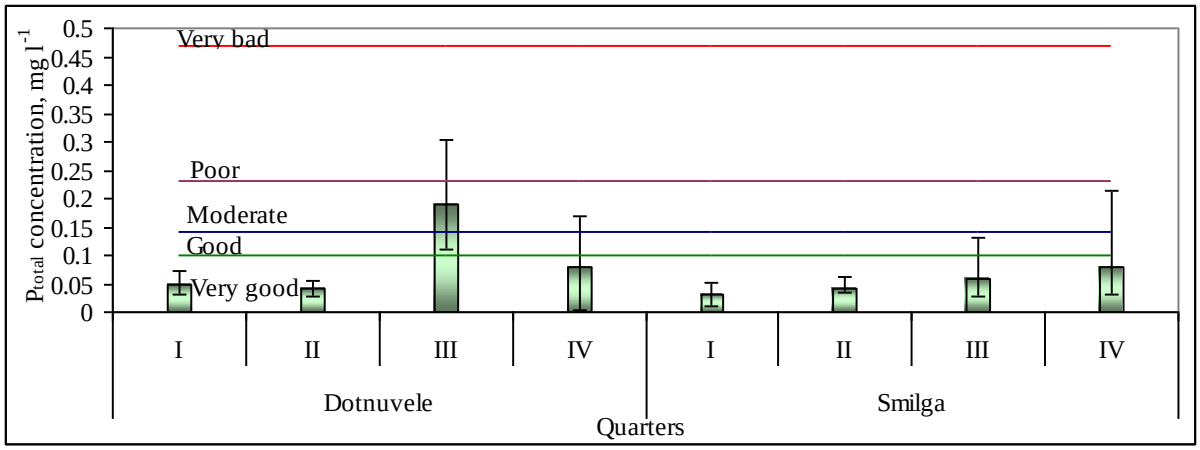

Figure 4. P_{total} concentrations change in streams' water

Due to meteorological conditions, the water speed in streams slows down significantly and the water temperature increases in August. In addition, a dam is located near the mouths of the Dotnuvele Stream, where sediments are deposited. Thus, secondary pollution from sludge accumulated in the streams could have had an effect (Howell, 2010).

On average 6.9 mm, 70.5 mm and 16.7 mm more precipitation than the climate normals has fallen in the I, III, and IV quarter of the year. The average air temperature was higher than the climate normals in every quarter of the year: I - 1.0 °C, II – 0.4 °C, III – 0.6 °C, IV - 1.5 °C (Table 1).

Table 1. Meteorological conditions of 2013-2017 study periods

Year	Quarters				Per year
	I	II	III	IV	
Precipitation, mm					
2013	87.0	142.2	224.5	112.5	566.2
2014	96.7	167.6	207.1	124.9	596.2
2015	125.9	128.2	129.2	119.8	503.1
2016	151.5	144.2	246.1	207.3	749.2
2017	78.2	123.7	440.6	204.1	846.6
Climate normals, mm	101	149	179	137	566
Average air temperature, °C					
2013	-4.5	13.1	16.4	5.0	7.5
2014	-0.2	12.1	16.9	2.9	7.9
2015	1.3	11.2	17.0	4.4	8.5
2016	-1.5	13.1	16.6	2.5	7.7
2017	-0.4	11.3	15.8	4.1	7.7
Climate normals, mm	-2.0	11.8	15.9	2.3	7.0

The statistical analysis of the data shows the relationship between the concentrations of the analysed biogenic substances in the streams' water and the meteorological conditions (Table 2).

Table 2. The influence of meteorological factors to the change in concentrations of nutrients in streams' water

Indices	Equation	r	n	$F_{fact.}$	p	r_1	r_2
NO_3-N	$z=10.2306-0.3850x_1-0.5815x_2$	0.67	40	15.06	0.0000	-0.66	-0.38
N_{total}	$z=13.6174-0.4095x_1-0.9015x_2$	0.54	40	7.64	0.0017	-0.51	-0.37
PO_4-P	$z=-0.0051+0.0034x_1+0.0042x_2$	0.45	40	4.64	0.0159	0.45	0.18
P_{total}	$z=0.0055+0.0045x_1+0.0128x_2$	0.52	40	6.86	0.0029	0.46	0.41

Note: z – concentration of indices; x_1 – water temperature ˚C; x_2 - precipitation amount mm; $F_{theor.95\%} = 3.3$; relationship is significant when $F_{theor.95\%} < F_{fact.}$

Meteorological factors influenced the increase of nitrogen and phosphorus concentrations in the researched streams' water: NO_3-N, N_{total}, PO_4-P and P_{total} respectively (r = 0.67), (r = 0.54), (r = 0.45) and (r = 0.52). Data analysis has shown, that concentrations of nitrates and total nitrogen were strongly influenced by the streams' water temperature: the lower it was, the higher amounts of the analysed indicators were found in the streams' water, respectively (r = -0.66) and (r = -0.51). This is confirmed by studies carried out by Tripolskaja (1995), which showed that nitrification processes are taking place even at low positive temperatures, which results in additional nitrate reserves in the soil. However, the autumn – winter period is very favourable for nitrogen leaching, because during this period the soil is bald and the precipitation is not too low. When the temperature is positive, though not high, mineralization processes in the soil are more intense, resulting in an increase in the amount of nitrogen that is washed into drainage, and via drainage it flows into the rivers (Clein and Schimel, 1995; McMahon et al., 2009).

In the third quarter of the year (2013 – 2017), the streams' water average temperature was high (in Dotnuvele - 21.6 °C, in Smilga - 20.5 °C), which affected the increase in the PO_4-P and P_{total} concentrations in streams' water, respectively (r = 0.45) and (r = 0.46). As claimed by Lintern et al. (2018), that the season of the year and the temperature have an impact on the fluctuation of nutrients in the streams' water.

Conclusions

When Dotnuvele and Smilga streams flow through the agricultural fields, they collect drainage water saturated with nutrients. In the winter, spring and autumn seasons, the ecological status of the streams according to N_{total} and NO_3-N concentrations are found to be poor or very bad. The smallest concentrations of these indicators in the analysed streams were determined in summer and corresponded to a good and very good ecological status of water and were due to the intensive assimilation of aquatic vegetation. The phosphates and total phosphorus levels in the winter and spring seasons were low, therefore the streams' water corresponded to a very good status of water bodies. However, in summer, due to the high temperature of the water. the increase in these indicators has been observed, therefore the ecological status of the water in Dotnuvele has been set to moderate.

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TRADITIONAL OLD FLOWER GARDEN IN LITHUANIA AND POLISH BORDER

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Abstract

Lithuanian-Poland border is a special territory, which belonged to Grand Duchy of Lithuania for 300 years long. Only in the 20th century state borders separated this territory. Still 20-30 km from the border on both sides the bigger part of inhabitants are Lithuanians. Decorative and medical plants were inventoried in 16 farmsteads (8 on each side) in Lithuania and Poland. The aim of this research was to assess how well the traditions of Lithuanian old traditional gardens were kept for around one hundred years in territories separated by state border. Altogether 95 species, now classified as traditional Lithuanian gardens plants, were found. These plants have been grown in flower gardens in Lithuanian villages from old times. 83 species of these plants grew on Lithuanian side of the border and 76 species on Polish side. There are no records telling when flower gardens have been established from in Lithuanian rural homesteads, but it is thought that it was done around 15th-16th centuries. According to their growth time plants were divided into three groups: 1) plants which were grown from 15–16 centuries – 14 species of such in Lithuania grown plants and 16 species in Poland; 2) from 17–18 centuries – 12 species in Lithuania and 13 species in Poland; 3) from 19–20 century – it was found 57 species in Lithuania and 47 species in Poland.

Keywords: *Lithuania-Poland border, traditional Lithuanian gardens; decorative and medical plants and herbs.*

Introduction

Flower garden is a traditionally formed usually is fenced composition of traditional assortment plants (Martynėnaitė, 2010). In Lithuanian villages flower gardens appear around 15th-16th centuries. Decorative, medicinal plants and herbs introduced from monasteries, rectories and manor parks spread around gardens in villages. Homesteads management has intensified after cancellation of serfdom in 1861. It is impossible to identify exact species, which were grown in Lithuanian gardens also to detect time when it was started to grow them. There are no remaining plants grown in monasteries gardens lists but there are some manor gardens descriptions from XVI century. First specific knowledges about plants spread in gardens were found in work about the flora of Grand Duchy of Lithuania by S. B. Jundzila in 1791 (Gudžinskas, 2010). More data from 19th century is in notes by J. Jundzila, S. B. Gorskis and J. A. Pabrėža in Vilnius University herbar. Flower gardens in the current territory of Lithuania and Punsk surroundings were most thoroughly explored by G. Žumbakienė (2016). For a long time, the variety of species grown in flower garden was not big. At first useful plants were grown such as: medicinal or flavoring plants, herbs, plants used for coloring or parasite-repelling or repulsive plants, plants used for religious rites. Only in the 19th century at the beginning of 20th century more attention was paid for aesthetic plants qualities such as: pleasant smell, beautiful flower blossoms or leaves. All times assortment of plants changed: one were liked more than the others, more ornate plants changed less ornate plants. Not many species were kept in flower gardens for couple of hundreds of years. This is why it is interesting to find traditional old plants growing next to newly spread plants in Lithuanian flower gardens.

It was interesting to compare the growing traditions and the variety of species grown in villages in both sides of the Lithuania-Poland border.
Lithuanian–Poland border is a special territory, which belonged to Grand Duchy of Lithuania for 300 years. Only in the 20th century state borders separated this territory. In Poland where there was no collectivization, lots of Lithuanian rural homesteads remained together with traditionally flower gardens. In Lithuania lots of bigger old homesteads were destroyed but flowers were loved and gardens with traditional plants were established in newer times. 20-30 km from the border on both sides the bigger part of inhabitants still are Lithuanians (Žemaitis, 2017). The object of present paper is to assess how traditional Lithuanian flower gardens at the Lithuania-Poland border.

Material and Methods

Decorative and medical plants were inventoried in 16 farmsteads (8 on each side) in Lithuania and in Poland. It was chosen the farmsteads 20 km from national border – Punsk Gmina in Poland and Marijampolė and Alytus County in Lithuania. Geographical position and altitude of every farmstead was detected.
Plants were inventoried only in such flower gardens which had around 50% of traditional/old gardens plants (which grew in Lithuania from 15th to 20th centuries).
Plants were observed three times during their vegetarian period (May, August and September). During observation plants were identified by species. We described plants based on plants description guides (Snarskis, 1968; Vilkonis, 2001; Botanica, 2006). They were assigned to medicinal plants or herbs according to literature sources (Dagytė, Penkauskienė, 1978; Smaliukas et al., 1992; Ragažinskienė et al., 2005; Gudžinskas and Balvočiūtė, 2007; Budnikas and Obelevičius, 2015). Collected data were compared to literature sources about all Lithuanian traditional flower gardens especially in Dzūkija and Suvalkija (Andriušytė et al., 2008; Kačinskaitė et al., 2008; Pabrėža, 2009; Gudžinskas, 2010; Žumbakienė, 2016) and according to these authors assigned to traditional Lithuanian gardens plants.

Results and Discussion

Altogether 95 species, now classified as traditional Lithuanian gardens plants, were found in 2017. These plants have been grown in flower gardens in Lithuanian villages from old times. Eighty three species of these plants grew on Lithuanian side of the border and seventy six species on Polish side. According to the date of plants planting in Lithuanian gardens, which were specified in literature sources (Pabrėža, 2009; Gudžinskas, 2010; Žumbakienė, 2016) we analyzed todays plants assortment at such specific territory as Lithuania-Poland border. Geographically friendly areas almost one hundred years belonged to a different country could have had different flower garden growing traditions. Although there are no exact data about the beginning of flower gardens in Lithuania's villages it is thought that first decorative and medicinal plants could have been grown in farmsteads next to dwelling houses from around 15th-16th centuries (Gudžinskas, 2010; Žumbakienė, 2016). Everywhere they spread from the nearest monasteries and manor houses later from one farmstead to another.

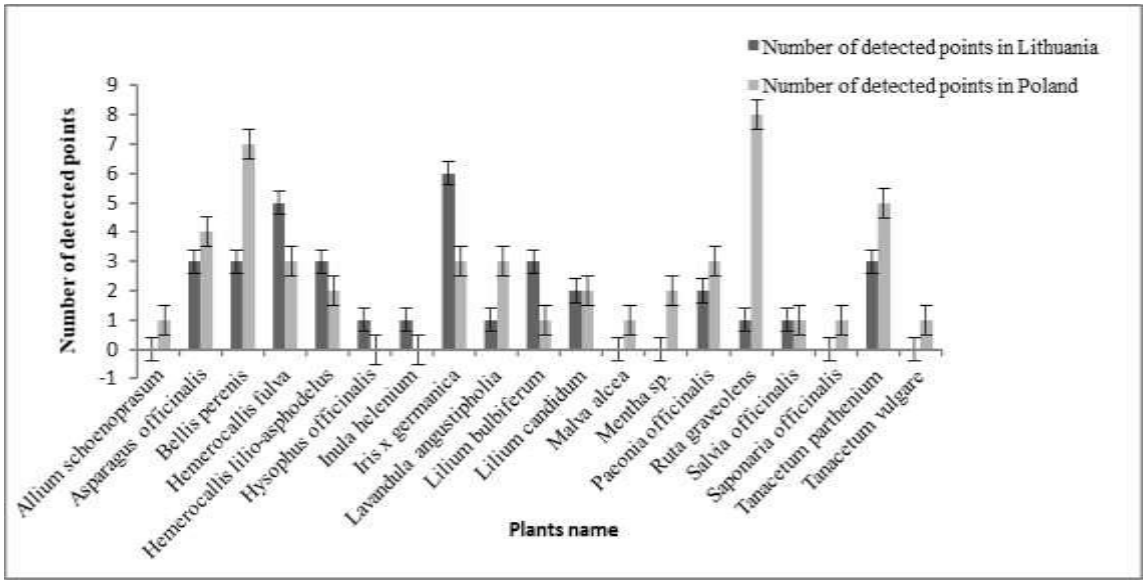

Fig. 1. Plants which were grown from 15-16 centuries in Lithuanian flower gardens in farmsteads at Lithuania-Polish border in 2017.

The variety of oldest plants is not big most of these plants are used for medicines or food (Fig. 1). Exceptions are *Hemerocallis* spp. and *Lilium* spp., which were very respected and spread together with Christianity (Gudžinskas, 2010). In Poland in one of the farmsteads *Hemerocallis fulva* (L.) L. var. *kwanso* Regel was grown. It's a long time introduced full-fledged plant, which is rarely grown now. A little elder species (16 species) are grown on Poland side (14 species in Lithuania). *Ruta graveolens* L. and *Bellis perenis* L. are grown almost in all farmsteads in Poland and less on Lithuania's side. Most of these plants grown in old Lithuania's flower gardens are not being grown everywhere now usually separate species in 1-2 farmsteads. Also, most of the plants which were grown couple of hundred years later are modest, mostly medicines, fragrant. Brighter blossoms or fruits have: *Tulipa gesneriana* L., *Dianthus barbatus* L., *Physalis alkekengi* L., *Calendula officinalis* L.

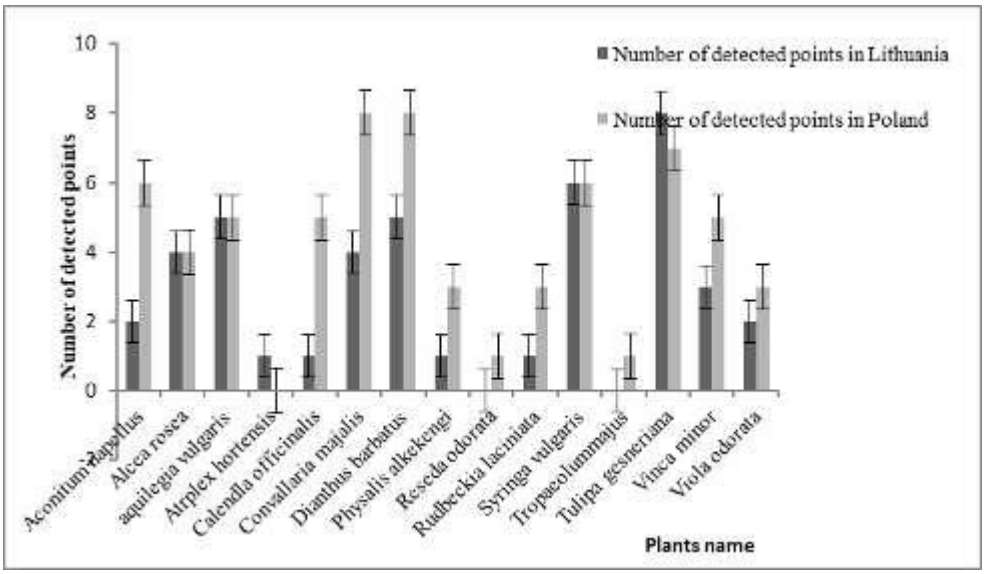

Fig. 2. Plants which were grown from 17-18 centuries in Lithuanian flower gardens in farmsteads at Lithuania-Polish border in 2017.

From those, which grew from 17-18 centuries, it was detected 13 species in Poland and 12 species in Lithuania now. *Aconitum napelus* L., *Convallaria majalis* L. grow almost in all farmsteads in Poland and in Lithuania - *Aquilegia vulgaris* L., *Syringa vulgaris* L. (Fig. 2).

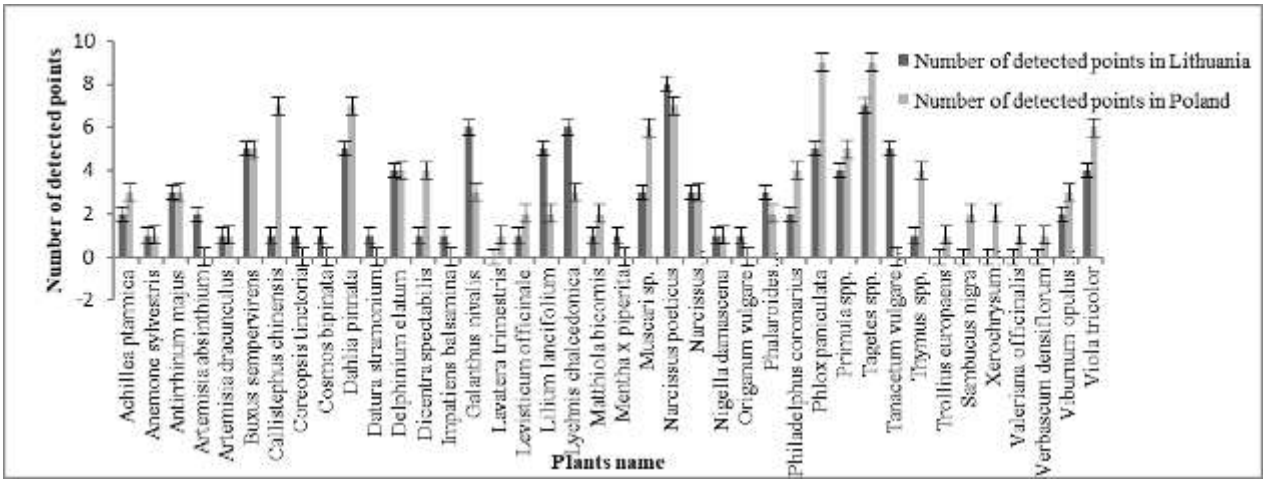

Fig. 3. Plants which were grown from 19 centuries in Lithuanian flower gardens in farmsteads at Lithuania-Polish border in 2017

During 19th century the variety of plants species got bigger in Lithuania's flower gardens (Fig. 3). Annual plants were planted next to already popular perennial plants. Until now these annual plants are grown at the Lithuanian-Polish border: *Antirrhinum majus* L., *Callistephus chinensis* (L.) Nees, *Coreopsis tinctoria* Nutt, *Cosmos bipinnatus* Cav., *Impatiens balsamina* L., *Lavatera trimestris* L., *Mattiola bicornis* (Sibth. et Sm.) DC, *Tagetes* spp., *Tropaeolum majus* L., *Xerochrysum bracteatum* (Vent.) Tzvelev and bulbous plants: *Galantthus nivalis* L., *Muscari* spp., *Narcissus* spp. are grown too. At the end of 19th century *Dahlia pinnata* start growing which widespread in 20th century. More and more plants are grown for their look and not for the use. Most of these plant species are still popular. Now other cultivars especially annual and bulb plants are grown but tradition remain in growing same species plants.

In the beginning of the 20th century Lithuanian flower gardens became more and more flowery, flowers which require more caretaking are grown: *Gladiolus* x *hibridus* Van Houtte, *Rosa* spp., *Primula* spp., *Hosta* spp. and *Paeonia lactiflora* Pall. cultivars. All these plants still grow at the Lithuania-Polish border. In Poland almost every flower garden has *Paeonia lactiflora*, various species and cultivars of *Primula*, couple of species and many cultivars of *Symphyotrichum* spp. (Fig. 4). These flowers also grow on Lithuanian side of the border but not so often.

On both sides of the border farmsteads are trim with lots of flowers but during last decades especially after the war different conditions had influence on farmsteads management in Lithuania and Poland.

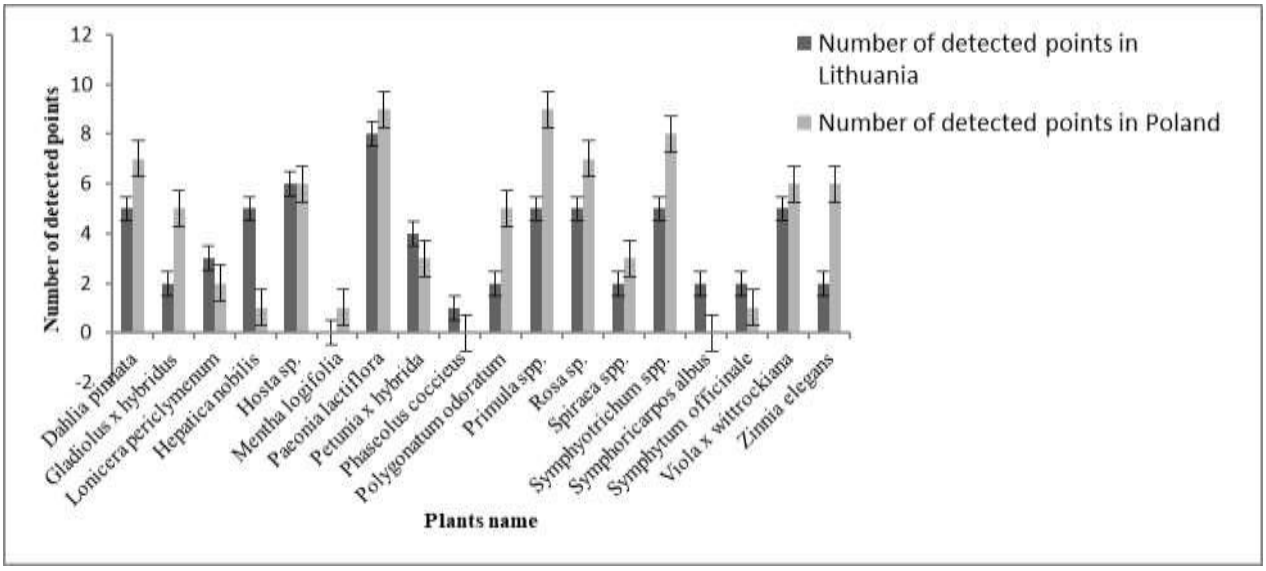

Fig. 4. Plants which were grown from the first half of the20 century in Lithuanian flower gardens in farmsteads at Lithuania-Polish border in 2017

In Punsk Gmina remain many old farmsteads which were established by ancestors of todays owners. These farmsteads are big and young people live in them. On Lithuanian side of the border after collectivization old and rich farmsteads did not remain. Many of current inhabitants are worthy age. Although farmsteads are under care their future is unclear. Lithuanians living on the Polish side of the border kept not only Lithuanian language but also Lithuanian traditions. It was harder to do that in Lithuania. During the last 100-120 years there were many events in Lithuania which influenced economic, political and cultural counties life. Also these events influenced the survival of traditional rural ethno cultural domain. Only some places kept ethnic architecture even less places kept traditional plantations characteristic to ethnographic regions (Misius, 2015). On both sides of the border owners of flower gardens spoke with love about their plants if the plants were medicinal or herbs people knew their uses. Usually people used folk names for the plants. In Poland they usually used Polish names. Traditional interwar mono-farm rural landscape lost its traditional peculiarity when it was changed into collective farmland landscape. Today for other reasons (globalization, destruction of granges system) but ethnic cultural heritage is still vanishing in Lithuanian villages. Together with ethnical architecture are disappearing the unique plantings, traditions of farmsteads landscaping and regional peculiarities (Misius, 2010; 2015).

Conclusions

2017 we inventoried 95 species of traditional Lithuanian flower gardens plants in rural gardens at the Lithuania-Polish border. On both sides of the border was detected similar number of species: 83 plant species on Lithuanian side and 76 species on Polish side. Half of all decorative and herbs/medicinal plants grown in 9 farmsteads on Lithuanian side of the border and 9 farmsteads on Polish side are traditional Lithuanian flower gardens plants which started growing in Lithuania from 15 to 20 centuries. Most of the plants in farmsteads at Lithuania-Polish border were starting to grow and spread in Lithuania in 19-20 centuries. These plants are still popular in flower gardens. Only newer cultivars of annual and bulbous plants species are grown.

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ENVIRONMENTAL USE OF WATER IN MEXICO

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Abstract

Mexico is located between 12° and 32° north latitude and 84° and 121° west longitude, in the world's tropical deserts area. With almost 2 million km^2, a third of its total surface lies in the desert and semidesert areas. Desert areas are characterized by: rainfall volume lower than 500 mm/year (in the northernmost parts of the country this is less than 200 mm/year), extensive hours of sunlight, real evaporation and evotranspiration (1500 mm/year); around 80 % of Mexico's population lives in desert areas, although 57 % of the total population lives in the meridional part of the country, a semidesert area, with rainfall volume ranging between 400 and 500 mm/year. The focus of the present analysis is on the geographic characteristics of the region in terms of water, lack of education and environmental awareness of its inhabitants, as well as the erratic government policies of neoliberal governments that keep the country in a chronic state of poverty, broken-down agricultural fields, unequal distribution of wealth, reasons that make groups of people live in extreme poverty. The methodological process used in the project was research-action, because, as members of society, we are part of the problem. Besides reviewing literature, informal surveys were carried out among the people involved, municipal authorities, school principals and teachers from the elementary and high school levels. The result is an educational proposal and a personal alternative to collect rain water.

Keywords: *shortage, arid areas, environmental education, chronic poverty*.

Introduction

Mexico has never had abundant water. It has always been a dry county. Why do people choose not to get involved with an issue that affects everyone? Why is the government expected to solve everything without any engagement from the citizens? It could very well be that Mexican culture has been thus developed: to ask and obey. The administrations that this country has had, following the Mexican revolution, are a clear example that officials in turn only have particular interests, acting always for their own benefit, forgetting about the citizens and the people who are always asking for handouts to make ends meet at the short-term, but not the long-term; such an issue is water supply. Government programs around water have not solved the problem, sometimes due to incapacity, others due to lack of knowledge, or just because it is convenient to keep capitalism running, a sistema that, through globalization, has caused that the State's functions are overrun, reinforcing interdependence for this political and economic system (Gómez, 2008).

In view of the above, it is important that people engage in shortage issues during dry seasons, analyze the government proposals aimed at diminishing the problem, and do not grow indifferent to such crisis. The objective of the current study is to analyze data about rainfall in Mexico to create strategies for the sustainable use of water, and to find a way that makes people engage in the solution to a problem that affects everyone.

Materials and methods

Methodology for the current research consisted of reviewing documents and data generated by Mexican government agencies such as the Secretariat of Environment and Natural Resources (SEMARNAT), National Water Comission (CONAGUA), and the Official Mexican Standards (NOM) 04, 05, 06, 07, 08, 09, 010, 011, published in the Federal Official Gazette (DOF, 2013). Through this data, the intention is to show to the people the relevance of water and also show them way for them to participate in the solution to the water shortage problem that affects several regions in this country; to bring them together through observing the water shortage matter and use of water in agricultural, livestock-related, industrial, household and urban activities. Data gathering in diverse social groups (housewives, farmers, municipal authorities, teachers, children) was conducted through participant observation (Spradley 1980), while attempting to identify the views on the water issue in its daily use, the shared responsibility that they acknowledge in the actions they take, the way they could participate in solving the problem, and the degree of commitment shown. A qualitative analysis of observed data was conducted as well as an inductive-deductive method to conclude the analysis and establish alternate proposals (Hernández *et al*., 2003).

The method of active investigation was adopted because of the researchers' involvement with the problem and its solution; they are acting for changes to take place around them. (Bisquerra, 1996). Work was carried out mainly in the states of Mexico (Tezoyuca, Acolman, Teotihuacán), Guanajuato (Dr. Mora), Michoacán (Zinapécuaro, Maravatio), central regions in the country, and also in the Peninsula of Baja California Norte (San Quintín, Ojo de Liebre, Ensenada), places where participant observation was performed as well as informal interviews with municipal authorities, farmers, water users, and elementary education principals, with whom data was gathered to conduct the research and analyze proposals.

Results and discusion

Regular rainfall in the country from 1981-2010 was around 740 millimeters. Normal values, according to the World Meteorological Organization (WMO), correspond to the average measures calculated for a uniform and relatively long period, which has to span at least 30 years of data gathering, which is considered as a minimum representative climate period (CONAGUA, 2016).

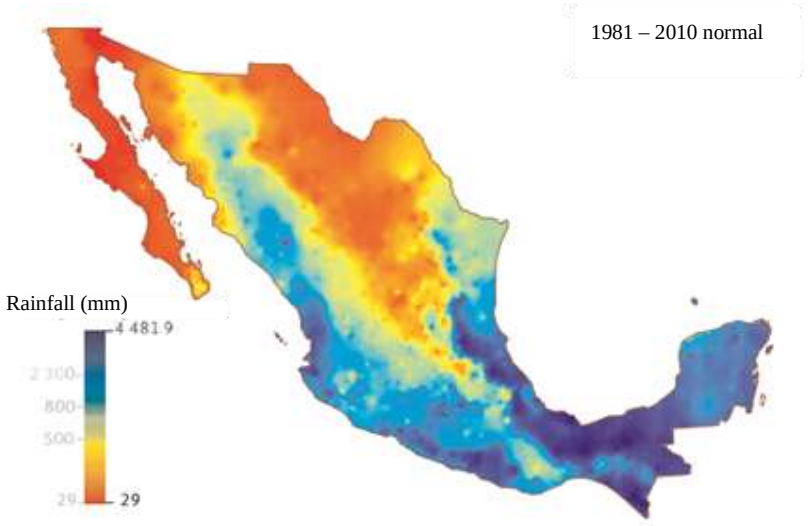

Image 1.- Map that represents the pluvial precipitation index in Mexico. Source: Elaborated by CONAGUA (2016, p. 35)

Tetumo (1993) registered that in Mexico, average yearly rainfall is 780 mm, equal to 1, 538.59 km^2, deposited in 1, 972, 447 km^2 and distributed as follows: 2/3 in the south of the

country, 1/3 in the north of the country, concentrated from the month of June to October. In the Mexican territory, because of its geography and climate, two large availability areas stand out, the first of them encompasses the south and southwest while the second encompasses the north, center and northeast of the country. Natural availability in the former is seven times greater than in the rest of the country. In the north and central areas, 77 % of the population is located, 85 % of the Gross National Product (GNP) is generated, and only 32 % of the average natural availability is at hand.

Surface-water Hydrology

The surface runoff value for the national territory, according to the first result obtained by the National Hydrologic Plan, was 410, 021 million m^3, and represents 27 % of the surface runoff volume. The largest runoff volume occurs in the south of the country, through the Grijalva and Usumacinta rivers, while in the Peninsula of Baja California runoff is around 1 %. In the rivers of the country, runoff approximately amounts to 410 km^3 each year, including water coming from other countries and excluding water going outside the country. Around 87 % of runoff occurs in the main 39 rivers listed below and whose basins occupy 58 % of the Mexican continental surface (CONAGUA, 2016).

Groundwater hydrology

For the purpose of water management, the country was divided into 653 aquifers, on January 31, 2013. Water availability in 188 aquifers was published in the DOF (2013). From these aquifers, 66 % of groundwater used in the country is extracted, and 79 % of underground water recharge is collected in them. From the 70s onwards, the number of overexploited aquifers has increased substantially, from 32 in 1975 to 36 in 1981, 80 in 1985, 97 in 2001, 102 in 2003, 104 in 2004 y 126 in 2010. From them, around 60 % of groundwater is extracted for all kinds of use (CONAGUA 2016). In the DOF of December 5, 2001 (DOF, 2013), 653 aquifers were published. The splitting of the Sonoita-Puerto Peñasco aquifer is not official; however, occasionally it is used for technical purposes. There are 17 aquifers with issues of salt water filtering in the states of Baja California, Baja California Sur, Colima, Sonora and Veracruz. Among them are Maneadero y San Quintín in Baja California, Santo Domingo in Baja California Sur; Caborca, Costa de Hermosillo y San José de Guaymas in Sonora.

Water distribution across Mexico in not balanced. In the current circumstances, large urban areas, climate change, and the lack of recycling and reutilization policies for water are making matters worse. According to Magaña (2006), by the year 2020, around 60 % of the world's population will live in cities, generating greater challenges for water supply. By the year 2050, it is likely that at least one in each four people will live in countries affected by chronic or recurrent fresh water shortages. Several hypothetical scenarios have been proposed based on the latest studies conducted by some agencies of the United Nations (UN) about population, and according to the worst possible scenario, almost 7000 million people in 60 countries might suffer from water shortages by the year 2050. According to the most optimistic scenario, less than 2000 million people in 48 countries will suffer from water shortages by the same decade.

Mexico's location on earth that makes it more vulnerable to climate change. Droughts are another major threat for the planet. Even though droughts are part of the weather's natural variability, growing demand for water has led them to turn into a serious danger for society, mainly hydrological and agricultural droughts in developing countries. The Mexican water sector needs to reformulate its strategies on development while trying to reach a balance between consumption, environmental demands, flooding control, as well as costs and benefits of climate variability in water-dependent sectors, such as the tourism, health and recreation industries.

Water availability is already low. The amount of water that people use in a given country does not depend only on minimum needs and on how much water is available for use, but also on the level of economic development and degree of urbanization.
On a global scale, out of the three common categories for the use of fresh water — or agricultural, industrial and household use (personal, familiar and municipal) — agriculture domains.
According to data from (CONAGUA, 2016), around 75 % of the water used in Mexico is for agricultural ends. Household use takes up to 14 %, while the remaining 11 % is for industrial use, *i.e.*, industry in Mexico has a relatively low consumption level according to international standards. In as far as the world becomes predominantly urbanized, agriculture grows more dependent on irrigation. According to the National Institute of Geography and Statistics (INEGI) and the National Water Commission (CONAGUA, 2016), in Mexico water consumption is distributed as follows:

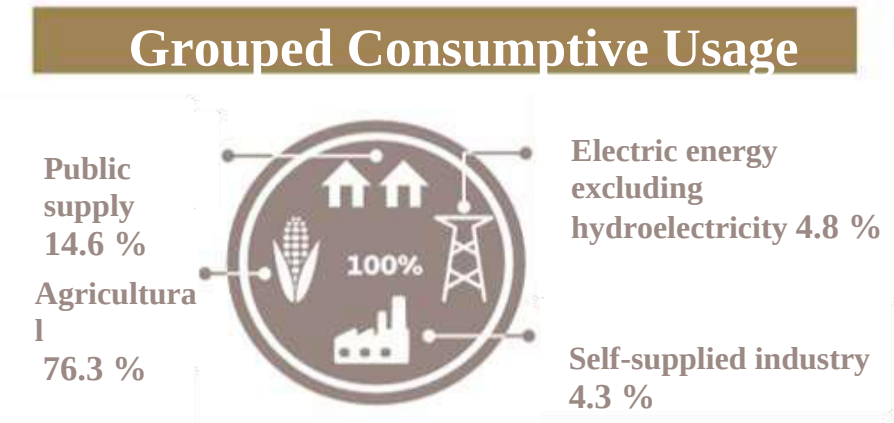

Image 2. Representation of water consumption in Mexico. Source: Elaborated by CONAGUA (2016, p. 69)

Among the many variables that will affect water availability in the country and that depend entirely on our choices are:

- Population
- GNP growth by sector and region
- Efficiency in the use of water
- Water consumption *per capita*
- Food demand
- Agricultura efficiency, both seasonal and irrigational
- Food imports
- Regional water extraction
- Tax regulations

Among the variables that are beyond our reach but that will affect our water availability are:

- Changes in temperature and rainfall
- Geological accidents
- Other natural causes

Official Political Tendencies

Through administrative and financial reforms and also by modifying policies and techniques about water management, authorities are trying to guarantee the provided services and preserve the environment.

Their goals include:

- Foster the efficient use of water in agricultural production
- Foster a greater coverage and quality of fresh water services, sewage and sanitation
- Achieve an integral and sustainable management of water in basins and aquifers
- Promote the technical, administrative and financial development of the water sector
- Consolidate engagement from users and organized society members in water management and foster proper use practices
- Diminish risks and attend the effects of floods and draughts

Their scheme for sectorial water management attempts to head on to a decentralized, integrated management that partakes in water resources, implementing crossed policies that consider concepts such as planification and territorial ordering on the scale of hydrographic basins (Wolfe, 2014).

Proposals at the household and personal level.

To acknowledge that water is not perennial, that it is depleting, not so much due to the water from the hydrologic cycle having been reduced, but rather because of heavy human pollution, which makes it take longer to recover water after it has been returned to nature in such a state. To employ mechanisms that decrease consumption, employ reductive devices in toilets' water tanks of six liters in two phases, one of low consumption and another of full consumption, as needed. These are not expensive. A toilet of this kind is around $ 50.00 USD. Employ water-saving shower heads, reduce time spent taking baths, shaving, brushing teeth, washing face, cars and doing dishes. To build water tanks and rain water containers. It is estimated that a water tank with a 20 000-liter capacity is around $ 1, 500.00 USD; water can also otherwise be collected in polyvinyl chloride (PVC) cisterns. Cisterns with a 10, 000-liter capacity are around $500.00 USD each. The aim is to use collected water for basic needs, which generally would otherwise go down the urban drainage system. This kind of water is very suitable for irrigation, laundry, and personal hygiene purposes. To recycle water, that water used in daily baths, in doing laundry, etc., is very convenient for later use in watering gardens, and if it follows an aerobic cleaning process it can be reutilized for doing further laundry and other household activities. To dramatically diminish the use of running water that fundamentally comes from underground deposits, from where great amounts of energy are needed to extract and use it. Conversely, to minimize residual water and provide less to a society that should also be capable of cleaning it before giving it back to nature. For a family of five members, it was proven that a decrease in running water consumption of 60 % was achieved by collecting rain water with a 20, 000-liter capacity tank, and it was enough to use through a five-month dry season. Once rain season arrived, the tank was re-filled to keep using that water. It was ascertained that only during a month of the year was it necessary to rely on running water to satisfy basic needs.

Conclusions

In terms of the geographical characteristics of the country, it is concluded that Mexico has never had enough water to sustain agricultural development, because the especial distribution of the territory is uneven. Where the best soil is to be found, there is not enough water on the surface or underground water, and where there are abundant water resources, there are not enough areas for extensive agricultural development, nor well developed soil. It is necessary to educate people about the water crisis, to raise awareness through constant advertisement campaigns, to implement the issue of water at the basis of environmental education, to

influence the youth to create in them life-long habits that care for water, to design and foster strategies that help in recovering residual water — in Mexico little water is recovered — not only with treatment plants or by irrigating fields with that same raw water, to design different collectors for graywater and sewage water. The former can be reutilized directly in gardens and for producing food. Sewage water, after being treated, can be reutilized or injected into aquifers. In agricultural production, simple flood irrigation must stop. Its efficiency in terms of conduction as well as in beneficial applications is low. The government must foster and provide the small-scale producer with economical support. Transitioning from traditional irrigation practices to advanced irrigation technology would yield more earnings, since water consumption is reduced by 30 % in comparison with traditional irrigation. Small-scale producers must also be provided with permanent technical support. It is necessary to return to proper water management for agricultural use in all related activities: it is not enough with coating irrigation canals; to guarantee that plots will yield 90 % efficiency and that growing crops will do the same with at least 70 % of direct application. If no action is undertaken for the sake of water in agriculture, which uses it the most, problems will become each time less manageable and more insurmountable.

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RESEARCH TRAINING IN THE MASTER'S AND DOCTORATE DEGREES ON ENVIRONMENTAL SCIENCES PROGRAM OF THE AUTONOMOUS UNIVERSITY OF THE STATE OF MEXICO

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Abstract

Research training on environmental sciences, in relation to climate change, is an issue that requires immediate attention. Around the world there is but a few researchers on environmental issues for every 100, 000 inhabitants. In Mexico, particularly in public universities, researchers working on the topic barely make up 0.9% of every 1000 inhabitants of the economically active population and, due to the neoliberal government that has ruled over this country, no medium-term improvement to the current situation is foreseen. Considering the circumstances, the present study addresses the case of research training in one of the most important state universities in Mexico: The Autonomous University of the State of Mexico (UAEMEX), regarding in particular the general experience in the Master's and Doctoral Degrees on Environmental Sciences Program. Through documentary research, a review of all theses developed during an eight-year period was carried out, using as indicators the lines of investigation that were developed, the number of theses presented each year, the type of study that was conducted in each of those papers, and the research techniques that were employed. The relevance of these programs in terms of research training in the field of environmental issues was analyzed , as well as its accomplishments, bringing it all to a conclusion with suggestions that may guide and improve the processes of research training on environmental issues related to climate change, in which the relevance of the epistemological construction of knowledge of environmental science with a holistic perspective is emphasized.

Key words: *knowledge generation, environmental research, environmental science, climate change.*

Introduction

The Master's and Doctoral Degrees on Environmental Sciences Program (PMDCA) of the Autonomous University of the State of Mexico (UAEMEX) was established in 2003 with the aim of specializing professionals to contribute in taking care of the environmental problems that the country faces in general and in particular in the State of Mexico (UAEMEX, 2003). It is housed in the School of Chemistry and emerged as a multi-and interdisciplinary effort that four other Schools of the University partake in: Geography, Engineering, Urban Planning, and Tourism. It is worth mentioning that most of the University's academic output is concentrated in this program (School of Chemistry, 2013).

One of the functions of any University's educational programs in postgraduate studies is to generate knowledge through the development of scientific research. The PMDCA does not possess a full research program; this is probably because all professors linked to the program belong to Academic Groups and, even then, some of them research environmental issues. Research conducted thus far belongs to the theses that alumni present to graduate; the lines of investigation followed in them are established by alumni or their lecturers. The objective of the current study consists in exploring the scope of the PMDCA in relation to the researchers

training in environmental field , its successes, as well as its problematics in generating new interdisciplinary environmental knowledge, with the overall aim of improving their processes in the researchers training and their medium and long-term achievements.

Materials and methods

With the aim of reflecting on the direction that the PMDCA is taking and the type of knowledge that it has been generating in a little bit over ten years since its creation, the research projects carried out by the alumni of the program in a period spanning from September 1, 2005 to April 13, 2013 were reviewed and analyzed in the present study. At that moment, the total number of theses registered in the program was 100, 78 of which corresponded to the master's degree graduated students and 22 to the doctorate degree graduated students. According to Sierra (2001), research can be divided into basic and applied. The first refers to the amount of possessed knowledge of the studied phenomenon, whereas the second implies the control, restructuring and transformation of the same. The following study lies in the basic investigation type due to the intention of only conducting an analysis on PMDCA research that is reflected in the postgraduate theses and developed in a determined time frame, with the overall aim of providing data relevant to the improvement of the PMDCA.

In an initial stage, through a deductive and quantitative approach to the results (Hernández et al., 2003; Pick and López, 2002; López, 1972), it was decided not to consider only the quantitative information, for which purpose a second stage of qualitative orientation was begun to find out the theorical quality and epistemological relevance of each thesis in generating new knowledge (Rojas, 1999; Victorina, 2015) in order to have a deeper knowledge of the outcomes. The assumption is made here that each of the 100 research papers are focused on understanding environmental processes and issues. The different fields belonging to each research were identified, as well as its objectives, considered topics, applied methods and techniques, as well as the methodic focus of each paper.

Results and discussion

The directors of the program insist that the purpose of the research that is conducted is to create comprehensive knowledge focused on understanding environmental processes. If it is to be considered that most of the analyzed theses address the environmental concern from both the natural sciences and social sciences perspectives, and that each, on a different depth level, refer to biophysical, social, cultural and political processes, it could be asserted that the aim of the program is met. However, a quantitative-type analysis of conducted research leads to a different conclusion. With the objective of carrying it out, all conducted research was grouped with basis on their covered topics. It was determined that the 100 theses refer to 11 topics or lines of generation and application of knowledge (formerly known as investigation lines): I. Climate Change; II. Pollution; III. Health Damage; IV. Sustainable Development; V. Environmental Management; VI. Environmental Impact; VII. Environmental Regulation; VIII. Hazardous Waste; IX. Environmental Risk; X. Environmental Technology; XI. Environmental Epistemology.

From those 12 lines of generation and application of knowledge, the most studied one was *Environmental Technology* (47 theses), followed by *Environmental Impact* (10) and *Environmental Regulation* (9). The least studied categories were *Environmental Risk, Climate Change, Health Damage, and Environmental Epistemology*, none of which caught the attention of more than three postgraduate students. If it were to be added to these results the fact that little research considers social concerns as components in environmental issues, the necessary conclusion is that the program is more apt to train in the technological than the social aspects.

Upon reviewing each of the covered topics, it was found that most research refers to problems related to chemical science, specifically, to problems about pollution. Such a tendency is considered natural since the program is housed in the School of Chemistry; it is due to this that most students and research are associated to this field of knowledge. What is striking is that those who have been trained in this field almost exclusively conduct studies on pollution; it is a tendency that seems to suggest that their view on environmental issues is rather limited, as they identify their involvement only in terms of such problems.

Additionally, aspects related to the graduation process were also reviewed. It was found that obtaining a degree is tightly adjusted to the compliance with criteria established by the authorities now in charge of postgraduate studies. It is clear without a doubt that emphasis has been made on keeping a level of efficiency superior to 80 percent; in such sense, this began two years after the start of the program, *i.e.*, in 2005. The years 2012 and 2011 stand out as the ones with greater output (22 and 19 respectively), and 2010 and 2005 as those with the least output (6 and 5).

Notwithstanding the erratic behaviour observed in the graduation process, which cannot be described as having established its own rhythm, and the dormant possibility that the program might disappear from the registry of the National Council for Science and Technology's (CONACYT) excellence programs (Román, 2018), the observed degree of compliance has thus far allowed it to be kept in the registry (CONACYT, 2015). The program's directors main concern in that sense is completely evident and is fundamentally due to how, in the current circumstances, budget availability is constrained by compliance with established parameters which are, to their fullest extent, quantitative in nature (Figure 1).

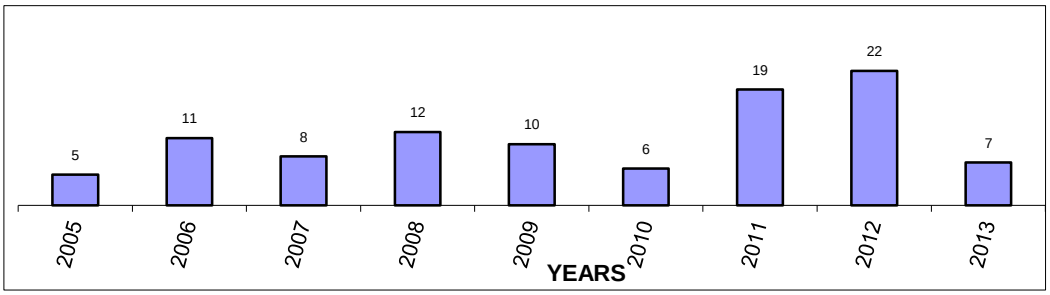

Figure 1. Master's and doctorate theses published yearly. Source: Elaborated by the authors.

The above goes to show how the program's directors have focused all their attention on fulfilling goals, which in turn causes other aspects of the program to fall into neglect, mainly those regarding knowledge production and environmental know-how (Leff, 1998). It is possible that for this reason most research carried out throughout the first 10 years of the program does not meet the understanding-explanatory level, which should otherwise be its primary characteristic since research conducted by postgraduate students is being dealt with; above all, theses corresponding to the doctorate degree should not leave any doubts in this regard. Nevertheless, only 15% of theses meet the alluded level of abstraction.

It can be said that generation of new knowledge is low, not only because little research meets the explanatory level, but also because the number of theses that meet the correlational level (39%) is still regarded as low, *i.e.*, the analysis of statistical relations with more than one variable, also because, for a postgraduate program, it is estimated that a very high amount of research remains at the descriptive (37%), exploratory (5%) and confirmatory (4%) levels (Figure 2)Another factor that allows for considering the generation of new knowledge as low is that related to the techniques used in conducted research. In most cases these are desk studies (42%), where data or information generated by other studies is used to conduct

laboratory simulations and replicate previously conducted experiments. This means that there is no direct investigation of environmental issues on any of its components or manifestations. However, it is fair to acknowledge that a tendency to conduct experimental research is observed (29%), where independent data is generated. Another tendency that is increasing in frequency is to carry out surveys (19%). The former is due to how, as has been said, a great number of master's degree students comes from the School of Chemistry itself, and they are used to experimental studies, which also explains the concentration of research conducted in that field of knowledge. The latter is subjected to how, inside the program, the belief is firm that environmental studies are not complete unless they reach a social knowledge of the issues at hand; the positive aspect of such conviction contradicts itself with the fact that social studies are being understood as the carrying out of little surveys, which blurs the purpose of including them when it comes to environmental studies.

A positive signal is that research that includes field work reaches up to 10%. This is a sign that the student's confronting of environmental reality is gaining preference, which allows to predict a substantial, medium term change. The globalization phenomenon, as part of capitalism, and its effects on education make up for a very extensive topic, which can be observed and analyzed from different perspectives. Currently, it is important to understand what is taking place in terms of both educational and environmental science studies issues, all of this within the global framework and its links to society (Velázquez, 2017). Let it be duly noted that the environmental problems which an increasing number of urban and rural communities go through because of the development of the neoliberal capitalist model do not play a part or are covered at all in the educational and research activities that are conducted (Figure 3).

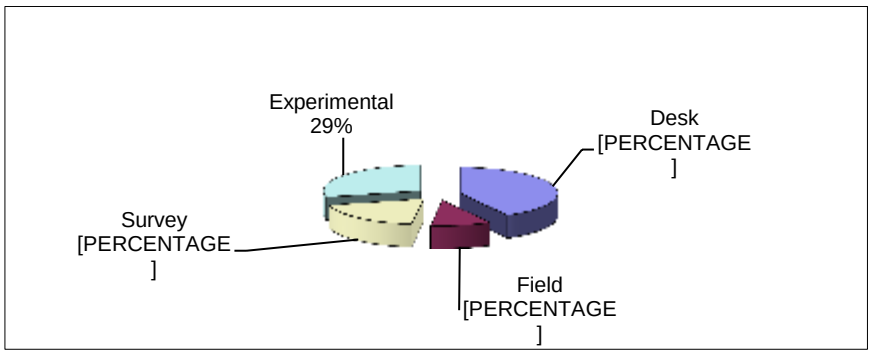

Figure 2. Theses according to study type. Source: Elaborated by the authors.

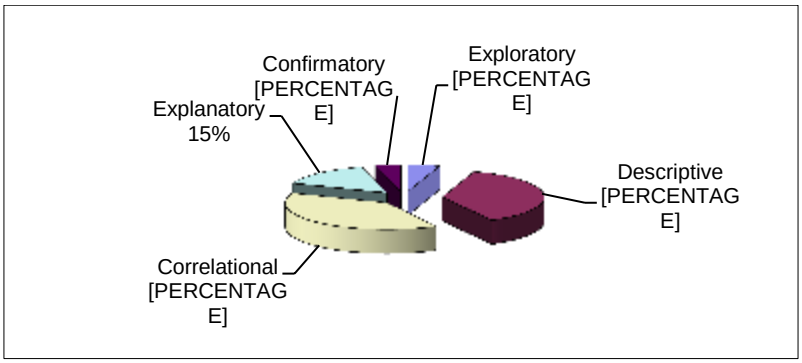

Figure 3. Theses according to used approach. Source: Elaborated by the authors.

Given that most research is technical in nature, studies that use a quantitative-type approach are predominant (59%), above those that offer a qualitative approach (9%). However, it is interesting to observe that a great amount of research is multimodal, or at least has a mixed

approach; *i.e.,* it uses both approaches (32%), so that it can be asserted that the program is moving forward in constructing environmental knowledge whenever both approaches are used (Table 1).

Table 1. Types of study according to approach

Approach		
Qualitative	Quantitative	Multimodal
9	59	32
TOTAL 100		

Conclusions

It is evident that the research that is conducted in the PMDCA focuses its attention on aspects related to environmental technology, such as: technology implementation, studies on damages to health arising from chemical pollution, water, soil and air pollution, while topics like climate change, environmental management, impact, risk and services as well as studies on environmental epistemology and ecological regulation are under-researched. It is a technical focus research rather than social. Such tendency matches studies conducted at a national level for this branch of knowledge.

Likewise, it is acknowledged that what is conducted within the PMDCA is primarily desk research since data or information generated in other studies is used to replicate experiments. The proportion of papers that stem from a direct approach to the studied phenomenon is low. Additionally, quantitative-approach research is being preferred, in which measuring data is what matters most. However, it is of the utmost importance to observe that there is a tendency to modify the referred trend. The propensity for specialized studies has not allowed for moving forward to a holistic view on environmental issues. To this it should be added that the desired level of knowledge has not yet reached the explanatory level that ought to belong to the advanced studies that the program alludes to. Studies historic in nature and with an interpretative approach are conspicuously absent or amount to only a few when all are considered. Although with limited relevance and faithfulness to its own objectives, it can be said that the PMDCA is contributing to the construction of environmental sciences in the state; more specifically, it contributes to the development of environmental chemistry, even though contributions are circumscribed to the field of environmental pollution. But little to no contribution is being made to the construction of environmental science as a whole; this is due to how, up until now, it has not been considered that environmental problems, more than just being technical or social ones, also represent problems in the construction of knowledge. An epistemological shift in the teaching and researching activities of the PMDCA is necessary and urgent.

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STUDY THE IMPACT OF CLIMATE CHANGES ON VEGETABLE CROPS FROM THE FARMERS POINT OF VIEW IN GAZA STRIP

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Abstract

This paper discusses the impact of climate changes on vegetable farmers in Gaza Strip. The objective of this study is to highlight the awareness of vegetable growers in different regions about the concept and elements of climate changes and how these changes effect on different types of vegetables in terms of growth rates, productivity and change of shape and color. Also, the objective of this study also is to highlight the impact of climate changes on the consumption quantity of water needed for vegetables growth. To achieve these objectives, the study relied on descriptive comparative analytical approach for various data collected from its primary sources through distribution of a questionnaire form to 104 farmers in the northern, central and southern Gaza Strip. This study has a lot of results, but the most important result is the existence of sufficient knowledge among the category of vegetable growers in Gaza Strip of the concepts and dimensions of climate changes, as 79.8% of them have sufficient knowledge of the concept and the dimensions of these changes. Also the results indicate that 96.2% of the vegetable growers have been affected by climate changes during the last period, 41.3% of them believe that the impact of these changes has been significant on the volume of agricultural productivity and on the quantity of water consumed for agriculture, 65.4% of them note that the climate changes have led to increasing in water consumption for agriculture. The results also indicate that 53.8% of vegetable growers believe that the wrong agricultural practices, as burning agricultural waste and excessive use of fertilizers and chemical pesticides have direct impact of climate changes, The study recommends the needed for facing the climate changes from vegetable growers by enhancing the concept of dealing with climate changes for these category of farmers and using new technologies to overcome these changes such as regular pressure irrigation techniques and water harvesting techniques.

Keywords: *climate change, vegetable farmers, pesticides, water harvesting, water consumption*

Introduction

Agricultural sector is one of the most important productive sectors in Palestine, it's contributing in leading the process of economic development because of its important role in enhancing food security and manpower. Also agricultural sector has an important role in contributing Palestine economic growth process over the past years, where it was the biggest economic sector in contributing Palestinian GDP with ratio 32% in 1993 which declined to be 5.6% during 2016 (Oumar, H. Qaoud, H (2016).

The observer of the general decline cause in agricultural sector in Palestine realize that this decline has a group of reasons which reflected on the productivity of this sector, perhaps the most important these reasons is the general political situation in Palestine represented by Israel occupation for the Palestinian territories which hinder any development in this sector. However, there are other reasons and factors caused this decline related to the general climate changes in the Middle East, which affected on Palestine in general and Gaza Strip in particular. The most important changes were temperature changes, wind, rain distribution and humidity, which have been associated with a change in agriculture times and type of

agricultural product, the productivity rate was reduced to more than 60% in most agricultural crops.

Materials and methods

First: Study Problem

Because of the importance of agricultural sector in achieving food security of the population specifically in Gaza Strip and the existence climate changes represented by sharp difference in temperature, humidity, rain distribution and wind, which directly reflected on the productivity of farmers in Gaza Strip especially vegetable farmers. The statistics indicates that 43% of farmers affected from high humidity levels and 45.3% of farmers negatively affected from rain intensity, 59.3% of them severely damaged from high temperature rate and high droughts and 50% of them negatively affected as a result of the intensity of wind during the last ten years in Gaza Strip. Therefore, the problem of the study can be formulated in the following main question:

What are the most important climate changes which have been affected on vegetable farmers in Gaza Strip?

A number of sub-questions are branched from this question:

1. Is there an impact from the changes of rates and distribution of rain on vegetable farmers in Gaza Strip?
2. Is there any effect from temperature changes on vegetable farmers in Gaza Strip?
3. Is there an impact from wind intensity changes and trends on vegetable farmers in Gaza Strip?

Second: Objectives of the Study

The main objectives of this study are as the following:

1. Identify farmers' awareness of climate changes and its different dimensions.
2. Identify the impact of climate changes on agricultural sector, both plant and animal.
3. Determining the relationship between climate changes and agricultural practices in Gaza Strip.
4. Identify the effect of climate changes on vegetable crops compared with fruit trees.

Methodology

First: Data collection methodology: Researchers depended on two tools for data collection, the first tool was the questionnaire which used to evaluate the impact of climate on a targeted group of vegetable farmers in Gaza Strip governorates specifically in Beit Hanoun - Gaza Valley - Deir Al Balah - Qarara - Abasan - Khuza'a - Rafah, while the second tool which used was the focus groups, 10 workshops were organized and 120 farmers were interviewed which chosen randomly to fill out the study questionnaire to clarify the impact of climate changes on them in terms of damages and it's economic dimensions.

Climate change in the Gaza Strip

Climate change is one of the most important global phenomena closely related to the agricultural sector in the world. These changes are related to a range of factors affecting the productivity of field crops and vegetables in general and in the Gaza Strip in particular. These elements are temperature, drought, The amount of rain and its various distributions, the rates humidity and wind , where these elements are the most important effects facing vegetable growers in the Gaza Strip because of the negative effects on the productivity of the agricultural sector of vegetables in Gaza Strip(Earth & Human Center for Researches and Studies,2018,P.5).Where the agricultural sector in general suffers from a range of negative effects of these changes and specifically during the past ten years, where the sharpness of climate change was concentrated in the lack of rain and high temperatures and drought, which reflected the effects on productivity in the agricultural sector where the productivity of seeds and vegetables decreased by 28.7% as a direct result of these changes and the inability of

farmers in Gaza Strip to adapt of these changes due to lack of advanced technologies that can be used in this process, which include water harvesting techniques, irrigation by regular pressure or the use of improved seed varieties adapted of this changed (Applied Research Institute Jerusalem, ARIJ, 2018, p. 19).

The reality of vegetable growing in Gaza Strip

Gaza Strip is characterized by the production of a variety of vegetables that meet the needs of the population of the Gaza Strip in a relative manner, where the production of tomatoes, potatoes and sweet pepper all colors, basil, mint, green onions, cucumber, spices and other types of vegetables and the total area cultivated in the Gaza Strip (400%), distributed over 330 thousand tons of vegetables and 70 thousand tons of fruits, including olives and citrus. The percentage of self-sufficiency in the production of vegetables in the Gaza Strip is 98%. Only a few crops are imported from vegetables at certain intervals According to the needs of the residents of the Gaza Strip (Al-Sakka, 2015).

With regard to the value of the Gaza Strip's production of vegetables, its value fluctuated in relation to a range of economic and political variables experienced by the Gaza Strip, where the maximum value of vegetables production in 2007 amounted to 199014 thousand dollars. This value was taken down in 2008-2010 by the Israeli war which In which agricultural land was affected by this war, which resulted in extensive destruction of the agricultural infrastructure, which led to a decline in the volume of vegetables production in the Gaza Strip, but the value of vegetable production was stabilized by the reconstruction projects that are called after the end The Israeli war for the rehabilitation of agricultural land, which averaged 115,200 thousand dollars during the years 2011-2015(Shawa, 2017, p. 27) .

The contribution of vegetables to the size of the plant production was related to the general economic and political variables, which were reflected in the size of the agricultural production of the agricultural sector as a whole in the Gaza Strip, where the maximum contribution of vegetables to the size of plant production in 2007 was 72.7%. Witnessed by the Gaza Strip, but stabilized at an average rate of 52% during the period 2008-2015.

The farmers in the Gaza Strip face many difficulties and challenges, the most important of which is the weakness of the infrastructure needed to grow vegetables in the Gaza Strip and the shortage of water needed for agriculture and the low quality due to the high salinity rates due to the increasing consumption of the reservoir that does not meet the needs of the population, and the lack of water in the Gaza Strip. The researcher interviewed Engineer. Wael Thabet, Palestinian MOA (4 July 2018)

Second: Systematic Analysis of Data:

Researchers used comparative descriptive analytical method as it's the most appropriate method for this study. This method used to study the demographic variables of the target areas, in addition to the different vegetable group which cultivated in Gaza Strip and farmers' culture about climate changes and how to deal with it, SPSS program has been used for data analysis and access to the targeted results. Al grad, K.M (2009).

Analysis and discussion the results of study:

Section One: personal information

In the framework of monitoring the attitudes and opinions of vegetable farmers regarding to the impact of climate changes on crops, the focus was on the most agricultural areas in Gaza Strip which contribute effectively to provide food security for the population in Gaza Strip. Central governorates of Gaza Strip was 43% from the total targeted respondents because it's the largest agricultural governorates in the strip, followed by southern governorates of Gaza Strip by 36% and finally the northern governorates of Gaza Strip by 21%. Rural women in the target group was 10% of the sample size, while male farmers was 90% of the total size of this category. The poor representation of rural women is due to the nature of agricultural holdings in Gaza Strip, where most of these holdings are for men, also the nature of

agricultural work is dominated by men in Gaza Strip, In addition, most of workers in vegetable category within the target group are more than 40 years, followed by the age group from 35 to less than 40 years, and the lowest age groups working in vegetable cultivation field are under the age of 25, which clearly indicates the weakness participation of young groups in agricultural work, this may be due to the fact that the nature of vegetable cultivation requires years of experience in agricultural work and heading the youth towards services jobs more than productive works which requires a high level of mental and intellectual effort. It is important to note here that the most of these workers are holding the General Secondary Education Certificate with ratio 33.7%, followed by the university graduates with ratio 21.2% and the ratio of intermediate certificate holders were close to the primary education certificate holders (20.2% and 19.2%, respectively) and finally the percentage of illiterate vegetable workers represented only 5.8%, These figures indicate that the cultivation of vegetables requires medium level of education which concentrated in general secondary school certificate holders, also the concentrate of workers in this category is due to the high unemployment rate among university graduates in Gaza Strip by 46% in Gaza Strip which led a large group of high school graduates, especially the children of farmers, to work with their family members because getting work has a priority more than university education because of lack of horizon for university graduates to provide jobs after graduation, also the orientation of university graduates to work in the agricultural sector has become limited because of their desire to work in comfortable jobs, especially in services sector, which justifies the low percentage of university graduates which working in vegetable growing field by 21.2% and may be most of those worker concentrated in agricultural colleges graduates.

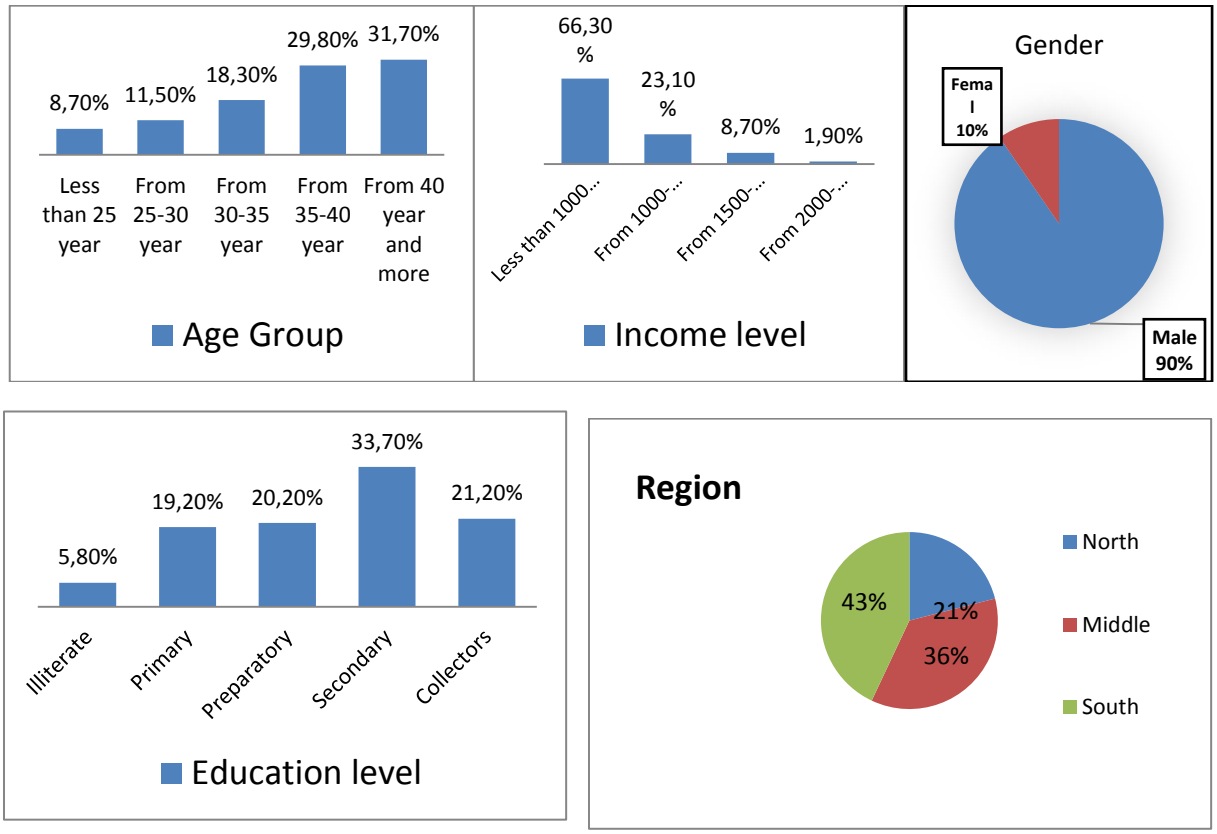

Figure (1) shows the personal information of the targeted group in the study

First: Awareness of climatic changes for vegetable farmers in Gaza Strip

In the framework of monitoring the awareness of vegetable farmers in Gaza Strip about the concepts of climate changes in its different dimensions, the results showed that most of vegetable farmers have a general awareness of the concepts of climate changes in Gaza Strip, as 80.8% of them heard about the concepts of these changes, the awareness of these changes differed between them according to their understanding of these changes, as most of these farmers believe that the concept of climate change is limited in the changes in the weather in terms of water level change as expressed by 93% of them, while 92.3% of them see that the concepts of climate change are limited in the changes in weather in terms of drought and storms, 90.4% believe that change in rain seasons represent the concept of climate changes, and 81.7% believe that temperature changes mainly represent the concept of climate changes, It is important to note here that the lowest percentage of vegetable farmers believe that all the dimensions like rain, heat and wind, combined to represent the concept of climate changes, which is expressed by 20.2% of vegetable growers. Hence, the awareness of vegetable farmers of the concepts of climate changes varies from farmer to farmer according to the effect these changes from region to region, but the final result showed the awareness of the concepts of climate changes between vegetable growers in Gaza Strip with differ in the awareness of the dimensions of these changes according to the impact of each dimension from one geographical area to another within Gaza Strip, where farmers in southern governorates suffer from higher temperatures and drought more than central and southern governorates, while the impact of rain changes in northern governorates is more than the other governorates. The following table shows the concepts of climate changes and its dimensions for vegetable farmers in the governorates of Gaza Strip.

Table (1) Awareness of the concept of climate changes for vegetable farmers in Gaza Strip

Answer	Number		%	
Did you hear about the climate changes before?				
Yes	84		80.80%	
No	11		10.60%	
Not Sure	9		8.70%	
What is the concept of climate change?	Yes		No	
	Number	%	Number	%
Change in temperature	19	18.30%	85	81.70%
Change in water level	7	6.70%	97	93.30%
Rain seasons change	10	9.60%	94	90.40%
Weather change (drought and storms)	8	7.70%	96	92.30%
All the above	83	79.80%	21	20.20%

Second: the reasons of climate changes and its relation with agricultural practices

In the framework of determining the causes that contribute the impact of climate changes in Gaza Strip, the wrong agricultural practices has the greatest correlation with the impact of these changes on vegetable farmers in Gaza Strip, as 80.8% of them believe that wrong agricultural practices is one of the main reasons for spreading the phenomenon of climate changes, where 46.2% of them believe that the burning of agricultural waste, excessive use of pesticides and chemical fertilizers and excessive use of water, are the most important forms of wrong agricultural practices that contributed in expansion the climate changes. The highest percentage of vegetable farmers considered that the excessive use of water and agricultural waste burning have the highest relative weight in affecting on the volume of climate changes

by 83.7% and 82.7%, respectively, then the excessive use of chemical fertilizers and pesticides by 81.7% and 76.5% respectively. Hence, all these dimensions are considered one of the most important variables related with climate changes in Gaza Strip. The following table illustrates the relationship between the different agricultural practices and the climatic changes in Gaza Strip.

Table (2) the relationship between wrong agricultural practices and climate changes

Answer	Number		%	
Are wrong agricultural practices have an impact on climate changes?				
Yes	84		80.80%	
No	20		19.20%	
If yes, choose the following:	Yes		No	
	Number	%	Number	%
Burn agricultural wastes	18	17.30%	86	82.70%
Excessive use of chemical fertilizers	19	18.30%	85	81.70%
Use agricultural pesticides	24	23.10%	80	76.90%
Overuse of water	17	16.30%	87	83.70%
All of the above	56	53.80%	48	46.20%

Third: The impact of climate changes on vegetable growers in Gaza Strip

The results indicate that the impact of climate changes on vegetable growers in Gaza Strip was significant, with 96.2% of farmers affected by these changes, while 3.8% of them were not affected by these changes. As for the degree of impact of these changes on vegetable growers, 41.3% of them indicated that the impact of these changes was significant, while 49% indicated that the effects was moderate, 5.8% indicated that the effect of these changes was weak, and the lowest percentage 3.8% were not affected by these changes. The following table illustrates the nature and the degree of these effects.

Table (3) The Effect of Climate Changes on Vegetable farmers in Gaza Strip

Answer	Number	%
Did the climate changes effect on your farm this year?		
Yes	100	96.20%
No	4	3.80%
How far is this effect?	Number	%
Significant effect	43	41.30%
Average effect	51	49.00%
Weak effect	6	5.80%
no effect	4	3.80%

It is note that the most affected crops due to climate change, especially in relation with the effect of temperature intensity as one of the most important dimensions of these changes, was potatoes as 77.9% of vegetable farmers see this. Then the eggplant by 69.2% and finally peppers and cabbage with the same ratio 65.4%, the following table shows the effect of these changes on different vegetable crops.

Table (4) the Effect of Climate Changes on Vegetable Crops in Gaza Strip

What kind of vegetable has been affected by the heat in the previous days?	Yes		No	
	Number	%	Number	%
pepper	36	34.60%	68	65.40%
Eggplant	32	30.80%	72	69.20%
Potatoes	23	22.10%	81	77.90%
Flower and cabbage	36	34.60%	68	65.40%

Agricultural seasons are considered the most important indicators of climate changes which associated with rainy seasons. The different agriculture periods have a direct effect on productivity. 95.2% of vegetable farmers believe that the different agriculture periods in the seasons had the highest impact on productivity. 73.1% believe that the degree of this effect was high, while 26.9% believe that the degree of this effect was weak, as shows in figure (2).

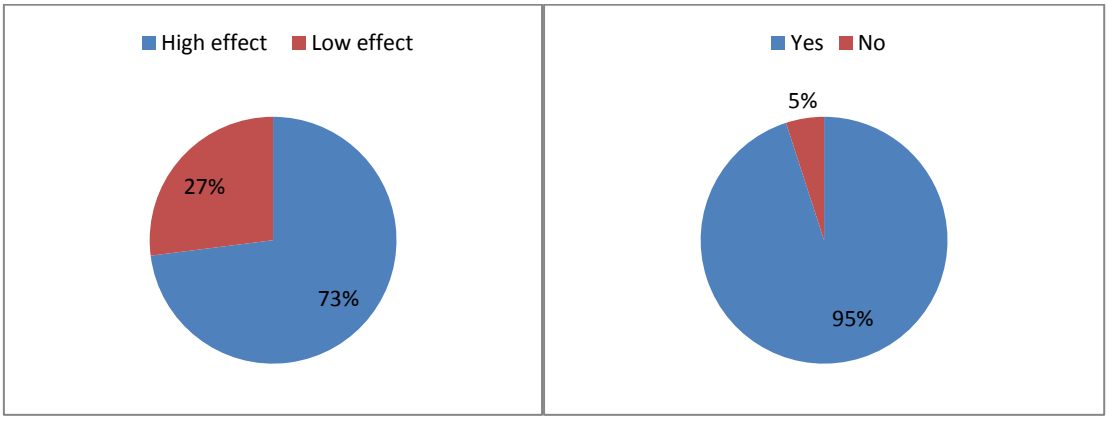

Figure (2): illustrates the belief of the agricultural periods have an impact on agricultural productivity

Fourth: The effect of climate changes on the characteristics of plant

Temperature is one of the most important forms of climate changes affected on the productivity of vegetable crops. The differences in temperature degree has a significant impact on these crops. 84.6% of vegetable growers believe that the differences in temperature degree has the greatest effect on plants in terms of shape. The impact of this differences on color was in the second degree with 83.7%, followed by the effect of temperature differences on plant growth by 66.3%. These changes have an effect on productivity as 59.6% of vegetable farmers seeing this. As for the effect of these changes on total characteristics of plant, 51% of Vegetable growers believe that the difference in temperature degree led to changes in the shape, color, growth rates and productivity of vegetable crops. As shows in Figure (3).

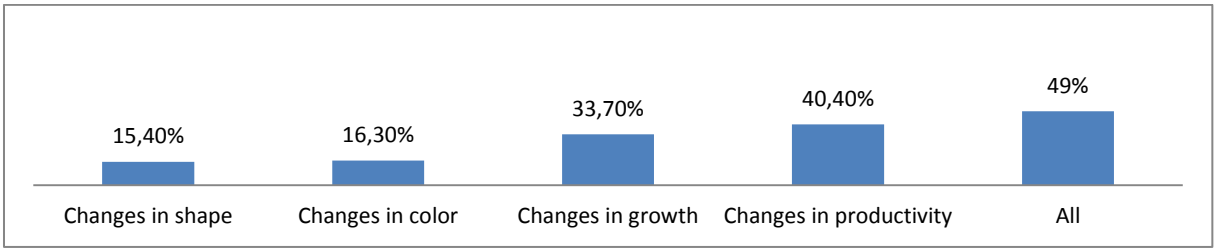

Figure (3) shows the effect of climate changes on plant characteristics

Fifth: The impact of climate changes on the productivity of vegetables

Productivity volume of agricultural crops is the most important factor that governs the economic empowerment of farmers. All measures taken by farmers in terms of technical aspects of plant care, are aimed to maximize the productivity. 65.4% of vegetable farmers believe that climate changes had a significant impact on the volume of vegetables productivity in Gaza Strip, while 27.9% believe that the effect of this changes was medium, 4.8% of vegetable farmers believe that the effects of these changes was weak on the volume Of productivity as shows in figure (4)

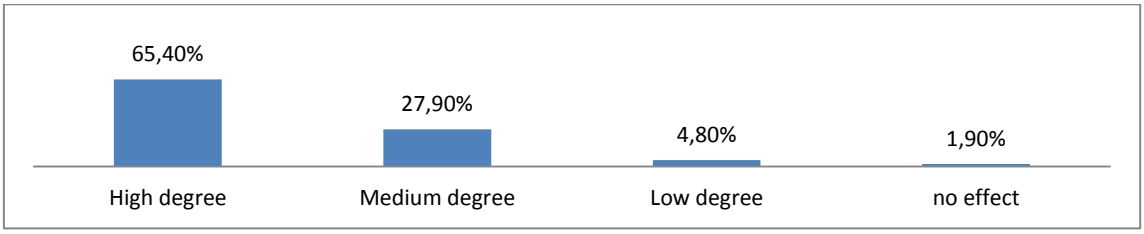

Figure (4) shows the effect of climate changes on the productivity of vegetables

Sixth: The impact of climate changes on water consumption rates

Water issue is one of the most important dilemmas face agricultural sector in Gaza Strip because of water scarcity and high salinity rates. Most reports indicated that water is not suitable for human consumption in Gaza Strip. The impact of climate changes on water consumption volume in the agricultural sector is considered to be one of the most important issues in Gaza Strip, the impact of climate changes on water consumption rate in Gaza Strip was significant. 65.4% of the farmers consider that there is an increases in water consumption for agriculture as a result of these changes, while 27.9% of vegetable growers found that the degree of impact of these changes on water consumption was medium, 4.8% believe that effect of these changes was weak, and finally, 1.9% did not see any effect of these changes on water consumption rates, The following table illustrates this.

Table (5) the Effect of Climate Changes on Water Consumption

Did climate changes effect on water consumption?	Number	%
High degree	68	65.40%
Medium degree	29	27.90%
Low degree	5	4.80%
no effect	2	1.90%

Seventh: The impact of climate changes on fruit tree farmers

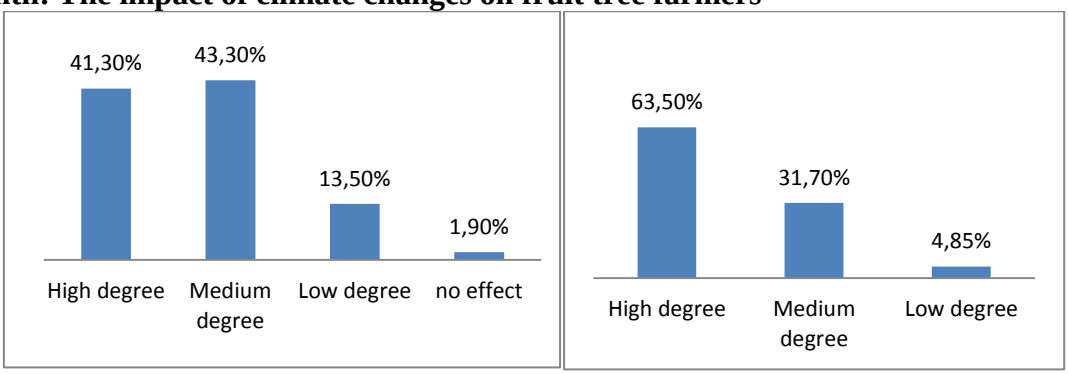

Figure (5) illustrates the impact of climate changes on fruit tree farmers

Climate changes has not only affected on vegetable growers, but has also extended to fruit farmers. The effects of climate changes was the highest on vegetable farmers, while this effect was lower on fruit tree farmers, 43.3% believe that this effect was moderate, 41.3% believe that it was significant, 13.5% believe that it was weak, and 1.9% did not see any effect of climate changes on fruit trees. As shows in Figure (5).

Eighth: The impact of climate changes on animal breeders

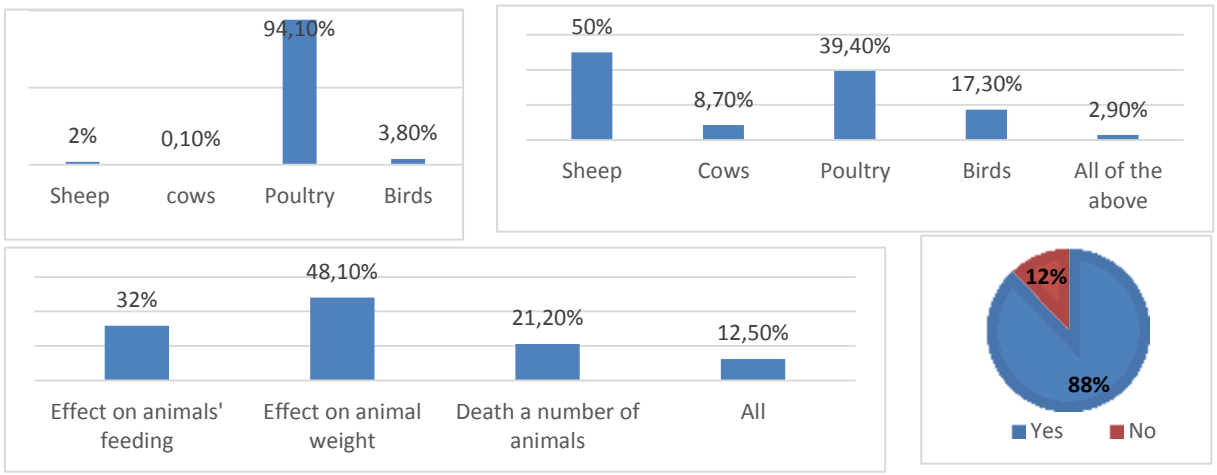

Figure (6) illustrates the impact of climate changes on livestock breeders

88.5% of vegetable farmers considered as livestock breeders, 97.1% of whom are breeders of cows, livestock and birds in various shapes, these farmers believe that the biggest effect of climate changes causing animal and bird aversion, where 78.8% of them see this, most farmers see that the aversion rates of was concentrated in poultry sector with ratio 94.1%, while 68.3% believe that climate changes affected on feeding of animals and birds. 51.9% see that these changes led to a decrease in the weights of these animals, figure (6) shows this.

Ninth: The impact of climate changes on the prices of vegetables in the markets

The effect of climate changes on vegetable and vegetable farmers in terms of productivity and plant characteristics has been reflected in the cost of production and therefore on the prices of vegetables in the markets, where 63.5% of vegetable farmers see that the climate changes led to increase the prices in the market, 31.7% see that the climate changes affected moderately on the prices of vegetables and finally 4.8% see that the reflection of these changes on prices was weak and the following table shows this.

Table 5: table caption

Because of climate changes (high temperatures), Did the prices of vegetables in the market has been affected?	Number	%
High degree	66	63.50%
Medium degree	33	31.70%
Low degree	5	4.85%

Results and discussion

First: Results

1- Most vegetable farmers in Gaza Strip are aware the concepts of climate changes, as 80.8% of them aware these concepts in terms of the different dimensions of it, and the difference is in the extent of their focus on these dimensions according to the different geographic regions within Gaza Strip

2- The highest percentage of vegetables farmers (91.3%) believe that the climatic changes in Gaza Strip are limited in changes in weather in terms of drought, storms and change in rainfall seasons.
3- Most vegetables farmers are linking between the spread of wrong agricultural practices and phenomenon of climate changes, with 80.8% of them see that the spread of wrong agricultural practices like burn agricultural waste and the excessive use of fertilizers and chemical pesticides, are the main cause that contribute the increasing of climate changes in Gaza strip.
4- Most vegetables farmers in Gaza Strip with ratio 96.2%, were affected from various climatic changes, while 49% of them see that the degree of impact of these changes on them was from medium to high degree, while 41.3% of them see that the impact of these changes was very high.
5- Most vegetable crops which affected by climate changes was potatoes. 77.9% of vegetable farmers are seeing this, followed by eggplant (69.2%) and peppers, cabbage (65.4%), all of these crops were adversely affected by these changes.
6- Temperature degree, as one of the elements of climate change, had the highest impact on vegetable growers, as 84.6% of vegetable growers believe that temperature had the highest impact on the plant in terms of shape, color, growth rate and productivity.
7- There is a relationship between the dimensions of the various climatic changes and water consumption rates. Climate changes has a significant impact on water consumption rates in Gaza Strip, with 65.4% of vegetable growers see increase in water consumption for agriculture due to these changes.
8- Increasing of phenomenon of climate changes in Gaza Strip led to a rise in vegetables prices in the markets, as 63.5% of the vegetable farmers believe that these changes led to a significant increase in prices because these changes are relating with the productivity volume and plant characteristics, which reflected on production cost, thus on the input and production prices.
9- Climate changes has been affected on fruit tree farmers and livestock breeders from vegetable farmers, where most fruit tree growers see that the effects of these changes was medium with ratio 43.3%. As for the impact of these changes on livestock breeders, it cause aversion of animals and birds, where 78.8% see this.
Second: Recommendations
1- The competent authorities should endeavor to find ways to support the adaptation of vegetable growers with climate changes and to reduce the impact of these changes on them by introducing new techniques for agricultural work which contribute in reducing the negative impact of changes
2- Introduce the techniques of water harvesting and irrigation techniques by regular pressure as one of the most important methods to combat the scarcity of water which result from low rainfall, increasing heat and drought, as these means will contribute the provision of water sources for agriculture, in addition, it will be reduce the volume of water losses which used in irrigation
3- Intensify awareness campaigns for vegetable growers to face climate changes, especially temperature, rain, wind and humidity, through using irrigation techniques, spraying pesticides, windbreaks and rainwater harvesting pools.
4- Work to educate farmers about the ideal methods of field management to reduce the wrong agricultural practices that contribute widely in spreading the phenomenon of climate changes in Gaza Strip.

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THE INFLUENCE OF LIMING WITH FLY-ASH OF KOSOVO POWERPLANTS ON CHEMICAL AND PHYSICAL PROPERTIES OF DISTRIC CAMBISOL

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Abstract

Increased acidity is very often a limiting factor for decreased soil fertility and unfavorable chemical and physical soil properties. Fly-ash of Kosovo power plants appears as the waste material after burning in the reactors. Material itself causes serious environmental consequences, especially because it is hardly remediated, due to its chemical and physical properties. Since 1963. Kosovo power plants have formed huge fly-ash dumps of very poor remediability, with over than 200.000mt of fly-ash. But, despite of its low remediability, the material has properties to be used in liming of acid soils. The fact that fly-ash of Kosovo powerplants is alkaline, with relatively high content of CaO and $CaCO_3$, recommends it as a material which can be successfully used in melioration of acid soils. In our experiment we have used soil type Distric Cambisol, as a typical acid soil for the region of Kosovo. The results show applied liming material as proper material for use in liming the acid soils. By use the fly-ash for amelioration of acid soil, we have reached significant improvement of pH and other chemical properties, as well as the stability of soil structure.

Key words: *Fly-ash, Kosovo powerplants, liming, acid soils.*

Introduction

Fly-ash occurs as a result of coal combustion in power plants poses a serious environmental problem. On the one hand, the ash landfill is deposited on agricultural land, which excludes them from agricultural production, and on the other hand EF ash is subject to eolation, which affects the quality of the environment kilometers around (Resulović, 1988.). Coal burning in Kosovo's power plants has been carried out since 1963 with the opening of a thermal power plant in Kosovo „A" power 449 MW, which was particularly intensified by the opening of another power plant Kosovo „B" power 580 MW 1980. year. During all the work of Kosovo's thermal power plants, ash dumps have been formed, which involve 2,000 hectares of arable land. Moreover, in EF ashes the dust fraction prevails, which allows its eolation, thus adversely affecting the environment and agricultural crops. According to the reccurability of ash deposits, they are hardly recurable (Cairney, 1987.), and therefore pose an additional problem in the living environment of the area. (Šmit, 1988). The cause of poor recurrence lies also in the chemical properties of EF ash, both in terms of high alkalinity and in terms of cementation of the surface layer of landfills (Đikić et al., 1995.). However, the fact that EF is an alkaline reaction with very high content of CaO recommends the research of its application in alkaline acid calcification. Calculation is a pedomeliorative measure of repair of acidic soils and the materials to which it is made may be different: CaO, CaCO3, dolomit, saturation sludge and others. This study recommends EF ash as a calibration material, given its chemical and physical properties. The available amount of ash is quite sufficient to consider its commercial use in the pessimism.

Materials and Methods

The research used the pedological map of the AP of Kosovo and Metohija (Institut „Jaroslav Černi", 1974.), as well as the soil type Distric cambisol from Klina near Pec. The chemical properties of ash were analyzed by standard methods (Black et al. 1965.). The experient was founded as an indoor one, in experimental pots of volume of 4 dm^3. Soil moisture was mainetned on the level of retentional capacity (33kPa).

The characteristics of the EF ash are presented in the table 2, as well as the properties of the soil used in the experiment (table 1). The use of EF ash was 10 (dosage 1), 30 (dosage 2) and 50 (dosage 3) tha^{-1}, with control without the use of calcification agent. Compost is used as an aditional fertilizer, for the purpose of achieving better solubility of the calcification agent (Banasova, 1989, Flaig, 1981). The culture of cultivation in this regard was barley. Barley was chosen as a typical culture of a given area.

The results were tabulated as well (Table 3). Statistic analyze have been done accordingly, and presented as well.

Results of research and discussion

The soil used in the survey was taken with k.r. Bica, Klina - Pec and by type belongs to distric cambisol. The chemical properties of the soil treated in the sample are shown in the table 1.

Table 1. Chemical properties of soil in the experiment

Depth	pH_{H_2O}	pH_{KCl}	Humus (%)	N (%)	P2O5 (mg/100g)	K2O (mg/100g)	H (meq/100g)	CaCO3
0-12	5,1	4,3	1,2	0,22	7,5	30	22,5	-
13-27	5,0	4,1	0,5	0,14	5,4	25	20,4	-
28-56	5,2	4,2	0	0,10	3,0	20	32,2	-
56-100	5,4	4,4	0	0,10	2,0	25	30,0	-

As seen from the table shown, the soil in the experiment is very acidic with very low humus, with a somewhat higher amount of phosphorus than expected because it was agricultural land as well as with a slightly increased amount of other nutrients for the same reason. Hydrolitic acidity, shows levels of necesarity of liming the soil. The characteristics of EF ash are shown in the table 2.

Table 2. Chemical properties of fly-ash from Kosovo powerplants

Depth	pH_{H2O}	pH_{KCl}	CaO (%)	Na (%)	P2O5 (mg/100g)	K2O (mg/100g)	CaCO3
0-10	10,52	10,0	47,5	1,22	8,5	>40	15,2
11-20	9,11	8,85	41,5	1,14	5,4	>40	18,4
20-30	10,00	9,21	37,2	0,90	3,0	>40	9,2
30-40	9,85	9,45	45,0	1,10	2,0	>40	8,5

The influence of liming material on amelioration of distric cambisol with fly-ash is statisticaly analyzed, and the results have are presented in Table 3.

By unifactorial univariant analysis of variance it was determined existance of statisticaly significant difference at parameters pH_{KCl}, N, K_2O, $CaCO_3$ and H depended on dosage. With the additional *Post-hoc* analyze with using of LSD test, it was determined that mean values of pH_{KCl} are significantly different at dosages 1 and 2, as well at the dosages 2 and 3, while at dosages 1 nad 3 there is no statistically significant difference. Median value of N(%) are significantly statisticaly diferent for dosages 1 and 3 and dosages 2and 3, but there is no statistically signifficant difference at dosages 2 and 1. Analyzing median values of K_2O, it is obvious that statistically signifficant difference exists only between dosages 1 and 3, while

mean values of $CaCO_3$, dosage 1 is significantly different from dosages 2 and 3. Analyzing mean values of hydrolytic acidity, signifficant difference exist at first dosage, compared to second and third.

Table 3. Influence of fly-ash on the soil type distric cambisol in the experiment

Dosage		pH (H2O)	pH (KCl)	Humus (%)	N (%)	P2O5 (mg/100g)	K2O (mg/100g)	CaCO3	H (meq /100g)
1	Mean	6,1000	5,8333	2,7667	,2367	8,4667	35,0000	1,0233	8,8667
	N	3	3	3	3	3	3	3	3
	Std. Deviation	,20000	,11547	,25166	,01528	,20817	3,00000	,02517	,63509
	Std. Error of Mean	,11547	,06667	,14530	,00882	,12019	1,73205	,01453	,36667
	Minimum	5,90	5,70	2,50	,22	8,30	32,00	1,00	8,50
	Maximum	6,30	5,90	3,00	,25	8,70	38,00	1,05	9,60
2	Mean	6,3000	5,2000	2,4000	,2233	8,0667	31,0000	2,1333	7,3667
	N	3	3	3	3	3	3	3	3
	Std. Deviation	,17321	,17321	,20000	,00577	,32146	1,00000	,32146	,15275
	Std. Error of Mean	,10000	,10000	,11547	,00333	,18559	,57735	,18559	,08819
	Minimum	6,10	5,10	2,20	,22	7,70	30,00	1,90	7,20
	Maximum	6,40	5,40	2,60	,23	8,30	32,00	2,50	7,50
3	Mean	6,5000	5,8667	2,5000	,3033	8,0333	25,0000	1,9333	6,9667
	N	3	3	3	3	3	3	3	3
	Std. Deviation	,26458	,05774	,30000	,03055	,25166	4,35890	,05774	,11547
	Std. Error of Mean	,15275	,03333	,17321	,01764	,14530	2,51661	,03333	,06667
	Minimum	6,20	5,80	2,20	,27	7,80	22,00	1,90	6,90
	Maximum	6,70	5,90	2,80	,33	8,30	30,00	2,00	7,10

As the table clearly shows the melioration of distric cambisol EF ash from Kosovo's thermal power plants, there has been a significant increase in pH. Changing the chemical properties of distric cambisol enabled better nutritional utilization, better cultivation of cultured culture (barley barley), as well as higher yields.

Conclusion

The results of the study of the impact of EF ashes on the repair of distric cambisol indicate that EF ash from the point of view of its chemical properties is a suitable material for the calcification of acidic soils of the distric cambisol type. This is particularly conditioned by the high content of CaO in ash, as well as by absence Na^+ ions.

The performance of the EF ash provided better feedability of the nutrients, which significantly influenced the yield of cultivated culture.

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THE INFLUENCE OF LIMING ON THE ACIDITY LEVEL OF DYSTRIC CAMBISOL AND CONTENT OF AVAILABLE FORMS OF: IRON, ZINC AND COPPER

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Abstract

Limited fertility of acid soils is caused by a high concentration of H^+ and Al^{3+}ions, presence of some organic acids and heavy metals, but also by a low accessibility of some nutrients (P, Ca, Mg, B, Zn, particularly Mo) and a low microbial activity. This study has been conducted to determine the effects of three levels of liming (partial – 1/3 Y1, half – 1/2 Y1, and complete liming) on the neutralization of the acid reaction, a high content of mobile Al^{3+}, and changes in the concentrations of available forms of Fe, Zn, and Cu in a Dystric Cambisol soil. The complete liming almost completely neutralized the acid reaction, and decreased the level of mobile Al^{3+} below 1.0 mg kg^{-1}. There has been a satisfactory degree of decrease in pH and Al^{3+} in partial (1/3 of Y1) and half (1/2 of Y1) liming. No level of liming had a significant influence on the content of available forms of Fe and Cu, while the content of Zn decreased in accordance to the level of applied lime material and it was the lowest at the maximum doses of CaO applied. The level of changes caused by partial and half-liming has justified these levels of acid repairing, which can be of significant ecological and economic importance.

Keywords: *Dystric Cambisol, liming, aluminum, micro nutrients*

Introduction

There are numerous factors that limit the fertility of acid soils. High concentrations of H and Al ions, some organic acids and heavy metals, as well as low accessibility of nutrients (P, Ca, Mg, B, Zn, particularly Mo) are just some of them. However, Al-toxicity is a major stress factor for plants on soils whose pH ≤ 5.5 (Poschenrieder *et al.*, 2008; Merino-Gergichevich *et al.*, 2010), and in these conditions, the prevailing pressure for the adaptation of cultivated plants is attributed to it (Ryan and Delhaize, 2010). The acidic environment serves to increase the presence of trivalent aluminum cations – Al^{3+} (Lidon and Barreiro, 2002; Kochian *et al.*, 2005), which is the most poisonous of all kinds of Al. More recognizable effects of Al-toxicity have been observed and well-described on the root (Barceló and Poschenrieder 2002; Panda and Matsumoto, 2007). However, damages may be present even on the upper parts of the plants (Merino-Gergichevich *et al.*, 2010), especially on the leaves, about which little is known. Today, there is growing evidence of the negative impact of aluminum on the light absorption, photosynthetic electron transport, gas exchange (Chen *et al.*, 2005a; Chen *et al.*, 2005b; Chen, 2006), photoprotective systems (Chen *et al.*, 2005a; Ali *et al.*, 2008), pigments (Chen *et al.*, 2005a; Mihailovic *et al.*, 2008; Milivojević *et al.*, 2000), as well as on other elements related to the structure or function of the photosynthetic apparatus. In addition to the direct effect, Al also affects plants indirectly, in such a way that aluminum ions, among other things, block the adsorption of phosphorus and potassium, thus disrupting the growth and development of crops (Zheng, 2010). The availability of microelements in different soils, apart from the presence of phosphorus and organic matter, is significantly conditioned by their pH value (Wei *et al.*, 2006; Asadu *et al.*, 2014). Thus, the availability of micronutrients

and toxic ions, as cations (e.g. Al^{3+}, Fe^{3+}, Mn^{2+}), increases with an increase of soil acidity (Porter *et al.*, 2004; Khabaz-Saberi and Rengel, 2010). After Al, an excess of Mn is the greatest factor of limited plant growth on acid soils (Rengel, 2000). Like Al, when manganese reaches the level of toxicity, it also limits the growth and function of plant roots, reduces the absorption of nutrients and water. Liming acid soils is one of the key measures that can preserve or increase their productivity (Mao *et al.*, 2008). That is why this research aimed to, by applying different levels of liming on Dystric Cambisol in the year of application, define their effect on the rate of change of acidity, the content of mobile Al, and some micronutrients (Fe, Zn, Cu) as important factors in fertility.

Material and Methods

The research was conducted in 2010 and 2011 on an experimental field near the town of Leposavic (43^0 16' N; 20^0 36' E), belonging to the southern and central part of the Ibar-Kopaonik region, and located in southwestern Serbia. Leposavic is located at an altitude of 545 m, and is characterized by a temperate continental climate. The soil on which the research was performed belonged to the Dystric Cambisol type. CaO of high degree of fineness was used for liming. In both years, the material was applied in September, more precisely before basic processing, by being properly distributed over the surface and entered into the soil by plowing. The applied rates of CaO were calculated according to the value of Y1 in the soil and the size of the experimental site (50 m^2). Three variants of liming were determined and applied: 1/3 Y1 CaO (V-3), 1/2 Y1 CaO (V-4), and Y1 CaO (V-5); as well as two variants without liming: the version with only the application of NPK (V-2), and the version without liming and fertilizer application – control (V-1). Fertilizers were also applied in the versions where liming was performed. In all cases, the doses of active compounds of nitrogen, phosphorus, and potassium were for N 120 kg ha^{-1}, and for P and K per 90 kg ha^{-1}. Fertilization was performed according to the standard technology for the wheat production. The experiment was set up as a random complete block design (RCBD) in four replications. The size of the base experimental site was 50 m^2, and the crop was wheat, Pobeda cultivar. In both years of research, during the stage of wheat tillering (T), 5 months since the liming and after the harvest (A. H.), 10 months since the liming, the pH was determined on a pH meter with a glass electrode in a 1: 2.5 suspension with water and 1 M KCl. At the same time, the content of exchangeable or mobile Al was determined by using Sokolov's method on a soil extract with 1M KCl by first determining the total substitutional acidity, and then by a deposition of aluminum with NaF and the share of Al^{3+} in the formation of substitutional acidity. Hydrolytic acidity, that is, Y1, was determined only after the harvest and by Kappen's method by treating a soil sample with $(CH_3COO)_2Ca$, and then the neutralization of excess acid was performed with 0.1 M NaOH. The content of available forms of soil microelements (Fe, Cu, and Zn) was determined by atomic absorption spectrophotometry. Germany. The content of available Fe was determined after the extraction into a solution of 1M CH_3COONH_4 (pH 7) in the stage of wheat tillering (T) and after the harvest (A.H.), and for the available Cu and Zn, only after the harvest, upon the extraction (A.H.) in 0.1 M HCl.
Statistical analyses were performed on SPSS software, variant 16. The effects of the treatment on all variants were tested by ANOVA. Statistical differences between the treatments were determined by using the t-test (95 and 99%) by Pearson for Fisher's LSD (SPSS, 2007).

Results and Discussion

The effect of three levels of liming on active, substitutional, and hydrolytic acidity has been in accordance with the applied dose of CaO (Table 1), the highest in the variants with complete liming (V-5), and the lowest in partial liming (V-3). The soil quickly reacted to entered CaO, and changes were obvious and complete already at the first check, 5 months after liming, i.e.

in the stage of wheat tillering. The differences between the treatments where CaO has not been used (V-1 and V-2) and the treatments in which CaO has been used (V-3, V-4, and V-5) are highly significant. The differences between the variants in which liming has been performed can clearly be observed. All the differences are highly significant ($p < 0.01$), except for the changes in active acidity in the tillering stage (T) in 2010, when a difference in the level of statistical significance ($p < 0.05$) has been found between the first (V-3) and the second level (V-4) of partial liming.

Table 1. The change in pH (H_2O and KCl) and Y1 after liming

Variants	pH H_2O				pH KCl				Y1	
	2010		2011		2010		2011		2010	2011
	T	AH	T	AH	T	AH	T	AH		
V-1	5.45	5.43	5.42	5.50	4.83	4.83	4.84	4,83	16.32	14.12
V-2	5.50	5.51	5.41	5.47	4.75	4.79	4.79	4.82	14.32	14.56
V-3	5.92	5.89	5.90	5.84	5.23	5.30	5.29	5.24	7.76	7.66
V-4	6.17	6.15	6.18	6.14	5.57	5.56	5.60	5.58	5.21	5.38
V-5	6.79	6.77	6.87	6.73	6.24	6.20	6.24	6.19	3.00	3.45
Lsd 0.05	0.243	0.081	0.098	0.094	0.120	0.099	0.136	0.086	1.69	1.565
Lsd 0.01	0.349	0.117	0.141	0.135	0.173	0.143	0.196	0.124	2.430	2.248

The resulting changes, both in active and substitutional acidity, are in favor of the claims of the need for liming acid soils (Busari *et al.,* 2008; Jelić *et al.,* 2011; Mao *et al.,* 2008), in order to perform the neutralization and create favorable conditions for smooth growth and development of plants. It particularly refers to a group of plants that are insufficiently tolerant to soil acidity. The observed changes in pH during one growing season should be emphasized. Namely, in the period between the first (tillering stage) and the second checks (after the harvest), there was a small decline in pH. This trend was expected because numerous processes in the soil, primarily flushing, as well as the adoption of Ca by the plants, led to losses of liming material, which inevitably led to a decrease in pH. For this reason, it is often talked about a time-limited (fixed-term) effect of this measure, which has to be repeated after a while for these reasons. Since the problem with acidity cannot be definitely resolved, a practical approach consisting of a regular application of moderate amounts of lime material, with which soil acidity would be maintained at an acceptable level, is completely acceptable (Garscho and Parker, 2001). The content of mobile Al^{3+}, in both years of research, was strongly changed in all the variants where CaO was applied (Table 2). Thus, trace aluminum content was found in the first measuring, in the stage of wheat tillering, in the complete liming variant. According to the results of other authors, liming had the same effect on other soil types, such as pseudogley (Jelić *et al.,* 2011; Pivić *et al.,* 2011).

Table 2. The changes in mobile Al content (mg · 100 g^{-1}) after liming

Variant	2010.		2011.	
	T	AH	T	AH
V-1	12.28	12.21	13.62	13.80
V-2	12.32	12.54	13.92	13.87
V-3	5.26	5.56	6.25	6.39
V-4	2.17	2.37	2.44	2.62
V-5	0.48	0.40	0.47	0.42
Lsd 0.05	0.346	0.277	0.346	0.072
Lsd 0,01	0.497	0.398	0.497	0.104

At the same time, also in the variants of partial (V-3) and particularly half-liming, the content of mobile aluminum was reduced to a level at which the risk of its toxic effect on the crops was significantly reduced. In V-3 and V-4 variants, a slight increase in the content of mobile Al was found during the growing season.

Despite an obvious improvement in the reaction of Dystric Cambisol, liming has not had a major effect on the content of available forms of Fe and Cu (Table 3). Namely, the content of Cu per variants, in both years of research, has not indicated any effect of entrained lime material, and the differences between the liming treatment and the control variant are not statistically significant. On the other hand, the content of Fe in the tillering stage, in the part of the experiment when amelioration was performed, did not sufficiently clearly indicate the real impact of lime. In particular, it was because the differences, in the part of the experiment when liming was performed and in the part without liming, almost entirely disappeared when the content of the element was measured after the wheat harvest. The observed difference in available Fe concentration during the growing season was most likely the result of changes in oxidation-reduction conditions, on which numerous processes that transform Fe into available forms and vice versa depend.

Table 3. The changes in the content of available forms of Fe, Zn, and Cu (mg kg^{-1}) after liming

Variants	Fe				Zn		Cu	
	2010		2011		2010	2011	2010	2011
	T	AH	T	AH				
V-1	17.43	9.73	33.48	21.28	3.78	3.18	2.40	3.65
V-2	14.78	12.30	30.15	19.20	3.30	3.75	2.49	3.69
V-3	14.45	9.78	30.18	20.40	3.13	2.83	2.49	3.66
V-4	14.61	10.05	30.25	20.21	3.05	2.75	2.51	3.64
V-5	14.50	10.78	30.60	19.55	2.95	2.55	2.55	3.62
Lsd 0.05	3.49	3.89	3.90	1.99	0.39	0.18	0.46	0.13
Lsd 0,01	5.01	5.59	5.61	2.86	0.56	0.26	0.66	0.19

The effect of liming has been observed only on the content of available Zn. Namely, lime material and an increase of pH have caused a decrease in the amount of available forms of this element. The differences of all the variants with liming, in both years of research, have been highly significant ($p < 0.01$) as compared to the control variant. Also, the amount of applied lime material has had an effect on the content of Zn. Thus, the lowest content, in both years, has been found in the variant where complete liming was applied (V-5), and the differences compared to the other two levels of liming (V-3 and V-4) had a statistical significance only in 2011. There is no complete consensus regarding the concentrations of mobile fractions of Zn after liming, so there are opinions that it is being reduced, or that the entered lime does not influence its content (Bošković-Rakočević and Bokan, 2005).

Conclusions

Liming has caused significant changes in Dystric Cambisol, and the changes were rapid and in proportion to the rates of applied CaO. Soon after the introduction, already in the stage of tillering, a decrease in acidity and in the content of mobile aluminum occurred, and of the analyzed microelements, only Zn reacted to entered lime material in such a manner that the content of its available forms was reduced. The degree of achieved changes was adequate to the level of applied liming. Complete liming almost completely neutralized the acid reaction, in traces it brought the content of mobile Al, but it also significantly reduced the availability of Zn, thus jeopardizing the regular supply of plants with this microelement. On the other hand, the level of changes caused by partial (1/3 Y_1) and half-liming (1/2 Y_1) has justified and

promoted these levels of acid soil repairing. The main benefits of lower levels of liming are lower investments in liming material, satisfactory pH neutralization, a reduction in mobile Al below the toxicity level and a lower risk of bringing some microelements to the deficit limits, which is of great ecological and economic importance.

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THE CONTENT OF TRACE ELEMENTS IN ALFALFA CULTIVATED ALONG THE HIGHWAY E75 - ROUTE SECTION LOZOVIK-GRDELICA (REPUBLIC OF SERBIA)

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Abstract

On the section of the highway E75 through Republic of Serbia from Lozovik to Grdelica, the content of trace elements in soils and alfalfa cultivated on Vertisol Eutric cambisol and Fluvisol was studied. The content of Pb, Ni, Cr and Cd were determined in soil and plant material. In all tested soil samples the content of trace elements was below the MPL (maximum permissible levels) values for agricultural soil. The content of analyzed elements in the samples of alfalfa varied depending on the pH value, the content of clay fractions and organic matter in soil on which it was cultivated. The obtained results showed that the values of trace elements in plant species of alfalfa were not above the toxic concentrations values (TC). The content of studied elements in plant material was increased in the soils with lighter texture probably because of the pH value, content of clay fractions and organic matter in soil contributing to their binding, indicating that the soil-plant translocation was affected by the physical and chemical properties of the soil, the location of accumulation as well as the physiology of plant.

Keywords:*Alfalfa, Trace elements, Translocation, Soil, Physico-chemical properties.*

Introduction

One of the basic factors of economic growth, certainly, is the developed road infrastructure which implies the existence of high frequency traffic. The E 75 motorway is one of such roads and through the territory of the Republic of Serbia it passes through zones where intensive agricultural production has been developed. It is important to examine the possible impact of the highway on the potential pollution, because of the location on which it is located, both on agricultural land and on cultivated plants along the motorway. The research covered the section of E 75 motorway from Lozovik to Grdelica. During 2010, samples of agricultural land and alfalfa were collected on land such as Eutric cambisol, Fluvisol and Vertisol, on parcels that are 10, 30, 50 and 400 m from the route of the motorway. A large number of authors (Wilson and Pyatt, 2007, Jankievicz and Adamczyk, 2010, Deska et al., 2011) lists data on potential soil contamination by heavy metals, one of the major causes of which is anthropogenic impact, which can be due to emission of exhaust gases, grease, oil and tire residues that are present along the motorway. Environmental risk assessment of soil contamination is particularly important for agricultural areas, due to the fact that heavy metals potentially harmful to human health exist in the soil and can be transferred in significant quantities to the food chain (Szynkowska et al., 2009). High concentrations of trace elements in soil can affect the soil's fertility and may represent an ecological and human health risk if they enter the food chain or leach into receiving waters (Daskalopoulou et al., 2014, Dinić et al., 2018). The mobility of the trace elements and their translocation in the plant are influenced by the content of the clay fraction, organic matter and pH value of soil (Dinić et al., 2018). In a pH range of soil from 5.50 to 8.00 the chromium (Cr) is almost insoluble, while the solubility of cadmium (Cd) decreases with increasing pH values so that at pH>7.50 comes to Cd immobilization (Kabata-Pendias, 2011). In soils with pH (1 M KCl) values from

3.80 to 7.10, Cd is less mobile than Ni (Adriano, 2001). The content of organic matter in the soil does not significantly affect the Cr and Ni mobility, while the mobility of Pb is equally affected by the content of clay and organic matter (The Dutch Environment Ministry, 2009). Assessment of plant bio-concentration factor, Soil-to-Plant bio-concentration factor (Biological Concentration Factor) is one of the ways to evaluate and monitor the translocation of trace elements from soil to plant. With increasing clay content, organic matter and increasing soil pH, BCF for Cd, Cr, Pb and Ni is decreasing. The largest reduction in the coefficient is for Ni (80 times) and at least for Cd (16 times). When assessing pH values for: pH less than 5.5; in the range of 5.5 to 6.5 and greater than 6.5; for the content of the clay fraction: less than 15%; from 15% to 40% and more than 40%; and for organic carbon content (SOC): less than 1%; from 1% to 3% and greater than 3% (Versluijs et al., 1998). Biological Concentration Factor (BCF) values for alfalfa range in a wide range and depend on agro-ecological conditions (McKone and Maddalena, 2007; Al-Rashdia and Sulaimanb, 2013; Solgi et al., 2017).

Material and

Sampling of soil and above-ground part of alfalfa plant material was carried out on the section of E75 from Lozovik to Grdelica during the vegetation period in August and September 2010., based on the coordinates of the sampling site registered with the GPS device, using the data from the pedological map of the Republic of Serbia (Institute of Soil Science, 2013). The locations of the soil type Vertisol, Eutric cambisol and Fluvisol (Škorić et al., 1985, Group of authors 2011, WRB, 2015) were determined, from which a plant species, alfalfa was sampled and studied. Soil and plant sampling spots in the section of the study with corresponding distances and coordinates presented in Table 1.

Table 1.-List of locations of samples and type of soil

Study site	Sample spots (m from route lanes)	Soil type	Coordinates	
			X	Y
1	D19(30)	Vertisol	7520908	4877018
2	D27(30)	Eutric cambisol	7548003	4830378
3	D27(50)	Eutric cambisol	7547990	4830558
4	L27(30)	Eutric cambisol	7548175	4830567
5	L27(50)	Eutric cambisol	7548193	4830583
6	D29(30)	Fluvisol	7559134	4820245
7	D29(50)	Fluvisol	7559115	4820228
8	D32(50)	Fluvisol	7566464	4799317
9	D33(50)	Vertisol	7565611	4792720
10	L33(50)	Vertisol	7565742	4792781
11	L39(50)	Fluvisol	7585241	4752772

Eleven soil samples in the disturbed state were sampled to a depth of 30 cm at a different distance from the highway, at 10, 30, 50 and 400 m from the road lanes. The alfalfa plant material, the above-ground part was sampled, with the remark that the year in which it was planted was not recorded. The average sample consisted of 15 to 20 individual samples, whereby the cut was carried out by hand cutting at a height of 3-5 cm of the plant.

In 11 composite soil samples prepared in accordance with SRPS ISO 11464: 2004 - Pretreatment of samples for physical-chemical analyzes, sieved through a sieve of 2 mm in diameter. Soil acidity (pH in H_2O and 1M KCl, v/v - soil:H_2O = 1:5, soil:1M KCl = 1:5) was analyzed potentiometrically, using glass electrode (SRPS ISO 10390, 2007), calcium

carbonate by volumetric method SRPS ISO 10693: 2005-Determination of carbonate content, total contents C was analyzed on elemental CNS analyzer Vario EL III (Nelson et al., 1996). SOM (soil organic matter) was calculated using the formula: SOM content (%) = organic C content (%) x factor 1.724 in carbonless soil samples, ie SOM content (%) = (organic C content (%) - 0.12 x% CaCO3) x factor 1.724 for carbonate soil samples (Džamić et al., 1996). Granulometric composition was analyzed by determination of particle size distribution in mineral soil material, using the standardized method by sieving and sedimentation (ISO 11277: 2009(E), 2009); according to granulometric composition the texture class was determined (ISSS, Baize (1993)).; determination of the total trace elements forms (Cd, Cr, Ni and Pb) was done by inductively coupled plasma-atomic emission spectrometry - THERMO iCAP 6300 Duo (radial/axial view versions) ICP-OES, after the digestion of the samples with aqua regia (ISO 11466:1995, 1995; ISO 22036:2008, 2008). Reference soils NCS ZC 73005, Soil Certificate of Certified Reference Materials approved by China National Analysis Center Beijing China, and reagent blanks were used as the quality assurance and quality control (QA/QC) samples during the analysis. The samples of plant material are air-dried and milled. The sampled plant material were dried at 105^{o}C for a period of 2 hours, using gravimetric method for determination of dry matter contents of plant tissues (Miller, 1998). The analysis of the plant material included the determination of Cd, Cr, Ni and Pb using THERMO iCAP 6300 Duo (radial/axial view versions) ICP-OES after the digestion of the plant samples with concentrated HNO_3 and redox reaction with 30% H_2O_2 for total forms extraction (Soltanpour et al., 1996). The results of the conducted soil analyze represent the arithmetic means of three replicates of each sampling.

Results and discussion

Alfalfa (*Medicago sativa* L.) is a perennial leguminous crop, which is regarded as the leading and most important forage crop for the production of high quality feed, and is used in the fresh state and conserved as well as hay, haylage, silage, meal, pellets and pasta (Vučković, 2004; Jakšić et al., 2013, Pivić et al., 2017). Alfalfa was grown on the following soil types Vertisole, Eutric Cambisole and Fluvisol (WRB, 2015). Some of the properties of these types of land are shown below (Glamočlija et al., 2013).

Vertisols are soils with unfavorable water-air and thermal regime, and some of their properties are the characteristics of hydrogen soils (formed on the substrates containing more than 30% of clay predominantly montmorilonite type). It swells in a wet state, and in dry cracks with the formation of cracks that can reach one meter in depth. The chemical properties of these soils are considerably more favorable. They are characterized by neutral to low alkaline reaction with high adsorption capacity. Base saturation is up to 90%. Humus, total nitrogen is easily accessible. These types of soils in terms of benefits for plant production belongs to the third rating class

Eutric cambisol is formed on different geological substrates. These are mostly medium heavy soils, with clearly expressed texture differentiation within the profile. The humus accumulation horizon is characterized by a very favorable water-air regime, as a result of favorable ratio of large, medium and fine pore. The chemical properties vary depending on the intensity of use, the degree of erosion, the chemical properties of the parent substrate and the degree of development. There are no carbonates and there is low acid reaction. Humus is medium-sized (in the interval from 2 to 5%). It is settled in soils of high ecological and production value. Regarding the benefits and limitations of plant production, Eutric cambisol belongs to second rating class of soils.

Fluvisol-similar soils are formed by transport and deposition of materials in river valleys during floods. The composition and stratification of alluvial varies considerably depending on the water regime of the watercourse, relief, geological substrate and soil. They differ in

mechanical composition, and the differences are accompanied by the mineral composition. These are layered recent river or lake deposits in which the sedimentation process plays a greater role than the genetic development of the soil. These are very fertile soils of a carbonate or neutral reaction. The humus content varies from low to high content. These soils have medium to high content of basic nutrients. They belong to a third rating class that includes soils used for plant production, but with certain limitations. The reaction of the tested soil was from acid to alkaline (pH in 1M KCl 4.90-7.30), according to the carbonate content, no calcareous to medium carbonate (IGDM-7.11), with medium to high organic matter content, SOM (1.65% -6.01%). The physical and chemical properties of the soil samples tested are shown in Table 2.

Table 2. Physical and chemical properties of tested soil samples

Study site	Clay fraction (<0.002 mm) %	Texture class	pH H_2O	pH 1M KCl	$CaCO_3$ %	SOM %
1	23.6	SCL	6.98	6.52	BLMD	4.30
2	8.1	LS	6.01	5.10	BLMD	1.96
3	12.1	SL	6.10	5.20	BLMD	4.51
4	8.9	LS	5.80	5.40	BLMD	2.66
5	21.3	SCL	6.30	5.60	BLMD	3.06
6	17.6	SL	7.80	7.30	3.74	5.24
7	7.8	LS	8.00	7.20	3.74	2.45
8	25.3	SCL	8.15	7.10	7.11	1.65
9	13.5	SL	6.05	5.10	BLMD	1.57
10	17.2	SL	6.11	5.70	BLMD	2.48
11	13.4	SL	5.64	4.90	BLMD	2.17

LS-loam sandy; SL-sandy loam; SCL-sandy clay loam;
SOM-soil organic matter; BLMD-below the limit of the method detection

The interpretation of data on the trace elements content in soil samples was done according to the Rule book of maximum permissible levels (MPL) of dangerous and hazardous materials in soil and water for irrigation and methods for analysis (Official Gazette of Republic of Serbia, 1994), where MPL for Cd is 3 mg kg^{-1}, MAC for Cr is 100 mg kg^{-1}, MAC for Ni is 50 mg kg^{-1} and MPL for Pb is 100 mg kg^{-1}. In the tested soil samples, the trace element content is within the limits of MPL (Table 3).

Table 3. The content trace elements in soil samples

Study site	**Cd** mg kg^{-1}	**Cr** mg kg^{-1}	**Ni** mg kg^{-1}	**Pb** mg kg^{-1}
1	1.95	86.71	49.40	98.19
2	0.38	31.42	33.54	28.08
3	0.35	37.97	30.02	26.17
4	0.42	46.42	38.47	30.02
5	0.41	45.67	37.59	28.37
6	0.56	44.36	36.75	41.49
7	0.58	49.07	38.51	46.28
8	0.44	34.38	26.05	32.18
9	0.41	45.78	40.69	27.53
10	0.41	43.08	40.25	26.65
11	0.93	62.16	23.66	43.11

The interpretation of data on the trace elements content in the samples of forage crops was done according to the reference data on toxic concentration (TC), where TC for Cd is 1 mg kg^{-1}, TC for Cr is 5 mg kg^{-1}, TC for Ni is 50 mg kg^{-1} and TC for Pb is 40 mg kg^{-1} (Adams, 1975; Kloke et al., 1984; Kisić, 2012). The results of the conducted plant material analyzes are shown in Table 4.

Table 4. The content trace elements in plant samples.

Study site	**Cd** mg kg^{-1}	**Cr** mg kg^{-1}	**Ni** mg kg^{-1}	**Pb** mg kg^{-1}
1	0.09	1.41	4.38	3.03
2	0.03	4.49	4.77	0.72
3	BLMD	1.45	3.53	0.20
4	0.01	0.64	3.25	0.13
5	0.03	1.32	2.15	0.32
6	0.06	4.60	3.63	0.11
7	0.06	2.12	3.31	0.45
8	0.03	4.38	2.98	1.93
9	0.51	3.35	28.92	9.26
10	0.01	2.73	3.48	0.23
11	0.26	4.89	4.23	10.84

BLMD - below the limit of the method detection

The content of Cr in plants varies and depends largely on the geological substrate. The source of Cr is also a significant factor that affects the solubility and availability of these elements (Adams, 1975; Kloke et al., 1984; Kastori et al., 1997; NRC, 2005). The content of Cr in the samples tested ranges from 0.64 to 4.89 mg kg^{-1}, which is below the toxic values.

Cd is one of the most toxic and harmful elements that adversely affects soil biological activity, plant metabolism and human and animal health. It is easily absorbed through the root system and accumulated in the aboveground plant parts. The pH of the soil solution is cited as the main factor in the adoption of Cd. The origin of Cd is also an important factor that affects the solubility and availability of this element. The content of Cd in the tested samples of plant material ranges in the range of normal values (up to 1 mg kg^{-1}).

The Ni content in the tested plant parts of alfalfa, also is below toxic levels (50 mg kg^{-1}) in all the analyzed samples, ranging in the range of from 2.15 to 28.92 mg kg^{-1}.

Pb is the least mobile element among the microelements of the soil (Kabata-Pendias, 2011). Pb is poorly adopted and transferred to the above-ground organisms of the plant, except on acidic soils. Plants can accumulate Pb either from the soil or absorbed from the air. Most of the Pb from the soil is not available to plants. Non-oganic Pb forms become accessible to plants only in acidic soils (Wiklander and Vahtras, 1977). Pb originating from the air is the main source of pollution by this element. According to some studies, about 95% of the total amount of lead in the plant can be originated from the air. In all tested samples, the Pb content is below the toxic values (40 mg kg^{-1}) and ranges from 0.11-10.84 mg kg^{-1}.

Conclusion

By analyzing the obtained results of the study of the content of trace elements (Cd, Cr, Ni, Pb) and their concentration in the plant material of the above-ground part of the alfalfa along the E75 from Lozovik to Grdelica, it is determined that content of trace elements are within tolerant values below the toxic for plant material. In the case of the soil sample at location D19 at a distance of 30 m from the traffic lane, compared to other sample tests, the content of

the total forms Cd, Cr, Ni, Pb is the closest to the maximum permissible levels. In the plant sample from this site, there was no increased translocation in the plant material of the examined elements, which is probably due to the neutral reaction (pH in 1M KCl is 6.52). In this sample the content of the clay fraction was determined from 23.6%, which is within the usual range, and the content of SOM is 4.30%, which is below the usual range (according to The Dutch Environment Ministry, 2009).

At the location L39 at a distance of 50 m from the motorway, in the soil zones with the lowest value of the medium reaction (pH in 1M KCl is 4.90), with the content of the clay fraction of 13.4% and the SOM of 2.17%, an increase in the values of Cr, Pb in plant material, but still below the toxic concentration . These results confirm the assumption and point to the conclusion that when interpreting the translocation of elements in traces from the soil to the plant material it is necessary to determine the content of the clay fraction, the content of the organic matter and the reaction of the environment, because only in this way full data can be obtained and the obtained results can be interpreted.

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PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE IN SERBIA

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Abstract

Serbia is characterized by a high level of genetic diversity and cultivated plant species which are used as food amount to 4.5% of used biodiversity (193 species). Plant genetic resources (PGR) can be used in breeding programs as a source of specific genes for the development of new varieties, adapted to the new environmental conditions and to expand the genetic base of breeding material, as well as food, in food industry. The paper presents the activities involving the conservation, management and utilization of Plant Genetic Resources for Food and Agriculture (PGRFA), and state of PGR in Serbia. What is the exact number of PGR in Serbia is not known precisely because there is no national inventory of PGR. It is estimated that about 25.000 samples of old landraces and cultivated plants are stored in the form of seeds and about 3.500 samples of fruit and vines are in *ex situ* collections. In Serbia, there are around 20 collections at the National Gene Bank and in institutes. The National Plant Gene Bank have 4.300 samples of 249 plant species: cereals and maize (2.983 sample-7 types), industrial crops (387 samples - 6 types), vegetables (214 samples - 11 types), fodder crops (285 samples - 9 species), medicinal and aromatic plants (389 samples - 216 species). In order to preserve genetic resources it is necessary to plan management activities and efficient and directed usage.

Keywords: *plant genetic resources, conservation, management, Serbia.*

Introduction

Our ancestors collected and started growing certain plants 10.000 years ago, which led to the domestication of almost all agricultural species that are in use today. About 7.000-10.000 different plant species have been cultivated, which accounts 3-4% of plant biodiversity (Hammer, 1995). At the beginning of the 20th century, Russian botanist Vavilov traveled around the world and noticed that the diversity within the agricultural plant species was not evenly distributed. He identified eight centers of diversification of cultivated plant species (Vavilov, 1992). Today, the world's largest plant gene banks are located in those centers of origin. FAO (Food and Agriculture Organization of the United Nations) has collected and kept information on plant expeditions since 1948, has prepared plant catalogs and oversees the collection of plant germplasm around the world. Today there are 1 750 individual genebanks worldwide, that have been established with the goal of collecting and conserving plant genetic resources (FAO, 2010).

Serbia is characterized by a high level of genetic diversity. The Serbia's flora contains about 4.300 species from a total of 11.000 plant species in Europe (Stevanović and Vasić, 1995). Cultivated plant species (193 species) used as food constitute only 4.5% of biodiversity (Prodanović et al., 2015). A significant part of the diversity of the species consists of local populations and old varieties.

According to the European Cooperative Program for Plant Genetic Resources (ECPGR), PGRFA are grouped into eight groups: cereals, fodder crops, vegetables, grain legumes, fruits, alternative plants, industrial plants and potatoes (http://www.ecpgr.cgiar.org). The number of plant genetic resources (PGR) has a tendency of continuous decline, agricultural varieties rich in diversity cease to be cultivated in certain regions, replaced by new uniform varieties and

hybrids that have higher market value and are adapted to modern agricultural practice. In order to preserve genetic resources, it is necessary to manage them in a planned and efficient manner and to be targeted use.
The paper presents an overview of activities that include the preservation, management and utilization of PGRFA, as well as their state in Serbia.

Collecting of plant genetic resources for food and agriculture

The main objective of collecting PGR is to include the maximum amount of genetic variation in the minimum number of samples. Collecting samples is primarily related to scientific expeditions for collecting and sampling, and then keeping them at certain conditions.
During the 20th century, several research missions were conducted around the world. Collecting of plants has a long tradition in Serbia: Josif Pančić has visited Kopaonik 16 times between 1851 and 1886 and has discovered many plant species, the most important being the discovery of spruce *Picea omorika* (Pančić) *Purkyne*.
After the Second World War, especially during the period from the 1960s to the end of the 1980s, numerous expeditions in former Yugoslavia were conducted in order to collect autochthonous genotypes. Collecting missions were organized for maize (Anđelković and Ignjatović Micić, 2012), fodder crops (Tomić et al., 2010), vegetables (Sabadoš et al., 2008), fruits (Keserović et al., 2007). Additional expeditions directed to maize and cereals were carried out in 2006-2010. within the SEEDNet (South Eastern European Development Network on Plant Genetic Resources) program in order to collect autochthonous, local populations and old varieties (Simeonovska et al., 2013).

Conservation of genetic resources

Plant genetic resources are held by conservation *in situ*, *on farm* and *ex situ*.
In situ conservation is applied for the preservation of autochthonous and old types of cultivated plants in their regions of origin or similar areas with favorable agroecological conditions.
By *on farm* conservation is possible to maintain the genetic diversity of locally developed populations in traditional farming systems, usually on farmers' fields.
Ex situ conservation is preservation of biological diversity outside natural habitats, includes botanical gardens, greenhouses and gene banks.
Herbal genetic resources are stored as seeds in gene banks, vegetative material *in vivo* as part of the plant (fruits and vines) or *in vitro* in tissue culture, and DNA material usually at -80°C or in DNA libraries.
Dominant strategy worldwide is *ex situ* conservation, with genetic resources of plants for food and agriculture of about 6 million accessions. Consultative Group on International Agricultural Research (CGIAR) has the largest collection of agricultural plants in the world of about 741.000 samples. About 45% of all accessions in the world's banks are cereals, followed by legumes with about 15%, vegetables with 10%, fodder plants 10%, fruits 6-9%, oils and fibers with 2-3% (FAO, 2010).
The precise number of PGRs in Serbia is not known, but it is estimated that about 25.000 samples of varieties, old and local populations are in the form of seeds. Out of them, the largest part belong to cereals (10.208 accessions, Table 1), then 5806 are maize accessions, 3569 vegetable accessions, 3323 samples of industrial crops, and 2375 samples of fodder crops. Also, about 3.500 fruit and vine samples are in *ex situ* collections (Drinic and Ivanov, 2017). The collections are mainly stored at the institutes and faculties.

Table 1. Number of *ex situ* samples of important crops in world and Serbia

Species	World	Serbia
Wheat	784 500	5759
Barley	485 000	2277
Oat	222 500	1015
Ray	27 000	93
Triticale	40 000	250
Sorghum	168 500	260
Soybean	174 500	1469
Bean	268 500	19
Sunflower	29 500	696
Sugar beet	24 000	390

In situ preservation mainly refer to the maintenance of wild relatives, since Balkan Peninsula and Serbia could be considered as secondary diversity center for some cereals, vegetables, fodder crops and fruit and vine trees.

On farm (on farmers' fields) in Serbia over 2.000 autochthonous and local populations of plants are grown nowadays. Most of them, predominantly cereals, are evaluated and characterized, and stored in seed bank. The number of fodder crops, medical plants and wild relatives are difficult to estimate, since they could be found both, in *in situ* and *on farm* conditions. Production of old varieties is not economical for farmers without significant financial support from the state. Exceptions are fruit and vine trees that are kept mainly with low maintenance costs, and local populations with specific properties, or for organic production, interesting for the market (Prodanović et al., 2015).

Management and utilization of plant genetic resources

Gene banks play an important role in the long-term preservation of PGRFA. However, in addition to conservation, they collect information about the material they store, which allows users to choose the most suitable genotypes for research.

Types of collections in gene banks are:

1. **Basic collection -** collection of samples and vegetative parts that are stored at -20°C, which ensures long-term sustainability of the material (more than 50 years)
2. **Active collection -** parts of the basic collections (also called working collection), regularly regenerated in the field, and which are immediately available for distribution, usage, multiplication, exchange and evaluation. They are stored at 4°C and 20-30% of relative air humidity, under conditions that ensure that the sample's viability remains above 65% for medium-term sustainability (10-20 years).
3. **Core collection -** contains representative samples, or subgroups that make about 10% of the entire collection but preserve its diversity.
4. **Duplicate collections -** are created when one institution sends samples from its collection to be stored in another institute, so the same samples are held in two places.
5. **Gen collections -** contain genotypes with specific traits of interest for research and development.

Local populations, genetically heterogeneous and adapted to specific local conditions, are a valuable source of gene for drought tolerance, salinity, high temperature, disease resistance or improved grain quality (Drinic et al., 2012). But breeding based on genetic resources is long-term, and opposit to the market demands for development of high yieldig commercial varietes for a short time. Today, genetic resources are valuable for genetic and biotechnological research, e.g. molecular characterization of autochthonous varieties and wild relatives and the

application of genomics can identify a number of genes and specific DNA sequences for desired properties that can be transferred to modern varieties by molecular breeding or genetic engineering.

Therewith PGRFA are increasingly important globally due to the crucial role in the context of climate change, human population increase, and secure food production. In the Svalbard archipelago between Norway and the North Pole, Svalbard Global Seed Vault was set up. It is an object built by the Norwegian Government designed to withstand every test, natural accidents and disasters caused by humans. About 4.5 million seed samples could be stored in it, and so far there are about 780.000 samples of 840 species. The purpose is to preserve seeds and vegetative parts of plants for future mankind's needs, as sources of genes and food (www.croptrust.org).

Plant Genetic Resources for Food and Agriculture in Serbia

Serbia is one of the most important areas of biodiversity in Europe. Wild relatives of cultivated plants, about 1.000 species in Serbia, are found in nature, and a smaller number is stored in germplasm collections. In Serbia, 193 types of plants are used for food and commercial agricultural production, divided into the following groups: cereals (12 species), industrial plants (19 species), fodder plants (43 species), vegetables (71 species), fruit and vines (48 species). Besides, more than 400 types of medicinal plants are officially registered (Ivanov et al., 2012).

The samples of the National Collection of Plant Genetic Resources were officially transferred to the cold rooms of Plant Gene Bank (PGB) on March 31. 2015, where all technical conditions for medium-term storage at + 4°C and long-term storage at -20°C were obtained. In this way, PGB of Serbia was activated, after more than 20 years since the facilities for the needs of the former Yugoslavia PGB were built.

There are 4.300 samples of 249 plant species in the PGB: cereals and maize (2.983 samples - 7 species), industrial plants (387 samples - 6 species), vegetables (214 samples - 11 species), fodder crops (285 samples – 9 species), medicinal and aromatic plants (389 samples - 216 species). Most of the accessions are characterized according to IPGRI/ECPGR descriptors for particular crop.

Draft of National Program for the Preservation and Sustainable Use of BGRHP in Serbia was prepared by the Special Working Group, according to the decision of the Ministry of Agriculture, and with the help of the FAO technical support project. The National Program for the Conservation and Sustainable Use of Plant Genetic Resources has been written in accordance with the stated programs and strategies, as well as with the Constitution of the Republic of Serbia. This is a strategy document describing the current state, outlining goals and proposing measures related to the conservation, use and development of plant genetic resources for food and agriculture. It is intended for numerous users: breeders, agricultural producers, researchers, gene bank, institutes and faculties, governmental and non-governmental organizations, various organizations and individuals, as well as farmers who conserve and use plant genetic resources, which require a stable and predictable policy in this area. It is written in accordance with international conventions, first of all with the Convention on Biological Diversity, the International Convention on Plant Genetic Resources for Food and Agriculture and the Global Action Plan for the Conservation and Sustainable Use of PGRFA (Drinic and Ivanov, 2017).

Conclusions

The issue of preservation of plant genetic resources is of particular importance due to the global climate change, the emergence of new diseases and pests, secure food production and the conservation of biodiversity. Local populations, old varieties and wild relatives represent

an necessary resource for securing progress in plant breeding and sustainable global food production of good quality and in sufficient quantities for today's and future human needs. Serbia is rich in biodiversity, and has about 25.000 accessions in *ex situ* collections in genebanks, mostly in research institutes. *In situ* and *on farm* conservation is applied mainly on fodder crops and medical plants and on some varieties that are interesting for the market nowadays (e.g. growing demand for organic production). Our state's representatives are involved in all international institutions responsible for the preservation, management and utilization of plant genetic resources, and all obligations in terms of biodiversity conservation are implemented through activities in institutions dealing with genetic resources.

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THE UTILIZATION OF FOOD WASTE IN FEED AND FERTILIZER PRODUCTION IN TURKEY: NEEDS AND CHALLENGES

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Abstract

Large quantities of food waste (FW) are emerging as a result of the increased production in the food industry, which often processes agricultural products. Reduction and utilization of FW is important not only for food security but also for the sustainable use of resources. The slow progress of reducing FW and developing effective waste management strategies can be a problem for almost every country. FW contains complex carbohydrates, proteins, lipids and nutraceuticals, and can be a source of raw materials for commercially important metabolites. The wastes generated during the food supply chain can be used to produce nanoparticles between biofuels, enzymes, bioactive compounds, biodegradable plastics and many other molecules. The utilization of FW for agricultural applications such as animal feed and fertilizer is a common waste management practice. These two waste management practices focus on the reuse and recovery of valuable components in the FW. However, the FWs obtained from different sources involve rich nutrients is accompanied by the risk of unbalanced nutrient composition and the risk of toxic substance presence as well. Topics are up-to-date in Turkey as well as on a global scale. Alternative utilization methods can bring risks as well as opportunities. Many countries are prohibiting or limiting, in particular, some animal feed production, by their legislations on the reduction of FWs. Increasing opportunities for fertilizer production continue with value-added practices. In this study the current situation in Turkey regarding the use of FW as animal feed and fertilizer has been examined. Moreover, the needs and challenges were evaluated by considering the practice in other countries.

Keywords: *Food waste, feed, fertilizer, Turkey*

Introduction

Along with the increase in the production of agricultural and food products, food losses (FL) and waste also reach large quantities. Definitions of FL and FW are not universally agreed upon, which makes studying FW difficult. Multiple terms have been used interchangeably, such as FL, FW, bio-waste, and kitchen waste. Also, often the same terms are used, but with different meanings (Thyberg and Tonjes, 2016). The Food and Agriculture Organization of the United Nations (FAO) defined FW as food appropriate for human consumption that is discarded (generally at retail and consumption stages) (FAO, 2013). FL and FW are often used in scientific literature to identify materials intended for human consumption that are subsequently discharged, lost, degraded or contaminated. Discharge of food material occurs along the entire Food Supply Chain (FSC) (Girotto et al.,2015). FW can also be defined as the outlets of the food industry, which are not currently used for defined end-products, not recycled or used in an alternative manner (Du, et al., 2018).

According to the FAO report "Global Food Losses and Food Waste", 95 percent of FL in developing countries occur "unintentionally" in the early stages of the supply chain (FAO, 2011). These losses refer to financial, administrative and technical limitations in harvesting techniques, transportation, storage and cooling, public infrastructure, means of transport, packaging and distribution systems. In developed countries, FW can be observed mostly at

retail and consumption stages. In these countries, roughly one-third of purchased food is wasted as household waste (Gustavsson et al., 2011; Premanandh, 2011; Prusky, 2011; Dorward, 2012; Wang, 2013). According to this approach, the difference between FL and FW is related to the phase in which the food supply chain has emerged. FL takes places at production, postharvest, processing and distribution stages of food supply chain, FW happens at retail and consumption (FAO, 2014). In this study, each stage of the food supply chain is considered together for wastage used in fertilizer and animal feed production.

Reducing FL and FW is considered to be one of the most promising measures to improve food security in the coming decades (Kummu et al., 2012; Demirbaş et al., 2017) and many international and nongovernmental organizations see reducing FL and waste as a priority for reducing global hunger and resource waste (Koester, 2014). At the same time, FW has been caused serious environmental issues, such as generating greenhouse gases and occupying land resources. With the increasing awareness of the problems associated with FW, different the FW management strategies have been improved (Du, et al., 2018). Some of these FW management alternatives are waste prevention, landfilling and incineration. In this study, animal feed and fertilizer production, which include FW management alternatives are evaluated for Turkey.

Generation stages of food waste

Discharge of food material occurs through the entire Food Supply Chain (FSC) and it involves all sectors of waste management from collection to disposal. A FSC initiates with the production of food from the agricultural sector where both farming and husbandry produce waste or by products that may be either organic waste, FW or FL (i.e. low quality fruits, damaged productions left in the field, good products or by-products with inferior commercial value). The food processing and manufacturing industry produces FL and FW throughout the entire production stage due to the following reasons: damage during transport or inappropriate transport systems, problems during storage, losses during processing or contamination, inappropriate packaging. The retail system and markets also generate FL and FW, largely due to problems in conservation or handling, and lack of cooling/ cold storage (Parfitt et al., 2010). The generation of FW by the end consumer is caused by over- or inappropriate purchasing, bad storage conditions, over-preparation, portioning and cooking as well as confusion between the terms ''best before'' or ''use by'' dates (Papargyropoulou et al., 2014). The main causes of FL and waste in low-income countries appear to be linked to financial, managerial and technical limitations in harvesting techniques, storage and cooling facilities in harsh climatic conditions, infrastructure, packaging and marketing systems. On the other hand the source of FL and waste in medium/high-income countries is due to consumer behavior as well as to a lack of coordination between the process stages in the supply chain.

The retail system may lead to the generation of FW throughout various process stages of food distribution and purchase i.e. damage during transport or non-appropriate transport systems, problems during storage, losses during processing or contamination, inappropriate packaging, problems in conservation or handling, lack of cooling/cold storage. The food supply chain is also affected by loss of products reaching their expiry date (Aiello et al., 2014).

The strategies applied in food waste management

The main action to be taken in FW management strategy is prevention of generation. It can be achieved either attempting to reduce losses and also decreasing the demand for food production, or diverting FL, exceeds, and still safe and edible FW to other end-consumers.

Gustavsson et al. (2011) stated that in developed countries, FW prevention has to focus on the consumer's behaviors at household level, on the other hand in developing countries it must focus on the retail and distribution system.

Several studies proposed that convenience in sorting, storage space at home, availability of sorting facilities, access to a curbside collection system and distance to collection points are determinant factors that can increase the recycling rate (Ando and Gosselin, 2005; Barr and Gilg, 2005; Rousta et al., 2015).

Kibler et al. (2018) classified the main FW management strategies as waste prevention, composting, anaerobic digestion, incineration, thermal conversion, land filling, drying for animal feed utilization, bio-valorization etc. and defined many detailed practices for these methods.

Additionally, agro-industrial residues and household FW no longer suitable for human consumption can be used as feedstocks for the production of bio-plastics and bio-fuels together with the extraction of high-value components. A considerable debate still continues on the manufacture of bioplastics from natural materials, raising the question as to whether they produce a negative impact on human food supply. From this perspective, the utilization of FW as a feedstock in the production of bio-fuels and bio-plastics seems a feasible option.

The utilization of food waste in feed and fertilizer production in Turkey

Regulations on the usage waste materials as animal feed and fertilizer

They mainly refer to rules on the regulations that can be related to FW generation and management in Turkey is shown below (OJ, 2005, OJ, 2011, OJ, 2015a, OJ, 2015b). All regulations are prepared in harmony with the EU.

Regulation on the control of waste vegetable oils (No: 25791, Date: 2005): To define the conditions for collection, transport, recycling/recovery, export, import, and disposal of waste vegetable oils.

Regulation of animal side products not used for human consumption (No:28152, Date: 2011): To specify the procedures and principles of animal by-products and their derivatives not offered to human consumption in order to prevent or minimize risks to public and animal health, food and feed safety.

Waste Management Regulation (No.29314, Date: 2015): To regulate the waste minimization, recycling, recovery, and decrease the usage of natural resources, and to provide waste management.

Compost Notification (No. 29286, Date: 2015): To decrease the biodegradable waste amount landfilled and identify technical criteria for compost plants and quality criteria for the compost products.

Needs and Challenges in the utilization of food waste as feed

FW can be used as animal feed directly or after a pretreatment step, such as dehydration, silage, liquid feeding, etc. In Turkey, although FW is used as animal feed no reliable data about the actual quantities. 85% of the animal feed in Turkey is produced by 162 enterprises registered to the Turkish Feed Manufacturers Association. 9.15 million tons of animal feed was imported in 2016 in Turkey and the imported feed rate increased between 2000 and 2016. Importing 31% of the consumption feed shows that the use of FW in feed production is limited in Turkey (Salihoğlu, et al., 2018). However, there is also a growing interest in organic feed milling. It is clear that these developments will affect the feed import positively.

When international developments are examined in this respect, it is seen that in many countries FWs are subjected to some restrictions and regulations in the production of feed due to the risk of toxic substances. For example, because household FW is vulnerable to contamination by foreign objects, it is not currently recycled in Japan. Again, the EU has the most stringent regulations governing the use of waste as animal feed. For instance, to prevent the spread of prion diseases, the use of processed animal protein, also known as meat and

bone meal, as animal feed has been banned in the EU (Mo et al., 2018). Turkey also continues to be a coordinated process of harmonization with the EU legislation.

Needs and challenges in the utilization of food waste as fertilizer

The main organic fertilizers that can be used instead of chemical fertilizers; compost, green manure, barn stover, chicken stew, horse stew, sheep and goat stew.

Composting involves the aerobic degradation of organic wastes. Under aerobic conditions, organic materials are converted to carbon dioxide, ammonia-nitrogen, or complex recalcitrant materials often referred to as humic substances (Saer et al., 2013; Kibler et al., 2018).

The number of existing active compost plants in Turkey is seven, and their total capacities are less than 200 000 tons/years. Since there is a target in the Turkish legislation for the reduction in the FW should be composted, anaerobically digested or incinerated instead of landfilled. Legislation defining compost criteria was issued in 2015 (OJ, 2015b).Although composting has been accepted as one of the main alternative treatment technologies for FW in turkey, the number of compost plant is still at a minimum. The main reason behind the closure of compost plants in Turkey is their economical unsustainability. The benefit-cost ratio of facilities in Turkey is generally low, and there is not a real compost market. However, there may be potential for compost products to be used in land remediation and restoration schemes (Salihoğlu, et al., 2018).

Conclusion

FW is a complex, interdisciplinary and international issue which has profound effects for global sustainability. Large quantities of food is currently wasted, and FW disposal has been increasing with time. The development of sustainable solutions for FW management represents one of the main challenges for society. A large variety of waste materials could be utilized by the husbandry industry to reduce the amount of conventional protein sources. There is an urgent need for research to fill the knowledge gaps on the usage and processing methods of FW.

Renewing standards and regulations in a timely manner would also help the industry to move towards the use of waste materials as animal feed and fertilizer. Being a major agricultural country and has a thriving food industry, the subject of alternative food waste management systems is becoming increasingly important in Turkey. The research on the subject continues to maintain its actuality for Turkey as well.

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SEASONAL IMPACT ON SOME QUALITY TRAITS AND BIOMASS YIELD OF COOL SEASON TURF GRASS GENOTYPES IN SAMSUN CONDITIONS

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Abstract

This study was carried out in order to determine adaptation and some quality traits of some cool season turf grass cultivars at randomized plot design with 4 replicates in Ondokuz Mayis University Experimental Area during 2010-2012. Plot size was 2 m² (1×2 m). In the research 5 cultivars of *Festuca arundinaceae* (Fa), 6 cultivars of *Lolium perenne* (Lp), 5 cultivars of *Festuca rubra commutate* (Frc), 6 cultivars of *Festuca rubra rubra* (Frr), 5 cultivars of *Festuca ovina* (Fo), 7 cultivars of *Poa pratensis* (Pp), 2 cultivars of *Poa trivalis* (Pt), 1 cultivar of *Agrostis stolonifera* (As), 2 cultivars of *Agrostis capillaris* (Ac), 1 cultivar of *Agrostis tenuis* (At) and 1 cultivar of *Festuca rubra trichophylla* were used. Leaf form (1-9), leaf colour and ranging of leaf colour depending on the seasons (1-9), regeneration power (1-5), fresh and dry biomass yields (g m^{-2}) were investigated. Cultivars of *Festuca arundinacea* were different from the others as coarse form (3.3). Two cultivars of *Agrostis stolonifera* were also the same coarse form group. There were pronounced colour change of the whole cultivars depends on the seasons. Compare to the others, colour change of *Festuca rubra* and *Festuca ovina* cultivars were limited. While the highest regeneration power was *Festuca rubra trichophylla*, the lowest was determined for *Poa trivialis*. Four cuttings were made in the second year. The highest biomass yield was obtained from the first cutting and it decreased by advancing cuttings. The lowest fresh biomass yields were obtained from *Poa pratensis* and *P. trivialis* cultivars (62,69 and 97,77 g m^{-2} respectively). *Festuca arundinacea* cultivars produced low and similar biomass yield for each cutting.

Keywords: *Turf grass, Seasonal change, Colour, Biomass yield, Quality.*

Introduction

In the contrary of urbanization and industrialization, recreation area is decreasing day by day. In order to supply human shelter requirement concrete apartment buildings are increased, view of the cities changed and consequence of those issues life quality decreased while stress of humanity is rising. Human is trying to live in congested areas, started to create small green areas inside the cities. After those researches on grass species that can be used in green areas increased (Avcioglu, 2014). The most important part of outdoor recreation areas is turf grass. These areas are used commonly for improve architectural, scenery and aesthetic quality. Heat and drought are the most important factors that restrict the growing of grasses during summer period (Jiang and Huang, 2001). Before choose the grasses, we have to know their usage aim, ecological conditions, sustainability and appearance of them (Harivandi *et al*., 1984). Turf grass has important functions on sports areas, in addition to around the houses. Turf grass species and cultivars selected for sports areas should be resist to pressing on. This trait is crucial for a turf grass, if it is used in sport areas (Yazgan *et al*.,1992). Before establish a sport or recreation area, turf grass cultivars should be suitable to climatic conditions of the region. To know the adapted cultivars in the region is definitely necessary. Cool season grass are suitable to North, East and Central part of Turkey, however warm season grass are more common in South and South-West regions. Province of Samsun is take place in Black Sea

region of Turkey. The area is very suitable for cool season grass due to its cool and rainy winter, mild and humid summer climatic conditions. Previous studies showed that turf grass cultivars belongs to *Lolium perenne L.*, *Poa pratensis L.*, *Festuca sp.*, *Agrostis sp.* are quite suitable to this region (Avcioglu, 2014). In the scope of this study, leaf form, biomass yield, regeneration power and variation of leaf colour according to the seasons of the cultivars that were improved different ecological conditions were determined.

Material and Methods

In this study 40 cool season grass cultivars in 8 species and 4 genus (Lolium, Festuca, Poa, Agrostis) were used. The experiment was established according to randomized plot design with four replicates in Samsun Conditions at November, 2010.

Plot sizes were 2x1=2 m^2, plot number were 40x4=80 and total experiment area was 40x2x4=320 m^2. Seeding rate was altered 25-90 g m^{-2} according to 1000-seed weight and plant characteristics. The seeds scattered with hand on plots and compressed. Leaf form (1-9), leaf colour and ranging of leaf colour depending on the seasons (1-9), regeneration power (1-5), fresh and dry biomass yields (g m^{-2}) were investigated.

Result and Discussion

Leaf form (1-9)

Leaf width of turfgrass plants is formed with the interactions of genetic and environmental conditions. For this reason, the highest and lowest leaf width of turfgrass plants should be determined and it should consider for selection process (Avcioglu, 2014). In terms of leaf form there were significant differences amongst the species. According to the result, coarse leaves were observed in *Festuca arundinacea* (3.3) and it is differentiated from the others (Table 1). The cultivars belong to *Festuca sp.* and *Poa trivialis* had thinner leaf form (Table 1 and Figure 1). These results are compatible to some previous findings, such as; leaf blades of *Festuca* plants twist on, thus their appearance is thin (Hubbard, 1992 and Beard, 1973); *Festuca arundinaceae* has the most coarse leaf form (Acikgoz and Basbug, 1993; Varoglu, 2000; Oztarhan, 2010).

Table 1. Leaf form, leaf colour and regeneration power of the species*

Species	Traits					
	Leaf form	Leaf colour				Regeneration power
		Spring	Summer	Autumn	Winter	
LP	4.96b	5.75ab	4.04bc	5.42b	3.08c	2.50b-d
FA	3.30d	6.20a	3.75c	5.45b	3.55c	2.10d
FRC	6.80a	6.50a	6.00a	6.75a	4.95b	2.20cd
FRR	6.9a	5.95ab	5.70ab	4.60c	5.20b	2.70bc
FRT	6.75 a	5.00b	4.75a-c	4.75c	5.25b	1.50e
FO	6.85a	6.90a	4.95a-c	6.65a	6.85a	2.80b
PP	4.79b	6.50a	1.83d	3.04d	6.75a	4.75a
PT	6.75a	6.75a	4.75a-c	2.75d	2.87c	5.00a
AG	3.91c	4.83b	6.08a	4.67c	5.50b	2.67bc

*There is no significant differences ($p \leq 0.01$) amongst the figures indicated in the same letter within the same column

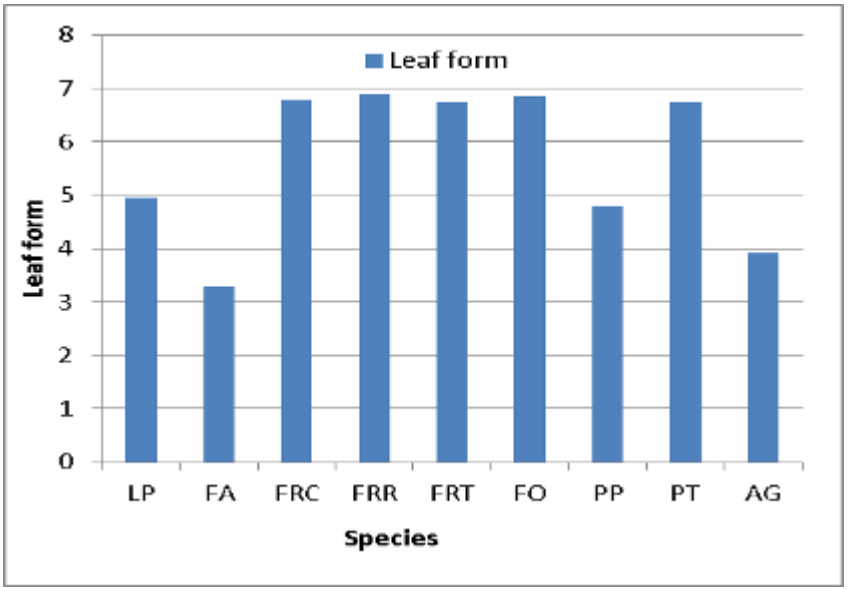

Figure 1. Leaf form of the species

Leaf colour and ranging of leaf colour depending on the seasons (1-9)

The colours of the species and colour variation depending on the seasons are given in Table 1 and Figure 2. The colour was changed significantly from season to season in all species. Colour change was limited in *Festuca rubra* and *Festuca ovina*, compare to the other species. All of the species were generally good appearance and their colour was dark green in spring. Responses of *Festuca arundinacea* and *Lolium perenne* to seasonal changing were similar. Only *Poa pratensis* has the darkest leaf colour in winter. The darkest leaf colour was determined in summer for *Agrostis sp*. cultivars. Though dark green leaf colour was lighten in summer for the whole plants, but the most effected from high temperature ones were cultivars of *Poa pratensis*. Leaf colour of the species was getting recovery in autumn, except for *Poa trivialis*. Yellowish green leaf colour of *Poa pratensis* turned to dark green in winter. Leaf colour of *Festuca ovina* cultivars was stabile throughout the year, except for summer. *Festuca rubra trichophylla* kept green leaf colour over the seasons. The results were compatible with previous findings (Varoglu, 2010; Oztarhan, 2010).

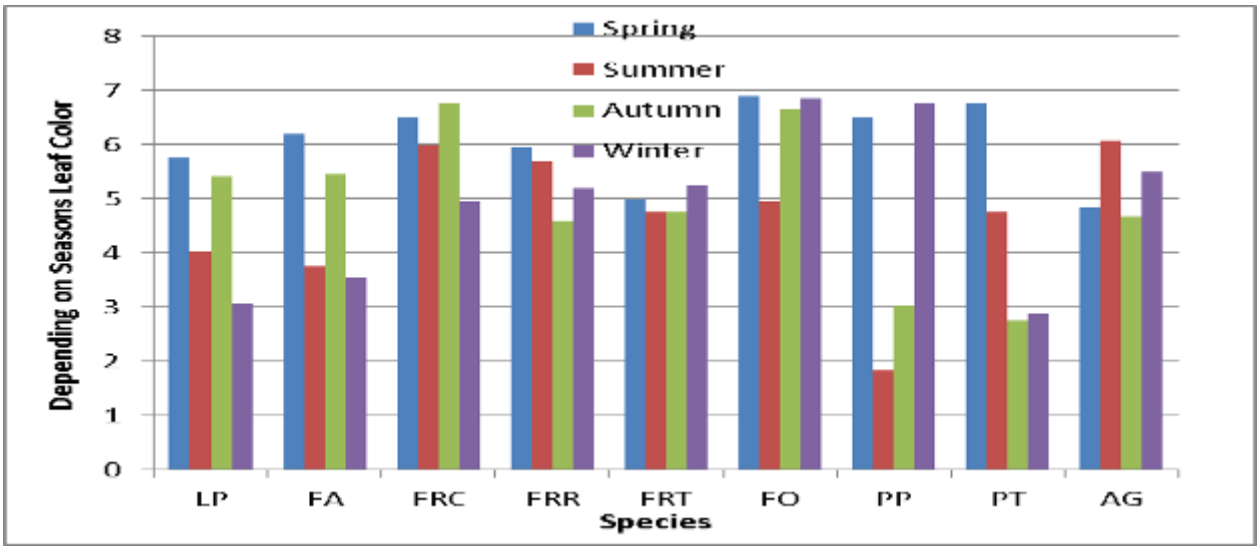

Figure 2. Leaf colour of the species depending on the seasons

Regeneration power (1-5)

Regeneration power data obtained from the cultivars as evaluating according to 1-5 scale (1= very fast growing, 3= medium growing, 5= very slow growing) in spring can be seen on Table 1 and Figure 3. Regard the regeneration power, statistically significant differences were identified amongst the cultivars. The highest regeneration power was in *Festuca rubra trichophylla*, while the lowest was in *Poa trivialis* and *Poa pratensis*. Similar results were obtained by Varoglu (2010).

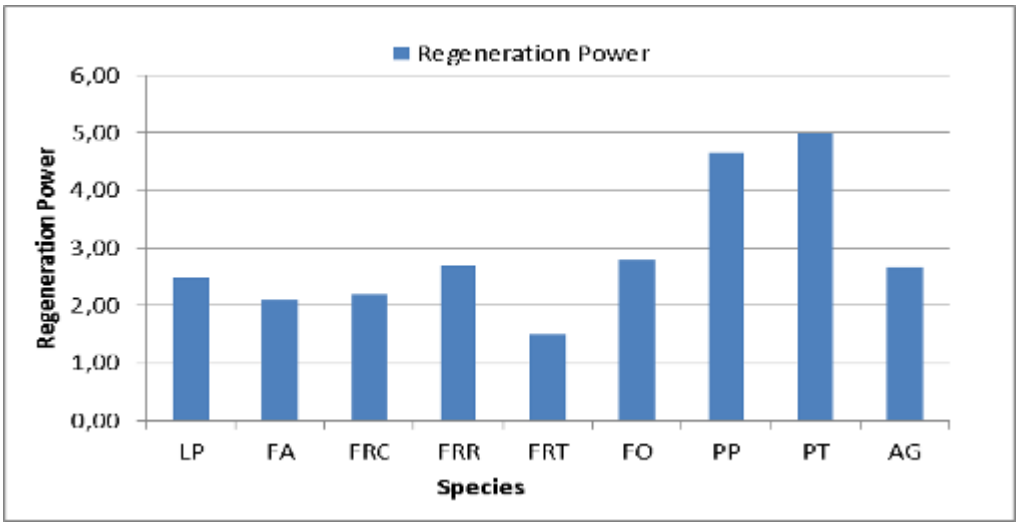

Figure 3. Regeneration power of the species

Fresh and dry biomass yields

Eventhough faster germination and emergence desire after sowing for turfgrass, in full covered turfgrass areas the slowest plant growing was demanded. After cutting faster covered but less biomass produce cultivars were prefered (Avcioglu, 2014). Four cuttings were made in the second year of the study and fresh and dry biomass yields were given in Table 2. There were significant differences among the species for all cuttings, in the point of fresh biomass yield. The highest fresh biomass yields per square meter were 1263.90 g from *Festuca rubra trichophylla*, 469.27 g from *Festuca ovina*, 323.87 g from *Lolium perenne* and 375.40 g, for 1., 2., 3. and 4. cuttings, respectively. On the other hand, the lowest fresh biomass yields were 237.80 g from *Poa trivialis*, 62.69 g from *Poa pratensis*, 97.77 g from *Poa trivialis* and 161.00 g from *Poa trivialis*, in order to consequtive cuttings. The lowest average fresh biomass yields were obtained from *Poa pratensis* and *Poa trivialis* in each one of the four cuttings. *Festuca arundinacea* gave similar and lower fresh biomass yields in the whole cuttings. Though the results have similarities with some previous findings, differentiated from some others. Hosaflioglu (2009) reported that in general the highest and lowest fresh biomass yields obtained from *Poa pratensis* and *Festuca rubra trichophylla* cultivars, respectively. Higher fresh biomass yields were obtained from the cultivars that have coarse tissue and wider leaf blade and it can be varied according to different environmental conditions (Zorer Celebi, 2009).

It is found that considering the dry biomass yield there were significant differences amongst the species in all cuttings. While the highest dry biomass yields for per square meter were obtained from *Festuca rubra tricophyla* with 320.13 g, from *Festuca ovina* with 110.67 g, from *Lolium perenne* with 68.16 g and from *Agrostis sp*. with 74.80 g, the lowest ones were determined for *Poa trivialis* with 50.01 g, for *Poa pratensis* with 12.22 g, for *Poa trivialis* with 18.58 g and for *Poa pratensis* with 32.98 g, respectively in successive cuttings.

Table 2. Fresh and dry biomass yields of the species*

Species	Fresh biomass yields (g m^{-2})				Dry biomass yields (g m^{-2})			
	1.Cut	**2. Cut**	**3. Cut**	**4. Cut**	**1. Cut**	**2. Cut**	**3. Cut**	**4. Cut**
LP	456.51c	282.80b	**323.87a**	210.67cd	87.59c	50.90bc	**68.16a**	40.87cd
FA	268.28c	267.80b	268.36ab	240.20cd	53.18c	49.19bc	56.122a	43.14cd
FRC	832.55b	377.99ab	258.27ab	329.60ab	183.00b	76.10b	51.44a	61.14ab
FRR	904.79b	341.87ab	238.77ab	**375.40a**	223.54b	68.81b	54.49a	**71.32a**
FRT	**1263.9a**	357.50ab	266.26ab	**352.00a**	**320.13a**	80.11ab	61.23a	62.40ab
FO	857.80b	**469.27a**	209.90bc	259.60bc	195.90b	**110.67a**	46.15a	52.12bc
PP	321.46c	62.69c	120.32cd	167.67d	71.82c	12.22d	23.77b	32.98d
PT	237.8c	111.61c	97.77d	161.00d	50.01c	21.29cd	18.58b	39.10cd
AG	1000.60ab	315.95b	288.25ab	382.67a	208.81b	65.84b	63.44a	**74.80a**

***There is no significant differences (p≤0.01) amongst the figures indicated in the same letter within the same column**

Conclusion

Species/cultivars have some superiority to each other because of their different morphological and physiological characteristics. When we consider weed competition, *Lolium perenne* and *Festuca arundinaceae* have to be added to the mixture in order to decrease weed competition because of their faster germination and higher regeneration power in Black Sea Region. There was colour variation distinctively depending on the seasons for all the species. But, compare to the others, cultivars of *Agrostis sp.* and *Poa pratensis* have the potential to keep leaf colour against to changing seasons. Leaf form and appearance of *Festuca rubra*, *Poa trivialis* and *Festuca ovina* are better than the others. In order to keep good conditions of turfgrass areas, especially after cutting in summer period, *Poa pratensis* and *Poa trivialis* should added in mixtures to get benefit their higher regenerating power.

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SUBSURFACE DRIP IRRIGATION SYSTEM

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Abstract

Irrigation is needed when rainfall is inadequate to meet plant water needs to increase efficiency in agricultural production. However, the water to be used in irrigation should be given to the root of the plant at the right time and in the right amount. For this purpose, various irrigation methods and systems have been developed. One of the systems that enables to save irrigation water within the developed irrigation systems and helps water directly reach the root zone is the subsurface drip irrigation (SDI) system. On the other hand, SDI systems, besides their many advantages, cannot provide expected benefits especially due to malpractices in operation, maintenance and installation of the system. Due to the simple faults and carelessness in the installation and operation of SDI systems generally installed with high costs and high expectations, SDI systems can sometimes become unusable. Designing a successful SDI system facilitates management and maintenance. It is not enough to design SDI systems properly. Besides the correct installation of the system, high efficiency can be obtained from the products grown under correct maintenance and operation conditions. In addition, it is possible to save irrigation water used in production. For this reason, the issues that need to be considered in the design and operation of subsurface drip irrigation systems (SDI), which have been widely used in irrigation in recent years, are discussed in this study.

Keywords: *Agricultural production, Irrigation method, Plant root zone*.

Introduction

In many parts of the world, global warming and the consequent irregularities that global warming caused in the precipitation regimes threaten the potential of available water resources. Irrigated agriculture is of vital importance in terms of meeting the needs of the rapidly rising human population. However, lands lose their qualities to be used for agricultural purposes due to the rapid industrialization and the effects of urbanization together with the inappropriate agricultural practices. In addition, increased freshwater demand from cities and industrial plants are putting more pressure on reducing the amount of water used in agriculture. Due to the limited water resources and increased water needs of non-agricultural sectors, it is necessary to develop new agricultural irrigation methods and systems so that they can use the available water in a conservative way. The need for this is increasing day by day. From this point of view, it is worth noting that subsurface drip irrigation (SDI) systems, which can save the amount of irrigation water compared to the other irrigation systems. However, the design, installation and management of SDI systems require relevant knowledge and experience. Errors in the installation and management stages cause the expected benefits of the SDI systems established with high costs and anticipations to fail. Today, the use of SDI systems is increasing. However, the system design, installation and operation must be done correctly. In studies earlier, subsurface drip irrigation systems were discussed (Howell at all., 1980; Bucks et all., 1982; Bucks and Davis, 1986). In some studies, the theory and different applications of the SDI were also discussed (Jorgenson and Norum, 1992; Ayars et al., 1999).
In this study, it is aimed to examine the recent developments in subsurface drip irrigation (SDI) systems. In addition, the issues that need to be taken into account in the establishment

and management of the system have been tried to be explained as much as possible. The system components and the issues to be considered in the system design stage are discussed.

Subsurface Drip Irrigation (SDI)

Subsurface drip irrigation systems have been part of irrigated agriculture in the United States for nearly forty years. However, the interest in this system has been increasing in the last 20 years. It is seen that the early drippers and pipes used in the SDI were quite simple and problematic compared to the drippers and pipes produced with the use of modern materials. In the early years, especially the clogging of the drippers and the inability to distribute the water in the desired amount caused some major problems. SDI has been becoming more popular thanks to the introduction of the plastic materials into the production systems and the developments in the dropper designs. However, the plant roots entering into the dripper and plugging the drippers are a problem that continued until recent years. SDI was the preferred system for growing high-value crops (such as fruits, vegetables, peanuts and sugarcane) in the beginning. As the system's stability and lifetime improved, the system started to be used more commonly for the plants with lower economic value. The fact that the system is for a multi-year purpose has come to the point to compensate for the high first installation costs. Over time, the SDI design criteria and equipment used in the installation (such as evacuation line, air vents) have developed. With the development of the special design criteria and usage equipment for different conditions (climate, plant, water quality, soil characteristics), the usage areas, system performance and system resistance of the SDI are also increasing accordingly.

Plant yield in the SDI applications is either the same or more when compared to the other irrigation methods. The use of water and fertilizers is the same or lower in some cases when compared to the surface drip irrigation and other irrigation methods. In the use of wastewater, the preference of SDI applications has increased in recent years. SDI can be a promising method especially in the areas where water quality is low and water saving is important for the future. SDI is an irrigation method (Camp, 2000) that provides a very precise and controlled application for the distribution of water and nutrients to the desired site at the desired time and frequency for optimal plant growth (Camp, 2000).

SDI System Components

Elements of the SDI systems are pump unit, check valve preventing water backpressure, flowmeter or water meter, fertilizer injection system, filter system, main pipeline, manifold pipeline, air suction cups, valves, pressure gauges, laterals, discharge valves. In Figure 1, the layout of the SDI system components is schematically shown.

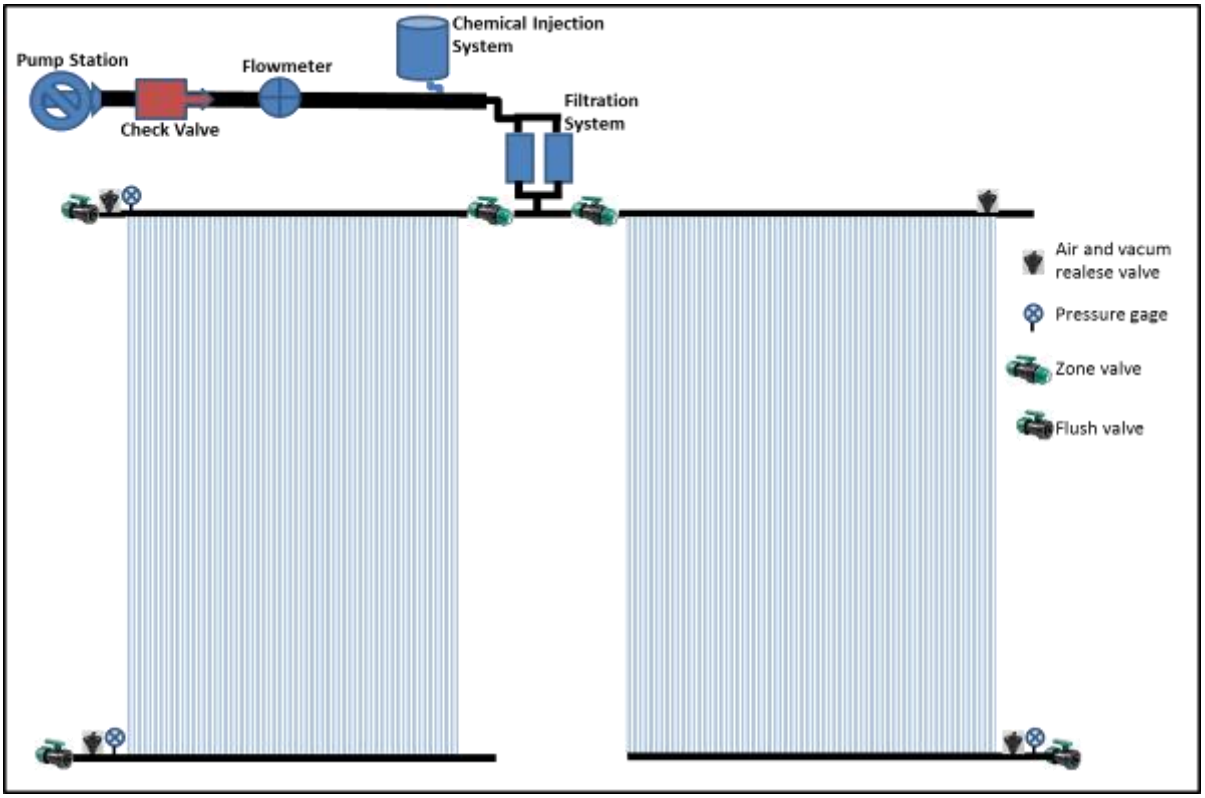

Figure 1. Subsurface drip irrigation system elements (adapted from Lamm et al., 2014)

Each of the system elements described in Figure 1 is handled separately, and each of them is seen to have a vital aspect in terms of management of the system. In the SDI system, the misuse of even one of these elements or ignoring any of them in the design stage can cause some serious problems.

SDI Design Features

In a successful SDI system, design, installation, management and maintenance are closely related to one another. Proper design of the SDI systems is not sufficient to have high yield and the system needs to be installed correctly, as well as the correct maintenance and operation of the system to save the amount of water used in the agricultural production. Prior to the design of the SDI systems, the characteristics of the water source should be assessed and analysis of the irrigation water quality should be made. The maximum and minimum amount of water flow that can be obtained from the water source should be calculated. The type of water source (deep well, irrigation canal, river, lake etc.) is very important. Some physical and chemical properties of the irrigation water should be determined.

At the start of the design process, two basic SDI design features need to be identified:

1) Selection of drip line (lateral diameter) and dripper
2) The shape of the drip line installation (depth to be placed in the lateral line)

The lateral pipe diameter affects the determination of the possible design patterns in the depth design process in which the interaction between the dripping element and the subsequent durability will be carried out. In other words, the choice of these criteria results in the design of the system.

Issues to be Considered in the Management of the SDI Systems

Since the lateral lines are buried under the ground in SDI systems, the risk of clogging is higher than that of the supersonic drip irrigation systems if relevant precautions are not taken. It is necessary to wash the laterals at regular intervals so that the various sized materials do

not accumulate in the drippers over time and cause clogging in the system. The frequency of the washing depends on the wash rate, irrigation water quality characteristics and filtration level of the filtration system. The minimum wash rate for micro irrigation systems is recommended to be as 0.3 m/s (ASEA, 2008). However, the recommended SDI systems are 0.3-0.6 m/s.

Both washing speed or washing line hydraulic properties affect the SDI system design. The high wash rate requires a large amount of water supply. The extension of the wash cycle contributes to an increase in the washing efficiency and a more economical process without requiring a greater wash rate. However, the velocity of the washing water should not be below 3 m/s (Lamm et al., 2014).

In SDI systems, the applied irrigation water is all buried underground. In properly installed and operating systems, wetting of the soil surface is rarely seen. For this reason, the flow meter and pressure indicators must be used to provide clues for feedback during the system start-up.

Some Examples of the Studies Examining the SDI Systems

Phena et al. (1987) reported that the SDI system provides advantages in five different ways when compared to ground surface drip irrigation on irrigated tomato plant: (1) The SDI system requires less labour use compared to the installation of ground surface drip irrigation due to its longer durability. (2) A decrease in soil-borne diseases and weed development due to the dryness of the soil surface has been observed. (3) It was found that the ability to move on the soil increased and the soil compaction rate decreased. (4) It was found that water and plant nutrients were used more effectively. (5) Improvement in the yield and fruit quality characteristics were found out.

Hanson et al. (1997) examined the effects of cablegation irrigation, surface drip irrigation and SDI applications on the lettuce plant. A slight decrease in the yield was found in the surface drop irrigation whereas similar results were obtained in the cablegation and SDI applications. SDI laterals were placed 15.2 cm deep from the soil surface. In both drip irrigation systems, the distance between the laterals was 101 cm. However, in the surface and subsurface drip irrigation areas, 43% and 74% of the water used in the mixed water irrigation was used.

In general, the increase in the plant yield and quality was observed with SDI application. For example, improved yields in cotton plant cultivation were reported under the SDI applications (Smith et al., 1991; Ayars et al., 1998).

Ayers et al. (1999) stated that some plants (generally small-seeded plants) may not be suitable for SDI irrigation during the germination period. However, it was reported that in the areas where the groundwater was near the surface and the groundwater level was high, efficiency and water use efficiency could be increased in the tomato, melon, maize plants by frequent and controlled SDI system irrigation.

Kassab (2005) tested sesame plants under different levels of irrigation using the surface drip irrigation, subsurface drip irrigation and surface irrigation methods. Irrigation was done 4 times in the surface irrigation method and 15 times in the ground surface irrigation methods. Seasonal amounts of irrigation water applied to the treatments; 437 m^3/da in the ground surface irrigation method and 237 m^3/da in surface irrigation methods. The highest yield values obtained according to the irrigation methods were 234 kg/da for the surface irrigation method, 224 kg/da for the subsurface irrigation method and 205 kg/da for the ground surface irrigation method. In the subsurface drip irrigation method, yield decreased by 4.3% compared to the surface irrigation method and 46% savings from the irrigation water.

Douh et al. (2013) conducted a study in Tunisia and examined the placement of SDI laterals in three different soil depths and their effects on the yields of the corn plant and soil moisture. It was stated that the yield of soil moisture and corn plants was higher in the case where the

soil was buried at a depth of 35 cm compared to the cases where laterals were buried at the depths of 5 and 20 cm from the soil surface.

Lamm et al. (2015) made a comparison of centre pivot (CP) and subsurface drip irrigation (SDI) systems with regards to their economic benefits. CP was found to be a more economical irrigation system in the corn cultivation when the cost of installation and service life were considered. However, it was reported that irrigation water used in SDI applications was saved by 25% compared to the CP applications.

Ayers et al. (2015) reported that the use of SDI in walnuts, almonds and tomatoes, which are commonly grown in California region in the United States, increased the yield and quality of the fruit. In addition, it was stated that the amount of water used under the SDI was reduced.

Robles et al. (2016) compared SDI and ground surface drip irrigation systems in terms of water use efficiency and efficiency at a 19-year-old lemon yard in a survey conducted in Spain. It was reported that the SDI system saved 19% of the amount of irrigation water without loss of productivity.

Conclusions

The studies conducted on SDI began to be studied intensively in the mid-80s and early 90s. These studies reveal that SDI applications provide some advantages in the use of fertilizer and water management compared to the other irrigation methods. As the increase in the plant yield and quality was observed, it was found that the amount of plant nutrient leaking into the deeper soil was decreased by the washing. The amount of irrigation water used was also reduced.

Compared to the other irrigation methods, SDI reveals good results in terms of crop efficiency and efficiency in water use. SDI is an irrigation system with a capacity that can enable fertilizer and water management to be carried out at the desired level, especially in the areas where water resources are limited or areas where the groundwater level is high. However, for the system design and installation should get help from a competent person. Adequate information on the use of the system should be provided.

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IRRIGATION IN MELON CULTIVATION

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Abstract

As in many cultivated plants, the irrigation of the melon plant when the water needs cannot be met in natural ways causes significant increases in crop yield. However, when the water resources are limited or the irrigation costs are high and when the amount of irrigation water used is reduced, the water requirement of the plant is incomplete. In such cases, there is a need to develop practices that can save irrigation water without causing a significant decrease in the yield and the yield quality. One of the practices that can save irrigation water is irrigation schemes. Particular irrigation schemes can be applied during the whole developmental period of the plant and at certain stages of the developmental period. There are many pieces of researches conducted on melon cultivation under deficit irrigation practices. The aim of this study is to determine the periods during which the plant is most sensitive to the water shortage. Thus, irrigation schedules that will save the most irrigation water used, with the least possible loss in yield and yield quality are determined. In addition, possible responses of the melon plant such as water consumption under furrow and drip irrigation methods have been investigated. Thus, it is aimed to determine the best irrigation method in melon cultivation. In this article, some researches on melon cultivation under different irrigation methods and different irrigation treatments have been evaluated.

Keywords: *Crop yield, Water resource, Deficit irrigation*.

Introduction

Turkey is the second country after China making the most of the melon production in the world. According to the data of 2016, 1.8 million tons of the total annual melon production of approximately 31 million tons in the world is produced in Turkey (Anonymous, 2018).

There are different opinions about the homeland of melon. Pitrat et al. (1999) claim that the types of wild plants which are considered to be Cucumis type are commonly seen in Africa, that the origins of melon come from Africa and the lands in Asia between Turkey and Japan are considered to be the secondary gene centre of melon plant. Günay (1993) recorded Anatolia, Iran, Afghanistan, Central Asia and Southwest Asia as origins of melon plant.

There are many types of melons that can be taken as wild and cultured and exhibit different morphological characteristics spreading over a very wide geographical area. This is exemplified by "Galia" cultivated in Israel and "House" melon cultivated in Japan (Pitrat et al., 2000). Melon species and varieties have significant morphological differences in terms of leaf and fruit characteristics in plants (Şensoy et al., 2007a).

A large portion of the melon produced in Turkey (85%) inodorous (Kırkağaç, Hasanbey, Slots and Winter Yellow) other parts (15%) is counted in the cantalupensis and reticulatus group (Baktemur, 2010). Melon cultivation in Turkey widely made under outdoor production conditions in the Mediterranean region and also can be produced in low tunnels and greenhouse conditions.

As in many plants, the irrigation of plant results in significant increases in yield when the water needs of plants cannot be met with the rainfall. However, irrigation of plants is neglected when there is not enough irrigation water and when water resources are limited or

irrigation costs are high. There are a number of studies aimed at finding out the amount of deficit that can be applied to irrigation water without causing significant decline in the yield and quality in such situations. In this study, some of the research on deficit irrigation in the melon carried out in the world and Turkey were discussed.

Literature Review

Deficit irrigation applications can be made during the whole developmental period of the plant in a single developmental stage. The aim of the study is to determine the stage or stages in which the melon is most sensitive to the water shortage. In this study, it is aimed to find out the irrigation programs that will save the most irrigation water with the least loss in the yield and quality after determining the periods when the melon is most sensitive to water shortage.

Yildirim et al. (2009) investigated the effect of full or partial irrigation applied on the yield and quality of melon in different periods of development (beginning of flowering, beginning of flowering, ripening and harvesting) using a Kırkağaç melon species (Cucumis melo L. To the water deficit treatments to which 50% and 75% of the water applied to the control group was applied. According to the results of the research, continuing the irrigation in maturation period in the treatments other than the control treatments increased vegetative growth and did not significantly affect the yield and dry material. However, when the irrigation level is taken into consideration, a higher water-soluble dry matter content and tastes were obtained in the case of 25% reduction in the irrigation water amount, compared to the 50% water restriction applied without water restriction. As a result of the research, it was suggested that irrigation should be maintained until the beginning of maturation, and irrigation in the ripening period should not be made to have higher yield and quality. As a result of the research, it has been suggested that 25% water reduction can be applied until the maturation period and the irrigation should be cut during the maturation period.

Li et al. (2012) conducted a study under greenhouse conditions, and the soil water content was brought back to the level of field capacity by irrigation when it fell to different levels (45%, 55%, 65% and 75%) of water content in field capacity. The amount of irrigation water applied in the study was found to have a significant effect on fruit thickness, water-soluble dry matter content, soluble sugar content, soluble protein content, vitamin C content and free amino acid content values. In the study, it was found that irrigation should be done at a rate that does not exceed the field capacity when the soil moisture content reaches 25% of the melon content in melon cultivation.

Wang et al. (2017) conducted another study on melon plant and found out that irrigations were planned to be carried out at different plant growth periods, at different levels of soil water content (soil moisture content fell to 45% and 55% and 65%, respectively) and brought to the field capacity by watering. According to the results obtained, it was reported that the effect of low soil water content conditions (when the irrigation water drops to 55% of the field capacity of the irrigation water) during the period from the flowering period to the fruit growth period had a negative effect on the yield.

Alizadeh et al. (1999) conducted a research, and it was found that irrigation of melon plant made with drip and cablegation irrigation methods led to the highest yield from full irrigation and drip irrigation system when the full, 25% deficit and 50% deficient conditions of the plant were considered. It was reported that the treatment irrigated with drip irrigation and 25% irrigation water deficit was applied did not cause any loss in the yield and the irrigation water consumption in cablegation method was three times higher compared to the value regarding the plant water consumption in drip irrigation system.

Kirnak et al. (2005) carried out a research in the province of Şanlıurfa using the Polidor hybrid melon species. In the study, irrigation treatments were arranged according to the evaporation values coming from the Class-A evaporation pan. Irrigation, nitrogen yield and

quality effects of melon plant were investigated together. In the study, it was found out that as the water content increased, fruit weight and size decreased while the amount of water-soluble dry matter increased. It is stated that as the water content increased, the value of leaf-proportional water content decreased, the amount of applied irrigation water and the amount of nitrogen increased, and the plant leaf area increased. In the study, it reported that while the pan coefficient was 0.75, it was found a loss of 14-17% in the yield of moderate water stress, in severe water stress it was observed 55% to 59% yield loss.

Sensoy et al. (2007b) used a Bonanza F1 melon variety as an experimental material in a study conducted in Van province. The effects of irrigation programs on different Class-A evaporation pan and irrigation intervals according to different irrigation intervals were investigated in irrigation methods. It was reported that the highest yields were obtained with a 6-day interval and using the evaporation coefficient of 0.9. The average fruit weight, fruit diameter, fruit length, water-soluble dry matter content and fruit juice pH were measured in the study. It was stated that the amount of irrigation water applied in the study affected the fruit diameter, fruit size, water-soluble dry matter content and fruit juice pH, and irrigation frequency had an effect on average fruit weight.

Dogan et al. (2008) conducted a two-year study in Şanlıurfa province, and different irrigation levels were investigated on the surface of the irrigated water using surface and superficial drip irrigation systems. Irrigation treatments were arranged to give 0, 25, 50, 75%, 100% and 125% irrigation water of the amount of evaporation coming from the Class-A evaporation pan. Pineapple F1 was used as plant material. There was no significant difference in the efficiency between the two irrigation systems used. However, in the calculation of the ideal irrigation water to be applied, it was proposed to use 83% of the evaporation from the Class-A evaporation pan in the under-surface drip irrigation system and 92% in the superficial drip irrigation system.

Wells and Nugent (1980) reported that the high soil water content during the ripening of the melon fruit reduced the amount of water-soluble dry matter in the fruit.

Leskovar et al. (2001) conducted a study in the US Southern Texas, Caravella melon was used as plant material in the Cantalop group. In this study, it was stated that the highest fruit yield and quality were obtained with irrigation systems and surface irrigation systems when compared to cablegation irrigation method, surface irrigation method and superficial irrigation method. In the same study, 53% less irrigation water was used in the drip irrigation method compared to the cablegation irrigation method.

In a study conducted in Spain, it was stated that the lack of moisture in the soil is sensitive to the lack of moisture in the early stages of fruit formation during the plant development. In addition, it was found out that the deficit irrigation condition applied during fruit ripening period increased the content of fruit sugar (Fabeiro et al., 2002).

Sharma et al. (2014) found out that the changes in the fruit yields were affected when the irrigations were applied to different melon species. During two breeding seasons in Texas, three types of foliage [Mission (muskmelon, reticulatus), Da Vinci (tuscan; reticulatus), Super Nectar (honeydew; inodorus)] were used in the conditions in which 100% and 50% of the irrigation water needs were met. In the study, the response to water shortage of varieties was different. In the study, it was stated that the water shortage saved between 37% and 45% in irrigation water in Mission and Da Vinci species, resulting in a moderate decrease in the economic efficiency. It was reported that 50% reduction of plant water consumption value in the Super Nectar variety was not suitable for the Super Nectar variety (Sharma et al., 2014), because 50% reduction of the plant water consumption caused a significant amount of productivity loss. Therefore, the 50% deficit in the water consumption value in the Super Nectar species was not found to be appropriate (Sharma et al., 2014).

Results and discussion

The response to water shortage and quality of melon species can vary considerably (Sharma et al., 2014). However, it is still possible to achieve some general conclusions based on the information summarized in the previous section, without completely ignoring the fact mentioned above. It can be suggested that the period in which the melon is most sensitive to water shortage is the development period from the beginning of flowering to the beginning of the fruit formation period (Fabeiro et al., 2002; Wang et al., 2017). However, it was found out that the deficit irrigation applied during the fruit ripening period increased the content of fruit sugar, and that the level of high soil water content decreased the fruit sugar content in this period (Wells and Nugent., 1980; Fabeiro et al., 2002). It can be suggested that the plant growth period in which water shortage causes the least loss in yield is fruit ripening period (Yıldırım et al., 2009). It is not recommended to reduce the amount of irrigation water to be applied by 25% (Alizadeh et al., 1999) and to apply the irrigation water at a rate of 50% or higher within the water deficit levels investigated (Kırnak et al. 2005). Similarly, irrigation should be done when less than 50% of the available water is consumed in melon cultivation (Wang et al., 2017). However, it can be suggested that irrigation is more appropriate when 25% of the available water is consumed (Li et al., 2012). It was found out that the drip irrigation method could save 53% of irrigation water compared to the cablegation irrigation method (Alizadeh et al., 1999) and it was found out that water consumption increased three times compared to drip irrigation method (Leskovar et al., 2001). In the studies conducted on melon plant; it was found out that the amount of irrigation water applied can change depending on the locality, climate where the fruit is cultivated, melon variety, the irrigation method used and the amount of irrigation water (Table 1). The amount of irrigation water given in Table 1 is the amount of irrigation water applied in conditions without any water deficit.

Table 1. Amount of irrigation water applied in melon cultivation

Group	Variety	Location/Country	Irrigation method	Irrigation amount (mm)	References
Cantalupensis	Bonanza f1	Van/Turkey	Furrow	549	Sensoy et al., (2007b)
Cantalupensis	Caravelle	Texas/ABD	Furrow	406	Leskovar et al., (2001)
Cantalupensis	Caravelle	Texas/ABD	Furrow/mulch	406	Leskovar et al., (2001)
----------	Huanghemi-3	Wuwei City/China	Furrow/mulch	218.1	Wang et al., (2017)
----------	Ananas f1	Şanlıurfa/Turkey	Drip	481.2	Doğan et al., (2008)
Kırkağaç	Kırkağaç	Ankara/Turkey	Drip	331.1	Yıldırım et al., (2009)
Cantalupensis	Caravelle	Texas/ABD	Drip/mulch	284	Leskovar et al., (2001)
Cantalupensis	Ananas f1	Şanlıurfa/Turkey	Subsurface Drip (30 cm)	481.2	Doğan et al., (2008)
Cantalupensis	Caravelle	Texas/ABD	Subsurface Drip (10 cm)/mulch	272	Leskovar et al., (2001)
Cantalupensis	Caravelle	Texas/ABD	Subsurface Drip (30 cm)/mulch	292	Leskovar et al., (2001)

Conclusions

Less water is used in surface and sub-surface drip irrigation methods than in cablegation irrigation method in melon cultivation. Generally, there is no significant difference between yield and surface irrigation methods. The use of mulch can be effective in reducing water evaporation in melon cultivation because it reduces the amount of evaporation from the soil surface.

When irrigation planning is carried out, it is appropriate to apply water deficit at the rate of 25% or less of the amount of irrigation water under normal conditions if water deficit needs to be applied. In this case, water saving can be achieved without causing a significant decrease in the yield and fruit quality. If the water deficit is applied in any part of the plant development period, it should not be ignored that the melon is sensitive to water shortage during the period from the flowering to the beginning of the fruit ripening period. The lack of irrigation in the period from the start of the maturation period to the harvest may lead to a significant increase in the sugar content in the fruit.

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ENDANGERED *COLCHICUM* SPECIES OF TURKEY

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Abstract

Turkey is one of the major centers of diversity in *Colchicum* species from Colchicaceae family. The genus *Colchicum* L. is represented by 47 species, of which, 35 are endemic to Turkey and some are under threat. Turkey has 3 *Colchicum* species in different categories of IUCN Red List. *Colchicum balanse* and *Colchicum troodi* are in Least Concern, *Colchicum leptanthum* is in Critically Endangered of IUCN Red List categories. Major threats of *Colchicum leptanthum* are dam and road construction. The threats to *Colchicum balanse* and *Colchicum troodi* are unknown according to IUCN Red List.

Collection of the all *Colchicum* species is prohibited in the wild at national level by regulation in Turkey. Some morphological and anatomical studies, description of new species, in-vitro propagation and karyotype studies on *Colchicum* were carried out in Turkey. *Colchicum* species have been used as a medicinal plant for more than 3000 years. *Colchicum* species have valuable alkaloids especially colchicine used in medicine. *Colchicum* species have also potential as ornamental plants. Their pharmacological usage and potential as ornamental plants increase their importance. Therefore, it is very important to protect genetic resources of these species. Some of these species are endemic. Lost of these endemic species in the Turkey flora, means their lost from the world. In this review, conservation actions and some information about the endangered *Colchicum* species in the flora of Turkey that are IUCN Red List categories were given and discussed.

Key words: *Endangered Colchicum spp, IUCN Red List, Endemic, Threats, Conservation*

Introduction

Colchicum is a perennial geophyte and belongs to Colchicaceae family. Because of the high frequencies of species and endemics, Turkey and the Balkans are the major centers of diversity and speciation (Persson, 1993; Karakaş Metin et al., 2014). There are 100 *Colchicum* species on the earth (Persson et al., 2011). Turkey has 47 *Colchicum* species and 35 of them are endemic and they are not spread to other countries (Persson, 2007; Kaya et al., 2013)

Colchicum species are very important because of being used for ornamental (landscaping, flowerbeds and rock gardens) and medicinal purposes (Karakaş Metin et al., 2014). However, many plants genetic resources, including *Colchicum* are under genetic erosion because of the environmental and other problems and therefore face with the danger of extinction. *Colchicum* species are one of the most important and endangered species of wild collected bulbs in flora of Turkey.

Turkey has 3 *Colchicum* species in different categories of IUCN Red List. *Colchicum balanse* and *Colchicum troodi* are in Least Concern, *Colchicum leptanthum* is in Critically Endangered of IUCN Red List categories. *Colchicum leptanthum* is endemic to Northeast Anatolia of Turkey (Ekim et al., 2014). There are also some other *Colchicum* species that are reported to be endangered (Tehditaltındakibitkiler, 2018).

International Union for Conservation of Nature and Natural Resources, IUCN

The IUCN Red List Categories and Criteria, is designed as an easily understood system for classifying species at high risk of global extinction (Allen et al., 2014). The IUCN Red List provides taxonomic, distribution, ecological, threat and conservation status information on

taxa that have been evaluated using the *IUCN Red List Categories and Criteria* (IUCN, 2012; Allen et al., 2014). These categories are; Extinct (EX), Extinct in the Wild (EW), Regionally Extinct (RE), Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC), Data Deficient (DD), Not Applicable (NA) and Not Evaluated (NE) (IUCN, 2012; Allen et al., 2014).

Colchicum Species of Turkey in IUCN Red List Categories

Turkey has three *Colchicum* species in various categories of IUCN Red List (Table 1).

Table 1. *Colchicum* species of Turkey in IUCN Red List Categories

Colchicum Species	Red List Category	Red List Criteria	Endemism
Colchicum balansae	LC	-	-
Colchicum leptanthum	CR	B2ab(iii,v)	Northeast Anatolia
Colchicum troodi	LC		-

Colchicum balansae

Red list category: Threat category is Least Concern (LC) for *Colchicum balansae.*
Endemism: It is not endemic (Düşen and Sümbül, 2007; Thacker, 2014).
Major threats: The threats to this species are unknown (Thacker, 2014).

Colchicum leptanthum

Red list category: Threat category is Critically Endangered (CR) (Eminağaoğlu et al., 2010).
Red list Criteria: B2ab(iii,v) (Ekim et al., 2014).
Endemism: Northeast Anatolia (Turkey) (Persson, 2001; Eminağaoğlu et al., 2010; Ekim et al., 2014).
Major threats: The species habitat is threatened by dam and road construction (Ekim et al., 2014), grazing (Eminağaoğlu et al., 2010).

Colchicum troodi

Red list category Threat category is Least Concern (LC) (Fragman-Sapir et al., 2017).
Endemism: It is not endemic (Fragman-Sapir et al., 2017).
Major threats: There are no known significant past, ongoing or future threats to this species (Fragman-Sapir et al., 2017).

Other *Colchicum* species that are under threat in Turkey

The other *colchicum* species that are reported to be under threat in Nezahat Gökyiğit Botanical garden are *C. baytopiorum, C. bornmuelleri, C. burttii, C. micranthum, C. micranthum*. These species are endemic to Turkey (Tehditaltındakibitkiler, 2018).

Discussion and Suggestions against Threats

Geophytes are the most preferred group among the ornamental plants due to their aesthetic features, suitability to be cut flowers and their fragrance. They have economic value due to their attractive flowers and usage in the drug industry (Karakaş Metin et al., 2014; Çığ and Başdoğan, 2015; Çakır, 2017). Turkey has rich genetic resources of geophyte including *Colchicum* species. However some of them are under threat. The important endangered *Colchicum* species are growing in different part of Turkey (Fragman-Sapir et al., 2017; Ekim, 2014; Thacker, 2014).

Colchicum species are very important due to their potential usage as ornamental plants and their pharmacological usage. It was reported that *Colchicum* species bloom in different times and can be used in landscape field (Kaya et al., 2013). The major alkaloid of *Colchicum* is

colchicine (Simanek et al., 1990; Düşen and Sümbül, 2007). Colchicine is an agent with antitumor properties used in chemotherapy (Fell and Ramsden, 1967; Düşen and Sümbül, 2007; Pırıldar et al., 2010, Sevim et al., 2010). Therefore, it is very important to protect these genetic resources of *Colchicum* due to their potential usage as ornamental plants and their pharmacological usage. In addition some of these species are endemic to Turkey. Lost of these endemic species in the flora of Turkey, means their lost from the world.

Grazing, dam and road construction are among the main threats for *Colchicum* species in our country, while the other threats to *Colchicum* species are unknown. These threats should be taken under control to protect the areas where *Colchicum* species naturally grown. In protecting the fauna and flora, restoring ecological balance, preserving and protecting rare and threatened plant species all over the world, several arrangements are being implemented (Akçakaya et al., 2000, Ibadullayeva et al., 2011). In order to save the flora Turkey, some measures should be taken. Prohibit or control the collection of all native plants without a special permit is of important. Collection of the all *Colchicum* species is prohibited in the wild at national level by regulations concerning bulbous plants. Nevertheless the government must increase the legislative measures.

Educating the local people about the importance of the native plants under threat is also important to increase the realization and care to flora (Çelikel, 2014). It was also reported that it is important increase awareness of local residents and visitors to reduce human impact (Manvelidze et al., 2009). The endemic and endangered species must be conserved.

Cultivation and propagation studies on these species should be encouraged and increased. In the frame of TUBİTAK project 47 *Colchicum* species (36 of which are endemic) were taken under conservation in Turkey (Kaya et al., 2009). Ellington et al. (1997) reported that corm of *C. autumnale* treated with paclobutrazol showed some basal protuberances. Corms of *C. luteum* were treated in vitro with soluble chitosan added to the MS tissue culture medium and chitosan increased the germination percentage (Wagh et al., 2015). In addition there are some studies about seed germination of *Colchicum* (Antonidaki-Giatromanolaki et al., 2008; Fukai et al., 2013). Studied on *Colchicum* species are mostly about their alkaloid content, especially colchicine activity on some diseases. It is clearly understood that vegetative and generative propagation methods of *Colchicum* species should be increased and improve to their sustainability and restore to the nature. Tissue culture techniques also are important for both propagation and in vitro conservation.

Conclusions

As a conclusion, *Colchicum* species have been of great economic importance from past to present due to their properties in medicine. It is also valuable for biodiversity and due to potential usage of ornamental plants. More research studies on biology, ecology, propagation methods and cultivating techniques of *Colchicum* species are among the main conservation measures for habitat and population of *Colchicum* species. In addition, management of grazing, cultivation in botanical gardens or maintenance in seed banks, reintroduction or reinforcement of populations are important to save endangered *Colchicum* species and other native plants of Turkey.

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SOME PERFORMANCE INDICATORS OF SPRINKLER IRRIGATION SYSTEMS ON THE SANDIKLI PLAIN (TURKEY)

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Abstract

Irrigation is one of the indispensable yield-increasing elements in agriculture especially in arid and semi-arid regions like Turkey. To obtain the benefit expected from irrigation, water use parameters, system designing-projecting criteria and system performances should be determined both at irrigation schemes and – on the parcel scale – in individual irrigation systems. For this purpose, this study was carried out to evaluate some performance indicators of sprinkler irrigation systems on the Sandıklı Plain in Afyonkarahisar, Turkey; to determine the performance of those systems; and to find out what precaution should be taken to increase the efficiency of the systems. The fieldwork of the study was performed on the field including the Koçhisar, Ülfeciler, Örenkaya, Kusura, and Ballık Villages of Sandıklı in 2012. For this purpose, 15 sprinkler irrigation systems were selected in regions where potato cultivation was intense, representing the Sandıklı Plain. Christiansen uniformity coefficient (CU), distribution uniformity (DU), sprinkler pressure as well as sprinkler head displacement and change were evaluated for each system. The water distribution uniformity was determined by doing single lateral line water distribution tests. Average sprinkler flow rates and soil infiltration rates were found between 6.84 and 37.46 mm/h and between 8 and 23 mm/h, respectively. Christiansen uniformity coefficient (CU) ranged from 44 to 86% and distribution uniformity from 25 to 84%. Furthermore, it was determined that the pipe diameters in 4 systems were poor and 11 operators were found within the acceptable level. Considering these obtained results, there may be increases in Christiansen uniformity coefficient and distribution uniformity values in the event that producers make improvements in their irrigation systems. These improvements in the performance indicators will enhance the efficiency in water use.

Keywords: *Sprinkler irrigation system, Christiansen uniformity coefficient, Distribution uniformity, Potato.*

Introduction

One of the most important inputs for the enhancement and sustainability of agricultural production particularly in arid and semi-arid regions is water. Achieving a water-based increase in yield depends on the providing of a plant with water at the time and amount the plant desires. The greatest problem of agriculture in Turkey, as in all arid and semi-arid regions, is the deficient irrigation water. Therefore, it is a must to make best use of the available irrigation water. Obtaining the benefit expected from the lands opened for irrigation depends on the planning, projecting, and operating of irrigation systems in agreement with soil, crop, and topographical conditions. Thus, the possible projecting and operating errors of the systems set up should be determined and, if available, these errors should be corrected. Of 112 billion m³ of annual average total available water in Turkey, 44 billion m³ are used in agriculture and industry and as drinking water. About 73% of this water is used in agricultural irrigation (Gökalp and Çakmak, 2016). The efficient use of water has gained importance as the demand for water is increasing. However, excessive water is still being wasted in agricultural irrigation (Akkuzu et al., 2011). Such cases as aridity, climate change, water

shortage, and technological developments in the recent years have made it inevitable to make a transition to pressurized irrigation methods instead of surface irrigation methods. The most common pressurized irrigation method is the sprinkler irrigation method (Kırnak et al., 2013). Negative cases occur in the sustainability of investments due to the use of materials with various properties in the irrigation area, different water distribution patterns, and low system performances. It is essential to reveal the reasons for this situation in systems with low efficiency and to take the necessary measures to this end (Dinç and Bahçeci, 2006).

Although sprinkler irrigation systems are used to irrigate different crops on the Sandıklı Plain in Afyonkarahisar, which is the study area, they are predominantly used to irrigate potato and sugar beet. This research was carried out to evaluate the performances of the sprinkler irrigation systems used in the potato growing areas out of the crops for which sprinkler irrigation was intensively used; to reveal how efficiently these systems worked; and to determine what kind of precautions should be taken to improve the system performances.

Material and Methods

The Sandıklı Plain, where the research was carried out, is at the geographical location between 29°50'-30°30' eastern meridians and 38°15'-38°45' northern parallels. The lowest mean temperature is 0.9 ^{o}C in January, whereas the highest mean temperature is 23.8 ^{o}C in August. The annual average relative humidity in the region is 61.9%. The annual average total precipitation is 445.2 mm (Anonymous, 2013). The irrigable area on the Sandıklı Plain is 49,372.9 hectares, of these 26,588 hectares (53.85%) were opened for irrigation. Sprinkler and drip irrigation methods are commonly used in Sandıklı plain. The field including the Koçhisar, Ülfeciler, Örenkaya, Kusura, and Ballık Villages, where potato growing was common and the sprinkler irrigation method was employed in irrigation, was determined as a pilot region and 15 sprinkler systems were randomly selected in this field.

The types of sprinkler systems, the number of laterals on the main pipeline, sprinkler spacing, the irrigation water source, the pumping unit, diameters of the main pipe and the lateral pipe and the types and nozzle diameters were determined in the research. Infiltration tests were done in all parcels that have sprinkler irrigation systems according to the Güngör and Yıldırım (1989). The lateral discharges were measured by the help of a water meter in the system. The average sprinkler discharge rate was found by volumetrically measuring the end sprinkler discharges on the laterals. The sprinkler pressures were determined by means of a pitot tube manometer (Figure 1).

Figure 1. Measuring sprinkler pressure and catch can

Christiansen's method was utilized to make a hydraulic evaluation of the lateral pipe diameters (Korukçu and Yıldırım, 1981). The singular lateral method was employed to obtain the water distribution patterns. After obtaining the water distribution patterns as a result of the experiments, Equation 1 was used to find Christiansen uniformity coefficients (Cu) based on

the catch volumes (or depth) but Equation 2 to calculate distribution uniformity (Du) (Christiansen, 1942; Korukçu and Yıldırım, 1981):

$$C_u = \left(1 - \frac{\sum_{i=1}^{n} |h_m - h_i|}{n \times h_m}\right) \times 100 \quad (1)$$

where h_m - the average water depth collected in all catch cans (mm),
h_i - the water depth collected in catch can (mm),
n - the number of observations in the uniformity test.

$$Du = 100 \frac{\overline{X}_{lq}}{X} \quad (2)$$

where X -the average volume of water received on the test area (mm),
$\overline{X}_{lq}$ -the average low-quarter volume of water received (mm)

The average sprinkler rate was calculated:

$$h_m = \frac{q \times 1000}{L_1 \times L_2} \quad (3)$$

where h_m - the average sprinkler flow rate (mm/h)
q - sprinkler discharge (m^3/h)
L_1- sprinkler spacing on the lateral (m)
L_2- lateral spacing (m)

Results and Discussion

Properties of the pumps and pipelines

Electric pumps were used in the parcels with an electric network but pumps with diesel fuel in the parcels with no electric network out of the selected parcels. The power of the pumps in the study area ranged from 27.5 to 85 HP. A 27.5 HP pump, a 30 HP pump, a 32 HP pump and an 80 HP pump were available. Of the rest, 3 were 35 HP pumps, 3 were 40 HP pumps, and 5 were 50 HP pumps.

The main pipelines varied between 45 and 1,000 m in length. All lateral pipelines were arranged as portable in the research area. It was seen that some of the main pipeline was buried in soil in sprinkler irrigation systems No. 12, 14, and 15. Pipes with diameter of 90 mm were used as the main pipeline in 13.4% of the systems, while pipes with diameter of 110 mm were used in 26.6% of them, pipes with diameters of both 90 mm and 110 mm in 46.6% of them, and pipes with diameters of both 110 mm and 125 mm in 13.4% of them. Although it is undesired to use pipes with two different diameters without any lateral separation on the main pipeline since it will cause different pressures, friction loss, and water speed in the system, two different pipe diameters were discovered to have been used in 9 of 15 sprinkler irrigation systems under examination (Table 1).

Table 1. Properties of the pipes used on the main pipelines in the sprinkler systems

Sprinkler System No.	Main Pipe					
	Type	Length	Diameter, mm			
	Plastic	5 (m)	90	110	90-110	110-125
Number	15	2	2	4	7	2
Rate (%)	100	13.4	13.4	26.6	46.6	13.4

Pipes made of two different materials were found to have been used on the lateral pipelines. Pipes manufactured from an aluminum material were used as lateral pipes in 8 sprinkler irrigation systems (53.4%), whereas pipes made of a polyethylene material were used in 7 of them (46.6%). All lateral pipe diameters were 75 mm in the sprinkler irrigation systems (Table 2).

Table 2. Properties of the pipes used on the lateral pipelines in the sprinkler systems

Sprinkler System No.	Lateral Pipe				
	Type		Length		Diameter, mm
	Plastic	Aluminum	5 (m)	6 (m)	75
Number	7	8	7	8	15
Rate (%)	46.6	53.4	46.6	53.4	100

Sprinklers

All sprinklers in the study area were double nozzle. As it will be seen in Table 3, the nozzle diameters of the sprinklers differ from each other. Sprinklers with nozzle diameters of 4.2-5 mm were used in 5 of the 15 irrigation systems, sprinklers with nozzle diameters of 5.5-4 mm in 3 of them, and sprinklers with nozzle diameters of 2-4 mm, 2.5-3.5 mm, 3.5-4.5 mm, 4.15-3.2 mm, 4-6 mm, 4.3-4.8 mm, and 4.5-5 mm in the other sprinkler irrigation systems (Table 3).

Table 3. Properties of the sprinklers used in the study areas

Type	Nozzle Diameter	Number	Rate	a	Discharge
Double Nozzle	(mm)	Piece	(%)	(m)	(m^3/h)
15	2 - 4	1	6.67	18	1.19
	2.5 - 3.5	1	6.67	20	1.12
	3.5 - 4.5	1	6.67	12	1.70
	4.15 -3.2	1	6.67	17.20	1.68
	4 - 6	1	6.67	10.80	2.93
	4.2 - 5	5	33.31	14	2.14
	4.3 - 4.8	1	6.67	13	1.96
	4.5 - 5	1	6.67	14	1.94
	5.5 - 4	3	20.00	12.50	1.90

Sprinkler flow rates

While the sprinkler flow rates ranged from 6.84 to 37.46 mm/h, their infiltration rates varied between 8 and 23 mm/h. The sprinkler spacing on the irrigation systems was designed as 6 m on 10 sprinkler systems, 10 m on 3 sprinkler systems, and 5 m on 2 sprinkler system. The lateral spacing was designed as 15 m on 7 sprinkler systems, 10 m on 6 sprinkler systems, and 12 m on 2 sprinkler systems. The sprinkler flow rate was higher than the soil infiltration rate at 12 sprinkler facilities (77.33%) (Table 4). These values indicate that the soil infiltration rate, one of the most important parameters of the project, was not considered when projecting the parcel-based sprinkler systems.

Table 4. Soil infiltration rates and sprinkler flow rates

Sprinkler System No.	L_1 (m)	L_2 (m)	hm_1 (1st sprinkler)	hm_2 (2nd sprinkler)	Average (mm/h)	Soil infiltration rate (mm/h)
1	10	15	8.19	8.58	8.39	9
2	6	15	20.99	20.54	20.77	23
3	6	12	14.92	27.78	21.35	18
4	6	10	40.88	34.03	37.46	20
5	5	12	27.73	29.87	28.80	18
6	5	15	27.24	23.53	25.39	19
7	6	15	22.41	21.77	22.09	12
8	6	15	17.02	16.54	16.78	11
9	6	15	18.44	16.81	17.63	14
10	6	10	23.45	22.28	22.87	21
11	6	10	36.37	35.18	35.78	16
12	10	15	7.29	6.39	6.84	11
13	6	10	18.82	23.00	20.91	8
14	6	10	33.45	31.97	32.71	13
15	10	10	9.21	8.97	9.09	23

*L_1: Sprinkler spacing on the lateral, L_2: Lateral spacing, hm: Sprinkler flow rates

Lateral designing

The lengths of the laterals in the sprinkler irrigation systems ranged from 50 to 200 m. Of the irrigation systems, 6.6% used laterals with lengths of 0–50 m, 40% used laterals with lengths of 50–100 m, 26.7% used laterals with lengths of 100–150 m, and 26.7% used laterals with lengths of 150–200 m. As known, it is not recommended that the lengths of lateral lines be greater than 250 m (Güngör and Yıldırım, 1989). No lateral lines longer than 200 m were encountered in the examination carried out in the study area. 40% of the lateral connection shapes were L-shaped, but 60% of them were T-shaped. Of the lateral pipe spaces, 40% were 10 m, 13.3% were 12 m, and 46.7% were 15 m (Table 5).

Table 5. The properties of the lateral pipelines in the study areas

Sprinkler System	Lateral Pipe										
	Number of Lines		Connection Shape		Length (m)				Arrangement spacing (m)		
	1	2	L	T	0-50	50-100	100-150	150-200	10	12	15
Number	12	3	6	9	1	6	4	4	6	2	7
Rate (%)	80	20	40	60	6.6	40	26.7	26.7	40	13.3	46.7

An evaluation of the lateral pipe diameters

The lateral inlet pressure ranged from 1.8 to 3.8 atm, whereas the lateral outlet pressure varied between 1.6 and 3.6 atm. The sprinkler pressures ranged from 1.4 to 3.8 atm. In addition, the pressure remained below 2 atm on Sprinkler Irrigation Systems No. 2, 3, 4, 12, and 13 (Table 6). In order for a lateral pipe diameter to be convenient, the sprinkler pressure losses to take place between the end sprinklers should not exceed 20% of the average operating pressure. Accordingly, the pressure difference turned out above 20% in Irrigation Systems No. 6, 8, 9, and 11 in the study areas. These results are an indication of the inadequacy of these systems in terms of pipe diameters. Nevertheless, the pressure difference was below 20% in the remaining 11 irrigation systems; hence, these irrigation systems were adequate in terms of pipe diameters.

Table 6. The pressure values of the lateral pipes and the sprinklers

Parameters	Sprinkler Systems														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
LIP	2.4	1.8	1.8	1.7	2.2	2.6	2.2	3.2	3.8	2.6	3.0	1.8	2.0	2.2	2.4
LOP	2.0	1.6	1.6	1.6	2.0	2.4	2.0	2.6	3.6	1.6	2.6	1.8	1.8	2.2	2.0
LAP	2.2	1.7	1.7	1.7	2.1	2.5	2.1	2.9	3.7	2.1	2.8	1.8	1.9	2.2	2.2
LPD	0.4	0.2	0.2	0.1	0.2	0.2	0.2	0.6	0.2	1.0	0.4	-	0.2	-	0.4
APL	0.44	0.34	0.34	0.33	0.42	0.50	0.42	0.58	0.74	0.42	0.56	0.36	0.38	0.44	0.44
IP1	2.2	1.6	1.6	1.6	2.0	2.4	2.0	2.8	3.8	1.8	2.6	1.8	1.9	2.2	2.2
OP1	2.1	1.6	1.6	1.4	1.8	2.4	2.0	2.8	3.6	1.8	2.4	1.6	1.8	2.0	2.0
IP2	2.2	1.6	1.6	1.6	2.0	2.4	2.0	2.8	3.8	1.8	2.6	1.8	1.8	2.2	2.2
OP2	2.1	1.6	1.6	1.4	1.8	2.4	2.0	2.6	3.6	1.8	2.4	1.6	1.8	2.0	2.0
AS1	2.2	1.6	1.6	1.5	1.9	2.4	2.0	2.8	3.7	1.8	2.5	1.7	1.9	2.1	2.1
AS2	2.2	1.6	1.6	1.5	1.9	2.4	2.0	2.7	3.7	1.8	2.5	1.7	1.8	2.1	2.1

LIP: Lateral inlet pressure; LOP: Lateral outlet pressure; LAP: Lateral average pressure; LPD: Lateral pressure difference; APL: Allowed pressure loss; IP1: Inlet pressure 1st sprinkler; OP1: Outlet pressure of the 1st sprinkler; IP2: Inlet pressure of the 2nd sprinkler; OP2: Outlet pressure of the 2nd sprinkler; AS1: Average of the 1st sprinkler; AS2: Average of the 2nd sprinkler.

Christiansen Uniformity Coefficient (CU) and Distribution Uniformity (DU)

The CU values ranged from 44% to 86% in the study area. The average CU was 63.33% in the evaluated irrigation systems (Table 7). Merriam and Keller, (1978) and Keller and Bliesner, (1990) reported that CU should be greater than 84% for in order irrigation to be regarded as uniform. According to these results, almost all Sprinkler Irrigation Systems has not been realized uniform water distribution. The CU found out higher than 84% only in Irrigation System No. 2. Furthermore, there were differences among the irrigation systems in the same village as well. It might be stated that the different wind speeds measured during irrigation, the different operating pressures and the positions of the sprinklers are the reasons why the CU so different. Another criterion used to evaluate in the sprinklers is the distribution uniformity. The DU values determined considering the water distributions between the laterals were in a quite wide range of 25%-84%. The average DU was calculated as 44.33%. Keller and Bliesner (1990) stated that DU should be greater than 75% for uniform irrigation. The average DU in the study area was approximately 30% lower than this value. When the irrigation systems are examined on an individual basis, it might be stated that an allowable level of uniform irrigation was performed only in System No. 2. The reasons why the distribution uniformity values differed among the irrigation systems are the same as the factors which affected the Christiansen uniformity coefficient. Such factors as the wind speed and direction during irrigation, the operating pressure and change, the distances between the sprinklers and the laterals, the different sprinkler nozzle diameters, their wear or their destruction by the farmers gave rise to the low DU coefficient values. Apart from them, Tarı and Yazar (2010) stated that such factors as not keeping sprinklers in an upright position, their clogging up or their turning would also negatively affect the distribution uniformity value.

Table 7. Christiansen uniformity coefficient and distribution uniformity values

Sprinkler Systems	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Average**
CU (%)	56	86	51	74	60	56	62	44	57	64	61	65	70	73	71	63.33
DU (%)	42	84	50	61	45	25	29	27	36	49	30	48	54	51	49	44.33

*Christiansen Uniformity Coefficient Values (CU), Distribution uniformity values (DU)

Conclusion

Many of the Irrigation Systems in the study area were discovered to have used only one lateral line when performing sprinkler irrigation. The power of the pumps varied between 27.5 and 85 HP, with 50 HP being generally used. Sprinklers with different nozzle diameters and properties were observed on the laterals. Some 11 irrigation systems were found convenient in terms of pipe diameters, whereas inconvenient pipe diameters were used in 4 of them. Moreover, the higher average sprinkler flow rate than the soil infiltration rate in 12 of 15 irrigation systems shows that the infiltration rate was not taken into consideration during designing. Additionally, the sprinklers were observed to have worn over time depending on the frequent use of the system and the exogenous factors. It was seen that sprinklers with different properties were used instead of the broken and deformed sprinklers and that nozzles with different diameters were used instead of the broken nozzles. For all these reasons, the Christiansen uniformity coefficient (CU) and distribution uniformity (DU) values remained below the limit values in almost all irrigation systems. The desired performance values can be obtained in the irrigation systems used at the locality in the event that the project and operating errors determined in this study are eliminated.

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HONEY PLANT NATURAL RESOURCES - A VITAL PART OF BIOLOGICAL DIVERSITY

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Abstract

The worldwide trend of declining biological diversity of plant resources exacerbates the problem of food security. Therefore, the study of the state of honey plants, which allows determining the full range of their species diversity, is relevant. Our studies of honey plants on the territory of the state of Mississippi, USA, found more than 180 species of 99 genera from 41 families, the most numerous of which are *Asteraceae* – more than 39 species (21%), *Fabaceae* – more than 20 species (11%), *Rosaceae* – 18 species (10%). The majority of honey plants studied – 123 species (near 68%) are native species, 53 (29%) – introduced, and 6 (3%) species widely distributed throughout the world. The most of the species are native forest plants, characterized by high level of endemism for the North American region. The bulk – 103 species of all registered (55%) – are evenly distributed throughout the territory of the state of Mississippi. By the place of growth, the registered honey plants are divided into: forestry ones – 79 species (37%), forest belts and ornamental plantations plants – 54 species (24%), field and fodder plants – 20 species (9%), plants of meadows, pastures and wetlands – 53 species (24%), garden and berry plantations plants – 9 species (4%), and plants of vegetable and melons fields – 4 species (2%). Totally, more than 87 species of honey plants from 57 genera of 35 families are cultivated. But the majority of species (more than 60%) are represented only in the wild state, mainly on forest lands.

Keywords: *Biological diversity, State of Mississippi, Honey plant.*

Introduction

Rational use and reproduction of natural resources is of great importance in addressing the problem of food security and sustainable development of agricultural production. These resources include honey plants, which are the food base of honey bees and other pollinators, as well as a source of production of beekeeping products that are important for human nutrition (Kostyleva and Zhitin, 2011). There is a growing recognition that the biodiversity of honey plants is a global asset of current and future generations (Wilson, 1992). At the same time, economic development of territories leads to a decrease in the species diversity of honey plants and scarcity of the honey resources (Naumkin, 2010). It is projected that almost two thirds of the world's plant species are threatened with extinction during the 21st century (Blackmore et al., 2000). It is known that the composition and productivity of honey plant resources depend on geographic conditions (Avetisyan, 1963). The richest in this respect are the humid subtropical regions (Ayers and Harman, 1994). Therefore, the study of the condition of honey plants in one of the representative regions of humid subtropics, which allows determining the full range of their species diversity, is relevant.

Materials and methods

The objects of the research were honey and pollen plants. The studies were carried out on the territory of the state of Mississippi, USA.

The body of honey plants was studied by a botanical survey of the region. All encountered species of honey plants were taken into account with the collection of herbarium material, if it

was necessary to clarify their exact affiliation. Beforehand, the location of counts was randomly determined on the map of the region, covering its northern, southern, eastern, western and central parts. The body of honey plants was studied by a botanical survey of the region. All encountered species of honey plants were taken into account with the collection of herbarium material, if it was necessary to clarify their exact affiliation. Beforehand, the location of counts was randomly determined on the map of the region, covering its northern, southern, eastern, western and central parts. The counts of honey plants were carried out by the route (linear transects) method (Krivtsov et al., 1999) comprising all typical plant associations on each of the dominant melliferous lands, where at least two or three routes were laid.

To determine the quantitative participation of the honey plants the trial plots (10-20) were laid on the routes, every 50-60 steps. Trial plots for wood and shrub plants were 25-100 m^2 (5x5 or 10x10 м) by the projection of crowns, for herbaceous ones - 0.25-1.00 m^2.

The species of honey plants were determined by Gleason and Cronquist (1991) and PLANTS Interactive ID Keys. The accuracy of the determination was checked by the samples of the herbarium of the Mississippi Museum of Natural Sciences. The taxonomy of species was indicated according to GRIN Database.

Results and discussion

In total, over 180 species of honey and pollen plants from 99 genera of 41 families have been registered in the region under study. Including families: *Asteraceae* Dumort. – more than 39 species (21% of the total number of species); *Fabaceae* Lindl. - more than 20 species (11%), *Rosaceae* Adans. - 18 species (10%), *Lamiaceae* Lindl. - 9 species (5%), (*Aquifoliaceae* Lindl.) - 6 species (3,3%); *Polygonaceae* Juss.), *Cornaceae* Dumort., *Magnoliaceae* Juss., *Scrophulariaceae* Juss., *Anacardiaceae* Lindl. - 5 species (2,7%) in each; *Bignoniaceae* Juss., *Ulmaceae* Mirb., *Lythraceae* St.-Hil., *Salicaceae* Mirb., *Cucurbitaceae* Juss. - 4 species (2,0 %) in each; *Verbanacea* St.Hil., *Ericaceae* Juss., *Asclepiadaceae* R.Br, *Oleaceae* Hoffmgg., *Violaceae* Batsch, *Theaceae* Mirb. ex Ker Gawl. – 3 species (1,5%) in each. The remaining families are represented by 1-2 species (1%). The classification of honey plant families by species composition is presented graphically in Figure 1.

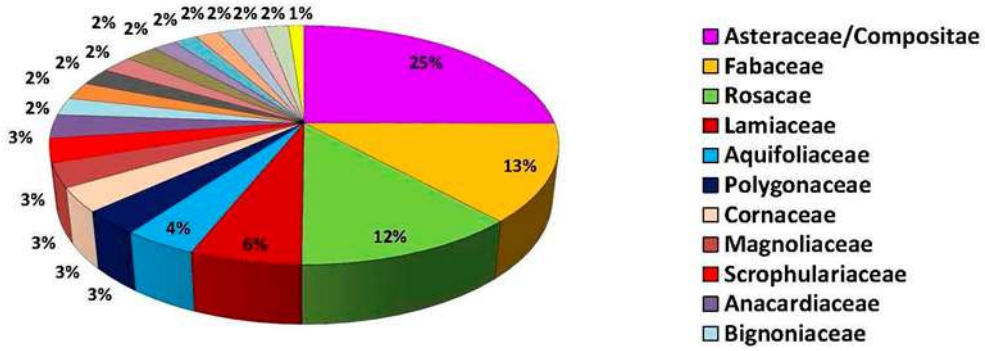

Fig. 1 Classification of honey plant families by species composition

Comparison of our data with other studies (Fulton, http: //www.entomology.msstate ...), conducted earlier in the same region, shows that the species composition of honey plants is more than 3.7 times broader than that given in the list of the indicated author, since our studies were performed more thoroughly, covering more biotopes and plant associations.

The majority of honey plants studied – 123 species (near 68%) are native species, 53 (29%) – introduced, and 6 (3%) species widely distributed throughout the world.
The largest proportion of native species is found in the families: *Bignoniaceae, Verbanaceae, Ericaceae, Ulmaceae, Salicaceae, Cornaceae, Asclepiadaceae, Magnoliaceae, Aquifoliaceae, Anacardiaceae* – 100% in each family; *Asteraceae* – 77%, *Lamiaceae* – almost 67%.
The largest proportion of species introduced from different parts of the world is found in the families: *Oleaceae, Cucurbitaceae, Theaceae* - 100% in each family, *Polygonaceae* - 80%, *Fabaceae* - 72%.
Both the general composition of the melliferous vegetation in a given locality and the entire complex of external meteorological and atmospheric influences, which affect the nectar production of plants, are largely determined by the geographical conditions of growth.
The honey plants in the study region are distributed unevenly (Fig. 2). The bulk - 103 species (55% of all the registered honey plants) are distributed throughout all the territory of the state of Mississippi. Of these, 6 species (more than 3%) were rare, 51 species (28%) were spread sporadically, the remaining 46 species (25%) were found almost everywhere on the territory under the study, i.e., were the most common species.

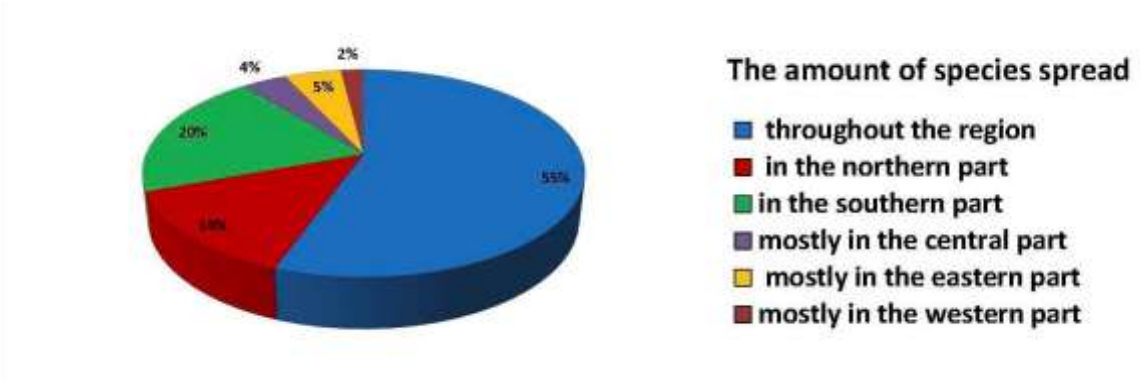

Fig. 2 Geographical distribution of honey plant species in the region

Twenty five species - (almost 14% of all the registered honey plants) are distributed only in the northern part of the region. Of these, 16 species (almost 9%) are rare: *Vernonia fasciculata* Michx., *Helianthus annuus* L., *Eupatorium purpureum* L., *Gaylussacia baccata* (Wangenh.) K. Koch, *Polygonum persicaria* L., *P. cuspidatum* Sieb., *Lythrum salicaria* L., *Decodon verticillatus* (L.), *Salix caroliniana* M., *Aesculus flava* Aiton, *Claytonia linearis* (Douglas), *Prunus avium* L., *Leonurus cardiaca* L., *Pycnanthemum verticillatum* (Michx.) Pers., *P. virginianum* (L.) T. Dur. & B.D. Jacks. ex B.L. Rob. & Fernald, *Nepeta cataria* L.; 5 species (almost 3%) are distributed sporadically: *Cichorium intybus* L., *Symphoricarpos orbiculatus* Moench, *Asclepias syriaca* L., *Scrophularia marilandica* L., *Verbascum blattaria* L. and 4 species (2%) are distributed throughout the north of the territory: *Glycine max* L ., *Ulmus crassifolia* Nutt., *Apocynum cannabinum* L., *Allium cepa* L.
Thirty six (almost 20%) of the species recorded are common in the southern part of the study region, including 13 species (7%) which are rare: *Acacia farnesiana* (L.), *Trifolium hybridum* L., *Vaccinium corymbosum* L., *Sinapis alba* L., *Scrophularia marilandica* L., *Crataegus spp*., *Cucurbita pepo* L., *Citrullus lanatus* (Thu.), *Cucumis melo* L., *Cucumis sativus* L.; 9 species (5%) are distributed sporadically: *Liatris spicata* (L.) Willd., *Parkinsonia aculeata* L., *Trifolium vesiculosum* Savi, *Cornus alternifolia* L., *Ligustrum japonicum* Thunb. *L. lucidum* Aiton, *Triadica sebifera* (L.), *Monarda punctata* L. and 14 species (almost 8%) are distributed throughout the south of the territory: *Baccharis angustifolia* Michx., *B. glomeruliflora* Per., *Gaillardia pulchella* Foug., *Trifolium nigrescens* Viv., *Verbena hastata* L., *Nyssa biflora* Walt., *Clethra alnifolia* L., *Magnolia grandiflora* L., *M. macrophylla* L., *Ilex glabra* L., *I.*

vomitoria Aiton, *I. coriacea* (Pursh), *Serenoa repens* Small, *Cliftonia monophylla* (Lam.) Britton, *Cyrilla racemiflora* L.

In the central part of the region, only 7 species (4%) were distributed, including 2 (1%) rare species: *Verbesina alternifolia* (L.), *Lifrum salicaria* L.; 3 species (2%) are distributed sporadically: *Coreopsis lanceolata* L., *Ilex verticillata* (L.), *Prunus persica* (L.) and 2 species (1%) are distributed throughout the center of the territory: *Helenium autumnale* L., *Monarda citriodora* Cerv. Some species are contemporaneously distributed in two parts of the investigated region: *Lythrum salicaria* L. - in the north and in the center, *Scrophularia marilandica* L. - in the north and south, though rarely found in the latter.

In the eastern part of the study region, we recorded 10 species (5%). Of these, 3 species (2%): *Eupatorium pilosum* Walter, *Aesculus glabra* Willld., *Fragaria* × *ananassa* are rare; 3 species (2%): *Salix interior* R., *Ptelea trifoliata* L., *Rhus / Toxicodendron vernix* (L.) - are distributed sporadically and 4 (2%): *Xanthium strumarium* L., *Alnus serrulata* (Aiton) Willd., *Asclepias verticillata* L. and Magnolia virginiana L. - are distributed throughout the eastern part of the territory.In the western part of the study region, we recorded 3 species (2%). Of these, 2 species (1%): *Sonchus arvensis* L. and *Catalpa speciosa* (Warder) - are rare and 1 (1%): *Taraxacum officinale* Wigg. – is distributed throughout the western part of the territory.

The occurrence of a relatively small number of honey plant species in the eastern, western and central parts of the area under study is evidently due to the fact that the territory of the region is strongly extended from north to south (the region is almost twice as long than it is wide).

We also found many species of honey plants, endemic to North American Atlantic Region (NAAR), which are preserved in the area to a large extent due to the pollination activity of the honey bee. Many of these endemics are threatened or endangered species.

For the Appalachian Province of NAAR (northern part of the state) endemic species include *Magnolia acuminate* (L.) L., *M. macrophylla* L., *Claytonia virginica* L., *Polygonum spp., Viola spp., Salix spp., Clethra spp., Rubus spp., Prunus spp., Crataegus spp., Tilia americana* L., *Gleditsia triacanthos* L., *Robinia pseudacacia* L., *Acer saccharum* L., *Aesculus glabra* Willd., *Catalpa speciosa* (Warder), species of *Penstemon, Solidago, Aster*, and *Eupatorium*.

For the Atlantic and Gulf Coastal Plain Province (southern part of the state) endemics are *Magnolia grandljlora L., M. virginiana* L., *Nyssa biflora* Walter, and species of *Asclepias.*

By the place of growth, the registered honey plants are divided into: forestry ones – 79 species (37%), forest belts and ornamental plantations plants – 54 species (24%), field and fodder plants – 20 species (9%), plants of meadows, pastures and wetlands – 53 species (24%), garden and berry plants – 9 species (4%), and plants of vegetable and melons plots – 4 species (2%).It should be noted that many forest honey plants (more than 33 species, i.e. about 40% of all forest species) are used in green construction and as ornamental plants: *Liquidambar styraciflua* L., *Symphyotrichum spp.*, *Baccharis glomeruliflora* Per. and *B. halimifolia* L., *Eupatorium purpureum* L. and *E. perfoliatum* L., *Catalpa bignonioides* Walter and *C. speciosa* (Warder), *Campsis radicans* (L), *Cercis canadensis* L., *Robinia pseudoacacia* L., *Gleditsia triacanthos* L., *Symphoricarpos orbiculatus* Moench, *Salix nigra* Marsh, *Cornus florida* L.. and *C. alternifolia* L., *Acer saccharinum* L. and *A. rubrum* L., *Aesculus glabra* Willd. and *A. flava* Aiton, *Tilia americana* L. *var. heterophylla* and *T. americana* L. *var. caroliniana, Liriodendron tulipifera* L., *Magnolia virginiana* L., *M. grandiflora* L., *M. acuminata* (L.), *Ilex opaca* A., *I. glabra* L., I. *vomitoria* Aiton and *I. coriacea* (Pursh), *Sabal minor* (Jacq.), *Crataegus spp, Viola sororia* Willd, *Callicarpa americana* L. Some forest species - *Gaylussacia baccata* (Wangenh), *Vaccinium corymbosum* L.) are used as a cultivated plant in the berry patches.

Many wild honey plants (more than 17 species, or about 8%) growing in the open spaces (meadows and pastures, clearing banks of rivers and streams, roadsides, and vacant lots, etc.) are also used as ornamental plants: some species of aster - *Symphyotrichum novae-angliae*

(L.) and others, *Gaillardia pulchella* Foug, *Helenium autumnale* L., *Coreopsis lanceolata* L., *Liatris spicata* (L.) Willd., *Parkinsonia aculeata* L., *Phyla nodiflora* (L.), *Asclepias syriaca* L.and *A. tuberosa* L., *Triadica sebifera* (L.), *Penstemon laevigatus* Aint, *Viola tricolor* L., *Monarda punctata* L. and *M. citriodora* Cerv. or as agricultural crops: *Helianthus annuus* L., *H. tuberosus* L., *Cichorium intybus* L. and the above 2 mint species cultivated as spices.
In the studied region, more than 87 species from 57 genera of 35 families of the honey plants registered by us are cultivated to a greater or lesser extent. The largest number of species of cultural honey plants belongs to the following families: *Fabaceae* - 16 species, *Asteraceae* - 12 species, *Rosaceae* - 6 species, *Aquifoliaceae*, *Cucurbitaceae*, *Magnoliaceae* - 4 species in each family, *Oleaceae*, *Theaceae*, *Lamiaceae* - 3 species in each family. In the remaining families, there are only 1-2 species of cultivated honey plants (Fig. 3).

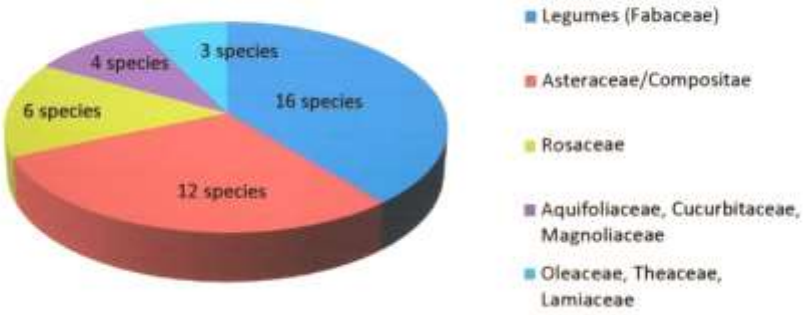

Fig. 3 Classification of the families of cultivated honey plants

In agriculture 33 species of honey plants belonging to 12 families are used: Fam. *Asteraceae* Dumort. - *Helianthus annuus* L., *Helianthus tuberosus* L., *Cichorium intybus* L.; Fam. *Fabaceae* Lindl. - *Vicia villosa* Roth, *V. sativa* L., *Melilotus officinalis* (L.) Lam., *Melilotus albus* (L.) Medik., *Trifolium repens* L., *Trifolium nigrescens* Viv., *Trifolium vesiculosum* Savi; *Trifolium incarnatum* L., *Trifolium pratense* L., *Trifolium resupinatum* L., *Trifolium hybridum* L., *Glycine max* (L.) Merr.; Fam. *Ericaceae* Juss. - *Gaylussacia baccata* (Wangenh.), *Vaccinium corymbosum* L.; Fam. *Vitaceae* Juss. - *Vitis rotundifolia* Michx.; Fam. *Brassicaceae* Burnett - *Sinapis alba* L.; Fam. *Liliaceae* Juss. - *Allium cepa* L.; Fam. *Malvaceae* Juss.: *Gossipium hirsutum* L.; Fam. *Rosaceae* Adans.: *Rubus aboriginum* Rb., *Malus domestica/pumila* Bork., *Pyrus communis* L., *Prunus persica* (L.), *Fragaria x ananassa;* Fam. *Cucurbitaceae* Juss.: *Cucurbita pepo* L., *Citrullus lanatus* (Thunb.), *Cucumis melo* L., *Cucumis sativus* L.; Fam. *Ebenaceae* Gurke: *Diospyros virginiana* L.; Fam. *Lamiaceae* Lindl.: *Monarda punctata* L. and *M. citriodora* Cerv.
55 species of honey plants from 23 families are used in landscaping and decoration: Fam. *Altingiaceae* Lindl. - *Liquidambar styraciflua* L.; Fam. *Asteraceae* Dumort. - *Symphyotrichum novae-angliae* (L.); *Baccharis glomeruliflora* Per., *B. halimifolia* L., *Gaillardia pulchella* Foug., *Helenium autumnale* L., *Coreopsis lanceolata* L., *Liatris spicata* (L.) Willd., *Eupatorium perfoliatum* L., *E. purpureum* L.; Fam. *Bignoniaceae* Juss. - *Campsis radicans* (L.), *Catalpa bignonioides* Walter., *C. speciosa* (Warder); Fam. *Fabaceae* Lindl. - *Cercis canadensis* L., *Gleditsia triacanthos* L., *Parkinsonia aculeata* L., *Robinia pseudoacacia* L.; Fam. *Verbanaceae* St.-Hil. - *Phyla nodiflora* (L.) Greene.; Fam. *Lythraceae* St.-H - *Lagerstroemia indica* L.; Fam. *Caprifoliaceae* Jus.- *Symphoricarpos orbiculatus* Moench; Fam. *Salicaceae* Mirb. - *Salix nigra* Marsh.; Fam. *Cornaceae* Dumort. – *Cornus florida* L., *C. alternifolia* L.f.; Fam. *Aceraceae* Juss.- *Acer rubrum L., A. saccharinum* L.*;* Fam. *Clethraceae* Klotzsc.- *Clethra alnifolia* L.; Fam. *Hippocastanaceae* DC. - *Aesculus glabra* Willd., *A. flava* Aiton; Fam. *Asclepiadaceae* R. Br. - *Asclepias syriaca* L., *A. tuberosa*

L.; Fam. *Tiliaceae* Juss. - *Tilia americana* L., *T. americana* L. var. *caroliniana*; Fam. *Magnoliaceae* Juss. - *Magnolia virginiana* L., *M. grandiflora* L., *M. acuminata* (L.), *Liriodendron tulipifera* L.; Fam. *Oleaceae* Hoffmgg. - *Ligustrum japonicum* Thunb., *L. lucidum* W.T. Aiton, *L. sinense* Lour.; Fam. *Euphoriaceae* Juss. - *Triadica sebifera* (L.); Fam. *Scrophulariaceae* - *Penstemon laevigatus* Aiton; Fam. *Aquifoliaceae* Lindl., *Ilex opaca* Aiton., *I. glabra* (L.) A., *I. vomitoria* Aiton, *I. coriacea*(Pursh); Fam. *Arecaceae* Schultz - *Sabal minor* (Jacq.); Fam. Rosaceae Adans. - *Crataegus spp.*, Fam. *Anacardiaceae* Lin. - *Rhus typhina* L.; Fam. *Violaceae* Batsch - *Viola sororia* W., *Viola tricolor* L.; Fam. *Theaceae* Mirb. ex Ker - *Camellia sinensis* L., *C. japonica* L., *C. sasanqua* Thunb.; Fam. *Lamiaceae* Lindl. - *Callicarpa americana* L., *Monarda punctata* L., *M. citriodora* Cerv.

But the majority of honey plant species (more than 60%) are registered by us only in the wild. Basically, they are represented by forest species, obviously due to the fact that most of the study area is covered with forests.

Conclusion

Honey plants registered by us compose a vital part of biological diversity of the state of Mississippi. The most of species are native forest plants, characterized by high level of endemism. Though numerous honey plants in the region are cultivated, the majority of species is represented only in the wild. Conservation and increase of honey plants resources can be achieved by protecting rare and endangered species of wild honey plants (Kostyleva, 2013), planting selected melliferous species around apiaries, along roadsides, railway tracks and power lines, on empty lands, and lands not having great agricultural value (Ayers et al., 1987).

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CONTENT OF NATURALLY OCCURING AND ARTIFICIAL RADIONUCLIDES IN CULTIVATED SOIL IN BELGRADE (SERBIA)

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Abstract

Radioactivity of soil is one of the main source of human exposure and an important subject of environmental monitoring. The present study was conducted in order to determine the content of naturally occurring radionuclides (U and Th series and ^{40}K) and artificial radionuclides (^{137}Cs and ^{90}Sr) in the surface layer of cultivated soil. The soil was sampled twice a year, in the spring and autumn season of 2017 on 5 locations in Belgrade (Serbia). The concentrations of naturally occurring radionuclides, as well as the artificial radionuclide of ^{137}Cs were measured using HPGe gamma detectors, while the concentration of artificial radionuclide ^{90}Sr was conducted by low level gas proportional counter. The average values of naturally occurring radionuclides were: 43.3 Bq kg^{-1} for ^{226}Ra, 45 Bq kg^{-1} for ^{232}Th, 552 Bq kg^{-1} for ^{40}K, 42.1 Bq kg^{-1} for ^{238}U and 2.1 Bq kg^{-1} for ^{235}U, while the average values of artificial radionuclides were 15.7 Bq kg^{-1} for ^{137}Cs and 0.74 Bq kg^{-1} for ^{90}Sr. The obtained concentrations of investigated radionuclides were in the range of the concentrations reported in the literature for the cultivated soil. No significant variation was noticed between concentrations of naturally occurring radionuclides with respect to the sampling period, while for ^{137}Cs, slight variations were noticed with respect to the location and season. Based on the average values of the activity concentration of gamma emitters in investigated soil samples, the health hazard indices were estimated. The values obtained are comparable with the corresponding world permissible values.

Keywords: *Radionuclide; Gamma spectrometry; Beta spectrometry; Cultivated soil*

Introduction

Radioactivity arising from natural sources is the main contribution to the annual dose received by the population, exposure resulting from radionuclides inherent in the earth's crust and from cosmic rays. The main sources of external γ-radiation are the radionuclides of the ^{238}U and ^{232}Th series, along with their progeny and ^{40}K (Mohery *et al*., 2014). Gamma radiation emitted from such naturally occurring radionuclides present in all soils represents the main external exposure to human body. With regard to terrestrial gamma radiation, the specific radiation levels are related to the content in the radionuclides of the soils, which in turn depends on the nature of the parent rock. The content of ^{238}U and ^{232}Th is large in the magmatic rocks as granite and basalt, but also from the sedimentary rocks as phosphate rock (Pehlivanovic *et al.*, 2017). The use of fertilizers in farming may result in enhanced levels of natural radioactivity in some particular areas. Studies of soil radioactivity can provide reference data in observing possible future anthropomorphic impact and associated radiological risk to human health (Santawamaitre *et al*., 2011).

Many radionuclides of artificial origin have been released to the environment by different processes. The radioisotopes of caesium and strontium are the most important ones that may be deposited by fallout over soil and plants as they are produced anthropogenically by several types of nuclear activities including past testing of nuclear weapons and nuclear accidents, with one of the largest was Chernobyl at 1986 (Prăvălie, 2014). The main mechanism of

deposition of artificial radionuclides is wet deposition (fallout), as well as dry deposition also (Krstić *et al.,* 2004). The fission products, ^{137}Cs and ^{90}Sr are biologically important, because of their behavior are similar to potassium and calcium, respectively. These radionuclides are deposited in biological systems, especially in sensitive tissues, followed the metabolic path of the mentioned ions. Measurement of natural and artificial radioactivity content in soil is very important to set a baseline level and then plot the trend with time as result of any radioactive release for environmental and public protection purposes (El Samad *et al*., 2013). Therefore, the main objective of this study is to analyze the distribution of naturally occurring radionuclides, as well as the artificial radionuclides in cultivated soil at the teritorry of Belgrade city (Serbia). This study will contribute to the better understanding of radionuclides behavior in the agricultural managed systems. Beside mentioned reasons, the knowledge of radioactivity present in soil enables one to asses any possible radiological hazard to mankind. Hence, based on the average values of the activity concentration for each sites, to estimate the health hazard indices by evaluate the gamma dose rate, the annual effective dose equivalent (outdoor), the external hazard index, the radium equivalent activity and the excess lifetime cancer risk.

Material and Method

In order to measure the content of the natural and artificial radionuclides, the cultivated soil samples were collected on 5 locations at the area of Belgrade city: Zeleno Brdo, Jabučki Rit, Dunavac, Obrenovac and Lazarevac. The soil sampling was conducted in the spring and autumn season of 2017 year. The coordinates of sampling sites and intervals of ambient dose rates are given in *Table 1*. The measurement of the ambient dose rate at sampling locations was performed using a scintillation probe (Model 6150 AD-b produced by Automess, Germany) 1 m above the ground.

Table 1. The GPS coordinates of sampling locations and the corresponding ambient dose rates

Location	**GPS coordinates**		**Ambient dose rate (nSv h^{-1})**
Zeleno Brdo	N: 44° 47'	E: 20° 31'	130 - 190
Jabučki Rit	N: 44° 55'	E: 20° 33'	120 - 130
Dunavac	N: 45° 03'	E: 20° 22'	120 - 130
Obrenovac	N: 44° 39'	E: 20° 12'	87 - 105
Lazarevac	N: 44° 23'	E: 20° 16'	115 - 130

About 2 kg of soil sample was taken from upper layer of 0-20 cm at each sampling site. The preparation of soil samples involved the following: removing plant residues from the basic soil mass, samples drying at 105 °C, grinding and sieving. About 600 g of prepared soil samples were placed in Marinelli beaker of 500 mL, sealed with beeswax and left to reach secular equilibrium for four weeks, before gamma spectrometric measurements. Also, 300 g of prepared soil samples were mineralized at 500 °C by the method of dry burning for radiochemical determination of ^{90}Sr.

The soil were analyzed for ^{226}Ra, ^{232}Th, ^{40}K, ^{238}U, ^{235}U and ^{137}Cs by direct gamma assay, using HPGe gamma detectors with a relative efficiency of 20 % and a resolution of 1.8 keV at 1.332 MeV (with associated electronics provided by Canberra Industries, Meriden, Connecticut, USA). The counting time was 17 h, because of this time providing a precision of

better than ±10 % and a 95 % level of confidence. The spectrum was analyzed using a GENIE 2000 Canberra software.

The geometric efficiency for soil matrices in the Marinelli beaker of 500 mL in volume was determined using standard reference material product a silicone resin matrix 9031-OL-208/08, type ERX, issued by Czech Metrological Institute (Prague, Czech Republic), spiked with a certified series of radionuclides (^{241}Am, ^{109}Cd, ^{139}Ce, ^{57}Co, ^{60}Co, ^{203}Hg, ^{88}Y, ^{113}Sn, ^{85}Sr, ^{137}Cs) with the energies that span from 59 to 1898 keV. The gamma spectrometers were energy-calibrated using the following standard point source gamma emitters to cover a wide range of photopeaks: ^{60}Co and ^{133}Ba (Czech Metrological Institute, Prague), which have traceable to BIPM-*Bureau International des Poids et Measures*.

The soil were analyzed for ^{90}Sr by validated radiochemical analytical method (Sarap *et al.*, 2014). This method is based on the radiochemical separation of ^{90}Y from the sample and consists of oxalate departing calcium from strontium, firing till oxide, and usage of aluminium as collector for ^{90}Y. The samples were stored for 18 days to reach the radioactive equilibrium between ^{90}Sr and its radioactive daughter radionuclide of ^{90}Y. After 18 days, the radioactive equilibrium is disturbed, ^{90}Y departs on collector $Al(OH)_3$, which is then firing till oxide and on that way, ^{90}Sr is determined via ^{90}Y. The measurements were performed in planchet of 59 mm diameter, using α/β low-level gas proportional counter Thermo Eberline FHT 770 T (ESM Eberline Instruments GmbH, Erlangen, Germany). The counting time was 1 h. The low-level gas proportional counter was efficiency-calibrated using standard point source of ^{90}Sr (Czech Metrological Institute, Prague), which has traceable to BIPM.

To assess the radiation hazards associated with the study soil samples, the following indices have been defined (Beretka and Mathew, 1985; Diab *et al*., 2008; UNSCEAR, 2010; Avwiri *et al*., 2012): The gamma dose rate in nGy h^{-1}, the external hazard index and the radium equivalent activity in Bq kg^{-1}, which were calculated based on the activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K; The outdoor annual effective dose equivalent in mSv, which was calculated based on the absorbed gamma dose rate, the conversion factor (0.7 Sv Gy^{-1}) and the outdoor annual exposure time (1752 h); The excess lifetime cancer risk, which was calculated based on annual effective dose equivalent, average duration of life (estimated to be 70 years) and the risk factor (Sv^{-1}), i.e. fatal cancer risk per Sievert, that amounts 0.05 Sv^{-1} for the public and stochastic effects (Taskin *et al*., 2009).

Results and Discussion

The results of measurements of gamma emitters and radiostrontium in soil samples collected at different sites at the area of Belgrade city are presented in *Table 2*. The activity concentrations of natural radionuclides in Bq kg^{-1} ranged from 25 to 71 for ^{226}Ra, 30-70 for ^{232}Th, 500-610 for ^{40}K, 25-81 for ^{238}U and from 1.4 to 3.7 for ^{235}U, while the activity concentrations of the artificial radionuclides were: between 3.1 and 40 Bq kg^{-1} for ^{137}Cs, and for ^{90}Sr the values ranged below of minimal detectable concentration to 1.04 Bq kg^{-1}. The obtained values in this study are typical for soil samples at area of the Republic of Serbia and can be comparable with world average values (UNSCEAR, 2010; Avwiri *et al*., 2012; El Samad *et al*., 2013; Milenkovic *et al*., 2015; Sarap *et al*., 2016). Having in mind the measurement uncertainties given in *Table 2*, seasonal variations of the radionuclides activity was not significant in the examined cultivated soil, except for the content of ^{137}Cs at Zeleno Brdo. There is exist possibility of re-location of sampling site. However, the differences in activity concentrations of some naturally occuring radionuclides, as well as the artificial radionuclide of ^{137}Cs by the locations are evident. Unlike ^{137}Cs, artificial radionuclide of ^{90}Sr did not showed a significant difference in activity concentration in relation to the sampling site.

Table 2. The activity concentration of investigated radionuclides (Bq kg^{-1}) in cultivated soil of Belgrade for spring (I) and autumn (II) of 2017 year

Location / Season		^{226}Ra	^{232}Th	^{40}K	^{238}U	^{235}U	^{137}Cs	^{90}Sr
Zeleno Brdo	I	45 ± 3	50 ± 4	590 ± 40	40 ± 5	2.4 ± 0.2	3.1 ± 0.4	< 0.51
	II	54 ± 3	52 ± 4	570 ± 40	42 ± 6	1.9 ± 0.2	21 ± 1	0.59 ± 0.16
Jabučki Rit	I	36 ± 2	35 ± 3	520 ± 30	34 ± 10	1.5 ± 0.1	6.4 ± 0.5	< 0,81
	II	25 ± 2	30 ± 2	510 ± 30	31 ± 4	1.7 ± 0.2	4.0 ± 0.5	1.04 ± 0.19
Dunavac	I	29 ± 2	35 ± 3	580 ± 40	38 ± 5	1.8 ± 0.2	6.7 ± 0.4	< 0.53
	II	37 ± 3	34 ± 3	560 ± 40	34 ± 7	1.5 ± 0.2	6.8 ± 0.7	0.64 ± 0.22
Obrenovac	I	35 ± 2	40 ± 3	610 ± 40	25 ± 8	1.5 ± 0.1	40 ± 3	0.75 ± 0.14
	II	36 ± 2	36 ± 3	570 ± 40	31 ± 4	1.4 ± 0.1	19 ± 1	0.64 ± 0.16
Lazarevac	I	71 ± 4	70 ± 4	510 ± 30	81 ± 6	3.7 ± 0.3	25 ± 2	< 0.99
	II	65 ± 4	68 ± 5	500 ± 30	65 ± 7	3.5 ± 0.3	25 ± 2	< 0.94

Based on the obtained activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K, the following health hazard indices were calculated: the gamma dose rate (*D*), the outdoor annual effective dose equivalent (D_E), the external hazard index (H_{ex}), the radium equivalent activity (Ra_{eq}) and the excess lifetime cancer risk (*ELCR*). The estimated values of these indices are showed in *Table 3*. All values obtained for radium equivalent activity are below than recommended value of 370 Bq kg^{-1} (UNSCEAR, 2010). The mean gamma dose rate in air (70.4 nGy h^{-1}) and outdoor annual effective dose (0.087 mSv) are insignificantly higher than the world average of 57 nGy h^{-1} and 0.07 mSv, respectively (UNSCEAR, 2010). The calculated values of external hazard index are lower than world permissible unity, which means that the radiation hazard is low and indicates that will not lead to respiratory diseases and skin. Average excess lifetime cancer risk is close to the average world value of 0.29×10^{-3} (Taskin *et al*., 2009). This implies that the radioactivity of these areas no health hazard effect on the populace, and therefore the chances of occurrence of cancer are insignificant in general.

Table 3. The assessed health hazard indices associated with the studied soil samples

Location	D (nGy h^{-1})	D_E (mSv)	H_{ex}	Ra_{eq} (Bq kg^{-1})	ELCR (10^{-3})
Zeleno Brdo	78.0	0.096	0.45	167.1	0.33
Jabučki Rit	55.4	0.068	0.32	116.6	0.24
Dunavac	60.0	0.074	0.34	126.2	0.26
Obrenovac	64.1	0.079	0.36	135.3	0.27
Lazarevac	94.3	0.116	0.56	205.6	0.40
Average	**70.4**	**0.087**	**0.41**	**150.2**	**0.3**

Conclusion

In this study, the distribution of natural and artificial radionuclides in cultivated soil was analyzed. The obtained results are confirmed the literature data that the most of investigated radionuclides are retain in the arable horizon of agricultural soil. Health hazard indices analysis is used in studies about radiation to arrive at a better and safer conclusion on the health status of a population and environment. The values of estimated health hazard indices were below or insignificant higher than the standard limits for such environment. Hence, to radiation exposure in investigated areas will pose no significant the health risk to population and to environment.

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EFFECT OF HERBICIDES ON CELLULOLYTIC ACTIVITY OF SOIL MICROMYCETES

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Abstract

This study examines the effect of different rates of 2,4-D and Paraquat herbicides under *in vitro* conditions on the cellulolytic activity of 20 strains of cellulolytic fungi isolated from the chernozem soil taken from Mount Fruška Gora. Results showed that high rates of 2,4-D (60, 120 and 240 μg/ml) inhibited the cellulolytic activity of *Fusarium aquaeductum* var. *dimerum, Fusarium solani var. argillaceum, Aspergillus candidus, Aspergillus ustus* and *Fusarium* sp. (L-7). Lower rates of 2,4-D (30 and 60 μg/ml) stimulated the cellulolytic activity of most *Penicillium* species. Paraquat exhibited higher toxicity to cellulolytic micromycetes and their cellulose degradation ability. Its inhibitory effect was observed at rates as low as those above 3μg/mL. Inhibition increased with increasing rates of the herbicide, with only eight fungi retaining at least some degree of cellulose degradation ability at 24 μg/mL Paraquat. Most species of the genera *Aspergillus* and *Penicillium* showed high sensitivity to Paraquat at rates as low as 6 μg/mL. At 24 μg/mL, none of them had the ability to degrade cellulose. A considerable degree of resistance to Paraquat was exhibited by *Fusarium* spp. (*Fusarium nivale* and *Fusarium solni var. argillaceum)* and *Hormodendrum sp.* (L-11), which retained their cellulolytic activity even at Paraquat rates of 6 μg/mL, whereas *Hormodendrum sp.* (L-10) retained its cellulolytic activity even at twofold higher rates of Paraquat (12 μg/mL).

Keywords: *cellulolytic fungi, 2,4-D, Paraquat*.

Introduction

The last six decades have been characterized by an abrupt expansion in the use of agrochemicals in crop production, with plant protection agents playing an important role in the process (Liu *et al*., 2002). Their absence has led to a decline in crop yield by 32-78% (Cai, 2008). However, in addition to their main role, once they enter the soil, pesticides may also have side-effects on the soil biological component and, indirectly, on the cycling of elements, as well as a range of other adverse health impacts (Djukic *et al*., 2007; Kalia and Gosal, 2011). These effects primarily refer to changes in the community composition, numbers and enzymatic activities of beneficial soil microorganisms, and alterations in the dynamics and direction of soil biological processes. Based on these considerations, herbicides can be classified into agrochemicals which have extremely variable impacts (Mandić *et al*., 2005; Riah, *et al*., 2014). Overall, low (recommended) rates of herbicides do not generally cause adverse effects on soil microorganisms. Initial depression is followed by the recovery of the microbial status of the soil. Microorganisms use herbicides as their source of energy, phosphorus, carbon and nitrogen, which often increases their biomass (Araujo *et al*., 2003; Zabaloy *et al*., 2008). However, most results show that herbicides have an adverse effect on soil microorganisms by reducing their numbers and enzymatic activities; therefore, these parameters are often used as bioindicators of the soil ecological status (Killham, 2002; Das

and Varma, 2011). The reduction in the numbers and activities of microorganisms is induced by changes in their biosynthetic mechanisms and cell membrane permeability, and disturbances in their enzymatic activities (Sannino and Gianfreda, 2001; Hussain *et al*., 2009). The degree of this impact is dependent on herbicide type, rate and application timing, duration of exposure, solubility, soil pH, soil organic content and cumulative co-metabolic effects (Zain *et al*., 2013; Mohiuddin and Khan, 2013). Herbicides have different effects on different types of microorganisms. The most common parameters of their active effect on the soil microbial status are the numbers and enzymatic activity of fungi (Zain *et al*., 2013), and their ability to degrade cellulose and other polymeric compounds in the soil (Lynd *et al*., 2002), even herbicides themselves (Zahid *et al*., 2016). Soil fungi are important cellulase producing microorganisms. However, their activity is heavily disturbed through direct or indirect exposure to herbicides (Smith and Mayfield, 1977).

The objective of this study was to examine the effect of 2,4-D and Paraquat herbicides under *in vitro* conditions on cellulose degradation by different species of micromycetes isolated from the chernozem soil taken from Mount Fruška Gora.

Material and methods

Under laboratory conditions (*in vitro*), different rates of 2,4 D (2,4-dichlorophenoxyacetic acid) and Gramoxone (Paraquat) (dichloro-1,1-dimethyl-4,4-bipyridine) were used to evaluate their effect on the cellulose degradation ability of 20 strains of cellulolytic fungi isolated from the chernozem soil sampled from Mount Fruška Gora (19°48'58" E and 45°09'25", altitude 507 m): *Aspergillus candidus, Aspergillus ustus, Penicillium sp. funiculosum, Penicillium nalgiovensis, Penicillium sp. purpurogenum, Penicillium piscarium, Penicillium* sp. (L-8), *Fusarium* sp. (L-7), *Fusarium* (tn-11), *Trichoderma* sp. (111), *Fusarium avenaceum* var. *herbarum, Fusarium solani var. argillaceum*, *Fusarium nivale*, *Hormodendrum* sp. (L-10), *Hormodendrum* sp. (L-11), *Nigrospora* sp., *Stachybotrys atra, Verticillium candelabrum, Mycelia sterilia* and *Fusarium aquaeductum* var. *dimerum*. The nutrient solution (3g $NaNO_3$, 1g K_2HPO_4, 0.5g $MgSO_4 \cdot 7H_2O$, 0.5g KCl, 0.03g rose bengal, 30μg streptomycin/1mL nutrient medium, 1L distilled water) was supplemented with 2% powdered cellulose (pure cellulose) and a single herbicide. The herbicide 2,4-D was used at 30, 60, 120 and 240 μg/mL, and Gramoxone (Paraquat) at 3, 6, 12 and 24 μg/mL, in three replications. The resulting solutions were inoculated with the spore suspensions of the tested micromycetes and incubated in a thermostat for 12 weeks at 28°C. Thereafter, the amount of cellulose (%) remaining in the nutrient solution was determined by potassium dichromate oxidation (Petkov, Markova, 1969). Cellulose residues were the result of difference in carbon content between cellulose-containing soil samples and soil samples without cellulose (control). The importance of differences in the level of cellulose degradation (%) for each individual fungus, as dependent on the herbicide application rate, was assessed by LSD test (Statistica SPSS 5).

Results and discussion

The results obtained in control treatments showed high variations in cellulolytic activity i.e. cellulose degradation ability across the micromycetes tested (Tab. 1, 2). The highest cellulolytic activity was exhibited by *Hormodendrum* sp. (L-11), *Fusarium aquaeductum* var. *dimerum, Mycelia sterilia* and *Fusarium* sp. (L-7). This result is in agreement with the findings of Tomas *et al*. (2011) reporting that *Hormodendrum* fungi had pronounced cellulolytic activity under optimal culture conditions. Similar data were observed for some species of *Fusarium* (Panagiotou *et al*., 2003) and *Mycelia sterilia* (Sunitha *et al*., 2013). The cellulolytic activity of some micromycetes was inhibited by the herbicide 2,4-D (Tab. 1), particularly when applied at high rates (60, 120 and 240 μg/mL) in *Fusarium aquaeductum* var. *dimerum, Fusarium solani var. argillaceum, Aspergillus candidus, Aspergillus ustus* and

Fusarium sp. (L-7). This result complies with the report of Joshi and Gupta (2008) on the significant inhibitory effect of 2,4-D on the growth and, hence, enzymatic activities of some *Fusarium* species and *Aspergillus ustus*. The low ability of these fungi to degrade this herbicide is another reason for its toxic effect (Vroumsia *et al*., 2005). In contrast, at almost all rates, this herbicide had no significant effect on the cellulolytic activity of *Fusarium avenaceum* var. *herbarum*. Low rates of 2,4-D (30 and 60 μg/mL) enhanced the cellulolytic activity of most *Penicillium* species examined. This may be associated with the marked ability of these micromycetes to rapidly mineralize 2,4-D and use it in their own metabolism (Vroumsia *et al*., 2005; Joshi and Gupta, 2008).

Table 1. Mean values of percent cellulose degradation by cellulolytic fungi (%) as dependent on 2,4-D rate (30, 60, 120, 240 μg /mL)

Strains of fungi	2,4-D, μg /mL				
	0	30	60	120	240
	Cellulose degradation, %				
Aspergillus candidus	20.3a	19.7a	18.6ab	15.3b	10.4c
Aspergillus ustus	81.2a	82.4a	66.5b	60.8c	49.7d
Penicillium sp. funiculosum	74.2ab	73.7ab	76.9a	72.1c	69.8c
Penicillium nalgiovensis	79.6c	86.1b	89.4a	80.4c	81.5c
Penicillium sp. purpurogenum	76.5b	83.7ab	87.5a	71.4c	50.2d
Penicillium piscarium	49.7a	50.7a	39.8b	35.4b	27.9c
Penicillium sp. (L-8)	60.9a	64.6a	63.8a	62.7a	53.1b
Fusarium sp. (L-7)	83.4a	68.2b	63.5b	61.4b	59.8b
Fusarium (tn-11)	79.8a	65.3b	57.4b	50.2c	40.1d
Fusarium aquaeductum var. *dimerum*	87.4a	73.6b	32.4c	– d	– d
Fusarium avenaceum var. *herbarum*	61.2b	64.8ab	63.2ab	66.5ab	68.4a
Fusarium solani var. argillaceum	75.3a	76.4a	59.7b	39.5c	17.2d
Fusarium nivale	38.4a	39.7a	29.3b	27.4b	26.5b
Hormodendrum sp. (L-10)	39.6a	41.4a	35.3c	37.6b	30.9d
Hormodendrum sp. (L-11)	90.4a	85.3b	80.7bc	82.5bc	78.8c
Nigrospora sp.	25.7a	25.6a	27.2a	21.3b	19.5b
Stachybotrys atra	47.3bc	50.4ab	56.2a	49.1bc	44.3c
Verticillium candelabrum	43.7a	41.5ab	34.8b	24.7c	18.4c
Mycelia sterilia	86.7a	71.2b	54.6c	39.7d	30.3d
Trichoderma sp. (111)	80.3a	79.5a	77.4ab	71.5b	53.2c

Means followed by the same lowercase letters in rows are not significantly different ($p > 0.01$) according to LSD test

As opposed to 2,4 D, Paraquat exhibited higher toxicity to the cellulolytic micromycetes and their cellulose degradation ability (Tab. 2). Its inhibitory effect was observed at rates as low as those above 3μg/mL. The degree of inhibition increases with increasing rates of Paraquat. At the highest rate (24 μg/mL), only eight fungi retained some degree of cellulose degradation ability. Most species of *Aspergillus* and *Penicillium* were highly sensitive to Paraquat at a rate as low as 6 μg/mL. At 24 μg/mL, none of them showed the ability to degrade cellulose. This result is consistent with Smith and Lyon (1976) who observed a linear decline in the growth and germination of *Aspergillus* and even some *Penicilluim* species as the Paraquat application rate was increased from 500 to 2000 mg/L, which consequently affected their enzymatic activity. The inhibitory effect of Paraquat on fungal growth and cellulose degradation was also reported elsewhere (Smith and Mayfield, 1977). As found by the authors, the application

of Paraquat above its recommended label rate reduced cellulose degradation and soil respiration by 39-58%. The degree of inhibition decreases with increasing content of organic matter in the soil i.e. its increasing sorption (Bromilov, 2003). In this regard, many authors have observed that the inhibitory effect of Paraquat on cellulolytic fungi is more pronounced under *in vitro* conditions than under natural soil conditions, even at rates twice lower than the recommended label rate (Zain *et al*., 2013).

Table 2. Mean values of percent cellulose degradation by cellulolytic fungi as dependent on Paraquat rate (3, 6, 12, 24 µg /mL)

Strains of fungi	Paraquat µg /ml				
	0	3	6	12	24
	Cellulose degradation, %				
Aspergillus candidus	20.3a	16.4b	11.7c	8.3d	– e
Aspergillus ustus	81.2a	67.2b	50.3c	17.2d	– e
Penicillium sp. funiculosum	74.2a	72.4a	53.5b	26.3c	– d
Penicillium nalgiovensis	79.6a	83.2a	42.1b	30.7c	– d
Penicillium sp. purpurogenum	76.5a	66.3b	31.5c	12.0d	- e
Penicillium piscarium	49.7a	53.4a	47.2b	14.8c	– d
Penicillium sp. (L-8)	60.9a	57.2a	43.4b	19.6c	– d
Fusarium sp. (L-7)	83.4a	73.4b	71.3b	48.2c	19.6d
Fusarium (tn-11)	79.8a	65.6b	60.3b	39.7c	10.2d
Fusarium aquaeductum var. *dimerum*	87.4a	76.4b	71.2b	60.3c	50.4d
Fusarium avenaceum var. *herbarum*	61.2a	55.6ab	47.4b	31.5c	– d
Fusarium solani var. argillaceum	75.3a	75.4a	73.4ab	67.5b	59.4c
Fusarium nivale	38.4a	40.2a	31.5a	18.7b	– c
Hormodendrum sp. (L-10)	39.6a	42.4a	40.5a	39.6a	18.7b
Hormodendrum sp. (L-11)	90.4a	90.3a	75.7b	29.6c	15.2d
Nigrospora sp.	25.7a	22.3a	15.2b	13.1bc	10.9c
Stachybotrys atra	47.3a	41.5b	36.4b	18.4c	– d
Verticillium candelabrum	43.7a	35.1b	24.9c	11.6d	– e
Mycelia sterilia	86.7a	82.3a	42.5b	11.4c	9.3c
Trichoderma sp. (111)	80.3a	75.4ab	72.1b	37.5c	– d

Means followed by the same lowercase letters in rows are not significantly different ($p > 0.01$) according to LSD test

This research (Tab. 2) showed that the cellulolytic activity of some species of *Fusarium* (*Fusarium nivale* and *Fusarium solni var. argillaceum)* and *Hormodendrum sp.* (L-11) was retained even at a Paraquat rate of 6 µg/mL, and that of *Hormodendrum sp.* (L-10) even at a rate twice as high (12 µg/mL). These results are supported by the findings of other authors who reported a decrease in the cellulolytic activity of some micromycetes in the soil only after the Paraquat application rate reached 500-1000 mg kg^{-1} soil i.e. above 12.5 mg l^{-1} under *in vitro* conditions (Bozarth *et al*., 1965; Wilkinson and Lucas, 1969).

The fact is that the cellulolytic activity of the micromycetes belonging to *Fusarium* and *Horodendrum* as important plant pathogens is resistant to Paraquat. This suggests that, under particular conditions, the use of this herbicide can exert a negative selection pressure on soil microorganisms for the dominance of the types of fungi which cause serious crop diseases.

Conclusion

The tested micromycetes exhibited various degrees of cellulolytic activity i.e. cellulose degradation ability. The highest cellulolytic activity was found in *Hormodendrum* sp. (L-11), *Fusarium aquaeductum* var. *dimerum, Mycelia sterilia* and *Fusarium* sp. (L-7). High rates of 2,4-D (60, 120 and 240 μg/ml) inhibited the cellulolytic activity of *Fusarium aquaeductum* var. *dimerum, Fusarium solani var. argillaceum, Aspergillus candidus, Aspergillus ustus* and *Fusarium* sp. (L-7). This herbicide had no significant effect on the cellulolytic activity of *Fusarium avenaceum* var. *herbarum*. Lower rates of 2,4-D (30 and 60 μg/ml) stimulated the cellulose degradation ability of most *Penicillium* species tested.

Paraquat showed higher toxicity to cellulolytic micromycetes and their cellulose degradation ability. Its inhibitory effect was observed at rates as low as those above 3μg/mL. Inhibition increased with increasing rates of the herbicide, with only eight fungi retaining at least some degree of cellulose degradation ability at 24 μg/mL Paraquat. Most species of the genera *Aspergillus* and *Penicillium* were highly sensitive to Paraquat at rates as low as 6 μg/mL. At 24 μg/mL, none of them had the ability to degrade cellulose

A considerable degree of resistance to Paraquat was observed in *Fusarium* spp. (*Fusarium nivale* and *Fusarium solni var. argillaceum)* and *Hormodendrum sp.* (L-11), which retained their cellulolytic activity even at Paraquat rates of 6 μg/mL, whereas *Hormodendrum sp.* (L-10) retained its cellulolytic activity even at twofold higher rates (12 μg/mL).

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UNAUTHORIZED (ILLEGAL) GOLD MINING IN RIAU PROVINCE, INDONESIA

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Abstract

Mineral resources as natural resources will contribute to the economic development of the country in accordance with the mandate of the 1945 Constitution. Governments as resource authorities must regulate their use to prevent wastage and optimize revenues from resource exploitation to gain the greatest benefit for the welfare of the people. The regulation on mining is referred to in Article 33 paragraph (3) of the 1945 Constitution and Law No. 4 of 2009 on Mineral and Coal Mining. In this case illegal mining in Riau based on 2014 were 12 cases, 2015 were 15 cases and 2016 were 16 cases.The main problem in this research is how to solve from the side of law and from the government side.There are many news about illegal gold mining in various places in Indonesia and especially Riau. Riau region has the potential of natural resources in the form of gold is large enough with good quality. This makes local entrepreneurs and communities start doing gold mining and most of them are illegal. These mining activities causes conflicts between communities in the areas near the mine with gold mining actors who did not care about the aspects of environmental sustainability and carried out without permission. The perpetrators of gold mining without permission is not only from the Riau region but begin to develop from outside the Riau region as well. Although efforts to control and prosecute perpetrators have been carried out, they do not have any significant effect on these illegal mining activities. Unauthorized mining business activities shall be subject to criminal sanction as set forth in the criminal provisions of Article 158 of Law No. 4 of 2009 concerning the mining of Minerals and Coal, states that: Every person who engages in mining business without Mining Business license, Mining Permit, Special Mining Business License as referred to in Article 37, Article 40 paragraph (3), Article 48, Article 67 paragraph (1), Article 74 paragraph (1) shall be imprisoned for a maximum of 10 (ten) years and a maximum fine of Rp. 10,000,000,000 (ten billion rupiahs).

Keywords: *Legal Mining, Gold Mining, Illegal Mining, Mining Law.*

Introduction

The Law of the Republic of Indonesia Number 4 of 2009 concerning Mineral and Coal Mining regulates the relationship between the state and a person or legal entity in the utilization of excavated materials and provides a prohibition as well as a sanction of imprisonment and a fine to a person or legal entity that violates it. In mining, the government provides opportunities for Indonesian legal entities, cooperatives and individuals and local communities to carry out mineral and coal exploitation based on permits, which are in line with regional autonomy, given by the government and / or regional government in accordance with their authority. each in accordance with the provisions of the legislation. Mining business must provide maximum economic and social benefits for the welfare of the people (Departemen Pertambangan, Indonesian Mining Department). If mining business activities are carried out without a permit, it can be subject to criminal law as stated in the criminal provisions of article 158 of Law Number 4 of 2009 concerning Mineral and Coal Mining. Every person who conducts a mining business without a Mining Business License as referred to in article 37, article 40 paragraph 3, article 48, article 67 paragraph 1, article 74 paragraph 1 and paragraph 5, shall be sentenced to a maximum of ten years in prison and a maximum fine of ten billion rupiah (UU 4/2009, Indonesian Mining Law Number 4 year 2009).

Material and Methods

Mining activity activities in Indonesia are now being questioned by various circles, including the Kuantan Singingi Regency which has the potential of natural resources in the form of large enough gold metal minerals with good quality, and its presence is almost spread throughout the districts namely Kuantan Tengah, Benai, Singingi, Singingi Hilir, Kuantan Mudik, Gunung Toar, Hulu Kuantan and Cerenti. This has made local entrepreneurs and communities begin mining in a crowd. The mining activity caused conflict between communities in the area around the mine and gold mining actors who were not concerned with environmental sustainability and carried out without permission (Salim HS, Mining Law in Indonesia, 2005). The perpetrators of gold mining without permission are not only residents who come from the area of Kuantan Singingi Regency but are starting to develop from outside the district area with large capital. Although the effort to control and prosecute the mining actors has been carried out, the effort does not have a significant effect on the illegal mining activities.

Results and Discussion

Basically the mining business has a positive impact on national development, namely increasing the country's foreign exchange, increasing local revenue, accommodating workers and improving the socio-economic, health and cultural conditions of the people who live in the vicinity of the mine. In addition mining activities are carried out in areas on land using heavy equipment in the form of excavators to explore minerals and sand that contain gold metal in the soil and enter into cavort boxes to filter gold metal mineral granules from sand and stone then using mercury to separate gold metal mineral grains from sand which resulted in ex-mining land becoming dead and damage to the forest in the area around the mine and many gaping holes in the ex-excavation and when it rained the hole was filled by rainwater which is very vulnerable to natural hazards such as floods and landslides. The perpetrators of gold mining without permission are not only residents who come from the area of Kuantan Singingi Regency but are starting to develop from outside the district area with large capital. Although the effort to control and prosecute the mining actors has been carried out, the effort does not have a significant effect on the illegal mining activities. Based on the findings in Kuantan Singingi Police during 2009, there were 4 criminal case files of gold mining without a permit which was carried out by the investigation process and was declared complete by the Public Prosecutor and the suspect was sent along with evidence to the Taluk Kuantan District Procuratorate to be prosecuted in court and The Rengat District Court which convened in Taluk Kuantan examined and tried criminal cases with a regular hearing at the first level court, stating that the defendant had been proven legally and convincingly guilty of committing a gold mining crime without a permit and imprisoned and fined the defendant in accordance with the article 158 of Act Number 4 of 2009 concerning Junto Mineral and Coal Mining Article 55 paragraph 1 to 1 of the Criminal Code in Law Number 8 of 1981. In 2010, there were 17 unlicensed mining case files that were investigated by the Kuantan Singingi Resort Police Investigator. Of the 17 prkara files, 2 case files about alleged criminal acts of manganese exploration mining were allegedly carried out without permission in the area of the manganese exploration exploration mining permit, which is currently in the process of being investigated to complete the Prosecutor's instructions. While 15 more cases about illegal gold mining practices committed by the perpetrators of mining in the Kuantan river and in the hilly / land area in the area of Kuantan Singingi Regency by sucking / digging the minerals of sand and stone containing gold metal in the Kuantan river in in the soil and put the excavated material into a slude box or box cavort then use mercury to separate gold metal grains from sand and water. Of the 15 case files, 13 case files have been declared complete by the Public Prosecutor then the investigator sends the suspect and evidence to the Taluk

Kuantan District Attorney to be prosecuted in court while 2 more cases are still under investigation to complete the prosecutor's instructions in order improvement of the investigation of the case file. Of the 13 cases, 7 case files were submitted by the Investigator to the Taluk Kuantan District Attorney to be prosecuted in a court hearing and the Rengat District Court convening in Taluk Kuantan examined and tried and claimed the defendant had been proven legally and convincingly guilty of gold mining without permission and impose imprisonment and fines on the defendant in accordance with Article 158 of Law Number 4 of 2009 concerning junto Mineral and Coal Mining Article 55 paragraph 1 of the Criminal Code, while 6 case files are still under prosecution of the public prosecutor (Department of Mining).
In 2010 almost all court decisions of perpetrators of gold mining without permission were sentenced to 1 year and 8 months as well as criminal penalties for each defendant of ten million rupiah.
In order to support sustainable national development, the objectives of managing minerals and coal include:

a. Ensuring the effectiveness of the implementation and control of mining business activities in an efficient, effective and competitive manner.
b. Ensuring the benefits of mineral and coal mining in a sustainable and environmentally sound manner
c. Ensure the availability of minerals and coal as raw materials and / or as energy sources for domestic needs
d. Support and develop national capacity to be more able to compete at the national, regional and international levels
e. Increase the income of local, regional and state communities and create jobs for thegreatest possible welfare of the people.
f. Ensuring legal certainty in the operation of mineral and coal mining business activities.

Law Number 4 of 2009 concerning Mineral and Coal Mining confirms that Mineral Mining is mineral mining in the form of ore or rock, outside the earth market, oil and gas and ground water, and mining is a mining business activity to produce associated minerals and minerals.
In Law Number 4 of 2009 concerning Mineral and Coal Mining provides provisions / rules that regulate the relationship between the state and a person or legal entity in the utilization of minerals, as follows:
1. In Article 36 of Law number 4 of 2009 states: business license
Mining consists of two stages:

a. Exploration mining business licenses include general investigation activities, exploration and feasibility study.
b. Production operation permits include construction, mining, processing and purification and transportation and sales.

2. In Article 40 paragraph 3 of Law Number 4 of 2009 states: Holder mining business license that intends to cultivate other minerals in its mining business license area, must submit a new mining business permit to the Minister, Governor and Regent / Mayor in accordance with its authority.
3. In Article 48 of Law Number 4 of 2009: Mining business license Production Operations are given by the Regent / Mayor, Governor and Minister.
4. In Article 67 paragraph 1 of Law Number 4 of 2009 states Regents / Mayors provide People's Mining Permits especially to local residents, both individuals and community groups and / or cooperatives.
6. In Article 74 paragraph 1 paragraph 5 of Law No. 4 of 2009 states Permit

Special Mining Businesses are given by the Minister with due regard to regional interests. Special Mining Business Permit holders are given 1 type of metal mineral or coal in 1 special mining business license area.

Based on data obtained from the Mining and Mineral Resources Office of Kuantan Singingi Regency, Owners and Companies that have mining business licenses within the Kuantan Singingi Regency are listed as follows:

1. Gold Mining Production Operations as many as 4 owners / companies.
2. Solid Bitumen Mining Exploration of 2 Company owners
3. Production operations Manganese mining as many as 5 company owners
4. Existing coal mining as many as 9 company owners
5. Group C materials (sand and stone) as many as 12 company owners.

Kuantan Singingi Regency holds maximum untapped wealth, namely gold content. From the data of the potential gold content that was recorded by the Mining Service of Kuantan Singingi district, the potential for gold content is estimated at around 64.2 tons with details of the location as follows: Logas Village, Singingi District, 2.4 tons, Sentajo Village 2.8 tons Kuantan District Tengah, Bawang River is 12 tons in Singingi sub-district, Sungai Jake in Kuantan Tengah District is 18 tons, Sungai Bulus is 13 tons, Bukit batabuh in Kuantan Mudik District is 10 tons. Ironically, although the potential of gold mining in this area is very large, until now this potential has not been managed properly. This potential then motivates the community to carry out gold mining activities without permission, as a result of which has a negative impact on the local environment. Although efforts have been made to approach and control the Kuantan Singingi district government and resort police, the activities of gold mining without the permit continue. Based on the findings, unlicensed gold mining in the area of Kuantan Singingi Regency is not only a mining business activity, some residents traditionally use hoes and crowbars to dig holes and then lift them with baskets and then separate gold metal mineral ores with a miner or by using just a panning tool in taking sand in the river then panning / separating gold metal mineral ore from sand, but the gold mining business without permission is done mechanically by using rafts and machines to suck the minerals of sand and stone containing the existing gold metal in the river, then put the mineral material into a slude box to filter gold metal mineral granules from sand and stone and then use mercury to separate gold grains from sand and water, which causes the river water to become cloudy and polluted disease outbreaks for people in the mine area.

In addition, mechanical mining efforts are also carried out on rock / hills in the mainland of Kuantan Singingi Regency by using heavy equipment in the form of excavators to explore the minerals of sand and stones containing gold metal in the ground and then put the excavation into the cavort box to filter gold metal mineral grains from sand and stone and then use mercury to separate gold grains from sand and water which resulted in ex-mining land becoming dead and damage to the forest in the area around the mine and many gaping holes were found very large in the former quarry and when it rains, the hole is filled with rainwater which is very vulnerable to natural hazards and floods and landslides.

Gold mining without the permit does not take into account environmental sustainability and prioritizes personal or group interests and does not have a mining business license from the Kuantan Singingi Regent. Mining activities without permits have a negative impact which results in the destruction of forests that are in the area of the mine, river contamination, disease contractions for people living in the area around the mine and conflict between communities around the mine and loss of state income or local revenue.

According to Kusnadi, the perpetrator of gold mining without permission, that gold mining activities carried out in the river without permission, use rafts and machines to suck up sand and use mercury to separate gold seeds from sand and water. On average one day can produce at least 1 ounce of gold and can reach 3 kilograms per month (Informan).

The same thing was also conveyed by Wahyudi, the gold mining agent without permission, that the gold mining activities he did in Petapahan without permission from the authorized official. Cases of gold mining without permits handled / processed by police in the form of (Information from Mining Department):

1. Mining carried out without a mining business permit document from the government in accordance with the provisions of the legislation
2. Mining outside the concession / area of the mining business license
3. Mining other minerals outside the mining business permit granted for one type of mineral.
4. Not yet have the completeness of the permit but has already carried out mining activities.
5. Mining carried out in prohibited places (Informan):

a. By using excavator heavy equipment which resulted in ex-mining land becoming dead and damaged forests and many gaping holes were found which were very susceptible to natural disasters such as floods and landslides.
b. By using rafts and machines to suck sand from the river which results in turbid and polluted river water as well as contracting diseases for the people in the mine's circumference
c. Use mercury to separate gold metal seeds / minerals from sand and water.

In its development, gold mining without permission always follows the times. This can be seen from the form of illegal mining of gold metal minerals which have the characteristics grouped as follows (Information from Police):

1. Viewed from business actors, consisting of:
a. Local people are people around the mining location who are working in the mining sector
b. Newcomers are outsiders from the mining location who try to make a living through mining
c. Local entrepreneurs are entrepreneurs who have capital that live around the location of the miners.
d. Newcomers are outside entrepreneurs from the mining area who come deliberately to mine.
e. Investors are capital owners who are working in the mining sector.
f. Person of the Apparatus, namely a person or person from the apparatus and the government who illegally supports the practice of mining without permission.
2. Judging from the status of its business activities carried out on:
a. Own land
b. State-owned land such as protected forests, nature reserves and others
c. Riverside
3. Judging from the commodity / quarrying material, consisting of Gold (Group A).

As for the factors that cause illegal mining are:

1. Law: Public ignorance of the laws and regulations that apply in the mining sector.
2. Economy
a. Limited employment opportunities and business opportunities in accordance with the level of expertise / skills of the community below
b. Poverty in various ways, economically poor, knowledge and skills.

Factors affecting illegal mining are:

1. Main factors:
a. The miners do not care about licensing regulations / provisions
b. Gold metal mineral deposits found on community land and in rivers can be found easily
c. Attractive gold metal prices and easy marketing
d. The economy of the community around the mine site is generally poor.
2. Supporting Factors:
a. There is support from strong investors.
b. Anticipatory policies from the government have not been effective
3. Driving factor:

a. The fee sharing system is very profitable
b. Misuse of permits granted for 1 type of mineral is used to cultivate other minerals
c. The economic crisis caused limited employment
d. Mining equipment is widely available and inexpensive at rentals such as excavators as well as mining equipment in rivers such as machines, rafts, slide boxes and cavort boxes are easily made.
e. There is a perception that illegal mining activities are very helpful in alleviating poverty.

The perpetrators of gold mining without most permits are not required to be maximized with the following considerations:

a. Most of these perpetrators were told to do or participate in the crime of gold mining without permission.
b. The perpetrators of gold mining without permission do not know the laws and regulations that apply in the mining sector
c. The defendant admitted frankly his actions
d. The defendant was polite during the course of the trial
e. The defendant admitted guilt, regret and promised not to repeat his actions later on.

Judges' consideration of imposing a criminal sentence on the perpetrators of gold mining without permission, among others:

a. Most of these perpetrators are told to do or participate in the crime of gold mining without permission and do not know the laws and regulations that apply in the mining sector.
b. Judging from the status and education of the convict.

Conclusion

Unlicensed gold mining activities in the area of Kuantan Singingi Regency are not only some residents traditionally separating gold metal mineral grains contained in the river but this activity is carried out mechanically by using rafts and other mining machinery and equipment to suck sand minerals and stones that contain gold metal and put mining material into the slude box to filter further using mercury to separate gold metal grains from sand and water which results in turbid and polluted river water. In addition, the activities of unlicensed gold mining are carried out on land mechanically by using heavy equipment in the form of excavators to dig and insert mining material into the cavort box to filter further using mercury to separate gold metal grains which result in damage to the forest and the large number of holes very vulnerable to the dangers of floods and landslides.

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ANIMAL HUSBANDRY

STATE OF PLAY AND RECOMMENDATIONS CONCERNING THE SANITARY QUALITY OF DRINKING WATER FOR SHEEP AND GOAT FARMS IN THE COMMUNE OF AIN ZAATOUT

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Abstract

The scientific and technical study on the sanitary quality of drinking water for farm animals is intended to assess the level of risk (identification of biological, chemical and physical hazards). In order to show the inventory of livestock (sheep and goats) of the municipality of Ain Zaatout, a field survey was carried out on a set of farms in terms of water and water installation (watering). We were interested in the origin of the drinking water, the number and the hygiene of the drinkers as well as their load. The evaluation of the sanitary quality of the water in the farms requires the carrying out of bacteriological and physicochemical analyzes at the level of the boreholes, the sources of water and the drinkers on these exploitations in order to detect the levels of contamination of the farms drinking water and degree of drinkability. The work carried out has allowed us to classify drinking water in this town as water of good bacteriological quality and of average physico-chemical quality which therefore requires giving recommendations and suggestions to ensure a good behavior of the farms through the improvement of the sanitary quality of water (nutrition) which protects animal health and animal production (sheep and goats) therefore.

Keywords: *drinking water, bacteriological quality, physicochemical quality, sheep, goats.*

Introduction

Livestock depend on a constant and regular supply of water; animal organisms are composed of 65 to 80% of water. That makes it a determining factor in the course of digestion and metabolism of food; it ensures the transport of nutrients in the body and it allows the excretion of metabolic end products (urine, feces, sweat) and the secretion of milk (dairy animals). In addition, water is a key element in regulating body temperature (breathing, sweating) and osmolarity (Jean-Blain, 2002).

A water deficit in animals leads first to a reduction in food intake and then leads to a decline in performance, increased susceptibility to disease and increased aggression of animals around water points (Jean-Blain, 2002; Steiger Burgos et al., 2001). Inadequate fluid intake can also lead to dehydration, heart rate and respiratory acceleration. These symptoms can lead to death of the animals in case of prolonged lack, especially in young and small subjects. In fact, they have very few body water reserves, which make them particularly vulnerable when the water supply is insufficient for their needs (Basselin, et al., 1999).

The relative importance of the different sources of water supplied to animals is poorly known, regardless of the type of farming (above ground or outdoors). Drinking water can come mainly from the public drinking water distribution network, from private wells that are subject to disinfection treatment or natural resources (Pierroux, 2008; Simonet, 2001; UPA-Estrie, 2002).

The regulations on drinking water are few and the inventory that follows cannot claim to be exhaustive. These regulations have two explicit objectives, the protection of human and animal health and that of animal welfare. They are most often general, limiting themselves to specifying that the water must be of "adequate", "appropriate", etc. quality. These formulations are often similar, whatever the objective (Dermaux, 1999; Medd and Brgm,

2004). The decrees relating to the controlled labels of origin do not set, in general, requirements on the quality of the water to be used throughout the chain, in particular for the watering of the animals (Medd and Brgm, 2004). The management of regulated products (ex Plant and Environment Directorate) of National Agency for Food Safety Environment and Labor, in charge of the evaluation of phytopharmaceutical products, evaluates their ecotoxicity, in particular their effects on birds and mammals via their presence in drinking water in accordance with the guide Consumer Health and Protection 4145/2000.

Some biological hazards can persist for weeks to months in water depending on temperature, exposure to sunlight, and competition with other aquatic microorganisms (Medd and Brgm, 2004). The degree of contamination of drinking water by micro-organisms varies greatly depending on the origin of the water. For example, surface water, streams, ponds, other natural water points, and sometimes very shallow wells (depth <1 m), can be highly contaminated due to runoff. Infiltration results in a large number of bacteria, viruses or parasites (Exova, 2009). The use of a water reservoir and direct watering in ponds or water holes particularly expose animals to microbiological pollution. In addition, flocks can be the cause of contamination of surface water or poorly protected catchment. For example, a cow can release 10 million Salmonella per gram of droppings, while the generally accepted oral infective dose for a domestic animal is at least one million bacteria (Dubreuil and Sauvageau, 1993; Medd and Brgm, 2004). Minerals such as arsenic, calcium, magnesium, selenium, chlorides, fluorides and sulphates can be naturally present in the water resources, and their concentrations vary due in particular to the geological nature of the lands crossed. These water resources are also subject to diffuse pollution by various chemical substances (nitrates, pesticides, etc.) related to human activities. Water used for livestock watering can be contaminated by these chemicals, leaching polluted soils for surface water, or by infiltrating pollutants deep into the groundwater after heavy rainfall (Métivet, 1996; Beilage, 2002).

Contagious diseases caused by pathogenic bacteria, viruses and parasites are very often linked to the consumption of water that does not meet minimum criteria of potability. They constitute for health the most common and widespread risk. It is therefore important to establish norms and indicators of potability and quality and especially to verify that they are respected, especially on points of access to water and if necessary to adapt them according to the zones and local peculiarities.

The aim of this paper is to study the sanitary quality of drinking water for livestock and to purpose assessing of the risk level for animal health, and for human health via the consumption of products derived from animals. The objective of the bacteriological analysis of water is not to make an inventory of all the species present, but to look for either those that are likely to be pathogenic, or those that are indicative of faecal contamination.

Material and Methods

In order to show the inventory of the farms of the commune of "Ain Zaatout" a field survey was conducted on all farms asking on water and watering facilities, we were interested in the origin of the drinking water, the number and the quality of the drinkers as well as their load.

We realized our practical study on the state of the breeding places and the recommendations relating to the sanitary quality of the water in the commune of "Ain Zaàtout", region of "El'outaya", city of Biskra, Algeria. It is within the Aurès known by the name of "Beni Ferah". To evaluate the sanitary, bacteriological and physicochemical quality of livestock watering, we have sampled water from boreholes, water sources and drinking troughs. Bacteriological and physicochemical analyzes were conducted to detect contamination levels and to determine the degree of drinkability of the drinking water at the farms of Ain Zatout commune.

The study area is divided into two regions because it contains two water wells. The first borehole in the first region is called "Ah M'nie" precisely to "yekhf n'souf" irrigates this first region and the region of "Afra" from which five samples were taken. Three water samples from "Ah M'nie" and two water samples from "Afra", from five internal waterers on specific and different farms. In this region there are many external watering places (springs of open water) from which we took two water samples, one to "Ah M'nie" and the other to "Afra" where sheep and goats water before and after their pastures. Only one water sample was taken from this first borehole. The second borehole located in the second area is called "Dachra" precisely behind the town hall, irrigates this entire second region from which we took five samples of water from five internal waterers on specific and different farms. In this area also are external drinking troughs from which we took a single sample from a single external water source where sheep and goats congregate before and after their pastures. Only one sample of water to be analyzed was also taken from the second borehole.

The bacteriological analysis of water was conducted to identify the rate of contamination by bacteria and make it unfit for consumption. The identification of faecal coliforms, enterococci, Heterotrophic Aerobic and Facultative Anaerobic Bacteria, and atypical colonies follows a scientific method for bacteriological examination of water samples, used in the laboratory recognized by the Ministry of the Environment (Laboratory Accreditation Program). The carefully completed analysis request form must always accompany the samples. The temperature plays a very important role in the solubility of the salts, and consequently the conductivity and the pH; it also has an influence on the biological activity of certain microorganisms in the water. The temperature has been measured in situ. Conductivity or pH meters usually have a built-in thermometer.

The pH (Hydrogen potential) measures the concentration of H + ions in the water; this is a characteristic that reflects both the acidity and the basicity of the water. It influences the form and availability of nutrients in the abatement water and should be between 6.5 and 8.4. At these values the solubility of most micro-elements is optimal. It has been measured in the field using a pH meter. Electrical conductivity (EC), expressed in dS/m measured at 20°C, is one of the most commonly measured parameters. Particularly in arid and semi-arid regions to estimate the total value of soluble salts in water.

Chemical analyses also have been performed according to National Agency for Food Safety, Environment and Labor standards. We dosed Ca^{++}, Mg^{++}, Na^{+}, K^{+}, Cl^{-}, So_4^{-}, Hco_3^{-}, Nh_4^{+}, No_3^{-}, Fe and Mn.

Results and Discussion

The results of the analyzes show a total germ concentration varying between a maximum of 30 on farms and a minimum of 0 to 7 in boreholes and water sources. The measured values of the total germs make it possible to classify drinking water in the study area as water of good bacteriological quality according to the norms of National Agency for Food Safety, Environment and Labor (Anses, 2010).

Trials show a complete absence of fecal germs, fecal streptococci or Enterococci and Staphylococci in the waters of the two boreholes and the three water sources and in the watering places of the ten farms. The samples were free of bacterial spores of the sulphitoréductive anaerobes and clostridium sulphito-reducing agents in the two boreholes and the three water sources and the ten farms according to the standards (Anses, 2010). There was no Salmonella sp. also in these samples, according to the same standards. The abatement water of sheep and goats in the study area had a satisfactory bacteriological quality, according to the standards (Anses, 2010).

Microbiological analyzes are based on the search for bacteria considered as indicators of faecal contamination: these bacteria have been chosen because they are present in large numbers in the stool of warm-blooded animals, which are frequent sources of fairly serious

contamination, they are easily detectable, and they do not grow in pure source. The indicator of choice is the search for Escherichia coli, or that of thermotolerant coliforms (bacteria of the same genus as E. coli) and is still widely used. Drinking water must not contain it. It is a good indicator of potability. Other indicators are added, such as the search for enterococci, and the spores of Clostridium perfringens. Livestock watering water analyzed in the commune of Ain Zaatout shows acceptable bacteriological quality, so we can say that there is good potability in terms of boreholes and water sources. On the other hand, this potability is average at the level of the drinking troughs on the farms. This deterioration in the quality of drinking water at farm level can be caused by the lack of hygiene that is not correctly practiced by about 50% of farmers, according to the results of the survey.

Knowledge of the temperature of the drinking water is important because it plays a role in the solubility of the salts, in the dissociation of the dissolved salts and thus on the electrical conductivity and on the determination of the pH. The temperature of the water taken from the sources and the boreholes was between 5°C and 8°C, and those of drinking water between 15°C and 26°C, which classified these waters as good for consumption.

The pH values of drinking water (boreholes, water sources, waterers) are relatively neutral to slightly alkaline; the averages are between 6.6 and 8.3. According to the drinking water quality grid, the analyzed waters intended for watering animals are of good quality.

The CE values of the water are between 2040 µm/cm and 1056 µm/cm; this is related to the presence of the ions, so it is the mineralization which depends on the temperature of the waters and the crossed lands.

Table 1. Results of chemical analysis of water from sourcing region 01

Location		Parameter										
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Cl^{-}	So_4^{-}	Hco_3^{-}	NH_4^{+}	NO_3^{-}	Fe	Mn
Region 01	Drilling 01	78	55	303	14	147	279	124	0	1	1	8,2
	Water source 01	92	62	285	16	174	271	126	0	3	0,8	7,9
	Water source 02	83	76	247	16	158	280	126	0	2	0,9	7,5
	Exploitation01	66	32	184	7	222	335	152	0 ,01	7	0,5	4,5
	Exploitation02	69	59	193	9	251	354	147	0	4	0 ,5	4,8
	Exploitation03	72	61	227	5	199	279	132	0	4	0,6	4,5
	Exploitation04	98	39	273	10	169	342	137	0	4	0,2	4,7
	Exploitation05	76	45	179	7	202	294	141	0	5	0,3	4,6

Table 2. Results of chemical analysis of water for sourcing region 02

Location		Parameter										
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Cl^{-}	SO_4^{-}	HCO_3^{-}	NH_4^{+}	NO_3^{-}	Fe	Mn
Region 02	Drilling 02	88	61	291	14	155	275	123	0	2	1	7,8
	Water source 03	99	54	278	13	188	298	125	0	4	0 ,8	7 ,6
	Exploitation06	79	46	155	6	367	301	152	0	5	0,3	6,2
	Exploitation07	148	85	230	8	404	283	143	0,03	5	0,4	6,4
	Exploitation08	118	76	161	7	289	293	147	0,02	6	0,2	6,7
	Exploitation09	76	57	129	7	255	352	132	0	9	0,2	6,9
	Exploitation10	81	67	133	10	303	312	155	0	7	0,3	7 ,0

A list should be drawn up listing the various physical and chemical parameters to be controlled and which define the potability of a water and the values limit not to exceed. From a chemical and physical point of view these different parameters make it possible to determine if a water is drinkable.

In addition to the usual parameters, certain specific parameters must be analyzed according to the region and the problems encountered. One distinguishes for the establishment of the chemical potability of a water:

- Undesirable substances: their presence is however tolerated as long as it remains below a certain threshold (fluorine F- and nitrates for example).
- Substances with toxic effects as lead (Pb), chromium (Cr), arsenic (Ar), cadmium (Cd) are part of it. The tolerated levels are extremely low, sometimes of the order of one millionth of a gram per liter.

Livestock drinking water subject of analysis in the commune of Ain Zaatout shows an acceptable physicochemical quality, so we can say that there is a good physico-chemical drinkability at the level of boreholes and water sources and average drinkability at the level of watering facilities.

Conclusions

Watering with contaminated water can lead to various organic disorders in the animal (possibly to the point of death) or loss of production or reproduction performance. That is why it is important to define what should be water of "adequate quality", as it is mentioned in the regulation. The bacteriological analyzes carried out on samples from different water points in our study show the total absence of total and faecal coliforms, coagulase-positive staphylococci, faecal streptococci or enterococci, spores of the sulphitoréductive anaerobes and clostridium sulphitrate reducers, and salmonellae, but also the presence of susceptible microorganisms (the total germs) at very low rates, not exceeding the limits. These results allow us to say that there is a bacteriological potability of drinking water in the municipality examined. The physico-chemical analyzes carried out showed normal levels of physical parameters and chemical substances at the level of boreholes and water sources, while in drinking troughs, the existence of minerals such as chlorides (Cl-), sulphates (So4-),

ammonium (Nh4 +), nitrates (No3-) was noted, with rates that exceed limits set by international legislation and standards. These results allow us to say that there is some physico-chemical drinkability of drinking water in the municipality. The results recorded allow us to classify drinking water in the commune of "Ain Zaatout" as a water of good bacteriological quality and of average physico-chemical quality. However, in these farms, the maintenance of the facilities of drinking troughs is recommended in order to guarantee the hygiene of materials in direct contact with drinking water. An annual analysis for the control of water quality is mandatory to meet good farming practices.

The survey carried out and informations got from the farms allows us to link this situation to several causes, like the absence of hygiene practices, the breeding methods, the number and the load of cattle, the systems of traditional installation of drinking troughs, the installation system of pipelines without maintenance, the lack of labor, the excessive use of pesticides in growing areas, the presence of these elements in organic matter of animal origin;, the development of farms, excessive fertilization of agricultural areas by fertilizers, droppings and various manure.

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STUDY OF POULTRY BREEDING EQUIPMENT IN BISKRA, ALGERIA

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Abstract

Poultry is a valuable and economical source of animal protein, particularly for developing countries, which has justified its very rapid development worldwide over the last fifty years. This evolution was the result of the industrialization of production thanks to the contributions of the various research carried out in the fields of selection, feeding, habitat, prophylaxis and end product technology. Our work was carried out at the level of Zâb-chergui in the city of Biskra (Algeria) and focuses on the diagnosis of buildings and equipments of poultry breeding in this region. Through a field survey, we aim to identify broiler breeders in this region and do either a quantitative and qualitative analysis of their activities. Our results reveal an inadequate situation of the majority of the farms visited in building quality and equipment. This situation has led to mortality rates above the acceptable limit for this type of farming. We propose recommendations for improving broiler farming to have better production under better conditions in this region. A good building should be equipped with dynamic ventilation, fluorescent tubes, air heaters, flushes and the presence of a sanitary lock.

Keywords: *equipment, broiler, building, production, Zâb-chergui.*

Introduction

In Algeria, the poultry sector is largely dominated by intensive modern poultry farming. The introduction of the intensive poultry model from 1975 onwards through the import of high-tech industrial poultry complexes has limited the development of traditional poultry farming, particularly the exploitation of local breeds. The annual national production of the poultry sector is considerable; it is estimated at more than 253 000 tonnes of white meat and almost 4.5 billion of eggs for consumption, thus ensuring more than 50% of the feed intake of animal products in 2011 (Ministry of Agriculture and Rural Development, 2012).

The poultry farms require from the farmer, a strict observation of the optimal ambient conditions (temperature, humidity, lighting, renewal of air), and otherwise serious troubles, both in terms of performance and on the sanitary level, do not delay in arriving. Lastly, rational management of poultry premises requires very precise indications regarding interior equipment. (Technical Institute of Poultry, 2001)

The choice of the location of the building is crucial because it defines the internal environment of the latter. During implementation in a valley, it is found a lack of wind, a lack of air renewal in static ventilation especially in hot weather, moisture and ammonia, resulting in health problems and a fall average daily weight gain (ADG) at the end of rearing (Boulakroune and Taleb, 2015; Djermouni and Fas, 2016). However, during implementation on a hill, there is an excess of air intake on the prevailing wind side, harmful especially during start-up (lack of heat regulation of chicks), insufficient ambient temperature and transverse air sweep resulting in diarrhea and dirty litter from the first day (Rosset, 1988; Djermouni and Fas, 2016).

The surface area of the buildings depends on the length and the period of rearing of the poultry and the type of production: For the chickens, one can retain as standard of density, 20 chicks / m² of building. This standard can be adapted according to the age at which the poultry is transferred to a farmhouse: the earlier the transition, the higher the density can be. So in practice, for a band of 200 chicks, it is necessary to have a 10 m² chick.

For breeding huts for broilers: it is advisable not to exceed the standard density of 8 chickens / m² (Fernard, 1992).
Static or Natural Ventilation is the simplest system; ventilation is provided by natural movements of the air inside the henhouse. Vertical ventilation is achieved by windows and horizontal ventilation is achieved by means of hatches placed on the façades (Bellaoui, 1990, Laraba and Lezzar, 2016).
Dynamic ventilation is much more efficient than natural and more recommendable for cold climates (Fernandez and Ruiz-Matas, 2003, Laraba and Lezzar, 2016). This ventilation requires the use of humidifier fans generating more expenses but effective in all seasons (Bellaoui, 1990, Laraba and Lezzar, 2016). The renewal of the air can be perfectly controlled by regulating the flow of the pressure and the speed of the air. This air is also extracted or pulsed by known theoretical flow rate (Djerrou 2006, Laraba and Lezzar 2016, Cherouana 2016). Fluorescent tubes (neons) perform better than incandescent lamps. The norm specifies 5 watts / m2 to be able to ensure 30 to 40 lux, the strongest intensity during the first days of the start, so as to allow the chicks to occupy well and use their living space (Castello, 1990; Bentchikou and Bachtarzi, 2015).
For heating two options are most often used: air heaters (heat guns) or radiant heaters. Unit heaters require precise adjustment for a room temperature. Heating power: 100 to 150 watts / m2 and capacity of the fans: from 2,000 to 5,000 m3 / hour. Radiants are much more common (and recommended) in the production of quality-differentiated chickens. Standard: 1 radiant of 1,400 kcal for 650 chicks or 1 radiant of 3,000 kcal for 800 chicks (Castello, 1990; Laouni and Dakhia, 2016). There are different models of drinking troughs, circular drinking trough still called "plasson", cup or mini drink, 1 per 100 animals, and nipple or "nippel" with or without salvage cup. The pacifier model is the most commonly used. In this case, there is 1 pacifier for 13 animals. The diet is almost always automated. There is then a plate for 70 animals (When feeding is manual, the standard is a hopper (20 kg) for 60 animals) (Bentchikou and Bachtarzi, 2015; Laouni and Dakhia, 2016).
Currently the intensive breeding of broilers in the city of Biskra becomes important, following the rise of private investments oriented towards the sector. For this, we propose the study of the equipment of poultry breeding in this region. The present paper is dedicated to the study of poultry farming equipment.

Material and Methods

Through a field survey, we aim to identify broiler breeders in the region of Zâb-chergui of Biskra (Sidi Okba, Loutaya, Djemourah, El Kantra, Biskra and Zribet el ouad), quantitative and qualitative analysis of their activities and the state of their livestock building (year of construction, orientation, density , etc.). In addition, to explore the equipment of the livestock building (water troughs, feeders, etc.), and also the mortality rate recorded by farmers in this region. The results of the survey are statistically processed by SPSS software.
According to statistics obtained from the Directorate of Agricultural Services of the city of Biskra (2018), the broiler buildings are scattered as shown in the following table.

Table 1. Distribution of broiler buildings in the city of Biskra

Areas		Number of buildings
Daïra	**Commune**	
Sidi okba	M'choneche	2
	Seriana	12
	Sidi okba	4
	Chetma	1
	Haouche	2
Loutaya	Complexe	4
Djemourah	Djemourah	3
	Ain zatout	7
El kantra	El kantra	1
Biskra	BISKRA	5
	El hadjeb	2
Zribet el ouad	Zribet el ouad	5
Tolga	Leghrousse	1
	Borje ben azzouz	1
	Bouchagroune	1
	Lichana	1
Ourellale	Mkhadma	2
	Liwa	1
	Oumache	3
Ouled djallel	Ouled djallel	3
	Doucen	2

The survey was conducted on a study area as most of the respondents were in the Zab-chergui region. Figure 1 shows the distribution of farmers in this area.

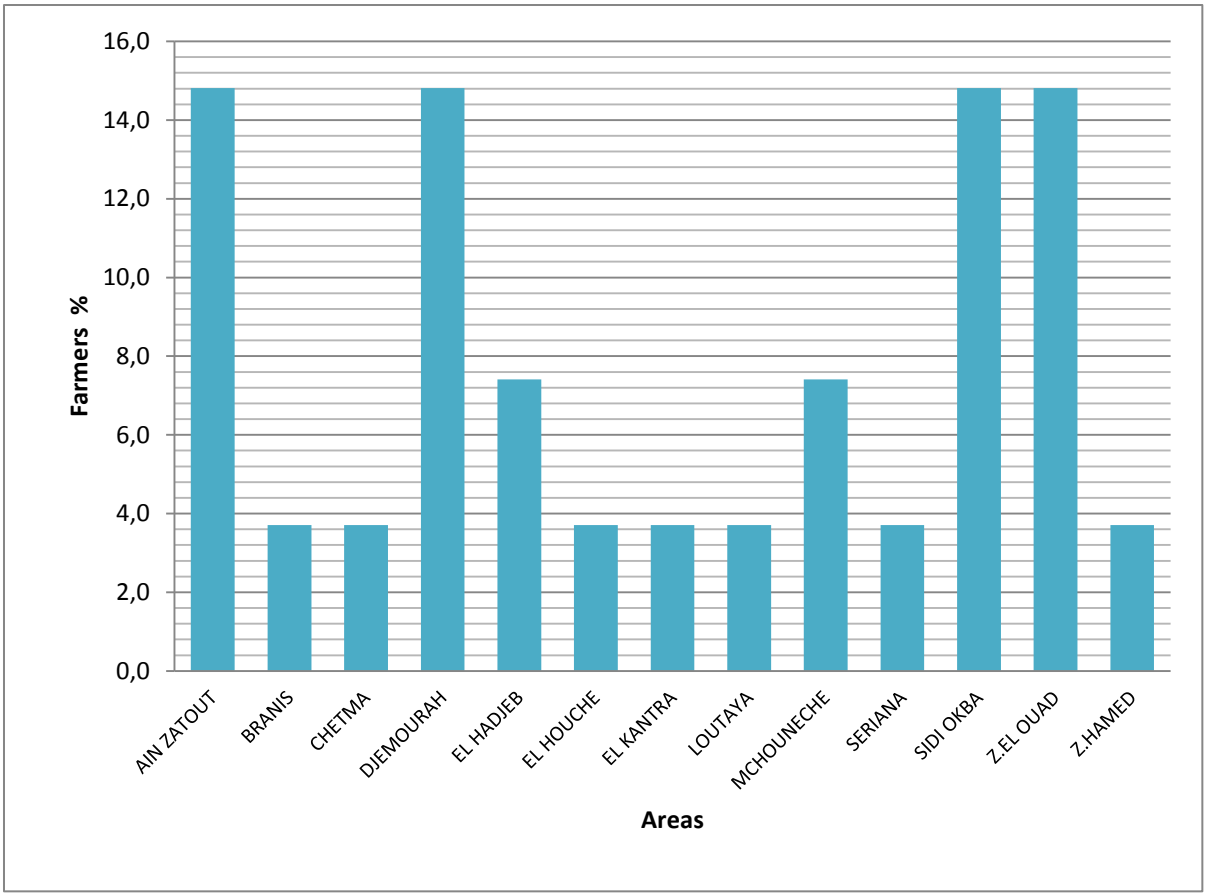

Figure 1. Distribution of farmers in area

Results and Discussion

According to the results recorded, the age of the breeders (27 breeders) is on average 43.88 ± 8.335. The most common age is 40 years old and ranges from 28 to 60 years old. 50% of breeders are under 45 years old.

The way in which buildings are asserted by the breeders is the majority owner (87.5%), the remaining breeders are tenants (12.5%). The majority of the buildings visited were permanently shut down (45%), 33% in production and the rest (22%) in crawl space.

During the survey, the majority of breeders (78%) were fattening, while the others (22%) were in the start-up phase. Most of the buildings visited (43.8%) were built between the years [2003-2010], compared to 31.3% between the years [2011-2016] and the rest of the buildings between the years [1984-2002].

Half (50%) of the buildings visited are badly oriented (towards the East /West) and the others (50%) are in a good orientation in North/South. Proper orientation of the building avoids exposure to prevailing winds, cold winter in the north and summer heat in the south.

We found that more than half of the buildings visited (56.3%) meet the standards of density of poultry breeding against other buildings have different and substandard densities. According to the results of the survey, the majority of the farmers considered (68.8%) have only one building, whereas the other breeders have more than 2 buildings which are in parallel. The majority of the hulls (88%) used belong to two types; traditional (hard) and light tunnel against the rest of these buildings (12%) is in the form of the rigid tunnel.

All of the poultry farm buildings in the Zab-chergui region are semi-dark, do not have chickens and have a dynamic ventilation system. It has been noticed that the majority of the livestock buildings visited (75%) do not have a sanitary lock at the entrance and that 25% of the buildings have a sanitary lock and that respect the sanitary standards. The majority of the

farmers leave their buildings in crawl spaces between 15 to 20 days with relative percentages of 25% and 43.8%.

The majority of the livestock buildings (69%) are equipped with incandescent lamps, 12% of buildings have low-consumption lamps and the rest of these buildings (19%) meet poultry farming standards and use fluorescent tubes (neon lights) as the best type of lighting.

It was found that all the farmers surveyed complied with the standards of poultry breeding in terms of heating. The majority of breeders (93.8%) use radiants in their buildings as a heating source but the other breeders (6.3%) use a type based on fan heaters.

We observed that 63% of the breeders use circular waterers (Plasson) in their livestock buildings, 31% of breeders use linear waterers, but only 6% of these farmers use teats (pipettes). Although the latter system is most commonly used in poultry farming standards. Half of the farmers (50%) have a private water network, but the others have based on public networks which are considered as their only source of drinking water used in their poultry farming. We found that more than 68% of the breeders use hoppers in their livestock buildings as a type of feeder, but only 31% of farmers use the plates.

During the visit of the livestock buildings, it was found that 56.3% of the breeders use wood chips as litter, 25% of the breeders use the ground straw and 12.5% of the breeders use the mixed litter (between wood chips, wood and chopped straw). On the other hand 6.3% of breeders do not use litter in their buildings since they are equipped with farm batteries.

According to the results of the survey on expensive chicken farming, the average mortality rate was found to be 5.52 ± 4.11 and with an interval of [1%, 15%]. More than half of the farmers (56%) have practices that do not respect poultry farming standards (they have a mortality rate of $\geq 3\%$), while 44% of the farmers have adequate practices (they have a mortality rate $\leq 3\%$).

The majority of the breeders with a mortality rate $\geq 3\%$ have light (greenhouses), semi-dark buildings with a dynamic ventilation system and poorly oriented (TSE / WEST) which causes an increase in the area of exposure to the prevailing winds, which is harmful especially during the start-up period (lack of thermoregulation of chicks, diarrhea and soiled litter from the first day). These breeders do not control the ambient conditions (ambient temperature, ventilation, hygrometry, litter quality, light):

- Improper light installation (do not respect the luminous intensity standards).
- Do not regulate the flow of pressure and air velocity.
- Use poor litter quality.
- Poor distribution of watering equipment and feeds on the building surface.

They do not respect the sanitary standards (lack of sanitary locks in their livestock buildings) and the density which influences the quality of the litter, which causes a decrease of the homogeneity then a reduction of growth. These parameters are all factors that apprehend the bioclimatic environment of birds and if they are not properly controlled and managed rationally, they cause an increase in the mortality rate.

To have a good building, well equipped and well prepared and for the success of poultry farming, it is necessary that the poultry farmer masters several components relating to hygiene, breeding standards, environmental conditions. Accounting and management elements , etc. For this purpose we propose the following recommendations:

1. Choose a good layout of the building (avoid obstacles and sites too exposed and too cashed to proscribe).
2. Ensure better insulation of the building to make the indoor climate conditions as independent as possible from external climatic conditions.
3. Building in good orientation (North/South) to avoid exposure to prevailing winds.
4. In poultry farming, the practice of the single band (only one age and one strain per farm) so as to respect the golden rule of "all-in-all-empty" farming.

5. Health protection requires the establishment of sanitary barriers and the practice of crawlspace since the quality of the latter must be related not to its duration, but to the effectiveness of disinfection.
6. The proper use of poultry equipment requires the application of certain accompanying measures, namely:

- Drinking and feeding equipment should be evenly distributed over the entire surface of the building.
- The change of the starting material by that of growth will have to be done gradually.
- Be sure to clean the waterers at least once a day at startup and twice a week thereafter.
- It is advisable to use only the fresh food and to distribute only small quantities in order to avoid the accumulation of the litter and droppings in the feeders and to add the food there as often as necessary.

7. The poultry farmer must master the ambient conditions (ambient temperature, ventilation, hygrometry, bedding quality, light) to achieve a better level of physiological comfort of the birds.

Conclusions

To obtain the best performance of broilers namely: a low mortality rate, a better weight growth and an improved consumption index, the efforts must be concentrated on: the design of the buildings, the rules of hygiene and the necessary equipment to create a suitable atmosphere inside the building.
The diagnosis of poultry farming equipment in the region of Zâb-chergui in Biskra wilaya reveals that most farmers in this region have badly-shaped buildings of hard or soft shell. The latter do not have sanitary gates and are equipped with incandescent lamps, a radiant-based heating system, circular waterers and feed hoppers. The majority of these breeders use litter made of wood chips. These farming conditions have resulted in mortality rates that exceed the standards recognized by the majority of broiler breeders in the region. Inside the building, the breeding standards must be required.
To succeed in raising broilers, it is necessary to have a building:

- Well oriented towards North/South (to avoid the prevailing winds).
- Rigid shell type.
- With a type of fluorescent tubes (neons) because they are more efficient than incandescent lamps.
- Based on unit heaters (heat guns) as a type of heating since they have the advantage of being compact and low maintenance.
- Equipped with nipples or "nippel" with or without recovery cups and hoppers. Because they are the most commonly used.
- Equipped with flax shives since they constitute an excellent litter as long as their price is reasonable. Straw (with reduced absorbency), sawdust (too dusty) and softwood or treated wood chips should be avoided.
- Has sanitary barriers (sanitary lock).

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CHARACTERIZATION OF CAMEL BREEDING IN SOUTHEASTERN ALGERIA

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Abstract

The study of camel breeding in southeastern Algeria during 2017 and from 30 breeders surveyed, showed that it was practiced in the majority of cases by small breeders whose herds did not exceed 25 heads (46.67%), sedentary (66.68%), age between 40 and 60 (75%), with a primary education level (37%) and 80% of whom practiced it for reasons of multiplication of the species between them. Reproduction was natural, the period of struggle was from November to April. The age of the breeding of the females was 4 years, of the males more than 5 years. We noticed predominance of reproductive females (66.90%) of the El Hamra breed (85%), raised especially for dress sought to manufacture the most famous Algerian Bernous and Kachabia. The production of milk and camel meat was low in the economy of the region and it was practiced only for self-consumption because of the free conduct of herds and the lack of routes in plant production and sources of water and the stringent climatic conditions of the current year.

Key words: *farm management, camel, herder, flock, range, arid zone.*

Introduction

The breeding system is an old activity that is part of a current economic context, it has a social and economic function by maintaining an activity, jobs and income in difficult regions and contributing to quality productions (meat, wool, skins ..) (Bencherif, 2011). About 200 million pastoral households raise almost 1 billion heads of camels, cattle and small ruminants in countries with large pastoral areas. Extensive pastoral production takes place on 25% of the world's land and provides 10% of the world meat production (FAO, 2001).

In Algeria, pastoralism is characterized by a great diversity of herds (sheep, cattle, goats and camels), huge rangelands stretching over 30 million hectares (land routes, Saharan, steppe, forest) and a large diversity of pastures (Bencherif, 2011).

The Algerian Saharan space is dominated by arid and semi-arid areas of more than 80%, through which animals are raised. Among these animals, the dromedary which had the power to take advantage of the essential food in this area. The environment with scarce resources and opportunities for agricultural development is limited (Adamou, 2008).

In recent years, there has been a fragility of rangelands in the Saharan environment (degradation of vegetation cover) due to the combination of several natural factors such as climate change (high temperature, scarcity of rainfall) (Aidoud, 1989) and anthropogenic notably anarchic and irrational grazing, which has led to a decline in camel breeding (Senoussi, 2011). Research on camel rearing systems and the management of this farming in the arid Algerian region are especially relevant to the Sud-Ouest regions (Ouargla, Ghardaia, Béchar) and aim at the knowledge of their diversity, the place of camel breeding and its role within the production units. On the other hand, dromedary has received little attention in the south-east region despite its specificities and abilities. We can make the current observation that this is a marginalized breeding, most of the time absent from development actions, despite the fact that it is the first victim of the degradation of pastures and which has a productive potential, still unknown.

In order to promote this animal of economic importance, this study aims to identify and to know the different camel farming systems existing in the south-eastern region of Biskra and to diagnose its different compartments.

Materials and Methods

From the 44 existing breeders in the wilaya (DSA, 2016), 30 were surveyed from January to April 2017. Regardless of the mode of rearing practiced, the majority of the breeders surveyed were chosen on the basis of their availability and cooperation, as well as the importance of livestock and herds in each location. The 30 selected breeders are distributed through the study region as follows; Besbess (22.23%), Doucen (19.94%), El Hadjeb (12.36%), Alhouche (13.65%), which represent the highest concentration followed by Ras El Miaad (4.63%), Ouled Djelal (7.43%), Chaiba (4.27) and Sidi Okba (5%). The low concentration is found in El Outaya (2.32%) and Zribet El Oued (1.61%) (Figure 1).

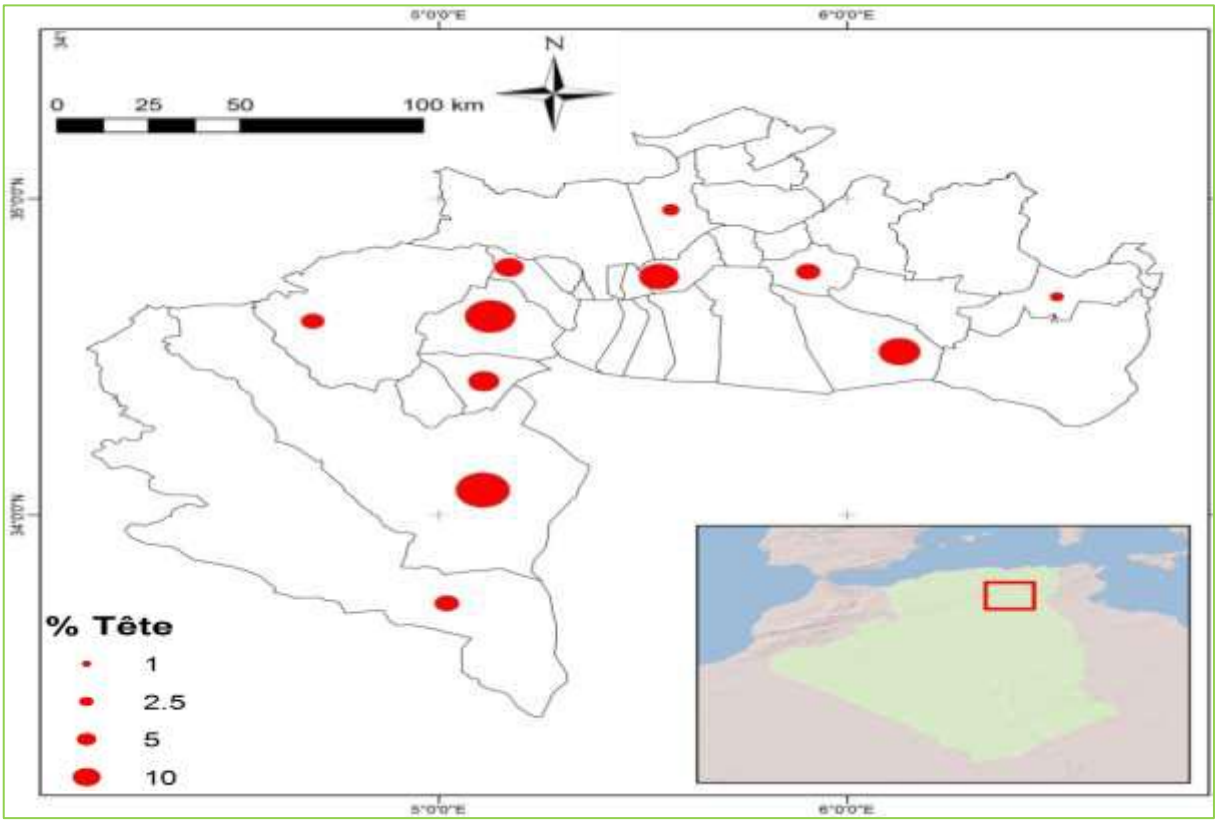

Figure 1: Importance of camel breeding in the region of Biskra

Conduct of the investigation

Our study is based on surveys conducted with herders, the herd is the main unit of investigation, supplemented by direct field observations and informations collected from different sources (breeders, veterinarians and livestock technicians).

Our survey was developed to highlight:

- the socio-economic status of pastoralists (age, sex, educational level, sources of income and way of life),
- the herd structure (size and reproduction),
- the rearing method (livestock management),
- the feeding system,
- livestock productivity.

Statistical analysis

Data processing was facilitated by the use of SPSS and Excel software. The descriptive statistical analysis of the data made it possible to produce synthetic results, to characterize and highlight the main indicators (socio-economic, production, reproduction, etc.) structured by theme already established.

Results and Discussion

To diagnose camel breeding in the South East region of Algeria, it is important to go through the identification of breeders, their typologies as well as the situation of herds and pastures through a survey.

Breeder

This part concerns the specificity of camel breeders, which is the central object of the production system, both as a decision-maker of the motivation of its breeding and as the main actor of this activity.

Habitats and lifestyles

Survey results showed that 66.68% of breeders are sedentary, while 26.66% are semi-nomadic and 6.66% are nomads (Figure 2). Three different types of housing have been identified among camel breeders. region of Biskra: the tent the house in hard and mixed housing tent and house hard.

Figure 2. Identification of livestock systems in the study area

Sedentary breeders predominate, because of the difficulty of moving their herds north in the spring (Achaba), the unavailability of the shepherd who moves with the herds and the high cost of transport. While nomads are represented by a low rate because they own a limited number of head (less than 10 head) able to transport them using their own vehicles.

Sex, age and level of education

Survey analysis showed that 100% of the surveyed breeders are male, indicating that camel breeding in the study area is a strictly male activity. Although women have dromedaries (inheritance), they entrust them to trustworthy men because of the difficulty of its breeding. 96.66% of these breeders are married, and 3.34% single. Married couples with children, on average 5 children for all the interviewed breeders.

Generally the age of breeders is for 75% between 40 and 60 years old. This age corresponds to the active period of an individual's life. Despite the small percentage (25%) of breeders over the age of 60, mainly nomads, they still remain a source of knowledge and know-how for young breeders. The transmission of the craft to children is a practice followed by all breeders, it remains a profession that is learned in family, passed from one generation to another, from father to son.

The level of education of the interviewed breeders is 16% of illiterates, 25% have not exceeded the average level and 37% have a primary level. The rest of the breeders, 22%, were deprived of any schooling except the Koranic school. This low rate of unschooled farmers (16%) represents the elderly and nomadic pastoralists whose permanent displacement does not allow them to follow schooling. As a result of the sedentarisation of pastoralists, young breeders are attracted more and more by the pursuit of the school curriculum, which explains the high rate of schooling 37% and 25%.

Sources of income

For the majority of breeders, camel breeding is considered as a means of saving and valorization in the region, for this reason that breeders practice different categories of profession. The results of our survey revealed a high rate (65%) of breeders with a liberal professional activity. They are either traders, merchants, transporters, or service providers (masons, mechanics, carpenters ...).

The rest of the breeders in our survey are either only camel drivers (7%) who live solely on the incomes of their breeding or practice a production system based essentially on the breeding-farming association (28%).

Herd

Guarding

Note that the camel breeding in the study area is characterized by a herd kept totally or partially. Depending on the composition and size of the flock and its destination, guarding (Figure 3) is done by the breeder and his children or by the shepherd, in other cases by both at the same time. 65% of the breeders surveyed are shepherds associated with the owner of herds who only help them during the breeding season and at the time of the watering. 35% of the breeders surveyed are owners of their herds.

Figure 3: Guarding the flock (Chaiba)

Size of herds

The treatment of survey results revealed 3 distributed classes as follows:

1st Class: groups small herds with a number of up to 25 heads. This class is met with 14 breeders, a rate of 46.67% of the total number of breeders.

2nd class: it groups herds from 25 to 49 heads. It is represented only by 5 breeders, ie a rate of 16.67% of the breeders surveyed.

3rd Class: groups herds of more than 50 heads, at 11 breeders, a rate of 36.67% of the breeders surveyed.

Structure of herds

In general, the composition of the camel herd varies according to a variety of factors, the most important of which is the vocation of breeding for either meat production or milk production. According to the survey, the total number of camels of the breeders surveyed (30 breeders) is of the order of 4,103 heads divided into 22.50% males with 66.90% breeding camels and 10.60% chameleons . The predominance of productive camels is due to the prohibition of female slaughter unless they are unproductive or reformed.

Reproduction

The majority of breeders surveyed (80%) take into account only the selection of males (fertility, height, disease resistance and tolerance to drought). Few selection (20%) is based on females (size of the udders, duration of lactation). All females are considered breeding stock for the maintenance and growth of the flock, but attention is always concentrated on males. According to the survey, all the breeders surveyed (100%) indicate that a single breeding male can ensure the fertility of 50 to 80 camels, in accordance with the results reported by Oueld Laid (2008), who reported that only one male can breed 80 females per season. This is why these breeders often keep one or two other males in their herds to use them when needed, according to Ben Issa (1989), these males who do not have to fight with the stallion, head of the herd.

The camel herd of the region of Biskra composed essentially by the El-Hamra (85%), Soufia (12%) and Targuia (3%) breeds, the latter being characterized by the scarcity of hairs, while the El -Hamra most wanted because of the color of her dress and the hairs are very appreciated and much requested by nomadic families for the making of khaïma, bernous and kachabia.

Livestock products.

Meat production

According to the results of our investigation, the sale of dromedaries is much more the males during the spring, the female is sold only if she is sick or old. The participation of camel meat in the economy of the region is low compared to that of sheep, beef and goat meat. This is explained by the fact that this breeding is practiced in the majority of cases (80%) for reasons of multiplication of the species between breeders.

Conclusion

Camel breeding in Algeria is obviously experiencing a revival of interest. However, this renewal must be maintained by a significant increase in the number of camel heads, but also by the increase in production (especially meat and more recently milk), by greater integration into the official and parallel trading systems, and by a certain intensification productions under the impulse development policies that are often voluntarist and without doubt, thanks to a better taking into account of this breeding by political authorities.

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FACTORS CONDITIONING GROWTH IN SHEEP IN SEMI-ARID REGION OF ALGERIA

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Abstract

Ruminant livestock is one of the key sectors of Algerian agriculture in which the component (small ruminant) predominates. This study was carried out in a semi-arid region of Algeria with a population of 200 lambs Ouled Djellal, with the aim of analyzing growth performance and specifying factors influencing this performance. The analysis of the least squared variance was carried out and the main factors tested were the parity, the size of the litter and the sex of the lamb. The results showed that the lamb weighed 4.19 kg at birth, 6.04 after10 days, 7.29 kg after 20 days, 8.44 kg after 30 days and 18.30 kg after 90 days. The mean growth rate was 179.55 g/d between birth and 10 days, 120 g/d between 10 and 20 days, 107.41 g/d between 20 and 30 days and 149.26 g/d between 30 and 90 days. Birth weight was very strongly related to weights at typical ages (10, 20, 30 and 90 days). Also, the average daily gains were highly correlated with each other. The litter size had significant influence on birth weight at 10 days, 20 days, 30 days, 90 days, and all daily average gains ($P < 0.05$). On the other hand, the sex and parity did not have significant influence on live weight at different ages or on the average daily gain from birth to weaning ($P > 0.05$).

Key words: *Ouled-Djellal lambs, growth performance, birth weight, ADG, sex, parity, and litter size.*

Introduction

The main problem of the sheep sector in Algeria is the low productivity ofthe flocks. The growth and viability of lambs, considered among the main components of sheep weight productivity, must be improved by selection. However, effective selection must be based on the genetic evaluation of animals. This, to be precise, requires knowledge of the factors of variation due to the environment and genetic and phenotypic parameters of the economically important characters.

Despite the exceptional growth of this sector, sheep meat remains inaccessible for a large part of Algerian households with medium and low incomes.

The low productivity of domestic herds is attributed according to Bencherif (2011) to a misbehavior of breeding and feeding of herds which is often of extensive type. The objective of this work is to study and analyze the impact sex of the lamb, and the size of the litter on measurable criteria (birth weight, weight at typical ages, average daily gains at ages types) in the Ouled Djellal lambs from birth to weaning in order to provide the breeder with assistance in managing the herd on the basis of reproduction and growth criteria on the one hand and to allow genetic evaluation ewes based on prolificacy and dairy value.

Materials and Methods

Animals

The study concerned a number of 200 lambs (male and female) of Ouled Djellal breed from multiparous and primiparous ewes that did not undergo heat synchronization. They were born between October 10th and December 9th, 2016. They were raised in semi-intensive, in addition to breast milk, they had barley hay at their disposal and they were previously identified with numbered earrings, they were housed in sheepfold with natural light.

Weighing the lambs was done using a scale with a maximum capacity of 50 ± 0.1 kg, and all information from birth to weaning is recorded on individual growth records for each lamb.

Checks carried out

Lambs are identified and weighed at birth. Then, they are weighed at the age of 10 days, 20 days, 30 days and weaning at 90 days. The average daily gain (ADG) between d0-d10; d10-d20; d20-J30 and d30-d90 were calculated from weights at the corresponding age-types.

The factors of variation considered:

- The sex of the product (male, female)
- The size of the litter (single, double)

Statistical analysis:

Descriptive statistics and one-way analysis of variance (ANOVA) were performed with the SPSS software (2013 version 20) for live weight and average daily gain analysis. The correlation results were calculated by the Pearson test for the evaluation of the relationship between live weight at different growth phases of lambs. All the averages of the results were calculated with their mean standard errors (mean ± S.E.M.). The statistical difference was reported at ($P<0.05$). - The generalized linear model (GLM) was used to test the effects of the factors on the variables, by the application of the T test for independent samples to estimate the meaning or the homogeneity between the different sets of data (comparison test between the averages). - The frequencies of each quantitative variable were graphically represented using Excel (2007).

Results and Discussion

Growth performance from birth to weaning

Depending on the size of the litter:

Weight at birth

The mean live weight of the lambs at birth is 4.19 ± 0.94 kg. Single lambs tend to have higher birth weights compared to doubles (4.70 ± 0.54 kg vs. 3.88 ± 0.30 kg) respectively (Figure 1).

Weight at typical ages (10, 20, 30 and 90)

Single lambs tend to have greater weights than twins throughout the experiment (Figure 2)
Mean weights observed at 10, 20, 30 and 90d correlate very significantly with birth weight ($r = 0.56$ $P = 0.009$; $r = 0.57$ $P = 0.008$; $r = 0.54$ $P = 0.013$ and $r = 0.63$ $P = 0.003$ respectively).
Birth weight and mean weights at 4 typical ages differ significantly with litter size ($P < 0.05$)

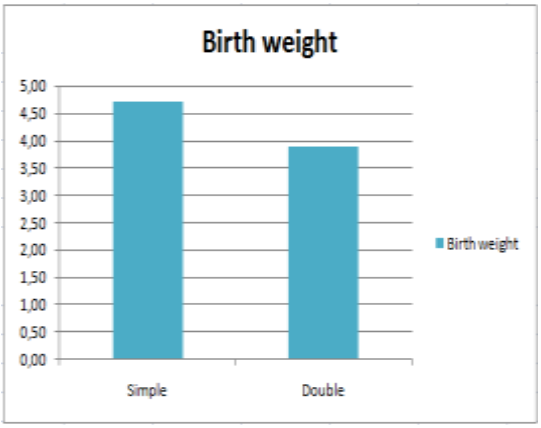

Figure 1: Difference in birth weight between single and double lambs

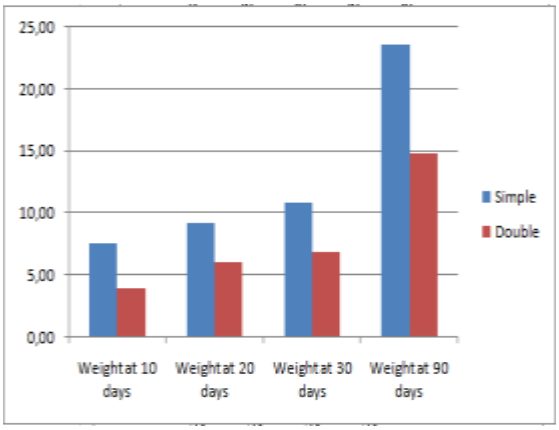

Figure 2: Variations in weights at different typical ages (kg) by litter size in Ouled Djellal lambs.

Average Daily Gain

It appears that singles tend to have higher ADGs compared to doubles (Figure 3). The correlations are significant between the average daily gains at the different typical ages, (the 30 day ADG is correlated with the 20-day ADG with a correlation coefficient r = 0.68 and P = 0.001, it is also correlated with the ADG at 90 days with a correlation coefficient r = 0.64 and P = 0.002, also the 90 day ADG is correlated with the 20 day ADG with a correlation coefficient r = 0.57 and P = 0.008). There is no significant correlation between birth weight and average daily gain at 30 days after birth (P> 0.05 / P = 0.30), whereas it is a milk production index.

Evolution of the weight:"Growth curve"

The live weight in single and double lambs evolves according to a first order polynomial regression curve, the equations are respectively y = 4.108x -1.163 and y = 2.478x - 0.362 of which (x is the time and y is the weight). Single birth lambs always grow larger than doubles. They tend to have higher live weights than doubles from birth to weaning.

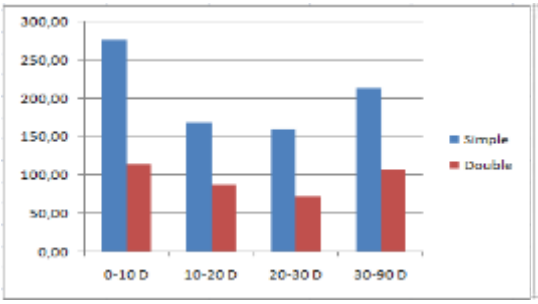

Figure 3: Variations in average daily gains (g) by litter size in Ouled Djellal lambs.

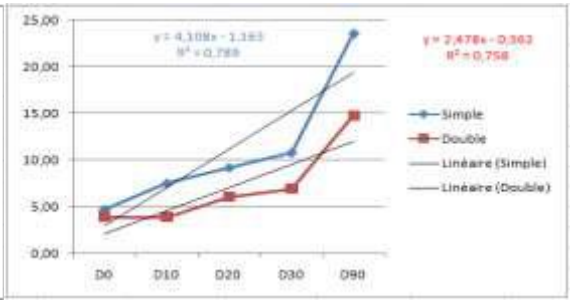

Figure 4: Evolution of weight relative to litter size in Ouled Djellal lambs

According to sex:

Birth weight:

The average live weight of lambs at birth is 4.19 ± 0.94 kg. Male lambs showed a slight difference in birth weight compared to lambs (4.37 ± 0.5 vs 4 ± 0.42 kg respectively) Birth weight did not differ significantly with lamb sex (p> 0.05)

Weight at typical ages (10, 20, 30 and 90days)
Male lambs tend to have higher weights than ewe lambs for the duration of the experiment (Figure 5). The weights at the different typical ages do not differ significantly with the sex of the lamb (P> 0.05 / P = 0.23, P = 0.16, P = 0.29, P = 0.86 respectively at the typical ages 10, 20, 30 and 90 days).

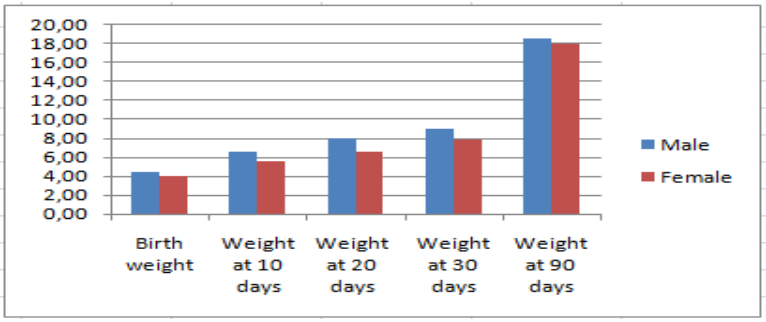

Figure 5: Variations in weight (kg) by sex in Ouled Djellal lambs

The average daily gains:
It appears that males have higher ADGs than ewes from birth up to 20 days, and then the opposite (Figure 6). Also at the typical ages, ADGs do not differ significantly with the lamb sex (p> 0.05 / P = 0.40, P = 0.34, P = 0.62 and P = 0.59 respectively at the ADGs at 10, 20, 30 and 90 days) (Figure 6)

Evolution of the weight «Growth curve»
Live weight in male and female lambs evolves according to a first order polynomial regression curve, the equations are respectively y = 3.092x + 0.027 and y = 3.031x - 0.699 of which (x is the time and y is the weight). Male lambs are still growing
faster than ewe lambs (Figure 7). They tend to have higher live weights than females from birth to weaning. (Figure 7)

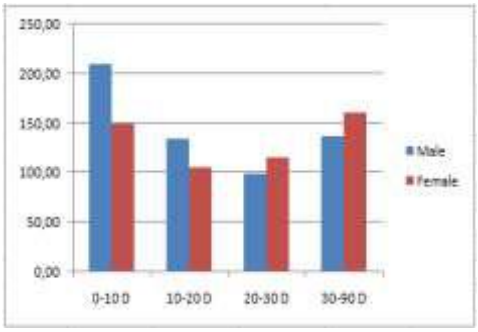

Figure 6: Variations in average daily gains (g) by sex for Ouled Djellal lambs.

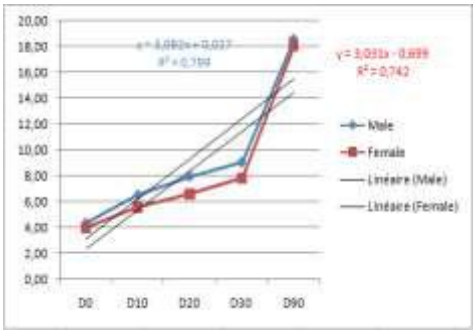

Figure 7: Evolution of weight by sex in lambs Ouled Djellal.

The mean live weight of lambs at birth (4.19 kg) is similar to the mean birth weight reported in lambs born from crosses between Ouled Djellal females and D'men males (3.99 kg) (Lebied et al. 2015) The weight results for the different typical ages found in this study are very similar to those reported by Bendiab and Dekhili (2012) for lambs of the same breed. The superiority in weight gain in favor of simple birth type has also been reported by Kerfal et al, (2005).

Conclusion

The results obtained at the end of this study allowed us to show that:

- Live weight and average daily gains of Ouled Djellal lambs from birth to weaning are closely related to litter size.
- Similarly, a very significant correlation was observed between mean weights at typical ages and birth weight.
- The growth performance of pre-weaned Ouled Djellal lambs is not related to the sex of the lamb.
- These weight and growth performances testify to the satisfactory growth potential of lambs of the Ouled Djellal breed.

At the end of these results, it appears that the "size of litter" factor has a significant influence on growth performance, which must be taken into account

Finally, this work should be supplemented by the identification and study of the effects of other factors which can induce the growth of lambs, future breeders, in other words the future of the Ouled Djellal breed.

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PERFORMANCE CHARACTERISTICS AND THE EFFECT OF TWO-WAY SELECTION OF THE BARBARY PARTRIDGE (*ALECTORIS BARBARA*) IN CAPTIVITY

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Abstract

Since 2000, an effort has been made to obtain a Barbary partridge population for restocking. We collected data on the characteristics of the performances of our captive population during three years of experimentation (2012, 2013 and 2014) from the program initiated for the Algiers region. In addition, we conducted a detailed study on the two-way selection, based on the expression of the slow feathering allele (KK) and the fast one (kk). Our results for the performance traits showed that during the year, the average number of egg / female in 2013 (17.46) was higher than the year 2012 and 2014, because of the disturbances recorded during these years, and the fertility of eggs during the three years of our experiment with an average rate of 82.27%. The effect of bidirectional selection based on the expression of the slow feather allele (KK) and fast allele (kk) was marked by the appearance of the fast feathering genotype determined by the recessive allele (k).The results obtained from three (3) generations F12-13-14 showed that 80% of chicks belong the fast line and 20% belong the slow line.

Keywords: *Barbary partridge, line, population, allele, generation.*

Introduction

The Barbary partridge is smaller than the other genera of *Alectoris* the difference between it and the Red-legged partridge is its red brown collar spotted in white bordering its bluish gray bib (Thonon et *al.*, 1977; Svensson et *al*. 2014). Also, it is more redhead than all *Alectoris* (Couzens, 2006). The total length of our bird is between 32 and 34 cm and its wingspan is from 46 to 53 cm (Geroudet, 1978; Heinzel et *al.,* 1995) and from 46 to 49 cm depending on the male weight compared to the female weight (Cramp and Simmons, 1980). species morphology Transformation is a fundamental part of evolution, for which the physical parameters as hatching are important in egg health and influencing the growth of nestlings until fledging (Benharzallah et *al.* 2015, Mezerdi et *al*. 2015 and Mezerdi et *al*. 2017).Our research consist to continue the selection of Barbary partridge restocking line F11 to F14 in the objective of obtaining samples with important genetic and sanitary quality, of both lines (fast and slow), raised and controlled at the hunting center of Zeralda.

Material and method

Our biological material, native of Beni-Slimane in the province of Medea (36 14'03.38LN ET 319426.20LE), was brought in the year 2000, at the hunting center of Zeralda. During three years of survey, the concerned breeders of our research (N=180 couples/year), represent the generations: F11-F12-F13. The main object purpose of a genetic amelioration program is to classify a restocking line (Boukrabouza et *al.*, 2003 and Belhamra, 2005). The breeders' preservation protocol in winter consists of avoiding the visual contact between males and females; they are separated in homo-sexed groups in parquets (GavardGongallud, 2000). The distance between these parquets is almost 100m. Earlier in the reproduction season, males start to sing and yell as signs to their females to start their coupling. During 91 days, we have collected the eggs, every Wednesday of the week. We measured many zootechnical

parameters such us: the number of the collected eggs, the rate of laying eggs the number of incubated eggs ; the unfertilized eggs ; the rate of the embryonic mortality; the production of chicks and the rate of egg hatching, to identify the quality of the eggs and the reproduction success. Also, we conducted a chicks selection by feathers, because the partridge chicks of the first day of the fast line present a developed and longer wing cover feathers than those of the slow line. (Fig.1).

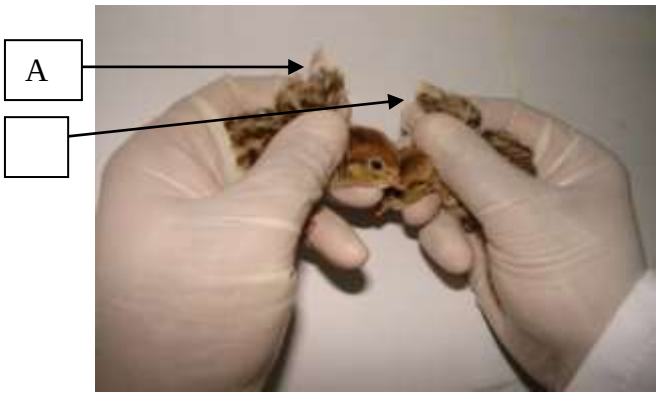

Figure 1: The feather form of the fast line (A) and the slow line (B).

Results and discussion

The characters of the performances of our Barbary partridge population

After three years of work successively 2012; 2013 and 2014, we have gathered some data concerning the performances characters of our population in captivity.

The zootechnical characters in 2012

The breeders of the year 2012, matches the generation F11 of our population. This year's birds are disturbed, because we have moved them into a smaller and a less enlightened place to develop the laying parquets, and we only had the chance to gather six series. Forthe first series of 19.04.2012. we have collected only 154 eggs. So, in six weeks we have incubated 980 eggs (Table1), whose size oscillates between a minimum of 0.38 and a maximum of 1.28 egg/week (Fig.2) We notice that there are two periods of laying averagefrom the first series to the third with a peak of 1.38 than a second one with a decrees of the laying, until the last series which corresponds to the sixth one with 0.38 egg/week.

Table 1: The laying performances' characteristics of Barbary partridge captive populations (N= 180 females) during 2012; 2013 and 2014.

	nlw	**nie**	**law**	**t.ue**	**t.fe**	**ave. rf (%)**	**t. em**	**t.cp**	**ave. hr (%)**	**ave. ehr (%)**	**t.v**	**ave. vr (%)**	**ave. pfs (%)**	**ve. pff (%)**
2012	6	980	5,44	156	824	78,3	147	677	63,27	78,87	617	86,93	22,28	77,72
2013	12	3142	17,46	310	2832	89,03	302	2530	78,82	88,46	2332	90,87	19,84	80,16
2014	9	1936	10,76	363	1573	79,49	99	1474	70,51	87,98	1397	92,47	17,18	82,82

law : laying average per week ; **ave :** average ; **t. :** total ; **nlw :** number of laying weeks ; **nie :** number of incubated eggs ; **ue:** unfertilized egg ; **fe :** fertilized egg **; rf (%) :** rate of fertility ; **em :** embryonic mortality ; **cp:** chicks' production ; **hr (%) :** hatchability rate ; **ehr (%) :** egg hatching rate; **v:** viability; **vr(%) :** viability rate ; **fpl:** phenotypic frequency of the slow line ; **fpr:** phenotypic frequency of the fast line

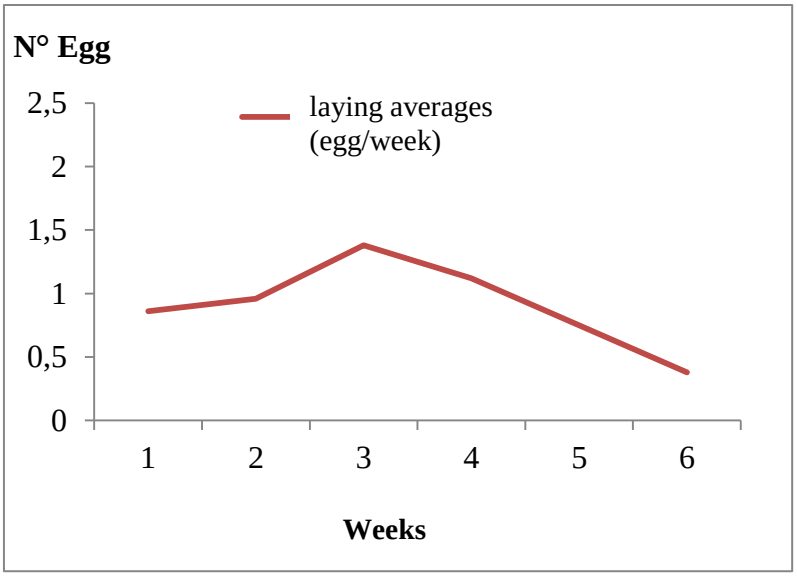

Figure 2: The variability of laying averages of the Barbary partridge during 2012.

During our experimentation in 2012, we noticed that the fertility rate and the egg hatchability rate starting from the 4th week reached a maximum value of 91.58% and 95.05%, and the minimum value in the last series with 26.47% and 36.76% successively. The same series of maximum values, we also registered a maximum rate of egg-hatching of 96.35%, but we registered the minimum value during the first series with 57.01%. We also notice that the rate of embryonic mortality is more important during the first week of laying eggs with a medium rate during 6 weeks of 15%.

For the phenotypic characteristics, we noted that the distribution of the phenotypic frequencies in 6 weeks (Fig3) shows that most of our population birds are at 80% of active phenotype. We have noted that during the first week, the frequency of both lines fast and slow express in the same way. But starting from the second week the phenotypic frequency of the fast line is more important until the last week with a rate of 93.75% and 6.25%for the slow line because this line's chicks couldn't resist in the last series.

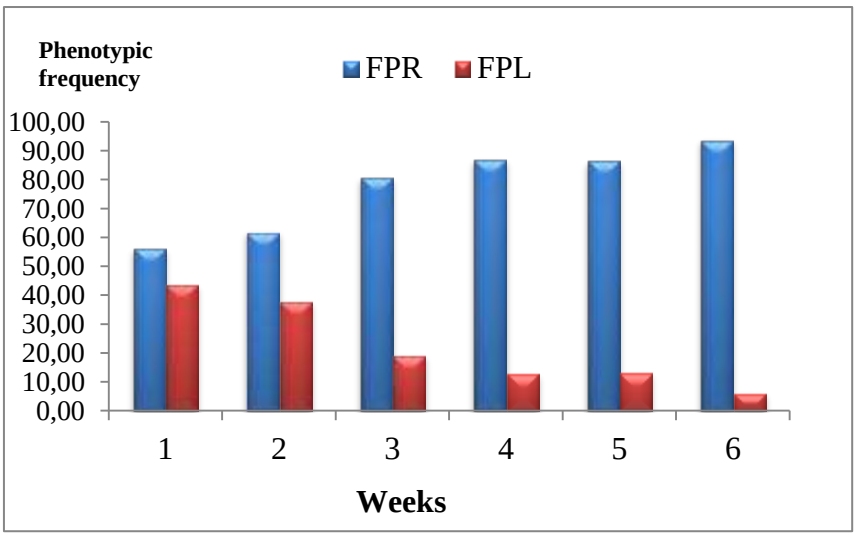

Figure 3: The variation of both lines' rates (slow and fast) in 2012.

Zootechnical characteristics in 2013

2013's breeders correspond to the F12 generation of our population. Our population is characterized by avariability in the laying size between a minimum of 0.33 and a maximum of 2.19 egg/week. During 12 weeks of follow-up , we have collected 3142 eggs (Table 1). In the middle of laying weeks which correspond to the 4th and the 8th week, we have registered a certain stability of laying average (>2 egg/week). This period synchronizes to the participation of all population females in the egg laying (Fig4). This explains that our population is well adapted to the captivity conditions. However, last year we have noticed a maximum of laying (>1.38 eggs/week). After the 8th week, a decrease of laying which reached 0.33 egg/week had been registered in the 12th week.

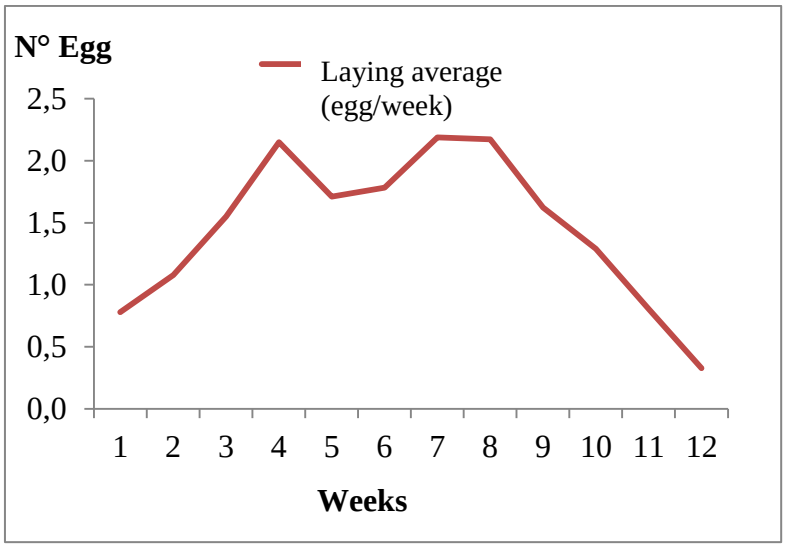

Figure 4: The average variability of laying of the Barbary partridge in 2013.

During 2013, we noted that the eggs' fertility rate is of 95.02%, in the 6th week, and a minimum value during the 12th week with 83.05%. Furthermore, the hatchability rate and the egg-hatching rate reached their maximum values during the 8th week with 86.96% and 95.51% and minimum values during the 12th week with 66.1% and 79.59% successively. The average embryonic mortality rate is 9.61%.

In 2013, the distribution of phenotypic frequencies over the 12 weeks (Fig5) shows that most of our population birds at 80% of active phenotypes. We notice that during the 1st week, the frequency of both lines fast and slow is expressed in a similar way with an advantage of the slow line. Starting from the 2nd week, the phenotypic frequencies of both lines is separated until the last week with a rate of 93.75% for the fast line and 6.25% for the slow one, in parallel with the year of 2012.

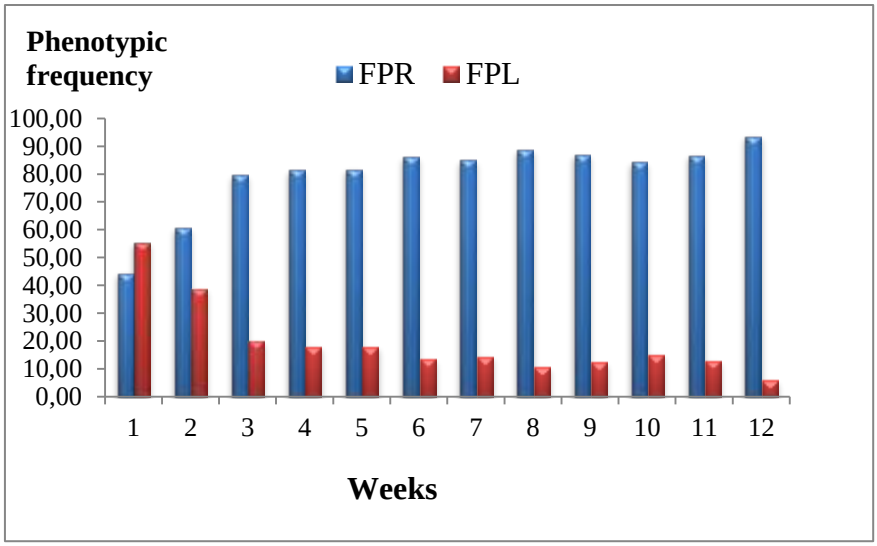

Figure 5: Variation of both lines'rates in 2013.

Zootechnical characteristics in 2014

The 2014 breeders correspond to the F13 generation of our population.

The laying size variability between a minimum of 0.21 and a maximum of 2.46 eggs/week. A planning during the reproduction period, affects the egg production, in the first weeks which is less than 0.35 egg/week. During 9 weeks of follow-up, we have collected 1936 eggs (Table1). Since the 3rd week, our population's females start to enter in the laying phase reaching in the 6th week 2.46 egg/week (Fig.6). In the last three weeks, we have noted a brutal decrease of egg laying reaching only 0.21 egg/week.

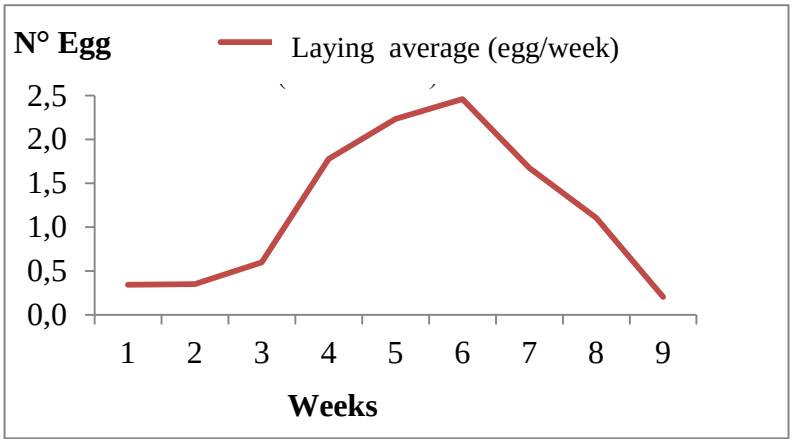

Figure 6: Variability of laying averages egg the Barbary partridge in 2014.

In 2014, we have registered that the fertility rate is about 92.46% during the 8thweek, and a minimum value during the 2nd week of 68.25%. Over the 1st week, the hatchability rate is too low with 45.16%, and then it starts to increase to reach a maximum value of 87.75% in the 7th week. Furthermore, the hatching rate in the 1st week is too low with 65.12% and it reaches a maximum value in the 6th week with 97.46%. the average rate of embryonic mortality 5.11%.

The distribution of phenotypic frequency in 2014, over 9 weeks (Fig.7), also shows that most of our population birds at 80% of active phenotypes. We note that during the three first weeks, there was a decrease in the slow line until the 3rd week and carry out 34.13%. Then, stability on both phenotypic frequencies.

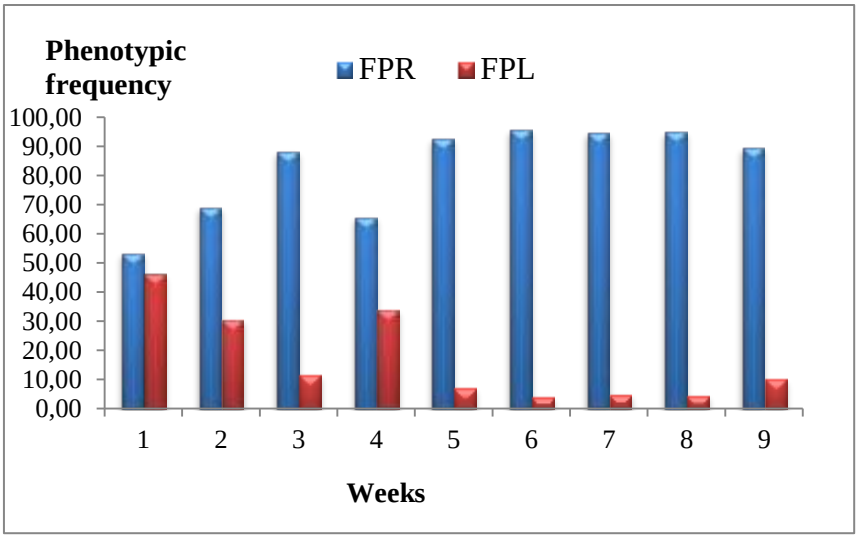

Figure 7: Variation of both lines' rates (fast and slow) in 2014.

Phenotypic effects on the frequency evolution of the allele k over three years of experimentation

The production of chicks in 2012 corresponds to the F12 generation of our population, which contains 617 birds, whose 77.72% of individuals of the fast line phenotype and 22.28% of the slow line, with an average of 6 weeks of laying. The production of chicks in 2013, corresponds to the F13 generation of our population, which contains 2332 birds, whose 80.16% of individuals of the fast line and 19.84 % of the slow line, on an average of 12 laying weeks. The chicks' production in 2014 correspond to the F14 of our population which contains 1397 birds, whose 82.82% of individuals are of the fast line phenotype and 17.18% of individuals are of the slow line phenotype, on an average of 6 laying weeks.

We propose a hypothesisthat if the males are homozygous (kk) and the females (k-) the descendants are at 100% fast phenotypes, and if the males are homozygous (KK) and the females are (K-) or (k-) the descendants are at 100% slow phenotype. But the case of our population and during three years of follow-up, the descendants F12-13-14 are at 80% individuals of the fast phenotype and 20% of the slow phenotype, so the parents are for heterozygous male sex (Kk) and female sex (k-).

The biological indicators and the performances' characteristics of the reproductive population of the generations F12-13-14

During3 years, we sampled 180 couples as a reproductive population. The conservation method of breeders in winter time, by the separation of males from females, has a positive effect on the egg fertility, over the three years of experimentation, with an average rate of 82.27%. The rate of fertility in 2011 was 89.09% (Mezerdi, 2011). This fertility rate is almost similar to the average fertility rate of 8 years for the Red-legged partridge with 84.1%, but more important than that of the gray partridge with 74.8% (Bay1975). The fertility rate is affected by the environment conditions and management, which is confirmed by Mourao et *al*. (2010).

In 2012, the F11generation; our population laid only 980 eggs in 6 weeks interval only. This laying fluctuation is caused by the disturbance during the reproduction period. The number of eggs per hen during the three years of our partridge remained inferior to that of their neighbours in the red-legged partridge, in 2012 (5.44 egg/hen/year), in 2013 (17.46 egg/hen/year) and in 2014 (10.76 egg/hen/year). But according to Cosse and *al.*, 2002, the red-legged partridge (49 eggs/hen/year) and achieved (53 eggs/hen/year) with females of 2

years old (Mourao et *al.,* 2010). These results exceed 2000 data (Khataoui and Oulmane, 2002). Over the period 2004 to 2010, Boukrabouza (2011) noted a strong laying fluctuation of 1400 to 3700 eggs. These fluctuations are caused by the breeding conditions and to the emergence of disease-related problems (Alitaleb and Bousaid, 2011).

The performances' results of our population of the Barbary partridge, over these three years from 2012 to 2014 are inferior to the one of Mezerdi (2011). According to these results, it presents to us the necessity to develop and ameliorate the adaptation techniques and to select a line of acclimatization on the basis of an encouraging character for the durability and the stability of the social links. This line presents a social motivation with the breeding conditions in groups (Belhamraet*al.*, 2007, Schweitzer, 2009 and Mezerdi, 2011), and a strong sensitivity to selection from one generation to another on the common pheasant (Boukhamza, 2007,BelhouaneandAbbou, 2010,Farhi,2011 and Larinouna,2011). The selection of the birds' lines allows to search for different characters and the quality of the birds' production in captivity, within different breeding problems. For our population of Barbary partridge F11-12-13, the egg-hatching rates are in the order of (78.87-88.46 and 87.98), respectively during three years of follow-up, we notice that there is an increase compared to 2012 and 2013. On the other hand we notice a decrease of the egg-hatching rate of birds from a year to another (Fasenkoet*al.*, 1992,Eliblet*al.*, 2002, Yilmaz and Tepeli, 2009). Also, the average egg-hatching rate of three year is of 85.1%. It is more important compared to the Red-legged partridge which is of 79.8% (Cosseet*al.*,2002). However, our results concerning the embryonic mortality rates over the 3 years are in the order of (15%-9.61% and 5.11%) respectively with and average of 9.04%. We noticed from these results, that the rate of the embryonic mortality decreases from year to another, in contrary to what had been noted by Yilmaz and Tepeli (2009) in Chukar partridge. Over the three years of follow-up, we have noted that the perturbation which presents during the reproduction period, affects the egg laying date. In addition to that, we have noticed an effect on the performance parameters of our population. Also the study of Gonzalez-Redondo (2006) on the Red-legged partridge, shows that the laying date has an effect on the fertility rate and the egg hatching. Many studies showed that the bad control of the physical parameters, during the phase of conservation and incubation, have a negative effect over the embryonic mortality (Le Bars and Simon (2005) *in* Belhouane and Abbou, 2010). The causes due to these technical factors can achieve an embryonic mortality going up to 16.63% (Mezerdi, 2011).

The selections in the descendants F12-13-14

During three years of follow-up, the majority of the descendants of the phenotypic frequencies of our population are of 80% for the fast line and 20% for the slow line. We propose the assumption that the genotype of the breeders is (k-) for the females and homozygous genotype (kk) majority or heterozygote (Kk) for the fathers. The animals of the active line are characterized by a low reactivity to a new stimulation (Faure, 1975) and are not very emotional and not very aggressive (Faure, 1982). Conclusion

Conclusion

Over the 3 years of this study, we have observed that in the year of 2013, we picked up 17.46 (egg/female). This production is superior to the two other years 2012 and 2014, but is remains far enough of the average of the egg laying acquired in the other species of partridge. We have also showed that the disturbance of adults, during the reproduction period, effects the date of the egg laying, the number of eggs and their fertility. With a regular follow-up of the physical parameters (Tp° and H %) while the phase of incubation, correspond perfectly, to the needs of the metabolism of the embryonic development without stress. These parameters affect negatively the egg hatching rate, which give a bad production of chicks. During 3 years and over 27 weeks of hatching, the average exceeds 80%. The phenotypic variability shows

dominance of the order of 80% of chicks from the generations F12-13-14 carry recessive alleles k- and kk, starting from females with fast feathering k- and recessive homozygote males kk. On the other hand, the slow bird morphology would be the descendants of the fast females' k- and the presumed heterozygote males Kk.

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CHARACTERISTICS OF BULLS' ACCOMMODATION FOR THE BOSNIAN-HERZEGOVINIAN BULLFIGHT

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Abstract

Contemporary livestock production is conceptually fairly "merciless" towards male animals.They are mainly used for the meat production, breeding or as a working animals. It happens really often that the conditions of their accommodation and exploitation are very difficult. The criteria for male specimens are defined by their production purpose and vary depending on whether they are fattening or reproductive animals. Reproductive males, comparing them with ather males are in the best position regarding living conditions. However, on the territory of Bosnia and Herzegovina there is however one category of animals that, thanks to the tradition over 300 years old, enjoys all the rights and privileges which, unfortunately, almost do not enjoy any other species and categories of animals in the world, except those in the wild. They are fighting bulls, in Bosnia known as „bak", who many either intentionally or out of ignorance, compare with bulls for Spanish Corrida. We must note that, apart from the species and sex of animals, they have nothing in common. The fighting bulls are the category of cattle whose goal is expressing their dominant patterns of behavior, such as mutual fights, which are otherwise happening in a natural environment. To make the fighting bulls a real alpha males, they must have, in addition to genetic and other predispositions, first of all good housing conditions, which is a topic of our work. In order to try to get closer to what conditions they enjoy, we tried to compared them to, according to livestock standards, the best positioned breeding bulls, used in breeding centers, about which almost no literature data exist. For this reason, comparative data are very poor, but from the aspect of the ethology and animal welfare it is very easy to conclude which accommodation parameters are better, because the animal welfare is compared with what it would have in a natural environment, which primarily implies the space they live in.

Keywords:*bull, Bosnia-Herzegovina bullfight, ethology, nutrition, accommodation.*

Introduction

The Bosnian and Herzegovinian bulls,called "fighting bulls" are extremely interesting national sports competitors, in the nation known as „bode" or bulls fight. Quite and totally wrong, these manifestations in the public are called corridas with which, besides the species and sex of the animals involved in them, they have nothing more in common. With the tradition of maintaining over 300 years of age, the Bosnian-Herzegovinian bull's fight is a traditional competition specially raised bulls from which are expected to be male individuals in the full, natural sense of that status.

Male animals in livestock production

Regardless of the characteristics, modern livestock production is conceivably rather "merciless" toward male animals. They are used mainly for the production of meat, for breeding or for working animals, often in extremely difficult conditions of exploitation. The male genotype, in relation to the female sex, determines physiological characteristics such as

rapid growth, realistic higher body weight and better food conversion, which is decisive for the desirability of introducing them to meat production (7; 11; 12; 25). This statement refers to all types of domestic animals, whether intensive or semi-intensive production. Male animals of some species, such as pigs, cattle or small ruminants, are castrated very early in order to further accelerate the growth of body weight and eliminate the undesirable smell of meat that is due to the presence of male sex hormones (7; 16; 18).

Zootechnical conditions for holding male cattle

Cattle (Bovinae) belong to the genus Mammalia, the line of two-aisles (Artiodactila), the subspecies of ruminant (Ruminantia), the family of cavalrymen (Cavicornia) (6; 13; 17; 19; 20). These are animals who are lonely, but they feel better in the group - the herd. Therefore, almost all of the wild cattle live in the herds. Within their own herd, they are rarely fighting each other except for food or water, when they move along a narrow path or when they leave the stable. Until they become accustomed to each other, they exhibit mutual intolerance (14; 15). Although cattle are relatively able to tolerate stable tipe of housing, recent zootechnical stances in exploiting this species of animals advice the principle of returning to the natural way of keeping under the clear sky. According to them (14; 15), the barn should only be "necessary evil" at a time of extremely unfavorable climatic conditions. This is explained by the facts, but also by the assumptions that the stable tipe of housing disturbs certain functions of the organism of animals, and changes the established social relations and established patterns of behavior.

Basic principles of hygiene accommodation of bulls

Bulls can be placed in indoor, classic stables with or without discharge, and in semi-open and open stables with mandatory drains (2; 4; 8; 9; 13; 23). According to the internal arrangement, stables are different, and in what kind of accommodation solutions will be applied, the number of animals and the size of the farm play an important role. Stables usually have a form of rectangles with frontal sides, which in our geographical circumstances are directed towards the south, southeast. The windows are located on the longitudinal sides of the stables, and the light coefficients, in the ratio of the surface of the windows and floors are 1:10 to 1:12 for the younger and 1:15 for older animals. The height of the stables is different. In small stables it is 2.5 m, medium size 2.8-3.2 m, and in large 3.5-4.0 m. Accommodation criteria for male animals are defined by their purpose and vary depending on whether they are fattening or reproductive animals. Male animals are located in closed stables on bearings on the connection or freely in group or individual boxes. Stables can have discharges that are mandatory for breeding animals. Fattening cattle bearing have a length of about 1.70 m and a width of about 1.10 m. The chain system is similar to the cows. The bearing are slammed, and the extermination is done through the extermination channel located behind the legs of the animals. Free hold of male heifer and bulls in fattening is organized in group boxes, and depending on local climatic conditions it is realized in semi-open stables. The floor area under these circumstances depends on the body weight of the individual and ranges from 3.5 to 4.5 m2 per bee (2; 7; 23).

Breeding bulls are usually located in artificial insemination centers. They are kept linked to a chain or more often freely. Linked to a chain means a long bearing and a secured outlet, while free hold is realized in individual boxes that also end up with outflow. When they are on chain, bulls bind around the door with a chain fastened to the front edge of the jasper. If necessary, the chain can be shortened. In this way, they are able to smoothly move back and forth and up and down. Individual boxing is 4.5 to 5.0 m long and 4.0 m wide. It consists of a moving part and a bearing. Regardless of whether it is free hold or bulls on the chain, the bearing is 3.5 m long, 1.5 m wide and lined with straw mats . The discharge dimensions are

usually 5.0 x 4.0 m. The bearings or boxes are separated by a solid fence 1,5 m high. The minimum surface area of the stables with associated infrastructure per adult breeding bull should be 10 m² (2; 23). Under the stables as well as bearing must be dry and clean. Bulls must not be located immediately near the door, nor in the dark parts of the stables from which they do not have a good view of the environment. In such circumstances they can become very irritable and aggressive (3; 4; 5; 13). The concern about the hygiene of the bulls should be guided by people who understand the work and what is very important people who loves this job.

Material and methods of work

Material

In the performance of the experiment, four bulls of different ages and categories were included.

Name: **Svilonja**
Location: Sanica;Unsko-Sanski Kanton
Breed: in the Bushas type
Age: 8 years
Body weight: 670 kg.
Category: medium
Participation in fights: yes
Victories: yes
Reproducibly active: yes
Accommodation: a long bearing on a link in a traditional barn; free grazing on pasture, walking and staying outside the barn whenever climate conditions allow it, including at least 2 days a week in the winter when there is a lot of snow

Ime: **Žujo**
Location:Bijeljina; regija Bijeljina
Breed: in the Bushas type
Age: 5th year
Body weight: 670 kg.
Category: medium
Participation in fights: yes
Victories: yes
Reproducibly active: yes
Accommodation:
- a long bearing on a link in a traditional barn; free grazing on pasture, walking and stays away from the stables throughout the year whenever climate conditions allow

Ime: **Baki**
Location: Kakanj; Ze-Do Kanton
Breed: in the Bushas type
Age: 8 years
Body weight: 800 kg.
Category: heavy

Participation in fights: yes
Victories: yes
Reproducibly active: yes
Accommodation:
- a long bearing on a link in a traditional barn; free grazing on pasture, walking and staying outside the barn whenever climate conditions allow it

Ime: **Čupo**
Location:Preočica-Vitez; Srednja Bosna
Breed: in the Bushas type
Age: 5 years
Body weight: 950 kg.
Category: heavy
Participation in fights: yes
Victories: yes
Reproducibly active: yes
Accommodation:
- a long bearing on a link in a traditional barn; free grazing on pasture, walking and staying outside the barn whenever climate conditions allow it.

Daily activities for all four bulls outside the standstill period:
- main training: walking with wooden load on the shoulders for up to 1 hour, and walking along and down the hill to 2 hours
- fitness training: walking along and down the hill to 2 hours
- Five to seven days before thefight without training
- reproductive engagement: after the season point in the period from November to January

To determine the ambient parameters we used:

a) VOLTCRAFT 4U1 ENVIRONMENTAL METER, technical data:
   - features of luxmeter: 0.01-20.000 lux, resolution:0.1/1 lux
   - features of sound level meter: 35-130 dB, resolution; 0.1dB;
   - features of thermometer: internal -20°C-+750°C
   - features of hygrometer: 25-95% RH
b) IR 800-20D infrared termometer Voltcraft, technical data:
   - response time:150 ms
   - resolution temperature: 0.1°C

Methods of work

Determination of zootechnical aspects:
Calfs after separating from the mothers at the age of about 3 months are held in joint boxes and outdoor. After separation of calves for fattening, future reproductive individuals from selected parents continue to live together in group boxes and on the outflows. During this period, the owners closely follow the behavior of the remaining male calves, that is, the bulls, in particular, the behavior of the dominant individual. After the separation of heifers from the age of 15 to 18 months, the mails under the control of the owner continue to stay together in mixed herds. After three years, one, two or at most three young bulls will be selected for future fighting bull who will be under special treatment. They are housed in individual bearing, ensuring the maximum stay on the open pasture, they start with a special diet

program, they are introduced into the training process and if they continue to show behavior characteristic of the dominant male animals, the owners translate them into the fighting buuls. With this passage, without any coercion in terms of stimulating their masculinity or aggressiveness by the owner, the bulls ensure their privileged position of the future players of the Bosnian and Herzegovinian bull fights. Thanks to this, they will spend a pleasant life with the expectation of the owners to be only what is essentially their natural task - to be the dominant male individuals (1; 21; 22; 24).

Determination of zoo-hygienic indicators:
In addition to the selection method, one of the important criteria for determining the zootechnical status of bulls for a bull fights is to determine the zoohigenic parameters of their accommodation in the stables. These parameters were determined by measuring the value with the help of appropriate measuring instruments. We determined the area and the volume of the accommodation space, the area of the bearing, the values of the most important ambient parameters - temperature, relative humidity and air velocity, noise in objects, light coefficient, and surface temperature of bulls at the level of the head and hull, to determine the degree of benefit in the stables.

Results and discussion

All observed bulls were kept in classical closed stables with secured outflows. The stables as well as their individual parts were built in accordance with the defined zoohigenic norms. The walls are made of solid building material, (to a coefficient of 0.9 to 1.1), the floors and bearings are made of concrete with a concave glaze made of lightweight concrete (the inclination of the floors and deposits to the channel for extermination was between 2% and 3%). The internal design and layout of technological parts and equipment were in accordance with the norms for free hold and holding bulls on the chain. The total useful area of the stables floor ranged from 19.25 to 51.84 m² per bull, and based on the determined ceiling height values, a useful volume of 48.125 to 184.03 m³ per bull was defined (Table 1).

Table 1. - Useful area and volume of stables where the bulls were located

Bull	category	floor area (m²)	volume of stables (m³)	number of bulls	the surface is given per bull (m²)
Svilonja	medium	19,25	48,13	3	6,42
Žujo	medium	51,84	184,03	6	8,64
Baki	heavy	40,50	141,75	1	40,50
Čupo	heavy	27,74	71,58	3	9,25

The surface of individual bearing for the observed bulls ranged from 4.07 to 8.00 m², and the volume of the associated area ranged from 10.17 to 20.00 m³. For stratification, pure straw was used in quantities of 3 to 4 kg (Table 2)

Table 2.- Surface area, volume of belonging area for bulls and daily straw for stratification

Bull	category	area of bearing (m²)	volume of space (m³)	quantity of straw (kg)
Svilonja	medium	4,07	10,17	3,5
Žujo	medium	4,86	17,25	3,0
Baki	heavy	8,00	20,00	4,0
Čupo	heavy	7,52	19,40	3,5

The determined interrelation of the surface of the light openings and the useful floor area, ie the light coefficient in the stables of the observed bulls ranged from 1: 9 to 1: 13.5 (Table 3 ..).

Table 3.- Luminous coefficients in stables for accommodating bulls

Bull	category	floor area (m²)	window surface (m²)	light coefficient
Svilonja	medium	19,25	2,10	1:9,16
Žujo	medium	51,84	5,76	1:9,00
Baki	heavy	40,50	3,00	1:13,5
Čupo	heavy	27,74	2,40	1:11,5

The average measured values of the temperature and relative humidity of the air in the stables, the surface temperature of the skin at the head and hull level, and the noise intensity and airflow velocity are shown in Table 4. The air temperature oscillated between 17.9 ° C (Čupo) and 26.8 ° C Baki) and relative humidity between 40% (Baki) and 73.5% (Chupo). The measured values of the noise level and the velocity of air flow in the stables were very uniform and the surface temperature of the head and hull skin oscillated from 21.9 ° C (Svilonja) to 26.5 ° C (Žujo) and from 28oC (Svilonja and Čupo) to 30 oC (Zujo and Baki).

Table 4.- Values of ambient parameters and surface temperature of the skin of bulls

Bull	air temperature (°C)	relative humidity (%)	noise (dB)	air flow rate (m/s)	surface of the skin of the head (C°)	surface of the hull skin (C°)
Svilonja	19,9	61,95	47,9	<2,3	21,9	28
Žujo	22,1	70,00	47,8	<2,3	26,5	30
Baki	26,8	40,00	47,8	<2,3	25,0	30
Čupo	17,9	73,50	47,8	<2,3	22,3	28

Results and discussion

In our earlier research (10) we concluded that it is primarily because of the human interest, the welfare of commercial animals in relatively acceptable limits, and that they have a higher index of well-being than most animals belonging to the category of pets. The stated position of these categories of animals in fact shows that the people from their holding primarily expects appropriate economic benefits, which is why it also ensures the relatively acceptable quality of the particular accommodation factors. Therefore, it is no surprise that reproductive individuals, and therefore reproductive bulls, enjoy zoo-hygienic and nutritional benefits that are inconceivable or difficult to achieve in commercial individuals. Through the relatively well-defined zootechnical standards of holding breeding bulls (2; 6; 8; 9; 23), we will try to compare the results achieved in our research. Future Bosnia and Herzegovina fighting bulls grow up in mixed herds. They are not forced to show their early sexuality only to brutal fights with same-sex animalsa, as is the case with bulls for Spanish coridas (26). On the contrary, their dominant social position can be fought and expressed in a completely natural way by numerous patterns of behavior, of which aggression and combat actions are only one part. In such circumstances, the behavior and position of the alpha male is clearer that it is a good basis for the owner to recognize the potential alfa mail and to pay special attention to him in the later period of life (1; 21; 22; 24). When it comes to the position of fighter, the bull gets a status that is unfortunately, however much it was unreasonable to compare, there are not many people in the world. Our results imply the conclusion that their good status and

preferences are almost impossible to be objectively compared with breeding bulls from commercial livestock production. In this sense, a slightly easier job is to consider only the hygienic indicators of accommodation. As we have already pointed out, it is not disputable that because of the human interest, reproductive animals in commercial production have a special, preferential status. They provide the best accommodation and ambient conditions, the best nutrition and the like. Zootechnical and zoohigenic standards are mainly referenced according to the position and needs of this category of animals. Through the results of our research, it is clearly visible that all four observed bulls- Svilonja, Žujo, Baki and Čupo are kept in accordance with the highest zoohigenic norms of combined holding in classical closed stables, with secured outdoors for free movement in the outer environment. With a much larger total useful area of barn floors per bull, but also with the total volume of space per bull, the observed bulls have provided much higher accommodation comfort than the norms for breeding bulls in the selection centers. Other micro-ambient parameters such as temperature and relative air humidity, room illumination, noise intensity and air flow velocity also reflect a well-kept hygienic environment that is by no means lagging behind the highest standards of accommodation of highly valuable breeding animals. This statement is most clearly confirmed by the measured values of the temperature of the surface of the skin on the head and the hull of the bulls, which indicate a harmonized thermal processing process as an indicator of the good benefits provided by the accommodation space. However, the data on practically completely free movement of observed bulls outside the stables on surfaces reaching more than one hectare significantly outweigh the recommended outflow area for breeding bulls that move within a few tens of square meters per one bull (2; 23).

Conclusion

Due to the issues of nutrition, reproduction and protection from predators, the larger accommodation space in relation to breeding bulls, guarantees a completely natural way of life in the most optimal conditions of its manifestation. Usually to natural death. And from this aspect, Bosnian and Herzegovinian fighting bulls belong to animals with the most privileged life.

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EFFECT OF NUTRITION ON HEMATOLOGICAL PARAMETERS OF LAMBS IN FATTENING

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Abstract

The objective of studie was to examine effect of nutrition on hematological parameters of cross-bred lambs fattening. The study was conducted on 20 cross-bred lambs from different pramenka types, divided in two groups. The lambs of one group were fed with mixture of cereals and sunflower meal without mineral-vitamin premix with 12.26% protein, but for the feeding of second group of lambs were used feed mixture for older lambs with 14.69% protein. During the fattening period the lambs from both group were fed hay *ad libitum*. Measurments of hematological parameters were measured on the 21st and 35th day of fattening. The results were statisticly analyzed, statistically significant differences and correlation coeficient were determined. The gain of hematological parameters was determined within the both groups according to age of lambs during fattening. The results indicated that the group lambs fed mixture of cereals and sunflower meal achieved better but not statisticaly significant ($p>0.05$) hematological parameters, comparing to lambs fed with feed mixture.

Key words: *fattening, hematological parameters, lamb, feed*

Introduction

Animal nutrition efficiency has a significant effect on the profitability of production systems and provides the ability to identify and select animals with enhanced conversion ability of food into the animal product (Basarab *et al.,* 2003). The economic importance of sheep farming is based on the biological characteristics of sheep, which enable relatively poor vegetation of pastures to become highly valuable products. A significant place in lamb feeding in the fattening, in addition to adequate energy supply, belongs to meal proteins or sources of protein in the meal. Protein nutrients, besides providing animals high value protein, also have a positive effect on production indicators (Memiši *et al.*, 2002).

In addition to nutrition, hematologic indications play a significant role in assessing the physiological, nutritional and pathological status of the animal, and their determination in the blood of the sheep completes a picture of the matabolic profile (Antunovic *et al.,* 2009b), which is a good indicator of health status and nutritional status. Amakiri *et al.,* (2009) have pointed out that it is very difficult to estimate the current health status of animals without a detailed blood test. Hematological indicators of sheep are affected by the breed, physiological state, season, altitude, breeding systems (Soch *et al.* 2010; Soch *et al.,* 2011). Changes in the hematological profile were established earlier in other species, both at birth and in the suckling period. Bornez *et al.* (2009) found a significantly higher number of erythrocytes, higher hemoglobin and hematocrit content, and a higher number of leukocytes, in the blood of lambs at 70 days old fed with concentrate and wheat straw, comparing to sucking lambs at the age of 30 days. Therefore, anything that affects blood, medicines, pathogenic microorganisms or nutrition will certainly affect the entire body negatively or moderately in terms of health, growth, maintenance and reproduction (Oke *et al.,* 2007). Lack of nutrients can affect blood hematologic parameters as well as evaluate the clinical and nutritional health status of the animals because the intake of nutrient components has measurable effects on blood

composition and can be considered as an appropriate benchmark for long-term nutritional status (Olabanji *et al.,* 2007; Daramola *et al.,* 2005).
The aim of the study was to investigate the effect of different percentage of protein in feed mixture on hematological parameters of crossbreed lambs.

Materials and Methods

The study was conducted on 20 cross-bred lambs from different pramenka types, divided in two groups. Initial body weights of lambs in the group I were 18.90±2.03 kg and in the group II 18.40±1.05 kg. The age of the lambs at the start of experiment was 55 - 60 days, and the fattening lasted for five weeks. The lambs of one group were fed with mixture of cereals and sunflower meal without mineral-vitamin premix with 12.26% protein (group I), but for the feeding of second group of lambs were used feed mixture for older lambs with 14.69% protein (group II). During the fattening period the lambs from both group were fed hay *ad libitum.*
Blood for laboratory analysis was taken after weighing lambs with a puncture of the neck vein (*v.jugularis externa*) in vacutainer tubes with ethylenediamine tetra-acetic acid (*EDTA*). Blood plasma of the examined animals was tested for the following hematological parameters: red blood cell count (RBC), white blood cell count (WBC), white cell differential count (WCDC), hematocrit (PCV), hemoglobin (Hb) and red blood cell indices: MCH (Mean Cellular Hemoglobin), MCHC (Mean Cellular Hemoglobin Concentration) and MCV (Mean Cellular Volume). Measurments of hematological parameters were measured on the 21st and 35th day of fattening. The obtained results were statistically analysed using Microsoft Excel 2010, module Data Analysis. A star system was used to indicate the level of significance and correlation coefficient ($p<0.05$*; $p<0.01$**, $p<0.001$***).
This study was in accordance with the Rules of procedure of Ethical Committee of Veterinary Faculty University of Sarajevo and was approved by the Committee.

Results and Discussion

Blood is a circulating tissue composed of cells suspended in a fluid intercellular substance (plasma), with the main function of homeostasis maintenance and animal growth (Etim *et al.,* 2014a). Nutrition, and the breeding system have an important influence on hematological parameters (NseAbasi *et al.,* 2014); Etim *et al.,* 2014b), as well as on the health status of farm animals (Oyawoya *et al.,* 2004). The results of the research show that the lambs of the group fed by mixture of cereal and sunflower meal without mineral-vitamin premix with 12.26% of protein in the 21-day fattening period, exhibited somewhat higher hematological parameters, but not statistically significantly higher ($p> 0.05$) than lambs that were fed with a complete feed mixture for older lambs with 14.69% protein. During the 35-day fattening period, the first group of lambs also exhibited somewhat higher hematological parameters, with justifiable statistical significance for differences in PCV and MCV at $p <0.05$. Lower values of hematological parameters were determined within both groups with the lambs getting older for fattening period, except the number of erythocytes (Table 1). In lambs, rumen does not function until 7 weeks of age, so lambs in the first weeks of age are labeled as functionally nonruminants (Antunović *et al.,* 2000) and are more sensitive to different stress conditions. By comparing the obtained results with the results of Novoselec *et al.,* (2015) we can conclude that the values of RBC, Hb, and MCHC and MCH are lower, while PCV and MCV values are higher, in both groups and for both fattening periods, where in his experimental design he used feed mixture with 17.73% protein.
Olayemi *at al.* (2000) conducted a study of haematological parameters in Western African dwarf sheep in various breeding systems, and found that animals that were intensively bred showed higher PCV, Hb and MCV values consistent with our results. The increased level of

Hb concentration is attributed to the ability to undergo infection, good nutritional status as well as animal breeding (Anwar *at al.,* 2012). According to Boronez *et al.*, (2009), MCV is affected by stress during blood taking in lambs.

Examining the production-hematological parameters of lambs fed on meals with the addition of pumpkin seed during the 70 days of lambs age, the authors (Novoselac *at al.,* 2017*)* found lower values for MCV, MCH, MCHC compared to ours in the experimental as well as in control group, and higher values of RBC and PCV, while the values of Hb and WBC were similar. Also, the mentioned authors in the 100-day period, which in our case approximate the 35 days of the fattening, found higher values for RBC, Hb, while the values of the other parameters are somewhat less or similar. The authors in this study used 16.13% protein in control and 15.50% in experimental group of lambs (Novoselac *at al.,* 2017).

The higher values of MCHC often occur in analytical errors (Jones and Allison, 2007), and in haemolysis, and are decreased in reticulocytosis or iron deficiency (Polizopoulou, 2010). The values of erythrocyte (RBC) of our study, in both groups and for both fattening periods, with a slight increase at the end of the fattening period, were generally below the reference value of Weiss and Wardrop (2010); https://www.merckvetmanual.com/special-subjects/reference-guides/hematologic-reference- ranges); Iyiola-Tunji e*t al.,* (2015); Antunović et al., (2010), which could be interpreted by the causes of different etiology, the consequence of regeneration, (Kraft 1999a), or in the emergence of anemia Awodi *et al.,* (2005).

Comparing the results with the results of Shek Vugrovečki *et al.,* (2017), erythrocytes values were lower, while the values of erythrocyte indices differed by less than or above the reference values, with the difference that the female lamb of Lika pramenka between two and seven years of age.

Table1. Values of some hematological parameters of lambs

21^{th} day fattening		
Parameters	Group I	Group II
WBC (10^9/L)	10.38	10.31
RBC (10^{12}/L)	4.90	4.89
Hb(g/L)	124.00	122.50
PCV (%)	32.00	32.00
MCV (fl)	67.85	68.16
MCHC (%)	38.04	37.67
MCH (pg)	25.95	25.97
35^{th} day fattening		
Parameters	Group I	Group II
WBC (10^9/L)	8.12	7.47
RBC (10^{12}/L)	5.91	5.93
Hb(g/L)	83.40	78.00
PCV (%)	39.00*	33.00
MCV (fl)	68.46*	57.77
MCHC (%)	21.41	23.54
MCH (pg)	14.61	13.66

*Statistical significance difference ($p<0.05$)

Results of White Cell Differential Count in lambs in two different fattening periods (Table 2) did not deviate from the reference values according to Weiss and Wardrop (2010). Comparing the values of white cell differential count in two periods of fattening, at the end of the fattening period, the parameters were lower except eosinophils, with no significance ($p> 0.05$) but all values were within the reference limits. Eosinophils are considered an important

indicator in response to invasion of parasites and their growth is often associated with the appearance of parasitic resistance (Balic *at al.,* 2000).

Table 2. Values of white cell differential count (WCDC)

21^{th} day fattening		
Parameters	Group I	Group II
Lymphocytes (%)	54.10	54.40
Neutrophils (%)	29.20	31.20
Eosinophils (%)	2.40	7.00
Monocytes (%)	1.90	1.82
35^{th} day fattening		
Parameters	Group I	Group II
Lymphocytes (%)	49.90	48.90
Neutrophils (%)	28.60	29.30
Eosinophils (%)	8.80	8.90
Monocytes (%)	1.40	1.60

The correlation coefficient values showed statistical validity on the level of very high significance of differences ($p < 0.001$) between RBC and PCV, RBC and MCV in both groups of lambs at the 21^{st} day of fattening period, while significant justification of the differences ($p < 0.01$ and $p < 0.05$) was established between RBC and MCH, RBC and MCHC, respectively. At the end of the fattening period, the correlation was very high ($p < 0.001$) for RBC and PCV, RBC and MCH, up to high ($p < 0.01$) for RBC and MCHC, RBC and MCV, and significant differences ($p < 0.05$) between RBC and Hb in the first group of lambs. A total correlation was obtained between RBC and MCH, and RBC and MCV in the second group of lambs (Table 3). According to the available literature (Mhori e*t al.,* 2005; Hrković-Porobija *et al.,* 2013; Nozad *et al.,* (2014); Antunović *et al.,* 2017) justifiably significant correlations have been established. We have to emphasize that above mentioned authors used adult sheep, or other types of animals in different experimental treatments.

Table 3. Correlation factors (r) between some haematological parameters

Ratio	21^{th} day fattening		35^{th} day fattening	
	Correlation with P-value		Correlation with P-value	
	Group I	Group II	Group I	Group II
RBC:PCV	0.881***	0.964***	0.899*	NS
RBC:MCV	0.888***	0.984***	0.743**	0.943***
RBC:MCH	0.703*	0.839**	0.864***	0.931***
RBC:MCHC	NS	0.653*	0.731**	NS
RBC:Hb	NS	NS	0.623*	NS

*Statistical significance difference ($p<0.05$)
**Statistical significance difference ($p<0.01$)
***Statistical significance difference ($p<0.001$)

Conclusion

The results of the research show that the lambs of the group fed by mixture of cereal and sunflower meal without mineral-vitamin premix with 12.26% protein, had achieved higher hematological parameters, but not statistically significantly higher ($p > 0.05$) in relation to the lambs that were fed with a complete feed mixture for older lambs with 14.69% protein, except

for PCV and MCV on the end fattening. Correlative values showed significant justified differences for individual hematological parameters in both groups of lambs in fattening.

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HATCHABILITY OF BROILER BREEDER EGGS STORED IN PLASTIC OR CARDBOARD FLATS

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Abstract

In practical conditions of hatching eggs production, different types of packaging for collecting, transporting or storing eggs are used. The packaging is made of different materials, mostly cardboard or various plastic materials. Cardboard flats are usually used only once, while plastic packaging materials allow multiple uses. The aim of study was to investigate the hatchability of broiler breeder eggs stored for 5 or 10 days before incubation, in plastic or cardboard flats. Study was conducted under the conditions of the commercial hatchery. A total of 1.200 eggs originated from Cobb 500 parental flock, were divided into four equal groups of 300 eggs depending on the length (5 or 10 days) and type of packaging (cardboard or plastic flats). Following parameters were analyzed: egg weight loss during storage and incubation, hatchability of all and fertilized eggs, as well as early, middle, late and total embryonic mortality. The variance analysis was used for statistical processing of egg loss, and a chi-squared test for other incubation indicators. The storage length and types of packaging has affected only egg weight loss during storage, but not during incubation ($p<0.01$). Hatchability indicators, as well as embryonic mortality had similar values among all groups ($p>0.05$). According to the data obtained from the research, it can be concluded that both types of packaging are suitable for storage of hatching eggs.

Keywords: *Hatching egg, Storage length, Storage material, Flats, Incubation.*

Introduction

Boiler meat production is associated with the production of day-old chickens, sector that includes broiler breeder farms responsible for production of hatching egg, as well as hatcheries responsible for incubation and delivery of day-old chicks to market. Fertility and egg traits, especially egg weight, shell quality, shape index and internal quality, are indicators monitored throughout entire period of exploitation of broiler breeders, due significant influence on hatchability and day-old chick quality (King'ori, 2011). Eggs are daily collected several times, mechanically or manually, and stored in flats of different materials, mostly plastic or cardboard. Advantages of plastic flats in regard to cardboard are numerous in practical production, e.g. eggs are more quickly cooled to adequate temperatures (13-19°C) after collecting due better air circulation, or sanitization and mechanical cleaning are possible contrary to cardboard flats which are not suitable for such purposes (Bell and Weaver, 2002). Egg storage in different flats on breeder farm or hatchery can vary from a few days to several weeks, which depends on organizational plan of day-old chick production. Hatchability and day-old chick quality usually are unaffected by storage in appropriate conditions up to 7 days, but further prolonging has a direct negative effect on these parameters (Fasenko, 2007).
The aim of this study was to compare egg weight loss and hatchability of eggs stored for five and ten days in plastic and cardboard flats.

Material and Methods

In this study was used a total of 1.200 hatching eggs from one 32 weeks old Cobb 500 flock grown at commercial farm in the northern part of Republic of Srpska. All eggs were laid and collected on the same day, and after transport to commercial hatchery were individually marked and divided, depending on flat type - cardboard or plastic, and storage length - five or ten days, in four equal groups with 300 eggs. Storage and incubation conditions were identical for all groups. Egg weight was individually determined using a technical scale before and after storage, and at transferring eggs in hatcher (day 18^{th} of incubation). Egg weight loss during storage and incubation was determined according to formulas given by Egbeyale *et al*. (2013). At hatching day, the number of hatched chicks was determined; all unhatched eggs were broken in order to determine embryonic mortality, while the eggs without signs of early death were categorized as unfertilized eggs. Dead embryos, depending on time of death, were assigned to one of three categories: early (1-7), intermediate (8-14) and late mortality (15-21 days of incubation). Fertility, hatchability of set and fertilized eggs, as well as early, medium, late and total embryonic mortality were calculated according to formulas given by Alasahan and Copur (2016). Data on egg weight loss during storage and incubation were subjected to two-way analysis of variance with *post hoc* Tukey test ($p<0.01$), while hatchability and mortality parameters were analyzed using a chi-square test.

Results and Discussion

Weight loss of eggs during storage and incubation are presented in table 1.

Table 1. Fresh egg weight (mean ± SD) and weight loss (mean ± SD) during storage and incubation of eggs stored in cardboard (C group) and plastic flats (P group)

Flat	Storage, days	Fresh egg weight, g	Egg weight loss during storage, %	Egg weight loss during incubation, %
C	5	60.07 ± 2.63	0.34 ± 0.07^{c}	10.48 ± 2.05
	10	60.12 ± 2.37	0.69 ± 0.15^{a}	10.31 ± 1.80
P	5	59.78 ± 2.72	0.40 ± 0.07^{b}	10.65 ± 1.52
	10	59.99 ± 2.62	0.71 ± 0.10^{a}	10.34 ± 1.30

^{abc} – values with different letter in the same column are statistically different ($p<0.01$)

Flat type and storage length were significant factors for egg weight loss during storage ($p <0.01$). Higher percentage of weight loss had eggs stored for ten days in plastic (0.71%) and cardboard flats (0.69%) compared to eggs stored for five days in cardboard and plastic flats, which was also significantly different (0.34 vs. 0.40%). Egg weight loss during incubation ranged from 10.31 (eggs stored for ten days in cardboard flats) to 10.65% (eggs stored for five days in plastic flats). This parameter was not affected by flat type and storage length ($p>0.05$) although it was relatively lower in eggs stored for ten days in both types of flats. A relatively higher egg weight loss during storage reported Khan *et al*. (2014) after five and nine days long storage of Rhode Island Red eggs (1.35 and 2.52%, respectively), Senbeta (2016) after five and ten days long storage of Leghorn eggs (1.63 and 3.27%, respectively) or Egbeyale *et al*. (2013) when stored commercial pullet egg in periods of six and nine days (0.87 and 1.95%, respectively). Reduction in egg weight during storage is related with water loss which occurs through shell pores depending on air temperature and humidity and storage length (Bell and Weaver, 2002). Relatively comparable values obtained Reijrink *et al*. (2009) during egg storage of same commercial hybrid as in this study (0.15, 0.43, 0.64 and 1.21% for 3, 5, 8 and 12 day long storage). Moreover, storage length did not affect egg weight loss during

incubation (10.45, 9.64, 9.47 and 9.84, respectively), which is also in agreement with results obtained in this research. Similarly, Goliomytis *et al*. (2015) did not confirm any effect of storage length (4, 12 and 16 days) on weight loss during incubation of commercial broiler strain (9.04, 9.01 and 8.83%, respectively). On the other hand, flock genotype and age, as well as storage length significantly affected weight loss during incubation according to Alsobayel *et al*. (2013). Weight loss during incubation of Japanese quail eggs is related to weight loss during storage, as reported Romao *et al*. (2008), since long stored eggs had a lower weigh loss during incubation compared to fresh incubated eggs or eggs stored a few days. Lacin *et al*. (2008) compared weight loss during incubation of Japanese quail eggs stored in perlite, hay or without storage material during three storage periods (1-3, 6-8 or 12-14 days). The average weight loss was higher in the environment in which no storage material was used (1.99%), compared to perlite (1.58%) and hay (1.67%). Weight loss of boiler hatching eggs during incubation in standard commercial practices varies in range 10.30-11.40%, with the best hatchability performances achieved when this parameter varies from 10.90 to 11.10% (Tona *et al*., 2001). Hatchability and embryonic mortality of eggs stored for five and ten days in cardboard and plastic flats are presented in table 2.

Table 2. Hatchability and embryonic mortality (%) of eggs stored in cardboard (C group) and plastic flats (P group)

Flat	Storage, days	EF, %	HSE, %	HFE, %	TEM, %	EEM, %	IEM, %	LEM, %
C	5	97.0	90.0	92.8	7.2	4.5	0.3	2.4
	10	97.7	88.7	90.8	9.2	6.1	1.7	1.4
P	5	96.7	89.3	92.4	7.6	5.9	0.3	1.4
	10	96.7	85.7	88.6	11.4	6.9	1.7	2.8

F – Egg fertility; HSE - Hatchability of set eggs; HFE - Hatchability of fertilized eggs; TEM - Total embryonic mortality; EEM - Early embryonic mortality; IEM - intermediate embryonic mortality; LEM - Late embryonic mortality

^{abc} – values with different letter in the same column are statistically different ($p<0.05$)

Egg fertility among groups had relatively uniform values and ranged from 97.0 (five days of storage in cardboard flats) to 97.7% (ten days of storage in cardboard flats). Hatchability of set eggs varied from 85.7 (ten days of storage in plastic flats) to 90.0% (five days in cardboard flats), whereas hatchability of fertilized eggs ranged from 88.6 (ten days in plastic flats) to 92.8% (five days of storage in cardboard flats). Egg fertility and hatchability were not affected by flat type and storage length ($p>0.05$). Total embryonic mortality had the lowest value in group of eggs stored for five days in cardboard flats (7.2%), while the highest value had group of eggs stored for ten days in plastic flats (11.4%). All embryonic mortality parameters were not affected by flat type or storage length ($p>0.05$). Reijrink *et al*. (2009) reported that hatchability of set and fertilized eggs stored for 5, 8 and 12 days was 62.7 and 86.3, 59.6 and 82.9, and 59.3 and 80.1%, respectively, with comparable hatchability of eggs stored for 5 and 8 days. In accordance with mentioned, Goliomytis *et al*. (2015) in incubation of eggs stored for 4, 12 and 15 days also obtain non-significant differences in hatchability of set (87.5, 87.5 and 80.0%, respectively) or fertilized eggs (89.7, 91.3 and 84.2%, respectively), as well as total embryonic mortality (10.3, 8.7 and 15.8%, respectively). Also, Egbeyale *et al*. (2013) have determined comparable hatchability of eggs stored for six (83.81%) and nine days (69.79%). Significant decrease in hatchability of set and fertilized

eggs stored for five days (58.78 and 74.90%) compared to storage of nine days (5.65 and 7.35%) reported Khan *et at*. (2014) or Senbeta (2016) after egg storage of five and ten days (68.23 vs. 54.69%). Duress *et al*. (2015) compared five different materials, available in conditions of traditional poultry production in rural area, as egg storage container (plastic egg trays - control group, bamboo baskets, cartons, clay pots, polythene bugs, and teff grains) for 4, 8, 12, 16 and 20 days long storage. Hatchability among the groups of eggs stored for four days showed comparable values, while after eight days it was still generally comparable. Longer storage periods resulted in significantly lower hatchability especially of eggs stored in clay pots. Higher hatchability (53.90%) of Japanese quail eggs stored in perlite compared to eggs stored in hay (46.20%) or without storage material (42.20%) reported Lacin *et al*. (2008), whereas embryonic mortality was unaffected by storage material. Long-term storage of hatching egg is associated with deterioration of internal egg quality, as well as reduction of vital cells in embryo which can affect development and metabolism (Fasenko, 2007). The absence of significant differences in hatchability of long stored eggs, according to Yassin *et al*. (2008), can be associated with flock age, since eggs of older flocks are more susceptible to negative effects of prolonged storage.

Conclusions

The storage of broiler hatching eggs in cardboard and plastic flats during five or ten days showed that egg weight loss during storage is significantly affected by flat type and storage length, while this effect was not determined for egg weight loss during incubation. Hatchability and mortality parameters showed comparable values irrespective of flat type or storage length.

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SELECTED QUALITY TRAITS OF TABLE EGGS ON BANJA LUKA (BOSNIA AND HERZEGOVINA) MARKET

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Abstract

The aim of the study was to analyze selected external and internal quality traits of table eggs of weight class large (L), originating from five different producers, present on Banja Luka (Entity of Republic of Srpska, Bosnia and Herzegovina) market. Three packs with 10 eggs, as repetition, for each egg producer were purchased, which encompass a total of 150 table eggs used in this analysis. All the eggs were produced in cage system and were seven days old on the day of analysis, which included determination of the following traits: egg weight, weight and proportion of main egg components (shell, albumen and yolk), egg shape index, unit surface shell weight, egg shell thickness, albumen height, Haugh units, yolk index, yolk color, and yolk albumen ratio. Data were analyzed using descriptive statistics and one-way analysis of variance (ANOVA). According to the obtained results, significant differences among the egg producers were found for egg weight, yolk weight, albumen weight, egg shape index, albumen height, yolk index, Haugh units and yolk color ($p<0.05$). However, shell weight, shell weight per unit surface, shell thickness, proportion of main egg components, and yolk albumen ratio had similar values among different producers ($p>0.05$). The samples of the eggs purchased on Banja Luka market generally showed satisfactory values regarding the external and internal quality parameters.

Keywords: *Table egg, Quality traits, Banja Luka, Market.*

Introduction

Eggs are characterized by high nutritional value, competitive price and availability on the market, as well as ease of preparation for consumption. They are a source of easy-digestible proteins of high biological value, polyunsaturated essential fatty acids, liposoluble vitamins, minerals and various antioxidants, ingredients that have a positive impact on human health; but also contain high level of cholesterol, which is why egg consumption has been reduced or limited in the past, due to the risk of cardiovascular disease (Zaheer, 2015). The quality of table eggs, as well as the conditions for packaging, labeling and storage of the eggs on the market of Bosnia and Herzegovina is regulated by the Regulation on standards for the placing of eggs on the market (Official Gazette B & H, 25/2010). Eggs are classified into A-class or fresh eggs and B-class, with A-class eggs divided in four weight categories: small (<53 g, S), medium (53-63 g, M), large (63 -73 g, L), extra-large (> 73 g, XL). Also, the Regulation defines the conditions for A-class eggs in terms of the quality of shell, albumen and yolk. Zelić *et al*. (2015), based on the example of Tuzla city, reported that consumers in Bosnia and Herzegovina eat eggs every day (57.53% of respondents), mostly bought in stores (32.19%) or super-markets (26.03%). Also, consumers prefer to buy eggs of domestic origin (97.95% of subjects) in the weight class L (50.00%) or M (35.62%). They consider ease of preparation and nutritional value as main benefits of eggs, and cholesterol levels, short shelf life and susceptibility to damage during transportation as their main disadvantages. Tolimir *et al*. (2017) also found the importance of egg quality parameters for consumers (73.28% of respondents) in Belgrade city, who mainly buy larger eggs in super- and hypermarkets. The quality of table eggs can be estimated by determining the indicators of internal and external

quality. Indicators of external quality refer to egg weight, index of shape and properties of the shell (weight, cleanness, color, strength and thickness), and internal quality to the properties of the albumen (e.g., weight, proportion, height, Haugh unites) and yolk (color, index). The initial quality of the eggs, determined by the above mentioned indicators, is influenced by a large number of factors, such as genotype, age, health status, nutrition and welfare of the layers, and production system (Roberts, 2004; Ledvinka *et al*., 2012). Egg quality analysis during three production cycles of one hybrid in five producers, according to Škrbić *et al*. (2006), showed differences in egg weight, height of the albumen, Haugh unit, yolk color and shell thickness among cycles, which can be attributed to differences in the parental flock and quality of feed. However, the quality of table eggs in the markets may vary a lot, depending on the factors that come to light during egg manipulation after delivery from the farm. Tolimir *et al*. (2017) found differences in the quality of eggs among super- and hypermarkets in the city of Belgrade, as well as differences on the level of market, regardless of producer. Abo Omar and Aref (2000) found that certain quality indicators vary depending on the place of purchase (super-markets, public markets or broiler selling shops). Similarly, Moula *et al*. (2013) found that the best quality of eggs in supermarkets, compared to public markets or shops. Storage length and conditions, in particular temperature, are a key factor in preventing deterioration and preserving the quality or freshness of table eggs in sales (Roberts, 2004). The quality of eggs in a market of four producers of similar production technology, reported by Youssef *et al*. (2014), differs in more than one indicator (shell weight per unit of surface area, albumen and yolk percentage, Haugh units, yolk index and color). Kralik *et al*. (2012) found that eggs originated from three different producers in the Osijek area (Croatia) do not differ in egg weight, albumen weight and height and Haugh units, but there is a difference in yolk weight, weight of the shell, shape index, shell thickness and yolk color. Recent study, conducted in the same city, also showed the influence of producers on certain egg quality indicators (Kralik *et al*., 2017). The aim of study was to analyze selected external and internal quality traits of table eggs of weight class large (L), originated from five randomly selected producers, present on Banja Luka (Entity of Republic of Srpska, Bosnia and Herzegovina) market.

Material and Methods

Three packages of 10 eggs of weight class L per manufacturer were sampled, so a total of 150 eggs were analyzed in April 2018. During the purchase of eggs, the eggs of the same age were chosen (determined on the basis of the data on the packaging). All eggs were packed in the same packaging - cardboard box and storage conditions were identical - refrigerated showcases. Quality analysis included the individual determination of selected indices of external and internal quality traits of each egg (Englmaierová *et al*., 2014; Youssef *et al*., 2014.) The egg weight (EW) was determined using a technical scale. The width and length of eggs was determined using a micrometer, in order to calculate the shape index (SI) according to the formula SI = (egg width / egg length) x 100. The analysis was continued after breaking eggs on a flat surface, so albumen (AH) and yolk height (YH) were measured using a tripod micrometer, and also a yolk diameter, using a micrometer. The yolk index (YI) was calculated as YI = (width of the yolk / height of the yolk) x 100. The shell weight (SW), as well as the yolk mass (YW), has been determined using a technical scale. The albumen weight (AW) was defined as AW = EW - (YW + SW). The proportions of shell (SP), albumen (AP) and yolk (YP) were calculated according to the following formulas: SP = (SW / EW) x 100; AP = (AW / EW) x 100; YP = (YW / EW) x 100. The yolk color is determined by the DSM yolk color fan (1-15 points). The weight ratio of egg yolk and albumen (YAR) was calculated as YAR = YW / AW. Haugh units (HU) were calculated as HU = 100 log x (AH + 7.57 - 1.7 x $EW^{0.37}$). The shell thickness (ST) was determined by a micrometer, on the equatorial area of the shell

with membranes at three different points. The shell weight per unit area (SWUSA) was determined as SWUSA = SW / SSA, and the shell surface area (SSA) was calculated as SSA = 3.9782 x $EW^{0.7056}$. The data were subjected to descriptive analysis and one way analysis of variance with *post hoc* Tukey test ($p<0.05$).

Results and Discussion

Values of external egg quality traits are presented in Table 1.

Table 1. External egg quality traits (mean ± stand. error)

Quality traits	Producers				
	A (n=30)	B (n=30)	C (n=30)	D (n=30)	E (n=30)
EW (g)	66.85^{b}±0.69	64.18^{d}±0.43	70.04^{a}±0.17	65.05^{cd}±0.41	66.13^{cb}±0.34
SI (%)	76.35^{b}±0.49	76.98^{b}±0.71	76.02^{b}±0.51	75.90^{b}±0.36	80.85^{a}±0.71
SW (g)	6.61^{a}±0.10	6.50^{a}±0.10	6.70^{a}±0.13	6.46^{a}±0.09	6.56^{a}±0.11
SP (%)	9.93^{a}±0.19	10.14^{a}±0.16	9.56^{a}±0.17	9.94^{a}±0.14	9.91^{a}±0.15
ST (mm)	0.41^{a}±0.00	0.40^{a}±0.01	0.39^{a}±0.00	0.39^{a}±0.00	0.40^{a}±0.01
SWUSA (mg/cm^2)	85.87^{a}±1.49	86.73^{a}±1.35	83.97^{a}±1.52	85.37^{a}±1.18	85.60^{a}±1.30

EW - Egg weight (g); SI - Egg shape index (%); SW - Shell weight (g); SP - Shell proportion (%); ST - Shell thickness (mm); SWUSA - Shell weight per unit of surface area (mg/cm^2)
^{abc} – values in same row with different letters are statistically significantly different ($p<0.05$)

The obtained results indicate there are significant differences for the egg weight and egg shape index ($p<0.05$) between the eggs of different producers. The eggs in the weight class L, according to the Regulation, must have a weight from 63 to 73 g (Official Gazette B & H, 25/2010). The egg weight is determined by genotype, weight and age of layers, intensity of laying, nutrition and ambient conditions (Ledvinka *et al*., 2012). The average egg weight varied from 64.18 (producer B) to 70.04 g (producer C). The obtained values of shape index indicates that analyzed eggs generally had a round shape, because their range is from 75.90 (producer D) to 80.85% (producer E), which is more than 74% in eggs of a normal or optimal shape (Kralik *et al*., 2012). The shape index varies between genotypes, but also varies during the laying cycle. As an example, eggs in the initial stage of laying often have an untypical shape (Ledvinka *et al*., 2012). The proportion of shell in analyzed eggs ranged from 9.56 (producer C) to 10.14% (producer B). The Obtained values of shell thickness, ranged from 0.39 (producers C and D) to 0.41 mm (producer A), indicates satisfactory shell quality, as well as values of shell weight per unit area (83.97-86.73 mg/cm^2 in producers C and B, respectively). These three shell characteristics are at the same time indicators for assessing the strength of the shell, very important egg quality trait, which influences percent of eggs broke during collection, transport or storage. Kralik *et al*. (2012) reported that the L-class eggs of three different manufacturers had a shape index of 80.70, 74.37 and 77.56%, as well as shell thickness of 0.43, 0.39 and 0.41 mm, and according to a recent study of same authors, analysed M-class eggs of two manufacturers, showed shape index of 76.67 and 79.04% and shell thickness of 0.362 and 0.380 mm (Kralik *et al*., 2017). The average shell thickness, according to Abo Omar and Aref (2000) varies from 0.372 to 0.395 mm, and Moula *et al*. (2013) reported values of 0.352-0.397 mm. Youssef *et al*. (2014) in their study found values of egg shape index of 74.7-75.7%, shell thickness of 0.358-0.393 mm, and values of shell weight per unit of surface area of 78.79-87.54 mg/cm^2. Casiraghi *et al*. (2005) in their egg quality survey of all weight classes in stores in northern Italy obtained values of 75.7-77.4%

for egg shape index and from 0.40 to 0.42 mm for shell thickness. Perić *et al*. (2016) found the shape index of 75.83%, and shell thickness 0.382 mm for the eggs from conventional production on the market in Novi Sad (Serbia). The quality of eggshells, as reviewed by Roberts (2004), varies depending on genotype and age of layers, nutrition, ambient conditions, especially temperature, and health and wellfare status. Ledvinka *et al*. (2012) points out that modern layer hybrids differ in weight and strength of the shell, while the shell weight ratio and its thickness are not closely related to the particular hybrid.
Values of internal egg quality traits are presented in Table 2.

Table 2. Internal egg quality traits (mean ± stand. error)

Quality traits	Producers				
	A (n=30)	B (n=30)	C (n=30)	D (n=30)	E (n=30)
AW (g)	43.85^{ab}±0.71	41.43^{d}±0.41	45.53^{a}±0.37	42.02^{cd}±0.38	43.55^{cb}±0.31
AP (%)	65.86^{a}±0.52	64.57^{a}±0.34	65.17^{a}±0.50	64.73^{a}±0.40	65.76^{a}±0.29
AH (mm)	7.29^{a}±0.23	7.38^{a}±0.25	5.54^{b}±0.16	7.45^{a}±0.20	7.90^{a}±0.17
HU	82.85^{a}±1.54	84.04^{a}±1.62	68.90^{b}±1.37	84.55^{a}±1.22	87.08^{a}±0.97
YW (g)	15.98^{b}±0.31	16.25^{b}±0.24	17.61^{a}±0.32	16.41^{b}±0.28	16.11^{b}±0.19
YP (%)	24.05^{a}±0.44	25.34^{a}±0.35	25.22^{a}±0.47	25.28^{a}±0.40	24.32^{a}±0.25
YAR	0.37^{a}±0.01	0.39^{a}±0.01	0.39^{a}±0.01	0.39^{a}±0.01	0.37^{a}±0.01
YI (%)	48.57^{a}±0.72	46.75^{ab}±0.65	44.47^{b}±1.60	47.43^{ab}±0.60	47.75^{ab}±0.43
YC	14.03^{a}±0.12	13.45^{ab}±0.21	13.73^{ab}±0.18	13.03^{b}±0.21	13.28^{ab}±0.21

AW - Albumen weight (g); AP - Albumen proportion (%); AH - Albumen height (mm); HU - Haugh units; YW - Yolk weight (g); YP - Yolk proportion (%); YAR - Yolk/albumen ratio; YI - Yolk index (%); YC - Yolk color

^{abc} – values in same row with different letters are statistically significantly different ($p<0.05$)

Significant differences in the albumen weight, Haugh units, as well as yolk weight, index, and color among samples from different producers have been identified ($p<0.05$). The weight and proportion of the albumen varied from 41.43 (producer B) to 45.53 g (producer C), and from 64.57 (producer B) to 65.86% (producer A), respectively. Yolk weight and share values were in the range from 15.98 to 17.61 g (producers A and C), and 24.05 - 25.34% (producers A and B), respectively. There is a positive correlation between egg weight and the weights of its parts, especially yolk and albumen, although their share in eggs of different weight can still be different (Ledvinka *et al*., 2012). The yolk/albumen ratio (YAR) varied from 0.37 (producers A and E) to 0.39 (producers B, C and D). This relationship is influenced by genetics, hen age and egg size. Suk and Park (2001) reported that YAR varied from 0.38 to 0.40 during the laying cycle, and also a negative correlation between YAR and egg weight. The higher values of YAR were found by Moula *et al*. (2013) (47.98%) and Youssef *et al*. (2014) (43.74-48.48%). The quality of the albumen is usually determined by measuring of its height. On the basis of albumen height and egg weight, Haugh units are calculated, as generally accepted indicator of egg quality and freshness (USDA, 2000). The higher the value of Haugh units, the better the quality of the albumen. The albumen height in this study varied from 5.54 (producer C) to 7.90 mm (producer E), while Haugh units ranged from 68.90 (producer C) to 87.08 (producer E). The average value for fresh eggs, according to Englmaierová *et al*. (2014), is 88.5 Haugh units. Firm albumen has 72.0 or more Haugh units, i.e. such eggs are in AA category, while eggs with 60.0-71.9 are in category A (USDA, 2000). Kralik *et al*. (2012) analyzed three egg producers, and found albumen height of 6.36, 6.17 and 6.17mm,

respectively, while Haugh units were 77.31, 75.31 and 75.43, respectively, while their recent study (Kralik *et al*., 2017) showed lower albumen quality (albumen height 5.03 and 5.27 mm, Haugh units 70.36 and 71.33). Abo Omar and Aref (2000) report that eggs on the market have average values of 77.8-85.9 Haugh unit, which is comparable to 79.92, found by Moula *et al*. (2013). Tolimir *et al*. (2017) surveyed egg quality in the super- and hypermarkets in the city of Belgrade and found albumen height values of 6.4-7.2 mm, or 78.3-84.3 Haugh units in eggs stored up to ten days. The lower quality of the albumen (albumen height ranged from 3.282 to 5.074 mm and Haugh units ranged from 50.23 to 68.2) was found by Youssef *et al*. (2014). The differences in the albumen height and Haugh units value, according to Roberts (2004), can be attributed to the storage conditions, in particular the length and temperature of storage, but also with genotype and age of hens, and the production technology (production system, nutrition, health). Jones *et al*. (2010), in their study of egg quality in the markets, did not establish clear relation between the albumen height and the Haugh units values from one, and the age of eggs on the other side. The yolk index in our study varied from 44.47 (producer C) to 48.57% (producer A). The yolk index in fresh eggs usually fluctuates around 45.0% (Englmaierová *et al*., 2014), with tendency to decrease during the storage period due to the weakening of the yolk membrane (Roberts, 2004). One of the most important quality traits, besides the egg weight and shell strength, is yolk color. This visual property is equally important to consumers and the food industry, and the intensity of the yolk color depends on the type and quantity of pigments in food (Beardsworth and Hernandes, 2004). In our study, yolk color values ranged from 13.03 (producer D) to 14.03 (producer A). Consumers prefer eggs with a yolk in a darker yellow shade, closer to orange, which corresponds to value of 14 according to DSM yolk fan (Beardsworth and Hernandes, 2004). The lighter shades of yellow (6.24-7.11) in domestic conditions of egg production were found by Pandurević *et al*. (2015), while on egg market in neighboring countries higher values of yolk color were found, such as 12.66-13.90 (Kralik *et al*., 2012), 13.16 (Perić *et al*., 2016) or 10.9-13.2 (Tolimir *et al*., 2017).

Conclusions

Based on the results of our study, it can be concluded that the values of the quality indicators vary depending on the producer. The obtained values of the egg quality indicators meet the standard criteria, except for one sample characterized by the relatively lower internal quality when compared to samples of other producers. Analysed eggs generally have a round shape and a shell of satisfying thickness. In terms of internal quality, Haugh units indicate high quality albumens, while the yolks are characterized by darker shades of yellow. Eggs included in our study originated from intensive production, which assumes that adequate accommodation, nutrition and health care of hens are provided, which are preconditions for good laying capacity and quality of eggs. Also, keeping of eggs for a long time under adequate conditions can significantly slow down the process of deterioration of egg quality. In order to better understand the quality of table eggs on the market from the city of Banja Luka, further research, with a longer period of monitoring and sampling from more producers, should be conducted.

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POPULATION STRUCTURE OF THE ENDEMIC NERETVA RUDD (*SCARDINIUS PLOTIZZA*) FROM THE DERANSKO LAKE, BOSNIA AND HERZEGOVINA

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Abstract

The Neretva rudd (*Scardinius plotizza*) is an endemic species, which lives in the watercourses of the Neretva river basin in Bosnia and Herzegovina and Croatia. Population structure was studied on 55 individuals caught by gill nets at Deransko Lake. The aim was to determine the population structure according to length and mass, and relationship between the length of gut and total body length. The results are presented in the form of frequency histograms, linear and mass structure, and the regression relation between total body length and length of gut. Individuals with total body length of 22-24 cm were dominant, with maximum total length of 40.4 cm. The maximum recorded weight was 1127.7 g. The frequency histogram of body mass showed that weight class 100-200 g dominated, too. Positive allometric growth with high b-value of 3.25 was determined. Coefficient of correlation (r=0.99**) shows full relation connectivity between total body length and length of gut. This research provided new results on structure of population of the endemic Neretva rudd.

Keywords: *Neretva rudd, population, length, body, Deran Lake.*

Introduction

The freshwater ihtiofauna of Adriatic basin is characterized by a large number of endemic species, which are distributed on a restricted area (Mrakovčić *at al*., 1995, 2006). For these species, there is a small number of published scientific papers, referring to their systematic position, distribution, problem of endangerment and biological-ecological character (Crivelli and Maitland, 1995a, b; Kottelat and Freyhof, 2007). The Neretva rudd (*Scardinius plotizza*) is also one of these interesting species (Heckel and Kner, 1858). It is an endemic species distributed on a very restricted area. The Neretva rudd inhabits lower watercourses of the Neretva river basin in Bosnia and Herzegovina and Croatia (Vuković, 1977; Mrakovčić *at al*., 2006; Kottelat and Freyhof, 2007). In Bosnia and Herzegovina, it was recorded in the Neretva River basin till the locality Čapljina and in the swamps of Hutovo Blato (Vuković, 1977; Mrakovčić *at al*., 2006; Kottelat and Freyhof, 2007). Neretva rudd is mainly feeding with water plants, detritus and invertebrates (Vuković, 1977; Kosorić, 1978; Mrakovčić *at al*., 2006). In the Croatian red list of freshwater fish species, and according to IUCN status (Mrakovčić *at al*., 2006), the data for *S. plotizza* is deficient (DD). Genius *Scardinius* (Bonaparte, 1873) is widespread in the whole Europe. According to Kottelat (1997) this genus covers five, and according to Kottelat and Freyhof (2007) ten species with restricted area of distribution. *S. hesperdicus* (Bonaparte, 1845) and *S. scardafa* (Bonaparte, 1837) are distributed in the area of Italy; in the area of Greec are endemic species *S. acarnanicus* (Economidis, 1991) and *S. graecus* (Stephanidis, 1937); *S. knezevici* (Bianco and Kottelat, 2005) inhibits the waters of Albania, Montenegro and Macedonia; in Turkey is *S. elmaliensis* (Bogutskaya, 1997); *S. racovitzai* (Müller, 1958) is in Romania and *S. dergle* (Heckeland Kner, 1958) and *S. plotizza* (Heckle and Kner, 1858) are distributed in the waters of Bosnia and Herzegovina and Croatia. The Neretva rudd is a species that is retained in flocks, and inhabits clean, shallow, peaceful river flow and lakes of lower Neretva basin with plenty underwater vegetation. The publications about Neretva rudd biology and ecology are rare and outdated, and they are mostly about the general biology issues (Vuković and Ivanišević, 1962;

Vuković and Ivanović, 1971; Vuković, 1977) and distribution (Kosorić, 1978; Kosorić *at al*., 1983). More recent data of Neretva rudd are related to length-weight ratio (Dulčić *at al*., 2009), morphometric and meristic characteristics (Prusina *at al*., 2009) and growth (Ivanković *at al*., 2017).

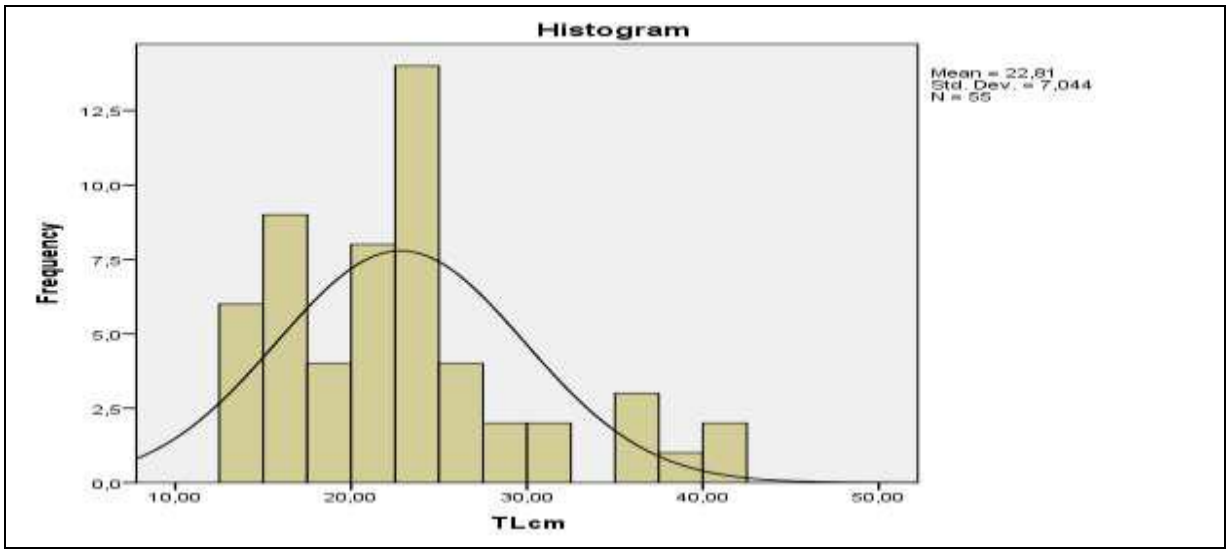
The aim of the present work was to provide data about length and mass structure of the Neretva rudd population, and relation between length of gut and total body length. The results are presented in the form of frequency histograms, linear and mass structure, and the regression relation between total body length and length of gut.

Material and Methods

The sampling was done in the area of Deransko Lake that is a part of the Hutovo Blato swamps in Bosnia and Herzegovina. Fishes were caught by anchored gill nets, mesh diameter 28-72 mm and 30 meters long, while net height varied from 1 to 3 meters. Nets were set up at night and checked the next morning. Fish population that has been processed was total 55 specimens of Neretva rudd and they weren't analysed for sex determination. Determination of species was done by system key for determination of fish species presented by Vuković (1977) and Freyhof and Kottelat (2007). Collected specimens were processed by standard ichthyologic methods where total body length (TL; cm) was measured by ihtiometar and the body mass (TW; g) were analysed by analytical scale. Digestive tract was extracted from the analysed fishes, after cutting out the oesophagus and anus. Thereafter, length of the digestive tract was measured (DP; cm).

Results and Discussion

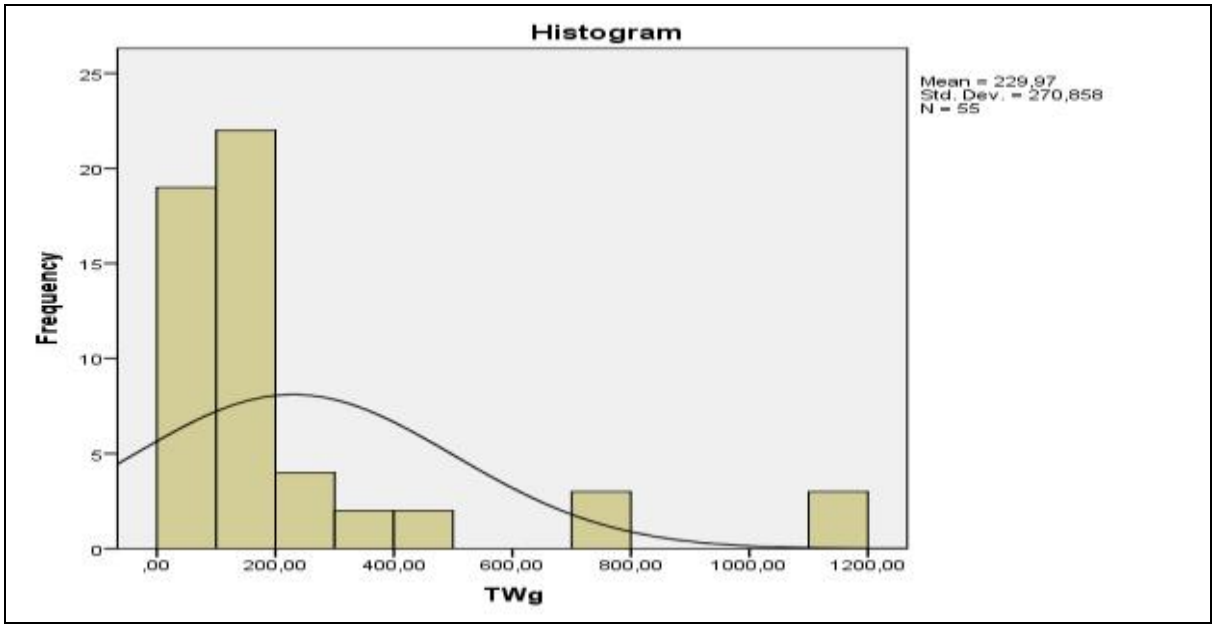
The maximum registered total body length of Neretva rudd was 40.4 cm and the minimum 14.2 cm. In the histogram: frequency of total body length Neretva rudd (picture 1), it's noticeable that the majority of specimens were in the fifth class with total body length between 22 and 24 cm. Truman *at all.* (2012) came to similar results, according to them; prevailing specimens of Neretva rud in the Hutovo blato are in the classes with total body lengths from 19 to 21 cm. The tenth size class with total body lengths from 38 to 40 cm was the least represented.



Picture 1. Frequency of Neretva rudd total body lenght

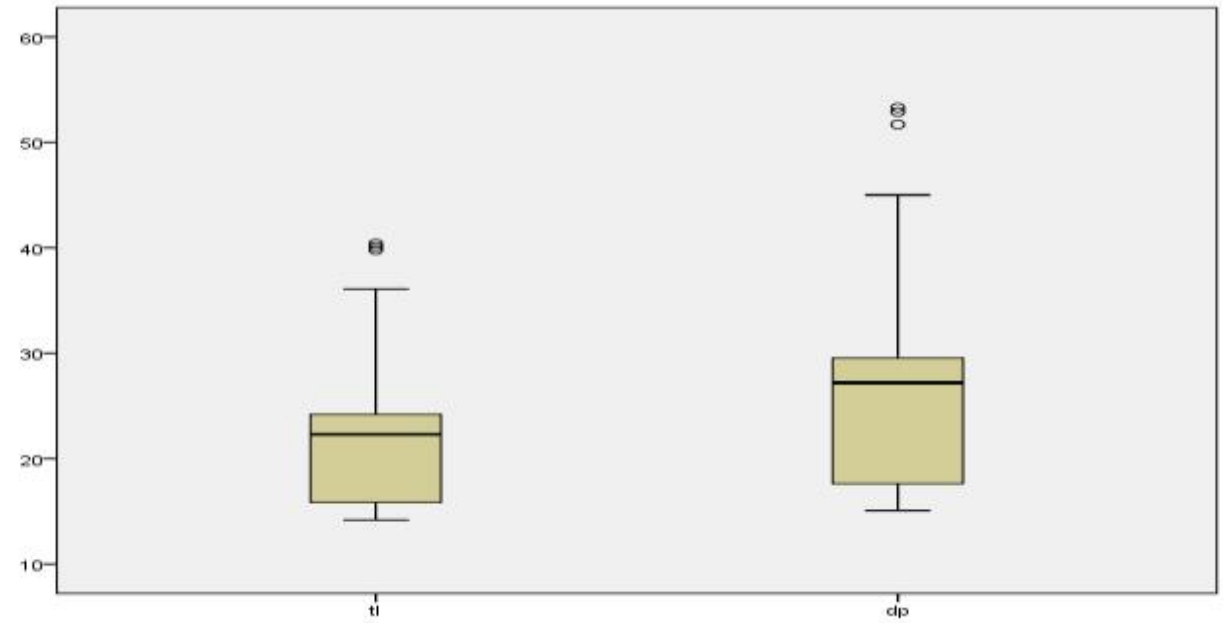
The maximum measured body weight of Neretva rudd was 1127.7 g., while the minimum was 36.9 g. The average body mass of 55 measured specimens was 229.97 g. The frequency histogram of Neretva rudd body mass showed that it is classified in seven classes according to

body mass, where the second class with a body mass of 100 to 200 grams dominated, while fourth and fifth mass classes, with body mass of 300 to 500 g had a minimum value (picture 2).



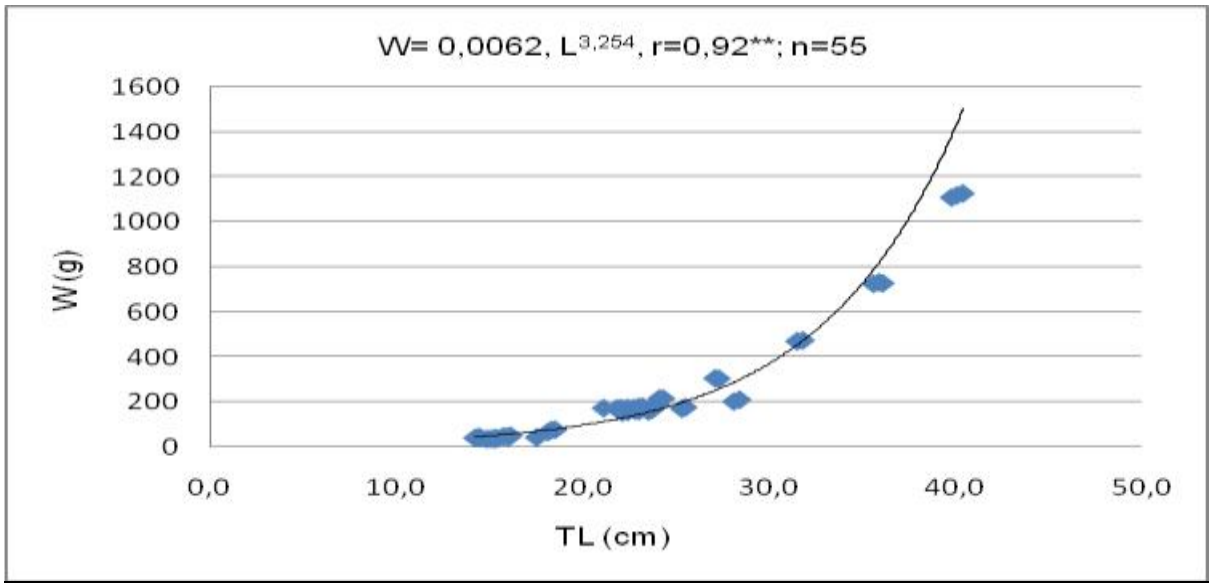
Picture 2. Frequency of Neretva rudd body mass

The gut length, of the Neretva rudd, ranged from a minimum of 15.1 cm to a maximum of 53.3 cm with the average value of 27.37 cm (picture 3).



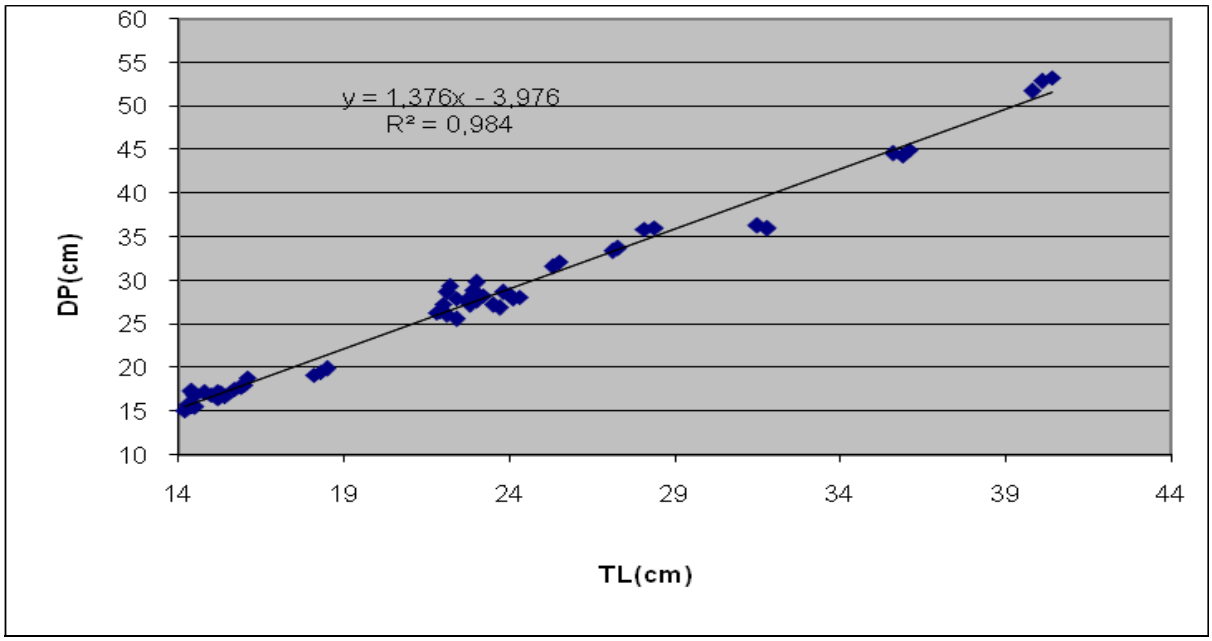
Picture 3. Minimum, maximum and average value ($\bar{x}$) of overall gut length (DP) and total body length of Neretva rudd

The function that best describes length-weight relationship specimens of Neretva rudd is: W = 0.0062 $L^{3.25}$ with the determination coefficient R^2 = 0.85. According to the obtained b-constant value of 3.25, demonstrated positive allometric growth for whole sample of Neretva rudd specimens (picture 4).



Picture 4. The length-mass relation between total length (cm) and body mass (g) of Neretva rudd from the Deran Lake

Correlation coefficient (r = 0.99**) shows full relation connectivity between total body length (TL) and length of gut (DP) (picture 5). With increasing of total body length for 1cm the length of gut will increase by 1.37 cm.



Picture 5. Regression relation between total body length (TL, cm) and length of gut (DP, cm) of Neretva rudd

In this research, the body length of the largest caught specimen was 40.4 cm, which is in accordance with the maximum length of 51 cm reported in Coast Pink (2016). Local fishermen's claims that this species can grow up to 3 kg body mass (Bistrobih.ba, 2016). Since the Neretva rud is relative with the rudd (*S. erythrophthalamus*) they can be compared, from the same Adriatic area. Rudd is the most numerous species in the Vransko Lake at island of Cres. In this lake, the largest caught specimen had the total body length of 61.7 cm, and

body mass of 3623 g (Šprem *at al*., 2010). Its fast growth has been confirmed by Scheibl (1998). Valić *at al*. (2013) are suggesting, according to the phylogenetic analyses, that in the Vransko Lake dunabe rudd was not introduced, but that it is an endemic species *Scardinius hesperidicus*, whoes morphological characteristics of maximal body length and body mass revealed new data about maximal values. Neretva rudd from the Deransko Lake, after first year reached the length of 12.76 cm (Ivanković *at al.*, 2017), similar to Tutman *at al*. (2012), who have caught one specimen of these species with body length of 12.5 cm, and they also concluded that all specimens, under one year of age, are less than 9 cm long. The rudd from Vransko Lake grows even faster, and reaches a length of 19.03 cm after the first year (Scheibl, 1998). The link between length of the rudd at the age of one year and its width was shown by Tarkan *at al*. (2010). They found generally faster growth at the specimens with lower width, but also the obvious larger variations in the body length at the one-year-old fishes from the southern populations (40-46^{0}N), where is also a habitat of studied Neretva rudd. Many researches who studied fish's diet found a link between the length of the digestive tract and the type of diet. Coefficient of correlation between total body length (TL) and length of gut (DP) shows full relation connectivity for the specified length of Neretva rudd from the Deransko Lake. Vuković (1963) also indicates the existence of a certain link between the length of gut and the standard fish length.

Conclusions

The rivers of Bosnia and Herzegovina are known for the existence of many endemic species and subspecies of fish. In the lower watercourses area of the Neretva river basin, which belongs to Adriatic basin, a certain number of endemic species has been recorded. The data about this species are limited. Only a few papers are covering their ecology, biology, taxonomy and their mutual relationship. One of these species is the Neretva rudd and according to IUCN red list it is registered as worryingly vulnerable species. The maximum registered total body length of Neretva rudd, from Deransko Lake, was 40.4 cm, and the maximum body mass was 1127.70 g. From the shown frequency histogram for the body length, it can be concluded that the majority of specimens were in the classes with total body length between 22 and 24 cm, while in the frequency histogram for the body mass dominated specimens of Neretva rudd between 100 and 200 g. Correlation coefficient (r = 0.99**) between total body length and length of gut shows full relation connectivity between the specified features. Relation between length of gut and total body length indicates omnivorous diet. Positive allometric growth, for the Neretva rudd, with high b-value of 3.25 was determined.

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EFFECTS OF TURKEY'S AGE ON THE MAIN EGG INCUBATION INDICATORS AND THEIR PHENOTYPE CORRELATION

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Abstract

With an aim to determine the phenotype correlation between certain incubation characteristics of the line hybrid British United Turkey (BUT) Big 6 eggs, a study was conducted of two groups of eggs laid by turkeys of 34 (TA_{34}) and 45 (TA_{45}) weeks of age. Average egg mass before incubation was 80.37 g (TA_{34}) and 85.71 (TA_{45}), and average mass of newly hatched turkey poults was 52.62 g and 55.80 g. Differences were statistically very significant ($P<0.001$). Absolute and relative egg mass loss until day 25 of incubation was also larger in case of the older turkey flock. Egg mass loss until day 25 was 9.20 g and 11.41% (TA_{34}), and 11.41 g and 12.38% (TA_{45}). Observed differences were statistically significant ($P<0.001$). In contrast to the previous indicators, difference in the relative share of the poult in the egg mass (0.38%) was not significant ($P>0.05$).

Turkey's age significantly affected egg mass and poult mass increase, absolute and relative loss of egg mass from day 25 of incubation, while the poult percentage in the egg mass was similar in both age groups of turkeys. Apart from the turkey's age having impact on the increase of the average egg mass, the egg mass also had significant impact on other observed indicators during the incubation period. These statements are confirmed by the calculations of the phenotype correlation coefficients between the observed indicators as well as most of the results of other authors (researchers) who studied this matter related to turkey and other poultry's eggs.

Key words: *turkey age, eggs, turkey poults, phenotype correlation, British United Turkey hybrid Big 6.*

Introduction

It is an established fact that appropriate rearing technology, nutrition and age of the parent poultry flock significantly affect incubation values of eggs for fertilisation and, consequently, the quality of the newly hatched offspring. Numerous studies were conducted in relation to this issue, with an aim to determine the impact of the age factor of different types of poultry on egg fertilisation, egg mass, offspring mass, egg mass loss during incubation period, and their mutual phenotype correlations. Most of related research refers to hens and quails, followed by turkeys, geese and ducks.

In relation to hen breeds, hybrids and mongrels, notable research was conducted by Abiola *et al.* (2008), Mitrović *et al.* (2010), Egbeyale *et al.* (2011), Yamak *et al.* (2015), Iqbal *et al.* (2016), on quails (Alkan *et al.* 2008), geese (Đermanović *et al.* 2008, Mitrović *et al.* 2018) and ducks (El-Hanoun *et al.* 2012, Đermanović *et al.* 2017). These authors mostly conclude that egg mass and offspring mass of the hens, quails, ducks and even geese, increase with their age. They also report strong, very strong and even total positive phenotype correlation between the egg mass of different types of poultry and the mass of newly hatched offspring.

Regarding turkeys, Rahn *et al.* (1981), Tullett (1981), Christensen and McCorkle (1982), Meir *et al.* (1984), Hristakieva *et al.* (2009), Mróz *et al.* (2010), Amir *et al.* (2015) and Hristakieva

et al. (2017) conducted much more extensive studies. They found similar effects of turkey's age and egg mass on the incubation indicators and their correlation.
Mróz *et al*. (2010) report that the egg mass of the Broadbreasted White Turkey grows linearly with its age from 81.9 g (turkeys 33 weeks old) to 101.4 g (turkeys 57 weeks old).Similarly, Hristakieva *et al.* (2009) report average egg mass of 84.67 g in 32 weeks old Heavy Meat turkey (HM), and 88.97 g at 44 weeks of age. Amir *et al.* (2015) observed two flocks of Converter turkey hybrids reared in the summer season and the winter season. During the production cycle lasting 24 weeks they observed a trend of egg mass increase with some oscillations, so that the average egg mass ranged between 75.98 g and 97.54 g.
Mróz and Orlovska (2009) report an inclination towards egg mass increase in Bronze turkey (81.9 g to 101.4 g) from 33 to 57 weeks of age, and the average mass of newly hatched turkey poults (59.1 g to 72.6 g), while the share of poults in the egg mass ranged between 72.8% and 87.2%. Hristakieva *et al*., (2017) who studied North Caucasus turkey, report average egg mass before incubation to be 82.04 g in turkeys 34 weeks old, and 84.22 g egg mass in turkeys 46 weeks old, while the relative egg mass loss until day 25 of incubation was 9.96% and 12.47% respectively. Average egg mass of one day old poults was 55.93 g and 54.45 g, and the relative share of curic in the egg mass was 68.16% and 64.58% respectively. Nestor *et al*. (1982) investigated incubation values of turkey eggs of different turkey hybrid line types (3 medium heavy hybrids + control and 6 heavy hybrids + control). Depending on turkey's age, average egg mass of medium heavy hybrids was 79.4 g (at the onset of the laying), 83.4 g (in the middle of the laying period) and 83,6 g (at the end), while in heavy hybrid lines average egg mass was 83.0 g (beginning), 90.6 g (middle) and 90.1 g (end).
Meir *et al*. (1984) report egg mass loss of 12% until day 25 of the incubation period. Tullett (1981) reports the same loss percentage of turkey egg mass, Christensen and McCorkle (1982) somewhat lower (11.5%), while Rahn *et al*. (1981) report considerably lower loss percentage (10%). Moran and Reinhart (1979) reached a slightly different conclusion regarding effects of turkey's age on egg mass and poults mass, *i.e*., on the absolute and relative egg mass loss until day 25 of incubation. Hristakieva *et al*. (2017) report positive correlation, $r_p = 0{,}77^{***}$ (young turkeys – 34 weeks old) and $r_p = 0.65^{***}$ (older turkeys – 46 weeks old), between the egg mass of the Nort Caucasus Bronze turkey and the egg mass of the one day oldpoults. The egg mass had significantly smaller effect on the egg mass loss until day 25 of incubation ($r_p = -0.31^{ns}$ and $r_p = 0.01^{ns}$), as well as the share (percentage) of the poult in the egg mass ($r_p = 0.14^{ns}$ and $r_p = -0.04^{ns}$).
The main purpose of this study was to determine the egg mass, egg mass loss during incubation, poults mass and share of the poult in the egg mass, as well as the phenotype correlation between observed indicators in BUT Big 6 turkey hybrids 34 and 45 weeks old.

Materials and methods

With an aim to determine the phenotype correlation between some incubation characteristics of the BUT Big 6 line hybrid eggs, a research was conducted of two groups of eggs laid by turkeys from different age groups. Two hundred (200) eggs coming from two different flocks were obtained from the farm „Bagrdan" in Jagodina, Serbia. The age difference between the flocks was 12 weeks, *i.e.* 3 months (one flock was 34 weeks old and the other was 45 weeks old). One of the flocks was in its maximum laying intensity period (peak), while the other was in the middle of the production cycle which lasted from the week 32 to week 56 of the parent flock's age. Egg incubation was carried out in the experimental laboratory of the Faculty of Agriculture, University of Belgrade.
100 eggs laid by the 34 weeks old turkeys (TA_{34}) and 100 eggs laid by the 45 weeks old turkeys (TA_{45}) was placed into the incubator. All eggs were individually measured, shells were marked with pencil and eggs were disinfected by the formaldehyde vapors before being

laid into the incubator. More specifically, egg mass was individually measured before laying into the incubator and on day 25 of incubation as well as the mass of the poults which dried after hatching. Based on the egg mass on the first and on the 25th day of incubation, absolute and relative egg mass loss was determined for this period and for each egg weight group. When eggs were moved from the laying tray to the hatching tray on the 25th day, they were individually placed into specially constructed partitions to make sure which poult comes from which egg.

At the end of the incubation period egg mass of one day old poults was determined which allowed the calculation of the relative share of the poult in the egg mass, *i.e.* the percentage of the poult (PP) in the egg mass, according to the following formula: PP = [(poult mass/egg mass) x 100]. Special attention was paid to those eggs from which healthy and vital poults were hatched. Basic data analysis was carried out with *Stat.Soft.Inc.* (2003) STATISTICA (data analysis software system), version 6, by implementation of the standard variational-statistics methods (descriptive statistics).

For most of the observed indicators in each of the egg weight groups, arithmetic mean ($\bar{x}$), arithmetic mean error ($s_{\bar{x}}$), standard deviation (S) and variation coefficient (VC) were calculated. Testing of the difference significance between observed incubation characteristics was carried out by implementation of the t-test according to the adequate formula. Furthermore, phenotype correlation coefficients (r_p) between observed characteristics were calculated according to the appropriate formula, primarily between egg mass and other incubational egg values (absolute and relative egg mass loss until day 25 of incubation), *i.e.* one day old poults (poult mass and the relative share of the poult in the egg mass).

$$r_{XY} = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2 \sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

Phenotype correlation coefficients strength was discussed on the basis of the Roemer – Orphal classification refered to by Tavčar (1946).

Results and discussion

Average values, absolute and relative variation measures of the basic studied indicators related to egg incubation of the BUT hybrid Big 6 are given in Table 1, and the difference significances of average values in Table 2. It should be noted that the analysis covers hatched eggs. The total number of hatched eggs was 76 (TA_{34}) and 72 (TA_{45}).

Table 1. Average values and variability of egg mass and one day old poults mass in turkeys of different age (TA_{34}) and (TA_{45})

Turkey age	Indicators	$\bar{x}$	$s_{\bar{x}}$	S	C.V.
TA_{34}	Egg mass before	80.37	0.63	5.47	6.81
TA_{45}	incubation (g)	85.71	0.62	5.33	6.22
TA_{34}	Loss of egg mass until	9.20	0.12	1.06	11.52
TA_{45}	day 25 of incub. (g)	10.62	0.14	1.21	11.39
TA_{34}	Loss of egg mass until	11.41	0.08	0.76	6.31
TA_{45}	day 25 of incub. (%)	12.38	0.16	1.34	10.82
TA_{34}	One day old poults	52.62	0.48	4.23	8.04
TA_{45}	mass (g)	55.80	0.47	3.97	7.11
TA_{34}	Relative share of the	65.44	0.15	1.21	1.85
TA_{45}	poult in egg mass (%)	65.06	0.14	1.20	1.84

TA_{34} = turkeys 34 weeks old; TA_{45} = turkeys 45 weeks old.

Table 2. Difference significance of average values of observed indicators during incubation

Indicators	TA_{34}	TA_{45}	Difference	Significance
Egg mass before incubation (g)	80.37	85.71	-5.34	***
Loss of egg mass until day 25 of incubation (g)	9.20	10.62	-1.42	***
Loss of egg mass until day 25 of incub. (%)	11.41	12.38	-0.97	***
One day old poults mass (g)	52.62	55.80	-3.18	***
Relative share of the poult in the egg mass (%)	65.44	65.06	0.38	NS

TA_{34} = turkeys 34 weeks old; TA_{45} = turkeys 45 weeks old.
NS = P>0.05; *** = P<0.001.

Average egg mass in turkeys 34 weeks old was 80.37 g, in turkeys 45 weeks old it was 85.71 g, while the average mass of one day old poults was 52.62 g and 55.80 g respectively. Average egg mass loss until day 25 of incubation was 11.41% (younger turkey flock) and 12.38% (older flock). In the younger flock the poult share in the egg mass was 65.44% and in the older flock it was 65.05% (Table 1).

It is evident from the data in Table 1 that the 34 weeks old turkeys produced eggs 5.34 g lighter than the 45 weeks old turkeys, and this difference was statistically very significant (P<0.001). Likewise, one day old poults of younger turkeys had statistically significant (P<0.001) smaller average mass. Eggs laid by the 34 weeks old turkeys also had smaller absolute (g) and relative (%) egg mass loss until day 25 of the incubation period. Differences were statistically very significant (P<0.001). In contrast to previous indicators, poult share in the egg mass was almost equal and the difference observed (0.38%) was not statistically significant (P>0.05). Based on these facts, it can be concluded that turkey's age made significant impact on the increase of the egg mass, poult mass, absolute and relative egg mass loss until day 25 of incubation, while the percentage of the poult in the egg mass was of similar value in both age groups. Nestor *et al.* (1982), Mróz and Orlovska (2009), Hristakieva *et al.* (2009), Mróz *et al*. (2010), Amir *et al.*(2015), Hristakieva *et al*. (2017) have established that the age of turkeys of different genotypes affects the increase of egg mass and poult mass. Similar conclusion related to other types of poultry (hens, quails, geese and ducks) were made by Abiola *et al.* (2008), Mitrović *et al*. (2010), Egbeyale *et al*. (2011), Yamak *et al* (2015), Iqbal *et al*. (2016), Alkan *et al.* (2008), Đermanović *et al.* (2008), Mitrović *et al*. (2018), as well as El-Hanoun *et al*. (2012) and Đermanović *et al*. (2017).

A slightly different conclusion was reached by Moran and Reinhart (1979) who report that the relative egg mass loss in fertilised eggs until day 25 of incubation did not depend on the age of the layer, being equal in both groups (13.7%). Average mass of one day old male and female poults coming from the older group was equal (54.2 g), while the average mass of female poults laid by the younger turkeys was 53.0 g, and the mass of male poults was 53.3 g (average 53.1 g). Relative poult mass at hatching (in egg mass %) was 62.8% (55th week) and 64.0% (48th week). Research demonstrated a strong correlation between the egg mass and one day old poults mass, as the heaviest poults hatched from the heaviest eggs, and *vice versa*, in both observed groups. Relative poult mass at hatching of the 91.3 g average egg mass was 64.2%, and in 78.2 g eggs it was 62.6%.

Moran and Reinhart (1979) report a significantly larger egg mass loss until day 25 of incubation (13.7%) in comparison to our results (11.41% and 12.38%). Similar egg mass loss until day 25 of incubation (12% - 11.5%) is reported by Meir *et al*. (1984), Tullett (1981), Christensen and McCorkle (1982), and significantly smaller (10%) by Rahn *et al*. (1981). Hristakieva *et al*. (2017) report smaller relative egg mass loss until day 25 of incubation in the younger turkey flock (34 weeks old) in comparison to the older flock (46 weeks old) as well as the larger percentage (share) of the poult in the egg mass, which is to some extent in line with our results.

Phenotype correlation, *i.e.* correlation coefficient matrix between observed indicators and correlation significance is given in Table 3.

Table 3. Correlations between mass and egg parameters

Indicator	***Egg mass (g)***	***Loss until day 25 (g)***	***Loss until day 25 (%)***	***Poult mass (g)***	***Poult% in egg mass***
Egg mass (g):					
Young turkeys (34w)	1.00	0.509^{***}	0.243^{*}	0.777^{***}	0.242^{*}
Old turkeys (45 w)	1.00	0.401^{***}	-0.122^{NS}	0.770^{***}	0.170^{***}
Loss until day 25 (g):					
Young turkeys (34 w)		1.00	0.612^{***}	0.589^{***}	0.217^{NS}
Old turkeys (45 w)		1.00	0.534^{***}	0.405^{***}	0.178^{NS}
Loss until day 25 (%):					
Young turkeys (34 w)			1.00	0.216^{NS}	-0.410^{***}
Old turkeys (45 w)			1.00	-0.121^{NS}	-0.214^{NS}
Poult mass (g):					
Young turkeys (34 w)				1.00	0.691^{***}
Old turkeys (45 w)				1.00	0.466^{***}
Poult % in egg mass:					
Young turkeys (34 w)					1.00
Old turkeys (45 w)					1.00

NS = P>0.05; * = P<0.05; *** = P<0.001.

Positive correlation was determined in both flocks (TA_{34} and TA_{45}) between the average egg mass before incubation and the absolute and relative egg mass loss until day 25 of the incubation period and between newly hatched poults and the relative share of the poult in the egg mass. Correlation between the egg mass and the relative egg mass loss until day 25 of incubation was negative (r_p = - 0.122), but the correlation coefficient was not statistically significant (P>0.05), Table 3. Other correlation coefficients were statistically significant (P<0.05), and very significant (P<0.001). Similar findings were made by Hristakieva *et al.* (2017), however, they report no correlation between the egg mass and poult share in the egg mass. Đermanović *et al.* (2008) report total correlation (r_p = 0.987) between the egg mass before incubation and one day old poults mass, Alkan *et al*. (2008) established strong phenotype correlation (r_p = 0.72) between the Japanese quail eggs and the offspring mass, Yamak *et al*. (2015) determined very strong correlation (r_p = 0.862) between the egg mass and one day old chicken, and Mitrović *et al*. (2018) report total correlation (r_p = 0. 986) between the egg mass and one day old goslings.

Data given in Table 3 demonstrate that strong correlation and medium positive correlation ($r_p = 0.691^{***}$ and $r_p = 0.466^{***}$) was established between the average poult mass and poult share, in both flocks, and the correlation coefficients were statistically confirmed at P<0.001 level. Negative correlation was found between the share of the poult in the egg mass and the relative egg mass loss until day 25 of incubation, in both groups of eggs, *i.e.* in both age groups of turkeys ($r_p = -410^{***}$ i $r_p = -0.214^{NS}$). Similar correlation between these indicators in North Caucasus bronze turkey aged between 34 and 46 weeks was reported by Hristakieva *et al.* (2017).

Conclusion

Based on determined average egg mass and newly hatched poults mass, absolute and relative egg mass loss until day 25 of incubation and the relative poult share in the egg mass, following conclusions can be made:

- Eggs and newly hatched poults originating from the older flock (TA_{45}) had statistically very significantly ($P<0.001$) larger average mass than those coming from the younger turkeys (TA_{34}). Average egg mass before incubation was 80.37 g (TA_{34}) and 85.71 g (TA_{45}), and the average mass of the newly hatched poults was 52.62 g and 55.80 g, respectively.
- Absolute and relative egg mass loss until day 25 of incubation were also bigger in the older flock. Egg mass loss until day 25 was 9.20 g and 11.41% (TA_{34}), and 10.62 g and 12.38% (TA_{45}). Differences were statistically significant ($P<0.001$).
- Contrary to the previous indicators, the difference in the relative share of the poult mass in the egg mass (0.38%) between the flocks was not statistically significant ($P>0.05$).

Calculations of the phenotype correlation coefficients between the indicators during egg incubation originating from two different flocks of turkeys (TA_{34} and TA_{45}), lead to the following conclusions:

- In the TA_{34}group, strong positive correlation ($r_p = 0.509^{***}$) was determined between the egg mass and the absolute egg mass loss until day 25 of incubation, very weak ($r_p = 0.243^{*}$) in case of the relative egg mass loss, very strong in case of the one day old poult mass ($r_p = 0.777^{***}$), and very weak ($r_p = 0.242^{*}$) between the egg mass and the poult share in the egg mass. Similar correlation was found in the TA_{45} group, except between the egg mass and the relative egg mass loss until day 25 of incubation, where the negative phenotype correlation ($r_p = -0.122$) was found, which was not statisticaly significant ($P>0.05$).
- Strong positive correlation was found between the absolute egg mass loss and the relative loss as well as one day old poults, which were statistically significant ($P<0.001$), while very weak correlation was found between the absolute egg mass loss and the poult share in the egg mass and the correlation coefficients in both egg groups ($r_p = 0.217$ and $r_p = 0.178$) were not significant ($P>0.05$).
- Very weak and medium negative correlation was determined between the relative egg mass loss and the poult mass, as well as the poult share in the egg mass in both groups, except between the relative egg mass loss and the poult mass where the positive correlation was found ($r_p = 0.216$) which was not statistically significant ($P>0.05$).
- Strong phenotype correlation (TA_{34}), and medium phenotype correlation (TA_{45}) was found between the average poult mass and the poult share in the egg mass and correlation coefficients were calculated ($r_p = 0.692$ and $r_p = 0.466$) which were statistically significant ($P<0.001$).

Based on this information, we can conclude that turkey's age had significant impact on the increase of egg mass, poult mass, absolute and relative egg mass loss until day 25 of incubation, while the percentage of the poult in the egg mass was of similar value in both age groups of turkeys. Apart from the significant impact of the turkey's age on the increase of average egg mass, egg mass also had a significant impact on the increase of absolute and relative egg mass loss during incubation (until day 25), average one day old poults mass and the poult share in the egg mass, but not on the relative egg mass loss in older turkeys (TA_{45}) where the negative coefficient beween the egg mass and the relative egg mass loss was determined ($r_p = -0.122$), which was not statistically significant. These final conclusions are confirmed by the calculations of the phenotype correlation coefficients between observed indicators, as well as most of the results of other authors (researchers) studying this matter in relation to turkey and other poultry eggs.

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EFFECT OF SUPLEMENTING CHAMOMILE DRY FLOWER MEAL IN WEANED AWASSI MALE LAMBS DAILY RATIONS ON BODY PERFORMNACE AND MEAT QUALITY

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Abstract

An experiment was conducted on 20 lambs during 2016 using chamomile dry flower meal (ch). Animals were randomly allocated into 4 groups [CGCH (0% ch), EGCH2 (2% ch, EGCH4 (4% ch) and EGCH6 (6% ch)] by 5 heads. Animal health as well as palatability of rations was visually acceptable. Initial average live body weight (LBW) of all animals was 26 ± 1.5 Kg. Average LBWG/week was significantly (P<0.05) the best in group EGCH2 (3.42±0.48 kg/week/head). FCR was higher at the end of the trial in EGCH2 (6.09±0.40) where animals were fed with 2% chamomile supplemented to daily ration in comparison with all other groups. On the other hand, after cooling L* meat samples of lamb were better in EGCH2 followed by EGCH4, CGCH and EGCH6. Redness of meat from animals slaughtered decreased insignificantly (P > 0.05) 24h after cooling in all groups CGCH (18.99 ± 4.53 to 15.78 ± 2.99), EGCH2 (21.6 ± 7.8 to 15.4 ± 1.14), EGCH4 (17 ± 2.7 to 14.01 ± 1.97), EGCH6 (20.2 ± 4.43 to 18.32 ± 2.16). Yellowness b* decreased after 24 hours of cooling in all groups. At 24 hours' *post-mortem*, pH of fresh meat at slaughter was significantly (P<0.05) more acidic (5.57±0.054) in CGCH and EGCH6 (5.59) than EGCH2 (5.75) and EGCH4 (5.67). After 1 month of freezing CGCH became less acidic (5.79) in comparison with EGCH2, EGCH4 and EGCH6 averaging the level of 5.72, 5.77 and 5.67, respectively (P>0.05). The study of water holding capacity showed that drip loss was minimal in EGCH6 where the loss was (17.86 % ±1.47). Furthermore, tenderness of cooked meat after 24 hours of cooling was higher (P<0.05) in EGCH6 (8.4mm±0.7) followed by EGCH2 (5.64 mm± 2.1) than CGCH (5.5mm ±0.4) and EGCH4 (5.2 mm ±0.9). It is concluded and recommended to use rations containing chamomile meal in the daily feeding of Awassi sheep.

Keywords: *Awassi lambs, Chamomile dry meal, Body performance, Meat quality.*

Introduction

A major concern in the farming of small ruminants is loss in production, of which one of the main causes has been identified as food deficiency, mainly caused by high cost. Major challenges in such agricultural businesses are to ensure an enhanced net yield and to minimize high expenditure of concentrate feed. Many recent research data (Busquet *et al*., 2005; Jordan *et al*., 2006; Chaves *et al*., 2008; Benchaar *et al*., 2011; Duval *et al*., 2011; Klevenhusen *et al*., 2011 and many others) has been done regarding this aspect, and various strategies have been studied in introducing feed supplements and feed additives. An additive that has been addressed severe criticism is antibiotics (Anthony *et al*., 2001; Anderson *et al*., 2003; Casewell *et al*., 2003; Collignon, 2003). There are some important reasons behind the restriction of the use of antibiotics, some of which are the drug resistance established in bacteria and the drug residues found in meat (Bager, 2000). Elimination of antibiotics from the diet has resulted in poor performance and in an increase in the susceptibility to diseases. Attempts were made to find other alternatives in order to overcome such challenges. One of

which has shown immense interest in recent years is the utilization of growth promoters of natural origin. Odoemelam *et al*. (2013) observed that there are strong indications that herbs, spices and their products exert antioxidative, antimicrobial and growth promoting effects in livestock. The antioxidative effects of some of the herbs and spices in protecting the quality of feed as well as that of food derived from animals fed these substances cannot be ruled out (Odoemelam *et al.*, 2013). Aromatic plants, have been used since ancient times as folk medicine and as preservatives in foods (Christaki *et al*., 2012). The best known aromatic plants, such as oregano, rosemary, chamomile, sage, anise, basil, etc., originate from the Mediterranean area. Currently, the demand for these plants and their derivatives has increased due to the fact that they are natural, eco-friendly and generally recognized as safe products. Therefore, aromatic plants and their extracts have the potential to become new generation substances for human and animal nutrition and health (Christaki *et al*., 2012). Lee *et al*. (2004ab), Ciftici *et al*. (2005), Erats *et al.* (2005) and Zhang *et al*. (2005) have reported that in the new century the use of antibiotic growth promoters as a feed additive has been banned. Such antibiotics have been added to animal feed rations for improving growth performance, disease prevention and for proliferation of useful microorganisms in the intestinal microflora. After banning the use of most antibiotic growth promoters' scientists looked for alternatives. Therefore, they focused on herbs, spices and plant extracts (essential oils- EO) as possible antibiotic alternatives. Their addition to the feed ration or water improved the feed intake, feed conversion ratio, and carcass yield action (Patel *et al*., 2018). From these aromatic plants we can mention chamomile (*Matricaria chamomilla*). It is important to take into consideration when fed to animals that improved animal health can be translated to improved food safety and quality, which benefits the consumer (Sacchetti *et al*., 2005). For this purpose, this study aims to investigate the impact of supplementing sheep with chamomile dry flower meal, as natural phytogenic compounds at various concentrations in basic rations instead of adding antibiotics and antioxidants. These groups of Awassi male lambs are observed for body performance and meat quality as compared to control antibiotic-free group.

Materials and Method

A total of twenty Awassi Sheep, 3 months old, were purchased from Bekaa valley not far from Beirut/Lebanon. The site was designed to study the impact of feeding male sheep different supplementation levels of chamomile dry flowers meal on body performance and meat quality during the fattening period at an ambient temperature of 25°C, water and feed were offered *ad libitum* for the duration preceding the experimental period. When the experiment initiated, all animals were fed the basal ration (BR) for 5 days and with chamomile after allotting them randomly by 5 heads into 4 groups. Animals of the control group (CGch) were fed free choice antibiotic-free and antioxidant-free basal diet in mashed form based on yellow corn-soybean meal mixture and hay. The remaining 3 groups were fed the following experimental rations: Animals of group (EGch2) were fed BR supplemented with 2% Chamomile dry flowers meal; EGch4 received BR with 4% Chamomile dry flowers meal; EGch6 consumed BR with 6% Chamomile dry flowers meal. All experimental rations fed to animals were *iso-proteinic* and isoenergetic not exceeding 18% of crude protein and 2671.7±40.1 MEkcal/kg of feeds. Daily observations of the lambs showed that no health problems were noted. Live body weight (LBW) was measured individually every week using electronic balance (kg). Initial average live body weight (aLBW) of the sheep was 26±1.5Kg with no significant difference ($P>0.05$) among groups. Live body weight gain (LBWG), feed conversion rate (FCR) and cost-price of 1kg mutton produced was calculated on weekly basis. The meat physical properties were studied *(post mortem)* after 24h of cooling and 1 month of deep freezing, where L*, a*, b* colours and pH level were determined using a chromometer and pH-meter by the method of Offer and Knight (1988). Cooking loss was examined

according to Honikel (1998), the thawing loss was determined and the hardness (tenderness) was estimated using a Penetrometer. One way analysis of variance (ANOVA)) of the results obtained were calculated to evaluate the statistical differences between treatments and replicate means using "Sigmastat software V. 3.5". Mean values ± SD were used. Significant effects were further explored using Tukey's multiple range tests to assert the interaction among treatment means. A significance level of P<0.05 was used. Pearson' correlation of different traits was measured whose results was recorded as p<0.05*, P<0.01** and P<0.001***.

Results and Discussion

The results obtained for feed intake (FI) at the end of the experiment were significantly different (P<0.05) in group CGCH where it attained its maximal level (112.8±12.9kg/head) and its minimal (89.9±9.13kg/head) in EGCH6 whose animals were fed basic ration supplemented with 6% chamomile i.e. Palatability of rations and appetite of animals were positively acceptable in all animal groups where no feed rejection was noticed. This can be explained by the fact that decreased feed consumption has attributed to higher flavouring effects of 4% and 6% chamomile flower meal supplementation to BR decreasing feed intake. These results were in opposite to those findings obtained by Kolacz *et al*. (1997), Kamel (2001), Alçiçek *et al*. (2003) and Panda (2005) who applied essential oils of herbs and spices in animal feeding resulting in increased feed intake in comparison with rations exclusive of any herb supplementations. The average live body weight at the initiation of the trial was calibrated (P>0.05) between 26.4±1.13kg, 26.1±0.67, 25.72±2.1 and 26.2±2.2kg in CGCH, EGCH2, EGCH4 and EGCH6, respectively. The highest LBW was recorded in group EGCH2 (42.5±0.89kg) after 8 weeks of feeding BR supplemented with 2% chamomile followed by CGCH (41.2±1.62kg), EGCH6 (40.2±2.43Kg) and EGCH4 (39.42±2.99kg). This was explained by the positive correlation (r=0.928***) of LBW with the FI since animals of CGCH and EGCH2 consumed more and hence there LBW was higher. This reading matches with the results obtained by AL-Kassie *et al*. (2010) and Kolacz *et al*. (1997), that showed a significant improvement of LBW due to the main constituents of the herbs; the supplementation of 2% chamomile dry flowers meals played a role to enhance the activity of thyroxin hormone that accelerates the nutrients metabolites and biochemical reaction in the animal body (Mahmmod, 2013).

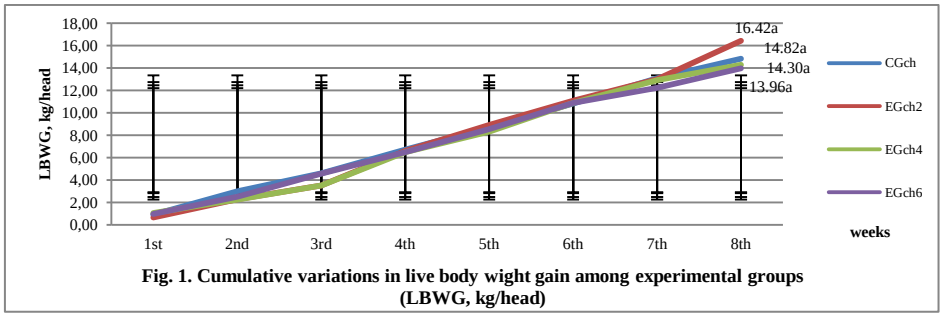

Fig. 1. Cumulative variations in live body wight gain among experimental groups (LBWG, kg/head)

Mean values with letter" a" are insignificantly different (P>0.05)

The recorded results (Fig. 1) of LBWG agree with the data presented by Spernakova *et al*. (2007), who had demonstrated that the addition of Chamomile to BR developed higher body weight gain compared to control group whose animals were fed basic rations with no herb addition due to the active health stimulating elements found in the herbs that show their positive effect on performance. Feed conversion (Fig. 2) was improved significantly (P<0.05) with the addition of chamomile dry flower meal to the basic ration where the most efficient FCR was obtained in EGCH2 (6.09±0.40) vs. CGCH (7.69±1.11). Even though, insignificant data (P>0.05) was obtained between EGCH4 (6.50±0.46) and EGCH6 (6.52±0.84) but still

was lower than CGCH. The obtained results are identical with the findings of Santurio *et al*. (2007) who concluded that when chamomile dry flowers meal are added at a level of 1% of the diet, the body weight gain and feed conversion ratio increased due to the positive impact of the biochemical active components of the herb in the form of antimicrobial, antifungal and antioxidant defense property against harmful body microorganisms.

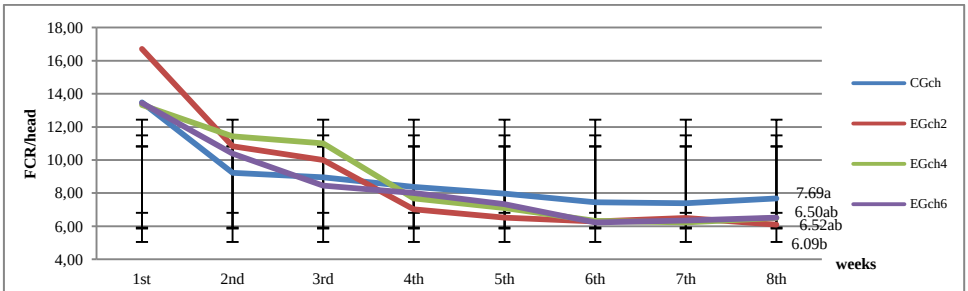

Fig. 2. Cumulative average of weekly variations in Feed conversion ratio among experimental groups (FCR/head)

Mean values with different letters "ab" are significantly different (P<0.05)

The PH have increased in CGCH meat from 5.57 after 24 hours of cooling and then attained the level of 5.79 after 1 month of freezing (P>0.05) in comparison with EGCH2, EGCH4 and EGCH6 averaging the level of 5.72, 5.77 and 5.67, respectively (P>0.05). The slow freezing process leads to development of crystals in muscle tissues. When defrosting, crystals tear the up cells, leading to a greater inter muscular distribution of salinity, thus a decrease in the acidity (P>0.05) was noticed in groups CGCH (5.79±0.045), EGCH4 (5.77±0.08) and EGCH6 (5.67 ± 0.06) and only a slight decrease in salinity in EGCH2 (5.72±0.02). The meat colour was affected by the interaction of myoglobin pigment with the absorbance and reflectance of light (AMSA, 2012). There was a significant difference of luminance L* between the groups, after 24h of cooling where, the highest one was for EGCH2 followed by EGCH4, CGCH and EGCH6. After one month of freezing, L* of mutton increased in CGCH (42.5±1.64 to 49.19±4.9) and EGCH4 (43.2±0.45 to 43.5±3.42), yet in contrast it decreased (P<0.05) in EGCH2 (50.24±3.34 to 39.92±1.07) and EGCH6 (40.118±0.9 to 39.65±1.64) but the higher was for CGCH (49.19 ± 4.9) followed (P<0.05) by EGCH4 (43.5±3.42) and EGCH2 (39.92±1.07). After one month of freezing, redness (a*) continued to decrease in EGCH4 to (13.86) and EGCH6 to (14.95) and increased in CGCH to (17.5) and in EGCH2 to (19.36) while yellowness b* value increased in all groups EGCH4, CGCH, EGCH6 and EGCH2 (13.5, 12.68, 12.3, 10.9, respectively) with a significant difference in EGCH2. The result was logical because of the meat tenderness after 1 month of freezing, where EGCH2 had the more tender meat. The denaturation of myoglobin proteins due to the high temperature effect and the formation of metmyoglobin, was the reason of this drop in redness a* of meat. This obtained result agrees with the study conducted by (Hanczakowska *et al*., 2015) revealing that adding natural antioxidants may improve oxidative stability of the meat, what can be the reason for smaller changes in meat colour. Changes in weight of the meat samples were due to the surface evaporation caused by the differences in temperature and relative humidity between the environment and the meat sample (Cano-Muñoz, 1991). The drip and thawing losses were due to the breakdown of the cell membrane and the diffusion of water outside the cell. After cooling in the refrigerator for 24 hours from slaughter, the mutton loss of their initial weight was significant (P<0.01), where EGCH2 loss was (28.1%±2.79) followed by EGCH4 (27.64%±0.76) and then CGCH (20.23%±2.72). The minimal loss in water appeared in EGCH6 where the loss was (17.86%±1.47). After 1 month of thawing the loss increased in all groups respectively EGCH4 (38.12%), EGCH2 (31.17%) and then CGCH (24.86%) followed by EGCH6 (20.47%), and the difference was highly significant (P<0.01),

especially between EGCH4 and EGCH6. Cooking losses of cooled and frozen samples shows that the cooking loss of frozen samples was higher (P>0.05) than the loss of cooled samples (32.8%>30.8% in EGCH4), (32.2%>23.1% in EGCH2), (31.9%>30.3% in CGCH) and (28.4% 22.4% in EGCH6). Nevertheless, water loss in samples after cooking frozen mutton were significant (P<0.05) between CGCH (higher loss) and EGCH6 (least loss). This decrease in weight or the high cooking loss was very normal due to the decrease in the water holding capacity by the effect of proteins 'denaturation'. Cooking loss and LBWG were found to be negatively correlated (r = - 0.468*). The tenderness of the cooked meat 24 hours after cooling was higher (P<0.05) in EGCH6 (8.4mm±0.7) followed by EGCH2 (5.64mm±2.1), CGCH (5.5mm±0.4) and EGCH4 (5.2mm±0.9). PND (Penetrometer values) of the meat 24 hours after cooling were significantly different between animal groups. Thawing and cooking after one month of freezing, the meat became heavier in all samples with no significant difference (P>0.05), but it was more tender consecutively in groups EGCH2 (5.56mm), EGCH6 (5.43mm), CGCH (5.36mm) and EGCH4 (4.88mm). Tenderness of meat was negatively correlated with the results of the variable L* (r = -0.408*). These results agree with those of Hergenreder (2011) stating that after freezing, cooked meat is tougher than the cooked meat after cooling and in contradiction with the results achieved by Koohmaraie *et al.* (1998) stating that frozen samples cooked after thawing are tenderer than those cooked after cooling.

Conclusion and Recommendations

Scarce results were found regarding local as well as international practical applications in comparing the influence of chamomile additions fed at different percentages: 2, 4 and 6% as a replacement to antibiotic and antioxidant additions. Beneficial effects of chamomile in small percentages mixed with basic rations and fed to Awassi sheep may arise from activation of feed intake and digestive secretions, immune stimulation, anti-bacterial, antiviral or anti-inflammatory activity and antioxidant properties. This situation requires the world to restrict using antibiotic growth promoters in sheep feed and push the Lebanese farmers to follow a new method of feeding their lambs rations supplemented with natural herbs and spices such as chamomile as growth promoters without using synthetic antioxidants.

As a result, this study shows that it is highly recommended to use 2% chamomile flower meals as a supplementation to 100 kg of basal diets in the daily feeds of Awassi sheep during the growth period, as a replacement to antibiotic and antioxidant additives, which has proved to give the meat a better quality mostly similar to that of organic meat which is a major demand of customers nowadays. Lastly, we suggest performing this practice on a commercial level to determine the real economic effect of using natural herbs and spices, such as chamomile, on the body performance and the meat quality. As future work, this study should be conducted on Awassi male sheep fed basic rations with more and less concentrated supplementation (%) revealing the appropriate concentration that leads to higher animal performance.

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EFFECT OF FEEDING THYME DRY MEAL TO WEANED AWASSI MALE LAMBS ON BODY PERFORMANCE

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Abstract

Animals were randomly allocated into four groups [CGth1 (0% th), EGth2 (2% th), EGth4 (4% th) and EGth6 (6% th)] by 5 heads and fed rations containing thyme meal (th). Results showed that initial LBW/head at the beginning of the experiment was insignificantly different averaging to 13.17±1.47 Kg (P>0.05). Average LBWG at the end of 1st week of the trial was insignificantly (P>0.05) lower (0.93±0.25 kg) in CGth1 group in comparison with all other experimental animal groups - EGth2 (1.37±0.29 kg), EGth4 (1.47±0.35 kg) and EGth6 (1.43±0.25 kg) /week/head. This increase became significant (P<0.05) at the end of the trial at 8th week attaining in EGth2 (1.67±0.58kg), EGth4 (2.00±0.00) and EGth6 (2.00±0.00) in comparison with CGth1 (1.00±0.00kg). Note that the highest increase (P<0.05) in weekly LBWG was noted in the 7th week in EGth4 (4.50±0.00 kg). Cumulative LBWG of animals in EGth4 at the end of the trial was significantly (P<0.05) higher (20.33±0.57 kg) than EGth2 (15.67±1.53 kg), EGth6 (15.70±1.15 kg) and CGth1 (14.67±1.16 kg). The most efficient FCR at the end of the experiment was in group EGth4 attaining the level of 2.92±0.16 which was significant to all other groups, CGth1 (3.75±0.52), EGth2 (3.68±0.24) and EGth6 (3.73±0.28). Based on our results, it is expected to have better quality of meat in EGth4 where 4% dry thyme meal was added to the basic ration. It is concluded and recommended to use rations containing thyme meal in the daily feeding of Awassi sheep.

Keywords: *Awassi lambs; Thyme dry meal; Body performance.*

Introduction

One of the main concerns in the farming of small ruminants is the loss in production, and primarily it is caused by the high costs which leaded to food deficiency. Major challenges in such agricultural businesses are to ensure an enhanced net return and to minimize high feed costs. Many recent research data (Busquet *et al*., 2005; Jordan *et al*., 2006; Chaves *et al*., 2008; Benchaar *et al*., 2011; *et al*., 2011; Klevenhusen *et al*., 2011 and many others) has been done regarding this aspect, and various strategies have been studied in introducing feed supplements and feed additives such as antibiotics, an additive that has been severly criticized (Anthony *et al*., 2001; Anderson *et al*., 2003; Casewell *et al*., 2003; Collignon, 2003). There are some important reasons behind the restriction of the use of antibiotics, some of which are the drug resistance established in bacteria and the drug residues found in meat (Bager, 2000). Elimination of antibiotics from the diet has resulted in poor performance and in an increase in the sensitivity to diseases. Attempts were made to find other alternatives in order to overcome such challenges. One of which has shown immense interest in recent years is the utilization of growth promoters of natural origin. Odoemelam *et al*. (2013) observed that there are strong indications that herbs, spices and their products exert antioxidative, antimicrobial and growth promoting effects in livestock. The antioxidative effects of some of the herbs and spices in protecting the quality of feed as well as that of food derived from animals fed these substances cannot be ruled out (Odoemelam *et al.,* 2013). Aromatic plants, have been used

since ancient times as folk medicine and as preservatives in foods (Christaki *et al*., 2012). The best known aromatic plants, such as oregano, rosemary, chamomile, sage, anise, basil, etc., originate from the Mediterranean area. Currently, the demand for these plants and their derivatives has increased due to the fact that they are natural, eco-friendly and generally recognized as safe products. Therefore, aromatic plants and their extracts have the potential to become new generation substances for human and animal nutrition and health (Christaki *et al*., 2012). Lee *et al*. (2004ab), Ciftici *et al*. (2005), Erats *et al*. (2005) and Zhang *et al*. (2005) have reported that in the new century the use of antibiotic growth promoters as a feed additive has been banned. Such antibiotics have been added to animal feed rations for improving growth performance, disease prevention and for proliferation of useful microorganisms in the intestinal microflora. After banning the use of most antibiotic growth promoters' scientists looked for alternatives. Therefore, they focused on herbs, spices and plant extracts (essential oils- EO) as possible antibiotic alternatives. Their addition to the feed ration or water improved the feed intake, feed conversion ratio, and carcass yield action. From these aromatic plans we have chosen the thyme. The Thyme or *Thymus vulgaris* is a medicinal herb in the Lamiaceae family, cultivated worldwide for culinary, cosmetic perennial and medical purposes. The growing interest of consumers in substances of natural origin in addition to the increasing concern surrounding potentially harmful synthetic additives has resulted in the use of aromatic plants, their extracts and essential oils, as functional ingredients in the pharmaceutical, food and feed industries. Plant derived components have considerable potential to fulfil such demands. It is also important to take into consideration that improved animal health can translate to improved food safety and quality, which benefits the consumer (Sacchetti et al., 2005). Therefore, this study aims to investigate the impact of supplementing sheep different aromatic plants such as the thyme, as natural phytogenic compounds at various concentrations in basic rations instead of adding antibiotics and antioxidants. These groups of Awassi male lambs are observed for body performance as compared to control antibiotic group.

Material and Methods

A total of twelve Awassi Sheep, 3 months old, were purchased from Rashaya - Bekaa valley for this experiment. The site was designed to study the impact of feeding male sheep different supplementation levels of thyme meal on body performance and meat quality during the fattening period at an ambient temperature of 25°C, water and feed were offered *ad libitum* for the duration preceding the experimental period. When the experiment initiated, all animals were fed the basal ration (BR) for 5 days and with thyme dry meal supplementation after allotting them randomly by 3 heads into 4 groups. Animals of the control group (CGth1) were fed free choice antibiotic-free and antioxidant-free basal diet in mashed form based on yellow corn-soybean meal mixture and hay. The remaining 3 groups were fed the following experimental rations: Animals of group (EGth2) were fed BR supplemented with 2% thyme dry meal; EGth4 received BR with 4% thyme dry meal; EGth6 consumed BR with 6% thyme dry meal. All experimental rations fed to animals were *iso-proteinic* and isoenergetic not exceeding 18% of crude protein and 2671.7±40.1 MEkcal/kg of feeds. Daily observations of the lambs showed that no health problems were noted, except a slight greenish diarrhea was noticed in CGth1 group. Live body weight (LBW) was measured individually every week using electronic balance (kg). Initial average live body weight (LBW) of the sheep was 13.17±1.08Kg with no significant difference ($P>0.05$) among groups. Live body weight gain (LBWG), feed conversion rate (FCR) and cost-price of 1kg mutton produced was calculated on weekly basis. One-Way analysis of variance (ANOVA)) of the results obtained were calculated to evaluate the statistical differences between treatments and replicate means using "Sigmastat software V. 3.5". Mean values ± SD were used. Significant effects were further

explored using Tukey's multiple range tests to assert the interaction among treatment means. A significance level of P<0.05 was used.

Results and Discussion

The results obtained for feed intake (FI) at the end of the experiment were significantly different (P<0.05) in group EGth4 where it attained its maximal level (59.31±12.9kg/head) and its minimal in CGth1 whose animals were fed basic ration i.e. Palatability of rations and appetite of animals were positively acceptable in all animal groups where no feed rejection was noticed. This can be explained by the fact that decreased feed consumption has attributed to higher flavouring effects of 4% thyme dry meal supplementation to BR decreasing feed intake. These results agree with those findings obtained by Manuel et al. (2009) who applied essential oils of herbs and spices in animal feeding resulting in increased feed intake in comparison with rations exclusive of any herb supplementations. The average live body weight at the initiation of the trial was calibrated (P>0.05) between 13.00±2.00kg, 13.67±0.58, 11.67±1.16, and 14.33±0.58kg in CGth1, EGth2, EGth4 and EGth6, respectively. The highest LBW was recorded in group EGth4 (32±1kg) after 8 weeks of feeding BR supplemented with 4% thyme dry meal and the lowest one in CGth1(27.67±1.155kg). This reading matches with the results obtained by AL-Kassie et al. (2010) and Frankie (2009), and Christaki et al. (2012), that showed a significant improvement of body weight (BW) due to the main constituents of the herbs; the supplementation of 4% thyme dry meal meals play a role to enhance the activity of thyroxin hormone that accelerates the nutrients metabolites and biochemical reaction in the animal body (Mahmmod 2013).

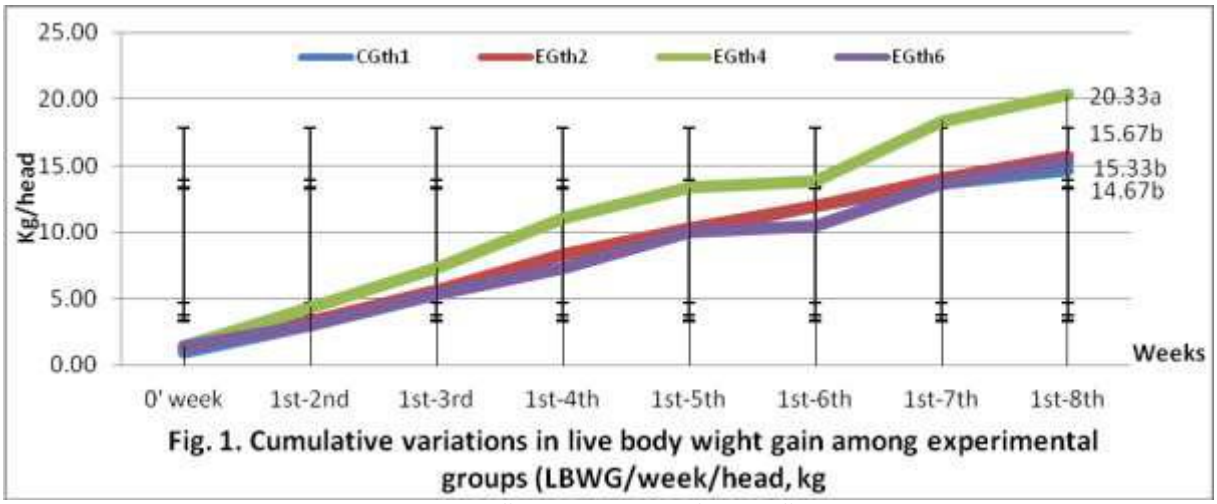

Fig. 1. Cumulative variations in live body wight gain among experimental groups (LBWG/week/head, kg

ab Mean values with different letters are significantly different (P<0.05)

The recorded results (Fig. 1) of LBWG had demonstrated that LBWG changed largely between the four groups during the experimental weeks where it was the highest in EGth4 in many stages of the experiment. Feed conversion (Fig. 2) was improved significantly (P<0.05) with the addition of thyme dry meal to the basic ration where the most efficient FCR was obtained in EGth4 (2.93±0.165ba) vs. CGth1 (3.75±0.524a). Even though, insignificant data (P>0.05) was obtained between EGth2 (3.68±0.24a) and EGth6 (3.83±2.283a) but still was lower than CGth1. The obtained results are identical with the findings of Windisch et al., (2008) who concluded that when thyme flowers are added at a level of 3kg/100kg of the diet, the body weight gain and feed conversion ratio increased due to the positive impact of the biochemical active components of the herb in the form of antimicrobial, antifungal and antioxidant defense property against harmful body microorganisms.

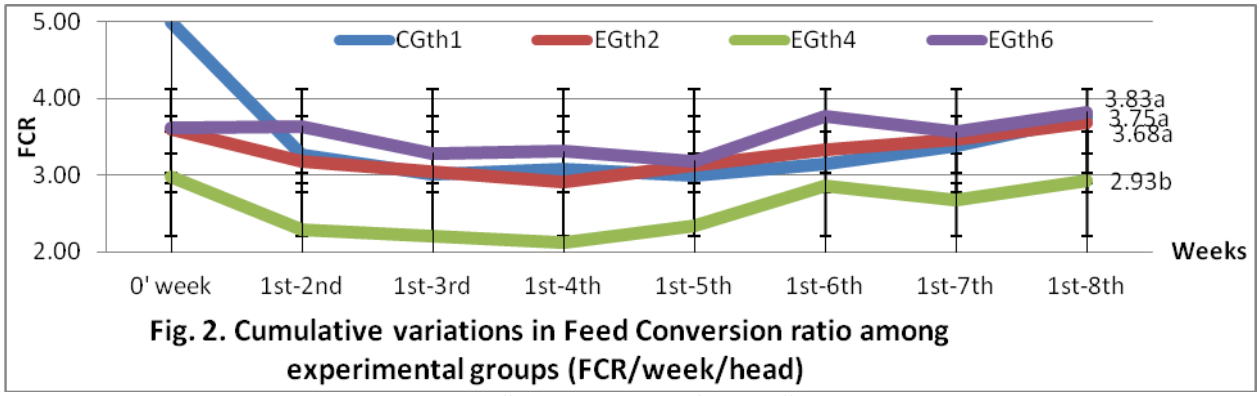

Fig. 2. Cumulative variations in Feed Conversion ratio among experimental groups (FCR/week/head)

ab Mean values with different letters are significantly different (P<0.05)

Conclusion and Recommendations

Scarce results were found regarding local as well as international practical applications in comparing the influence of thyme additions fed at different percentages: 2, 4 and 6% as a replacement to antibiotic and antioxidant additions. Beneficial effects of thyme in small percentages mixed with basic rations and fed to Awassi sheep may arise from activation of feed intake and digestive secretions, immune stimulation, anti-bacterial, antiviral or anti-inflammatory activity and antioxidant properties. This situation requires the world to restrict using antibiotic growth promoters in sheep feed and push the Lebanese farmers to follow a new method of feeding their lambs rations supplemented with natural herbs and spices such as thyme as growth promoters without using synthetic antioxidants.

As a result, this study shows that it is highly recommended to use 4% thyme dry meals as a supplementation to 100 kg of basal diets in the daily feeds of Awassi sheep during the growth period, as a replacement to antibiotic and antioxidant additives, which has proved to give the meat a better quality mostly similar to that of organic meat which is a major demand of customers nowadays. Lastly, we suggest performing this practice on a commercial level to determine the real economic effect of using natural herbs and spices, such as thyme, on the body performance and the meat quality. As future work, this study should be conducted on Awassi male sheep fed basic rations with more and less concentrated supplementation (%) revealing the appropriate concentration that leads to higher animal performance.

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COMPARISON OF PHYSICAL-CHEMICAL INDICATORS OF DIFFERENT MUSCLES OF CARCASS OF FATTENED CALVES OF HORNLESS HERFORD, ABERDEEN-ANGUS AND LIMOUSINE BREEDS

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Abstract

A comparative analysis of physical-chemical indicators of different muscles [*m semitendenosus, m. semimembranosus* and *m. longisimus dorsi*] was made from the carcass of fattened calves of Hornless Hereford, Aberdeen-Angus and Limousine in the meat farm of the Experimental Base at the Research Institute of Mountain Stockbreeding and Agriculture in Troyan, Bulgaria located in the mountain region. Breed and sex affected the main indicators characterizing the physicochemical and quality composition of the meat. Based on the water-holding capacity indicator in %, the best results were found in the fattened calves of Aberdeen-Angus in the three studied muscles. They surpassed Hornless Hereford calves, respectively with 4.09%, 7.48% and 2.36%, and Limousine calves with 11.90%, 17.38% and 13.50%. The muscles of calves from Hornless Hereford had the highest significance of tenderness in penetrant units, followed by the calves of Aberdeen-Angus and Limousine ($P<0.001$).

Protein content had higher values in Limousine calf muscles. The superiority of *m. semitendinosus* is 1.85%, with *m. semimembrenosus* by 0.92% and at *m. longisimus dorsi* was 2.21% of Aberdeen Angus breed. Compared to the muscle content of calves of Hornless Hereford, the values were relatively the same for *m. semiteenosenosus*, and the superiority is 0.92% for *m. semimebranosus* and 1.07% for *m. longisismus dorsi* ($P <0.001$). The fat in the carcass muscle of Limousine calf had the highest values 1.61%, 1.71% and 2.49%, followed by the fat in the muscles of calves of Aberdeen Angus and Hornless Hereford ($P<0.001$).

Keywords: *meat, collagen, calves, meat breeds, muscles, samples*

Introduction

The quantity and quality of beef and veal depend on a number of factors: type, sex, age, diet, breeding system, market and marketing interest (Todorov, 1987; Videv, 1994; Kalev, 2012; Polskih, 2015). The type and breed of animals greatly influence the fattening capacity and meat productivity. In the process of ontogenetic and phylogenetic development, the individual productive types and breeds of cattle have formed such a constitutional type that corresponds to their main productivity (Kadiiski et al., 1987; Todorov, 1987; Videv et al., 1987; Kalev, 2004; Kalev and Dimov, 2012). Allen (1990) and Mandell et al. (1997) assume that the significant differences in the growth potential of cattle of the same age depending on the breed are mostly related to the fat deposition phase. The accumulated valuable information from the experiments carried out so far has to be supplemented by new studies of biological and technological character (Videv, 1994; 1996; Chamboza et al., 2003; Mancini et al., 2005; Miroshnikova et al., 2005; al., 2007; Marinova and Popova, 2011; Tanana et al., 2016). Atanasov et al. (2009) compare fish meat (trout and carp) with veal, pork, lamb, turkey and chicken and report higher protein and vitamin A. The fat content of trout and carp ranges in broad limits. Pinkas and Marinova (1984) in a study of *m. longisismus dorsi* on 484 bulls of different breeds prove that pH values (6.19) are significantly higher than normal and the collagen content has average values that vary widely. Xiang Xie et al. (2012) compare the quality of male calf meat to five breeds – three local Chinese and two foreign – Simmental

and Limousine, slaughtered at 18.5 months of age, taking samples of *m. longisismus dorsi* and found that the breed had a relatively weak effect on most meat parameters. In Bulgaria for veal is considered the meat of fattened male and female cattle up to 2 years of age. The physico-chemical composition is not a constant value during the life of any animal. The physical and chemical composition of veal depends on the breed, sex, age, anatomical location and animal fattening ability. Agricultural establishments in the agriculture and food industry in the Republic of Bulgaria are obliged to comply with the basic requirements of Regulations EC 2073/2005 and EC 1441/2007 on microbiological criteria for food products. The regulations are still recommendatory for Bulgaria (Enikova, 2008). The aim of the present study is to compare the physical-chemical composition of muscles of beef of different genotypes of meat cattle raised in the region of the town of Troyan.

Material and Methods

The subject of the study were three groups of beef calves: 5 male calves of Hornless Hereford, 5 male calves of Aberdeen-Angus and 5 males of Limousine breed raised to 15 months of age in the Experimental Base of RIMSA in Troyan, Bulgaria. The animals were being bred and fed at free will following a suitable meat technology. The groups were equally aligned and raised under the same conditions. The control slaughter of calves from each group was performed according to the method of Zahariev and Pinkas (1979). For the purpose of the experiment, a material was taken from 3 muscles according to a fixed scheme from the left carcass half [m. longissimus dorsi (LL)] obtained from the back, from the cranial edge of the ninth thoraic vertebra to the caudal edge of the 10th thoracic vertebra, [m. semimembranosus SM] taken from the lateral part of the leg and [m. Semitendinosus (ST)] prepacked from the caudal part of leg of fattened calves with a live weight of 450 kg of the three breeds. The carcass weight of the calves varies from 270 to 290 kg. It is compact, rounded and well developed. Its hip portion is broad and level. The thighs are well-formed muscles. The back and loin are broad and dense, almost to the withers. The shoulder-blade, sternum, and vertebrae are significantly muscled. Samples of the above-mentioned muscles were taken after slaughter, placed in a refrigerated bag and transported to the meat laboratory of RIMSA-Troyan for analysis. Three samples were taken from each muscle from the same topographic sections. Separate physicochemical parameters were analyzed with "FoodScan-2000" apparatus (USA). Standard methods for water holding ability of meat (WHC) were used, the tenderness was determined by the method of Grau and Hamm (1953), modified by Pinkas and Marinova (1984) and the mineral substances following Poppandopul et al. (1956).
All data were processed statistically by using MS Excel and presented in a table.

Results and discussion

The results of the investigation of physical and chemical composition of different calf muscles are presented in Table 1.

Table 1. Physical-chemical composition of different muscles of carcass of fattened calves.

Indicators	Veal of different muscles groups											
	Hornless Hereford (n =5)				Aberdeen Angus (n =5)				Limousine (n=5)			
	m.s.t. (I group)	m.s.m. (II group	m.l. d. (III group)	P	m.s.t. (I group)	m.s.m. (II group)	m.l. d. (III group)	P	m.s.t. (I group)	m.s.m. (II group)	m.l. d. (III group)	P
	Physical composition											
pH, (24 hours)	5,39± 0,01	5,51± 0,04	5,48± 0,03	***	6,3± 0,02	6,4± 0,02	6,4± 0,01	***	5,31± 0,02	5,48± 0,02	5,38± 0,03	***
DM, %	23,02± 0,01	22,50± 0,01	21,70± 0,04	**	22,00± 0,14	22,70±0,02	21,32±0,02	**	25,08±0,01	24,64±0,02	26,59±0,01	*
Water, %	76,98± 0,02	77,50± 0,05	78,30± 0,03	*	78,00± 0,01	77,30±0,02	78,68±0,03	**	74,92±0,02	75,36±0,01	73,41±0,02	*
WHC, %	33,99±	32,69±	39,24±	***	38,08±	40,17±0,07	41,60±0,03	***	26,90±0,01	22,88±0,33	27,75±0,22	***

	0,02	0,02	0,33		0,01							
Tenderness penetrant units	161,1± 0,73	141,3± 0,22	167,0± 1,17	***	140,2± 0,13	151,2±0,29	153,6±0,16	***	146,7±0,12	134,2±0,22	135,4±0,13	***
DM / Water	0,30:1	0,29:1	0,28:1	**	0,28:1	0,29:1	0,27:1	**	0,34:1	0,35:1	0,35:1	**
Chemical composition												
Protein, %	22,57± 0,15	20,48± 0,02	21,13± 0,03	**	20,65± 0,14	20,34±0,19	19,99±0,04	**	22,58± 0.02	21,49± 0.02	22,23± 0.03	**
Collagen, %	1,49± 0,02	1,11± 0,02	1,89± 0,02	*	1,47± 0,03	1,1± 0,01	1,1± 0,09	*	1,38± 0.02	1,35± 0.02	1,35± 0.01	*
Fat, %	1,00± 0,17	1,20± 0,21	1,46± 0,02	***	1,12± 0,17	1,44± 0,15	0,92± 0,24	***	1,61± 0.02	1,71± 0.15	2,49± 0.017	***
Ca, mg/%	0,47± 0,01	0,51± 0,03	0,40± 0,01	*	0,45± 0,02	0,40± 0,02	0,60± 0,03	*	0,46± 0.01	0,53± 0.01	0,57± 0.02	*
P, mg/%	0,021± 0,002	0,037± 0,002	0,021± 0,002	***	0,04± 0,002	0,04± 0,002	0,06± 0,003	**	0,023± 0.002	0,04± 0.002	0,06± 0.003	***
Mineral substances, mg/%	0,92± 0,14	1,08± 0,14	1,08± 0,21	**	1,08± 0,14	1,08± 0,13	1,07± 0,14	**	1,08± 0.15	1,07± 0.17	1,08± 0.21	*
M/proteis	0,044:1	0,052:1	0,069:1	**	0,054:1	0,071:1	0,035:1	**	0,072:1	0,080:1	0,112:1	**
M/Water	0,013:1	0,015:1	0,019:1	**	0,014:1	0,019:1	0,012	**	0,021:1	0,:022:1	0,377:1	**

P<0,05* P<0,01** P<0,001***

The polydisperse system is characteristic for veal. Death in the slaughter of cattle leads to the breakdown of the buffer system of their organism. The normal pH of carcasses of calves after slaughter was 7-7.2, gradually decreasing to an acidic reaction. pH affects the meat colour. At pH 5.6 beef had a bright, colourful colour. With an increase in pH above 6.5, the colour usually changes to dark with different variants. pH 24 of the medium showed higher values for all three samples of *m. semltendenosus*, *m. semimbrannosus* and *m. longisimus dorsi* in Aberdeen-Angus breeds, respectively for the first with 0.91, the second muscle with 0.89 and for the longest muscle with 0.92 points in comparison with the breed Hornless Hereford and 0.99 for the first with 0.92 for the second and 1.02 points for the third compared to Limousine, but it was within the permissible (Pinkas and Marinova, 1984).

The dry matter expressed in % was the highest in calf muscle Limousine for *m. semiteenosenosus* by 2.5%, for *m. semimembrenosus* by 2.1% and for *m. longisimus dorsi* with 4.18% compared to the calf of the breed Hornless Hereford and with 2.5% for *m. seitendenosus*, 2.1% for *m. semimranranusus* and with 4.8% for *m. longisimus dorsi* for Aberdeen Angus calves.

The water content was with the highest values in the muscles of the Aberdeen-Angus calves, respectively 78.00%, 77.3% and 78.68%, and with the lowest values in the muscles of Limousine calves, respectively 74.9%, 75.3% and 73.4%.

The ratio of dry matter: water had a direct relation to the juice of meat in the examined muscles. It varied from 0.27: 1 to 0.35: 1 in the tested samples of the three breeds. It was most favourable in the tested muscles of Limousine breed: 1: 0.34, 1: 0.35 and 1: 10.35.

Water holding capacity of water in % was higher for muscles of Aberdeen-Angus calves with 4.09% for *m. semiteenosenosus*, with 7.48% for *m. semimembranusus*, and 2.36% for *m. longissimus dorsi* compared to the muscles of the calf of the breed Hornless Hereford. Higher were the differences in the values between (WHC) in the muscles of calves of Aberdeen-Angus breed compared to those of Limousine breed respectively for *m. semiteenosenosus* 11.9%, *for m. semimranranusus* 17.37% and for *m. longisimus dorsi* 13.5%. Water holding capacity is a factor reflecting the quality of veal. Juiciness, tenderness, taste, as well as some technological properties depend on the amount of bound water (P<0.001).

Meat tenderness was higher in the muscles of calves of Hornless Hereford for *m. semitendenosus* with 20.90 penetrant units and for *m. lomgisimus dorsi* with 13.4 penetrant units. In *m. semimembranusus*, however, had this advantage for Aberdeen-Angus breed with 9.9 penetrant units. There were significant differences in values compared to muscles of Limousine calves, respectively 14.4 penetrant units for *m. semiteenosenosus* (ST), 7.2

penetrant units for *m. semimranranusus* and 31.6 penetrant units per *m. longisimus dorsi* (P<0.001).

Collagen is a connective tissue protein, incomplete protein, insoluble in water, and salt solutions associated with the formation of sarcolemma. It was the most common in the body, up to 1/3 of all available proteins. The muscles of Hornless Hereford showed superiority – at m. semitendinosus (ST) of 0.2%, at m. semimembranosus (SM) with 0.01% and in the longest muscle with 0.79%, compared to the muscles of Aberdeen-Angus calf, the differences being relatively minimal.

Protein content had higher values in Limousine calf muscles. The superiority of *m. semitendinosus* (ST) was 1.85%, for *m. semimembrenosus* was 0.92% and for *m. longisimus dorsi* is 2.21% compared to Aberdeen-Angus breed. Compared to the muscle content of Hornless Hereford's calves, the values were relatively the same for *m. semiteenosenosus*, and the superiority was 0.92% for *m. semimebranosus* and 1.07% for *m. longisismus dorsi* (P<0.001).

The amount of fat is an indicator that is involved in determining the nutritional value of veal. Fat in muscles in Limousine breed surpassed those of the calves of Aberdeen-Angus breed. It was with 0.49% for *m. semitendenosus*, for m. semimembranosus with 0.27% and for *m. longisimus dorsi* with 1.57%, the differences were larger with the examined muscles of Hornless Hereford – 0.61%, 0.51% and 1.03%.

The fat: protein ratio was the highest in muscles of Limousine breed, as it is 0.072: 1 for *m. semiteen,* 0.080: 1 for *m. semimeterranus* and 0.112: 1 for *m. longisimus dorsi*. Aberdeen-Angus breed occupied an intermediate location with the following results for the tested muscles: 0,072: 1 for *m. semiteenosus*, 0,080: 1 for *m. semimebranosus* and 0,112: 1 for *m. longisimus dorsi*. The lowest values of the ratio were observed in the muscles of Hornless Hereford, 0.044: 1 for *m. semiteenosenos*, 0.052: 1 for *m. semimranranusus* and 0.069: 1 per *m. lõngisimus dorsi*.

The Fat: Water Ratio determines the "Maturity" index in the muscles of the three breeds tested. This indicator ranged from 0.012: 1 to 0.377: 1, with a significantly higher value in the muscle samples of Limousine calves – 0.021: 1 0.022: 1 and 0.377: 1.

The investigated mineral substances ranged from 0.92 mg /% to 1.08 mg/% in the muscles of the three breeds and were within normal range. Beef is one of the most important sources of phosphorus in the human body. Its level in the examined muscles ranged from 0.021 mg /% to 0.06 mg/%. Calcium (Ca) was involved in various metabolic processes of the human body. Its level in the examined muscles of the three breeds ranged from 0.40 mg/% to 0.51 mg/%.

The obtained by us data are similar in value and correspond to the results of Xiang Xie et al. (2012), Polskih (2015) and Tanana et al (2016).

Conclusion

Breed, sex and genotype affect on the main indicator characterizing the physical-chemical and qualitative composition of the various muscles of beef calves. The physical-chemical properties of veal determine its importance for human nutrition, diet and culinary use of people.

The water-holding state of water in % is higher for calve muscles in Aberdeen-Angus breed, respectively 4.09% for *m. semitendenosus*, with 7.48%, for *m. semitendenosus,* with 7.48% for *m. semimembranusus*, and 2.36% for *m. longissimus dorsi*, compared to the muscles of calves of Hornless Hereford breed. Higher are the differences in the values between in the muscles of calves of Aberdeen-Angus compared to those of Limousine breed respectively for *m. semitendenosus* 11.9%, for m. semimembranosus 17.37% and for *m. longisimus dorsi* 13.5%. Meat tenderness is higher in the muscles of calves of Hornless Hereford for *m. semitendenosus* with 20.90 penetrant units and for *m. lomgisimus dorsi* with 13.4 penetrant

units. The tenderness in *m. semimembranusus*, has 9.9 penetrants units higher in Aberdeen-Angus breed compared to the other two breeds. Protein content has higher values for Limousine calf muscles. The superiority of m. semitendinosus is with 1.85%, in *m. semimembrenosus* is with 0.92% and for *m. longisimus dorsi* is with 2.21% compared to Aberdeene Angus breed. Muscles in Limousine breed are superior in fat content in comparison to muscles groups of calves of Aberdeen-Angus breed. In *m. semitendenosus* with 0.49%, for m. semimembranosus with 0.27% and for *m. longisimus dorsi* with 1.57%, the differences in the examined muscles of Hornless Hereford are greater – 0.61%, 0.51% and 1.03%.

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EFFECT OF FEEDING WEANED AWASSI MALE LAMBS WITH FABA BEANS (*VICIA FABA*) AS COMPARED TO SOYBEAN MEAL ON BODY PERFORMANCE

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Abstract

As the world population continues to increase, so does the demand for protein. Researchers predicted that traditional sources of protein for livestock would become increasingly scarce because of the need to feed a progressively larger human population that will compete with an expanding intensive livestock industry that is being forced to exclude animal protein sources in animal feeds. As a result, a considerable effort has been made to explore the use of plant proteins that can be grown in areas where soybeans are not agronomical successful. A trial was conducted in "Al Jarrah animal farm" during May- July of 2016 at Bekaa Valley for 8 weeks to fatten fifteen male Awassi sheep lams after weaning fed concentrate mix containing soybean meal (SBM) and Fava seed meal (FSM) as legume-protein source in different proportions in isocaloric and iosprotienic rations. Five different rations were under investigation were fed to five groups by three lambs each under the same management and environmental conditions: A1 (25% SBM: 75% FBS), A2 (50% SBM: 50% FBS), A3 (25% SBM: 25% FBS), A4 (100% FBS: 0% SBM) and A5 –control (0% FBS: 100% SBM). Best results were obtained in group A2 and A3 with feed conversion ratios (FCR) 4.6 and 4.4, final live body weight gain (LBWG) 10.9 and 10.8 kg and profit of 1.27 $/1 kg of LBWG and 1.19 $/1 kg of LBWG, respectively. No signs of antinutritional or gastro-intestinal disturbances were observed among all experimental groups.

Keywords: *Awassi lambs; Faba beans; Soybean; Body performance.*

Introduction

As the world population continues to increase, so does the demand for protein. Farrell (1997) and Magoda and Gous (2011) predicted that traditional sources of protein for livestock will become increasingly scarce because of the need to feed a progressively larger human population that will compete with an expanding intensive livestock industry that is being forced to exclude animal protein sources in animal feeds. As a result, a considerable effort has been made to explore the use of plant proteins that can be grown in areas where soybeans are not agronomically successful. Evans *et al*. (1972) noted that Vicia faba is an important crop in those areas of the world where cereal monoculture is practiced and soybeans cannot be produced economically as it requires a cool season for best development.

Duc (1997), Haciseferogullari *et al*. (2003) and Hossain and Mortuza (2006) published that the nutritional value of faba bean has always been traditionally attributed to its high protein content, which ranges from 27 to 34% depending on genotypes. Most of these proteins comprise of globulins (79%), albumins (7%) and glutelins (6%). They added that, Legume seeds contain several comparatively minor proteins including trypsin inhibitors, lectins, lipoxygenase and urease, which are relevant to the nutritional quality of the seed.

Many researchers as McDonald *et al*. (1973), Liener (1976), Cerioli *et al*. (1998), Antongiovanni *et al*. (2002), Matthews and Marcellos (2003)), Martinez *et al*. (2005), Morbidini *et al*. (2005), Dvořák *et al*. (2006), Esenwah and Ikenebomeh (2008) who studied

the effect of using FBS as an alternative to SBM found no adverse effect in including it in the daily rations of ruminants.
The objective of the present paper is to evaluate the influence of replacing totally or partially soybean meal with faba beans in rations fed to weaned lambs of local Awassi sheep on cost price of meat and some traits of body performance.

Material and Methods

This trial was conducted in Bekaa Governorate/Lebanon 5 Km of Zahle, 75 km from Beirut during May-June 2015. Relative humidity (RH%) and environmental temperature (ºC) recorded during this period of the year ranged between 50.3 - 76.9 and 16.6 - 24.6, respectively.
Fifteen 100-120 days weaned Awassi male lambs weighing 23.33 ± 0.52 kg/head started a fattening experiment. Animals were dipped and treated for all kinds of helmentic worms and vaccinated against all prevailing diseases in the area. The animals were in good health. Each animal-group was fed free choice forage feeds (about 1 kg/head of wheat straw) and around half kg/head of the experimental mix-rations and free access to fresh water. All rations were isocaloric (2.9 Kcal/kg ME) and adjusted to the same level of crude protein (17%) as recommended by NRC (1989) and based on cotton seed meal (CSM), wheat bran and corn, fed continuously with different ratios of Soybean meal (SBM) : Dry milled Fava bean seeds (FBS) for the whole experimental period as shown in table 1. A5 animal-group was fed basic ration (BR) exclusive of FBS, however A1, A2, A3, A4 received BR containing different ratios of SBM to FBS- 75%SBM: 25% FBS, 50% SBM: 50% FBS, 25% SBM: 75% FBS and 0% SBM: 100% FBS, respectively (table 1).

Table 1. Composition of the experimental rations (% as fed basis) fed to each animal-group with their cost-prices ($/ton)

		A1	A2	A3	A4	A5 (Control)
Cost price of Ingredients ($/ton)	**Ingredients**	**75% SBM+ 25%FBS**	**50% SBM+ 50%FBS**	**25% SBM+ 75%FBS**	**100% FBS**	**100% SBM (Basic ration-BR)**
600	SBM	11.1	7.4	3.7	0.0	14.8
325	CSM	7.4	10.0	13.0	15.8	5.1
350	FBS	3.7	7.4	11.1	14.8	0.0
150	Wheat bran	16.0	15.0	12.7	12.3	14.7
250	corn	61.7	60.1	59.4	57.1	65.4
	Total	100.0	100.0	100.0	100.0	100.0
	CP	16.8	16.8	16.8	16.8	16.8
	ME Mcal/kg	2.9	2.9	2.9	2.9	2.9
	Cost price of rations ($/ton)	282	276	271	264	291

Data obtained was analyzed using the analysis of variance (ANOVA) procedure of Sigmastat v.3.5 (Jandel corp. 2004) with $P<0.05$ statistical significance of treatment effects. Live body weight gain of each animal (LBWG/head), Feed intake of each animal-group (FI/group) and apparent feed intake of each lamb (aFI/head) and food conversion ratio of each group (FCR/group) and aFCR/head, were weighed and calculated weekly. Interaction of treatment means was tested using the All Pairwise Multiple Comparison Procedures (Duncan's Method) at 5% significance level. Mean ± SD (Mean values of the traits ± Standard Deviation) was

used in all obtained statistical studies. Cost-price of LBWG obtained ($/1 kg) was calculated to explore the feasibility of the study.

Results and Discussion

Random distribution of all animals resulted in almost equal average weight among the five animal groups. Difference in the initial Live Body weight (W_o) was statistically insignificant ($P>0.05$) where it averaged between 22.8 ± 0.41 kg in group A4 and 23.7 ± 0.35 kg in group A2.

No health problems were noticed and palatability of feeds (FI) was acceptable as inspected daily where no left behind ration remaining was collected. No signs of indigestion or diarrhea or any blood signs in manure were observed.

The amount of concentrate mix fed to animals did not exceed 49.6 kg/animal group for the whole period of the experiment. In 1st week the apparent daily feed intake (adFI/head) per animal in all groups averaged to 729 g/head whereas, at the end of 8th week this value increased to 1032 g/head. At the beginning of each consecutive week all animals of each group were weighed and fed daily mix concentrates (kg) in calculation to 3% of total live body weight obtained.

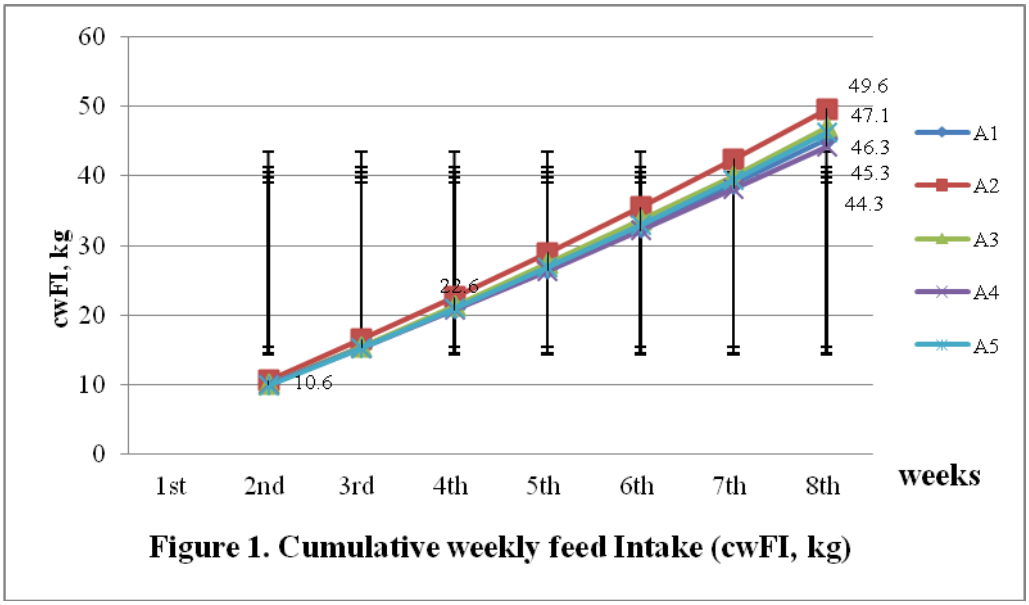

Figure 1. Cumulative weekly feed Intake (cwFI, kg)

Fig. 1 shows the weekly cumulative feed intake among all animal groups. Even though FI at the end of the 1st week was the same among groups it is shown that with the beginning of the 3rd week, 2nd group started to consume feeds (16.5 kg) by 8% more than all others and continued to attain this increase in all weeks of the experiment.

At the end of 1st month of the experiment animals were consuming 20.9 kg, 22.6, 21.3, 20.7 and 20.9 kg in groups A1, A2, A3, A4 and A5 respectively. Besides, it was recorded a decrease in feed consumption in group A4 with 100% inclusion of FBS to ration in all consecutive weeks beginning from 4th week by 0.8% and ending by 4.3 % in comparison with the results obtained in group A5 with 100% SBM inclusion. Moreover this tendency was also observed at the end of the trial in group A1 (75% SBM: 25% FBS) attaining the amount of 45.3 kg Vs 49.6, 47.1, 46.3 kg in groups A2, A3 and A5, respectively.

Looking deeper at the results obtained it was noticed that at the end of the 1st week of the experiment a significant increase ($P<0.05$) in body gain for group A2 (225 g/day) was observed in comparison with A4, A1, A5 and A3 attaining the levels of 106.7 g/day, 131.7, 166.7 and 173.3 g/day, respectively. Although in the 2nd week there was a small decrease (1.5%) in dLBWG in animal group A2 but still the gain was higher ($P<0.05$) than any other

group by 50.4 %, 34.6 %, 23.3 % and 15.8 in A4, A1, A5 and A3, respectively. Even though the health status of animals in all groups was acceptable with no signs of malnutrition and infectious diseases this decrease in dLBWG continued in A2 where it became stable beginning from week six, attaining the level of 183.3 g/day. Results obtained at the end of the experiment showed ($P<0.05$) an overall LBWG of 6.5 ± 0.3 kg/head in group A4 followed by A1 (8.5 ± 0.2), A3 (10.8 ± 0.9) and A2 (10.9 ± 1.1 kg/head). Although, body gain in A5 (9.9 ± 0.8 kg/head) was higher than A1 (8.5 ± 0.2 kg/head) but this increase was insignificant ($P>0.05$).

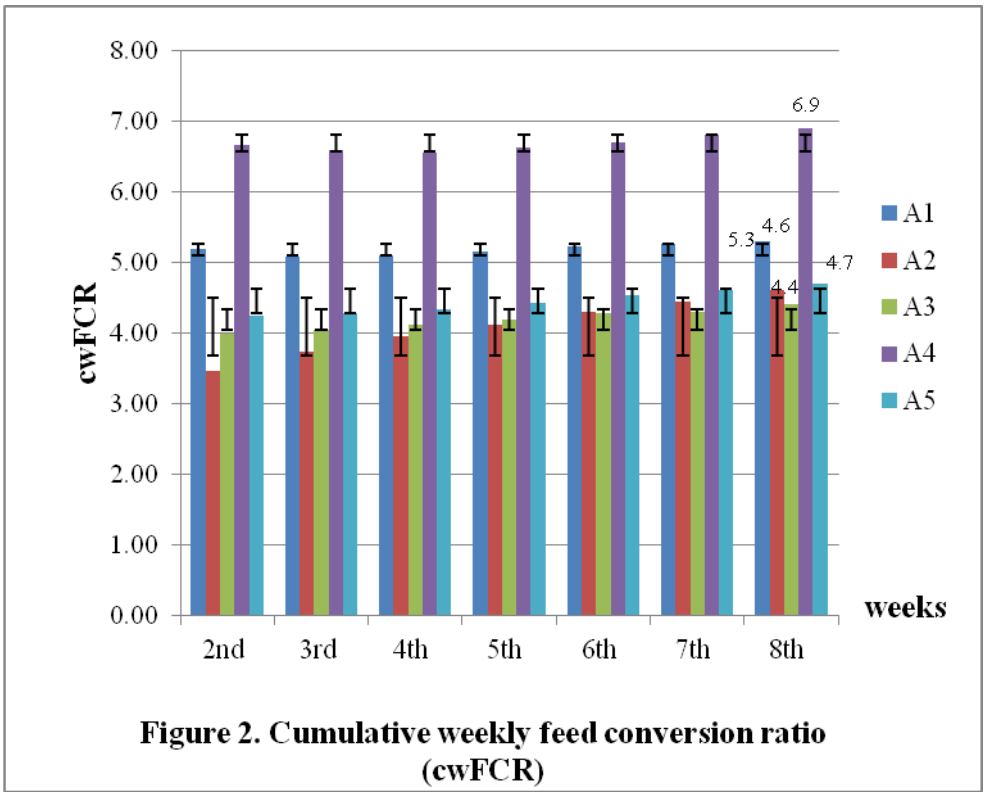

Figure 2. Cumulative weekly feed conversion ratio (cwFCR)

Fig. 2 illustrates the over-all cumulative FCR among experimental groups. The most effective FCR value ($P<0.05$) was recorded in A2 animals (4.6 ± 0.45) in comparison with A1 (5.3 ± 0.14) and A4 (6.9 ± 0.39). Moreover, the least FCR value ($P<0.05$) was observed in animal group A4 (6.9 ± 0.39) in comparison with groups. This can be explained by consuming more feeds to convert them to body weight gain and at the same time getting less live body weights due maybe to the overall effect of antinutritional factors found in fava bean seeds which was included as a sole legume ingredient (100 % FBS) in the basic ration fed to A4 lambs. Moreover, more efficient results were achieved in groups A2 and A3 whose animals were fed a combination of SBM: FBS in different proportions. This can be explained by the findings reported by Miller (1980) that fava bean protein is highly soluble in the rumen and rapidly degraded. Whereas, Emiola and Gous (2011) agreed that Fava bean feeding at various levels did not affect digestibility. However, Fulpagare (1993) concluded that as the level of fava bean increase (from 25 to 100%) in the diet of lambs, the digestibility of dry matter (Ether extract and crude fiber) increase, while that of nitrogen-free extracts (NFE) decreases.

After calculating the cost ($) of FI and LBWG it was found that feeding rations containing 100% FBS exclusive of SBM and 25% FBS in combination with 75% SBM to Awassi lambs is totally unfeasible as in group A4 (1.82 $/1 kg of LBWG) and A1 (1.50 $/1 kg of LBWG). The most efficient and feasible use of FBS was obtained in groups A3 (25 % SBM: 75 % FBS) and A2 (50 % SBM: 50 % FBS) where there cost price was the lowest, 1.19 $/1 kg of LBWG and 1.27 $/1 kg of LBWG, respectively.

Conclusion and recommendations

To simplify our discussion it was concluded, that there are opportunities for researchers to increase animal productivity through the application of appropriate technologies, particularly in production systems, nutrition and feeding potential used of local feed resources for ruminants. Researchers have emphasized the utmost importance of using local feed resources as the key driving force to increase the productivity of animals in Asia (Wanapat, 2009; Wanapat *et al*., 2011).

As it was observed, neither in all our findings including dry fava seed meal (FSM) coarsely grind in combination with the traditional soybean meal (SBM) has no adverse effect neither on live body weight gain (LBWG) and feed intake (FI) nor on feed conversion ratio (FCR). For that reason we recommend a combination of 50% SBM: 50% FBS (as in group A2) or 25% SBM: 75 % FBM (as in group A3) to be included in concentrate rations resulting in higher live body weight gains and feed conversion rate as well as profit. Further investigation is needed to detect the influence of such combinations on mutton quality (chemical and physical) of lambs and goats as well.

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THE EFFECT OF FEEDING WEANED LOCAL MALE KIDS GOATS "BALADI BREED" WITH FABA BEANS (*VICIA FABA*) AS COMPARED TO SOYBEAN MEAL ON BODY PERFORMNACE

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Abstract

The fattening study was carried out during May- July of 2017 in "Ghattas goat farm" located in Bziza, North-Lebanon, for 10 weeks and involved 15 intact male kids of the local "Baladi" goat breed after weaning. The trial included a 2-week adaptation period to the diet followed by 8 weeks of fattening by feeding five experimental diets. The goats were at 13-14 weeks of age and had at the beginning of the study 14.90 ± 0.259 Kg of live body weight (LBW). Each animal group Kids (n=3 replicates) was fed with one of the five dietary treatments: Ag1- FBS (Faba bean seeds) partially replaced 25% of the soybean meal (SBM); Ag2 - FBS partially replaced 50% of the legumes (SBM); Ag3- FBS partially replaced 75% of the legumes (SBM); Ag4 - FBS totally replaced 100% of the legumes (SBM) and Ag5 (control) – this ration was composed of 100 % SBM. Apparent feed intake (aFI), live body weight gain (LBWG) and apparent feed conversion ratio (aFCR) were calculated weekly. It was found that dry fava seed meal (FBS) coarsely grind in combination with the traditional soybean meal (SBM) had no adverse effects either on live body weight gain (LBWG) and feed intake (FI) or on feed conversion ratio (FCR). A combination of 25% SBM: 75% FBS (as in-group Ag3) to be included in concentrate ratios resulted in higher live body weight gains and feed retention as well as profit.

Keywords: *Awassi lambs; Faba beans; soybean; body performance.*

Introduction

Faba beans though are less consumed in western countries as human food, it is considered as one of the main sources of cheap protein and energy in Africa, parts of Asia and Latin America, where most people cannot afford meat sources of protein (Duc, 1997; Haciseferogullari *et al*., 2003). The crop is also becoming increasingly important in Saudi Arabia diets due to the high lysine content of the seed, which encourages the use of faba bean as a protein supplement for cereals (El-Fiel *et al*., 2002; Alghamdi, 2003). The need for alternative protein sources to soybean meal (SBM) in domestic animal feeding has recently gained focus. The main reasons include the attempt to limit SBM import from extra-EU countries, which represents a negative voice of the commercial balance; an effort to decrease costs of animal production and contemporarily reduce the loss of N compounds in the environment and the search to prevent the presence of GMO (genetically modified) in the food chain (Wilkins and Jones, 2000; Mordenti and De Castro, 2005; Mordenti *et al.*, 2007). Among the possible protein sources, lupins, peas and faba beans were successfully used in ruminants and non ruminants (Burel *et al.*, 2000; Bonomi, 2005; Masoero *et al.*, 2006; Vandoni *et al.*, 2007). Outstanding among other legumes, faba bean plays a critical role in crop rotations. The nutritional value of faba bean has always been traditionally attributed to its high protein content, which ranges from 27 to 34% (Duc, 1997; Haciseferogullari *et al*., 2003) depending on genotypes. As reported by Hossain and Mortuza (2006) most of these proteins comprise of globulins (79%), albumins (7%) and glutelins (6%). The aim of our experiment was to inspect the influence of replacing totally or partially soybean meal with

faba beans in rations fed to local "Baladi" weaned kid goats on health and some traits of body performance as well as economical feasibility. To our knowledge, the present study is among the firsts to focus on the effect of feeding FBS on body performance in local goat "Baladi" breed in fattening production.

Material and methods

This study was carried out with the approval of an Ethical Review Process and under the Animals (Scientific Procedures) Act 1986. The trial was conducted during May-June months-2017 for eight consecutive weeks on fifteen weaned male kids of local "Baladi" goat kids in Bziza at "Ghattas animal farm" in North-Lebanon, 100 km from Beirut. The experimental animals were distributed randomly into five groups by 3 animals each under typical ecological and management conditions of environment (air speed, humidity and temperature) and fed five experimental rations as shown in Table 1. Good quality wheat straw was fed *ad libitum*; clean fresh water and mineral blocks (lickers) were available all the time inside the animal pens (2 x 2 m^2)**.** Kids with 13-14 weeks of age were used having an average live body weight (LBW) at the beginning of the trial (data collection) of 14.90±0.259Kg. During the preparatory period (2 weeks) of adaptation the animals were ear tagged and vaccinated against Anthrax and FMD; Albendazole was administered as prevention for digestive tract parasites. Veterinary inspection was repeated every week where intramuscular injections of multivitamin dozes (A, D & E) were performed. Each animal-group was fed free choice forage feeds (about 1 kg of wheat straw) and around half kg of the experimental mix-rations. Ag1- FBS (Faba bean seeds) partially replaced 25% of the soybean meal (SBM); Ag2 - FBS partially replaced 50% of the legumes (SBM); Ag3- FBS partially replaced 75% of the legumes (SBM); Ag4 - FBS totally replaced 100% of the legumes (SBM) and Ag5 (control-basic ration- BR) – totally composed of 100 % SBM. All rations were isocaloric (2.9 Kcal/kg ME) and adjusted to the same level of (CP) crude protein (17%) as recommended by NRC (1981) and based on cotton seed meal (CSM), wheat bran and yellow corn, fed continuously with different ratios of Soybean meal (SBM): Dry grind Faba bean seeds (FBS) for the whole experimental period. Data obtained was analyzed using the analysis of variance (ANOVA) procedure of Sigmastat v.3.5 (Jandel corp. 2004) with $P<0.05$ statistical significance of treatment effects. Live body weight gain of each animal (LBWG/head), Feed intake of each animal-group (FI/group) and apparent feed intake of each lamb (aFI/head) and food conversion ratio of each group (FCR/group) and aFCR/head, were weighed and calculated weekly. Animals were fed on the base of 3% of live body weight recalculated as an average after weighing all animals at the beginning of each following week. Interaction of treatment means was tested using the All Pairwise Multiple Comparison Procedures (Duncan's Method) at 5% significance level. Mean±SD (Mean values±Standard Deviation) was used in all obtained statistical studies. Cost price of LBWG obtained ($/1 kg of meat produced) was calculated to explore the feasibility of the study. This level of CP and energy was selected on propose to make it easier to compare the results obtained with those achieved in another fattening trial on Awassi lambs that will study the physical and chemical quality of meat in both trials. Animals were assigned to the following five experimental rations as shown in table 1 below.

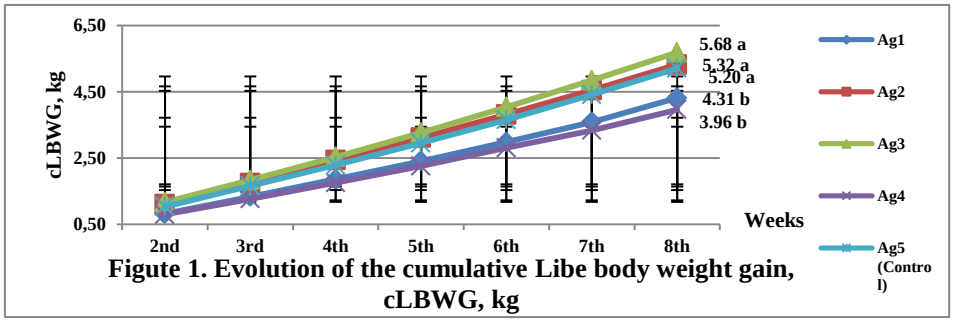
Results and Discussion

The difference in the initial live body weight (LBW) of all experimental animals (14.90±0.26Kg) were statistically non-significant ($P>0.05$) calibrating from 14.77±0.32kg in Ag5 group to 15.03±0.21kg as in-group Ag1.

Table 1. Composition of the experimental rations (% as fed basis) fed to each animal-group with their cost-prices ($/ton)

		Ag1	Ag2	Ag3	Ag4	Ag5 (Control)
Cost price of Ingredients ($/ton)	Ingredients	75% SBM+ 25%FBS	50% SBM+ 50%FBS	25% SBM+ 75%FBS	100% FBS	100% SBM (Basic ration-BR)
600	SBM	11.1	7.4	3.7	0.0	14.8
325	CSM	7.4	10.0	13.0	15.8	5.1
350	FBS	3.7	7.4	11.1	14.8	0.0
150	Wheat bran	16.0	15.0	12.7	12.3	14.7
250	corn	61.7	60.1	59.4	57.1	65.4
	Total	100.0	100.0	100.0	100.0	100.0
	CP	16.8	16.8	16.8	16.8	16.8
	ME Mcal/kg	2.9	2.9	2.9	2.9	2.9
	Cost price of rations ($/ton)	282	276	271	264	291

As for health, no problems were noticed and the animals were in good health. No signs of indigestion or diarrhea or any blood signs in manure were observed. Moreover, the appetite as we noticed in all groups was acceptable where no residues or left behind concentrate mixtures were collected. The amount of concentrate mix fed to animals did not exceed 30 kg/animal group for the whole period of the trial. At the end of 1st week, the apparent daily feed intake (adFI) per animal in all groups averaged to 450g, whereas at the end of 8th week this average value increased to 550-600g. The overall accumulation of feed intake for the whole period of the trial (cFI/head) in-group Ag4 was 27.88kg. Whereas, animals of Ag3 consumed the largest amount (29.58kg). This might be related to the fact that rations fed to animal-group Ag4 did not contain SBM and only 100 % FBS. As SBM increases in rations we observe an increase in feed intake as in Ag1 (28.53 kg), Ag2 (29.24 kg) and Ag3 (29.58 kg). Most probably the inclusion of big amounts in rations fed to goat kids has a negative effect on feed consumption relating this to the anti-nutritional factors contained in Faba bean. Figure 1 shows that, at the end of the trial group Ag3, whose animals fed a ration containing 25% SBM and 75% FBS gained the highest weight gain (LBWG) attaining the level of 5.68±0.49kg ($P<0.05$) in comparison with Ag4 (3.96±0.29kg), and Ag1 (4.31±0.25kg) and insignificantly ($P>0.05$) with Ag5 (5.20±0.75kg) and Ag2 (5.32±0.44kg). Antongiovanni *et al.* (2002), Martinez *et al*. (2004) and Morbidini *et al*. (2005) obtained results similar to ours on young growing lambs fed faba bean seeds (50-60% of the diet) as the sole protein source in concentrate mix increasing daily weight gain, demonstrating the already high protein value of faba beans for growing lambs.



Figute 1. Evolution of the cumulative Libe body weight gain, cLBWG, kg

ab Difference in the mean values with different letters is statistically significant (P<0.05)

Figure 2 illustrates the apparent FCR. The most efficient feed conversion rate (aFCR/head) was recorded in Ag3 animals (5.23±0.45) which was significant (P<0.05) with Ag1 (6.63±0.38) and Ag4 (7.06±0.51) and insignificant (P>0.05) with Ag2 (5.53±0.44) and Ag5 (5.69±0.81).

This can be explained by the overall effect of antinutritional factors found in faba bean seeds which was included as a sole legume ingredient (100 % FBS) in the basic ration fed to Ag4 goat kids. Moreover, results that are more efficient were achieved in groups Ag2 and Ag3 whose animals were fed a combination of SBM: FBS in different proportions.

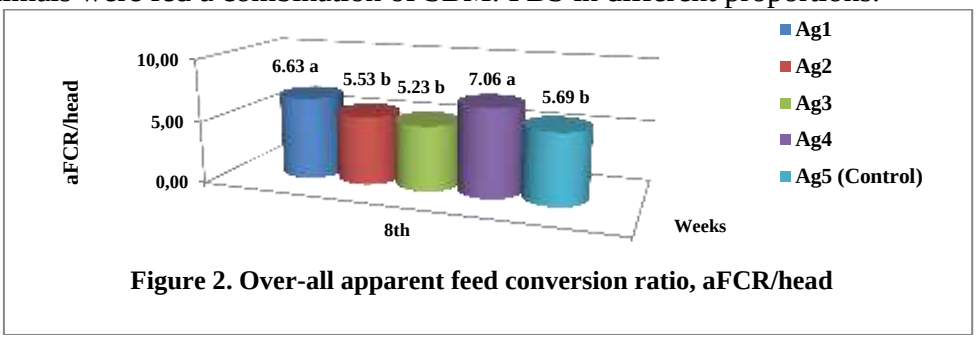


Figure 2. Over-all apparent feed conversion ratio, aFCR/head

ab Difference in the mean values with different letters is statistically significant (P<0.05)

This can be explained by the findings reported by Miller (1980) that faba bean protein is highly soluble in the rumen and rapidly degraded. Whereas, Emiola and Gous (2011) agreed that Faba bean feeding at various levels did not affect digestibility. However, Fulpagare (1993) concluded that as the level of fava bean increase (from 25 to 100%) in the diet of kids, the digestibility of dry matter (Ether extract and crude fiber) increase, while that of nitrogen-free extracts (NFE) decreases.

Duke (1981) suggested the fact that to reduce reliance on imported soybean meal (SBM) in temperate environments, faba bean may be alternative protein sources for small ruminant diets. Faba bean is used as an important source of protein rich food in developing countries and as both food and feed for animals in industrialized countries. Rations containing no SBM like in group Ag4 have negative effect on conversion of meat by eating more feeds and gaining less weight. On the contrast treatment, Ag3 whose animals were fed rations containing only 25% of SBM with 75 % FBS has maximum positive effect on conversion of feeds to meat, this can be explained by the findings reported by Miller (1980) that faba bean protein is highly soluble in the rumen. Whereas, Emiola and Gous (2011) agreed that Faba bean feeding at various levels did not affect digestibility. However, Fulpagare (1993) reported that as the level of faba bean increases (from 25 to 100%) in the diet of animals, the digestibility of dry matter (Ether extract and crude fiber) increase, while that of nitrogen-free extracts (NFE) decreases. Although in the trial conducted by Brand *et al*. (1995) performance was not reduced when faba beans were included at 0.2 kg of the diet, which was in contrast with our findings. Nevertheless, Results obtained by Guillaume (1977), Reddy *et al*. (1985), Wiseman & Cole (1988), Marquardt (1989), Jansman *et al*. (1995) and Knox *et al*. (1995) were in agreement with our findings. Where performance has been reduced by the inclusion of faba beans this has been attributed to the content of condensed tannins and non-starch polysaccharides (NSP) in the seeds. After calculating the cost ($) of FI and LBWG it was found that feeding rations containing 75% SBM: 25% FBS (Ag1) and 50% SBM: 50% FBS (Ag2) to Awassi lambs is especially unfeasible attaining the levels of 1.93 and 1.78 $/1 kg of LBWG, respectively. The most efficient and feasible use of FBS was obtained in groups Ag3 (25 % SBM: 75 % FBS) and Ag4 (50 % SBM: 50 % FBS) where there cost price was the lowest, 1.65 and 1.67 $/1 kg of LBWG, respectively.

Conclusion and Recommendation

To simplify our discussion it was concluded, that there are opportunities for researchers to increase animal productivity through the application of appropriate technologies, particularly in production systems, nutrition and feeding potential used of local feed resources for ruminants. Researchers have emphasized the utmost importance of using local feed resources as the key driving force to increase the productivity of animals in Asia (Wanapat, 2009; Wanapat *et al*., 2011). As it was observed neither in all our findings that included dry faba seed meal (FBS) coarsely grind in combination with the traditional soybean meal (SBM) has no adverse effect neither live body weight gain (LBWG) and feed intake (FI) nor on feed conversion ratio (FCR). We recommend a combination of 25% SBM: 75% FBS (as in-group Ag3) to be included in concentrate rations resulting in higher live body weight gains and feed retention as well as profit.

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IMPACT OF FEEDING HERBS - PEPPERMINT AND THYME ON BROILER CHICKENS BODY PERFORMANCE DURING THE GROWTH PERIOD

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Abstract

An experiment was conducted in 2017 to test the effect of using thyme, peppermint and their combination in the broiler diet on the performance of the birds. 250-one day old unsexed Ross chicks were divided randomly into five groups. For the first 19 days, all chicks were fed with the same starter basal diet (BD) without antioxidants and antibiotics. At age of 20 days, groups were subdivided into three replicates. Group I was fed on a basal diet free of any antibiotics and antioxidant, group II was fed on a basal diet and 15mg/kg of virginiamycin and commercial antioxidant, group III was fed on a basal diet without antibiotics and antioxidants supplied with 10g/kg peppermint meal, group IV was fed on BD without antibiotics and antioxidants supplied with 10g/kg thyme meal, group V was fed on BD without antibiotics and antioxidants supplied with a mixture of 5g/kg peppermint and 5g/kg thyme meals. As a result of the experiment all groups showed higher results compared to group I that have been fed BD without any additives. However group III recorded the highest live body weight (1219.4g) only through the growing period. Based on the overall period of the experiment, from day 1 till slaughter age, the feed conversion ratio (FCR) did not indicate any significant difference between the different groups. However, group III recorded the most effecient FCR (1.27) and group I had the worse (1.45). In conclusion, this experiment showed promising potential of thyme and peppermint as natural growth promoters.

Keywords: *Peppermint; Thyme; Broiler chicken nutrition.*

Introduction

Farrell (2013) reported that, due to the ever increasing demand, the poultry sector is probably the fastest growing market between live stocks especially in the last 15 years in countries of all different income levels. During the past few decades, Petracci *et al.* (2015) also observed a notable increase in the demand for poultry meat due to its low cost, good nutritional profile and suitability for further processing. However, antibiotics used in food animals for treatment, disease prevention or growth promotion, allows resistant bacteria and resistant genes to spread from food animals to humans through the food chain. This is why the use of antibiotics as growth promoters is being banned in several countries and natural product to replace these growth promoters were subject to many studies and experiments. Herbs and spices had shown strong potential as alternatives to antibiotics. Many plant products and their constituents have a broad antimicrobial activity and antioxidant properties. Scientists discovered that the performance of broiler production could be enhanced by the appetizing and stimulating effect of herbs and their extracts on the animal digestive system (Salih and Gurbuz, 2015). Therefore, the aim of this study is to investigate the impact of natural herbs (Peppermint and Thyme) as phytogenic compounds on feed intake, growth rate, feed conversion ratio, carcass production, breast cuts and physical quality of some edible internal visceral organs on broiler production during the growth period.

Materials and methods

A total of 250 one day-old unsexed broiler chicks (Ross breed) were used for 31 days in an opened poultry house in Lebanon. The birds were housed on floor and routinely managed as any other commercial broiler flock. Chicks were randomly allotted into 5 treatments by 50 in each until slaughter (10 birds/1m^2). Chicks in 1st phase (starter period 1-19 days) were kept in an area fenced with a circular cardboard (40 cm height) and littered with wood shavings and equipped with bell drinkers and feed troughs. Water and feed were offered *ad libitum*. A continuous light program was provided by incandescent 75- Watt Tungesten pulp lamps to achieve light intensity between 5-10 lux at floor level. All diets were formulated to meet minimum nutrient requirements established by the NRC (1994). Vitamin and mineral Premix were provided to basic diet (BD). The birds were vaccinated at hatching and growing period due to a programmed schedule against Marek's and Newcastle diseases, Gumboro and Infectious Bronchitis. General health status of the birds was checked daily and number of dead chicks was recorded per pen at 19 and 31 days of age. Antibiotic-free and antioxidant-free BD in mash form based on yellow corn-soybean meal mixture (230 g CP/kg and 13.1 MJ ME/kg), was offered *ad libitum* to all chick groups in the 1st starter phase (0-19 days) whereas, grower diets (2nd phase, growth period: 20-31 days) were fed with 210 g CP/kg and 13.4 MJ ME/kg distributed as follow:

- Control diet (I) - BD antibiotics-free and antioxidants-free;
- Control diet (II) - BD with antibiotics and antioxidants supplementations;
- Group (III) - as in control diet I supplemented with 10g/kg peppermint meal;
- Group (IV) - as in control diet I supplemented with 10g/kg thyme meal;
- Group (V) - as in control diet I supplemented with a mixture of 5g/kg peppermint and 5g/kg thyme meals.

Average live body weight (LBW) at 19 days of age was calculated by weighing from each group 3 batches by 3 birds each, where the average from each batch was recorded and then the total average of the 3 batches was registered. At 31 days of age, 3 batches by 3 birds from every replicate were weighed before and after slaughter by vein incision till complete bleeding after which the feather was manually plucked and the troops eviscerated to get carcass weight. Breasts (left and right) were cut and then heart and liver removed and weighed. Feed intake (FI), rate of body weight gain (LBWG) and feed conversion ratio (FCR) were calculated at the end of each feeding period (19 and 31 days of age). One way analysis of variance (ANOVA) of the results was applied to evaluate the statistical differences between the treatments using "Sigmastat software V. 3.5". The results were presented as Mean values ± SD. Significant effects were further explored using Tukey's multiple range tests to assert the interaction among treatment means. A significance level of $P<0.05$ was used.

Results and Discussion

The mortality in the first period varied from 3.9% in group V to 5.9% in groups I and II and 7.8% in groups III and IV. Since all the birds were fed the same antibiotic-free and antioxidant-free basal diet, mortality rate can be attributed to management and breeding factors. 100% livability was observed at the end of the second physiological phase in groups III and IV followed by group V (98%) and I (97.9%). The highest mortality rate (%) was attained in treatment group II (4.2%) fed with antibiotics-antioxidants basal diets. The average feed intake (FI) at 19 days of age (end of starter period) of the broiler chickens was 657.14g where no feed rejection was noticed, and remaining was negligible. The appetite of the birds was good and the same for all birds of all groups. No significant difference was noted between the different replicates, which may reflect that the distribution of all treatments was not affected by environmental or external elements that might interfere in the performance of the birds in replicates (wind, sun light, temperature, humidity). For the first 19 days of age

where all birds where on the same starter basal diet, the highest and least efficient use (P>0.05) of feeding basal diet also known as feed conversion ratio (FCR) was observed in group III (1.4) followed by group II (1.33), group IV (1.27) and group I (1.22). Group V showed the lowest FCR (1.18) in the mean values. The FCR calculated in the period from the 20th day until the end of the experiment showed significant difference (P<0.05) between treatments of the group I (average of the 3 replicates: 1.58) and group III fed with the addition of peppermint (1.11), but did not differ from the groups II (1.30), IV (1.31) and V (1.57). These results confirmed the ones from Abdulkarimi *et.al* (2011), as they noted that FCR improved by adding thyme in drinking water, and matched with the results of El-Ghousein *et al.* (2009) that formulated diets by adding 0.5, 1.0, 1.5 and 2.0% of crushed thyme. Rahimi *et.al* (2011) showed similar results as the addition of plant extracts showed significant difference in broiler performance. This could be due to the volatile oil components of thyme (thymol, carvacolo, borneol and geraniol). Thymol is the most important and primary volatile oil of thyme. Those oils and components of thyme affect the performance of the digestive system by enhancing the secretion of enzymes and endogen (amylase and chemotripsin). By this, the absorption rate of the intestine will increase and will positively affect the FCR and general performance of the birds (Feizi *et.al*, 2013). On the overall period of the experiment, from day 1 till slaughter age (Fig. 1), FCR did not indicate any significant difference between the different groups. However, group III did register the lowest FCR (1.27) and the control group had the highest FCR (1.45).

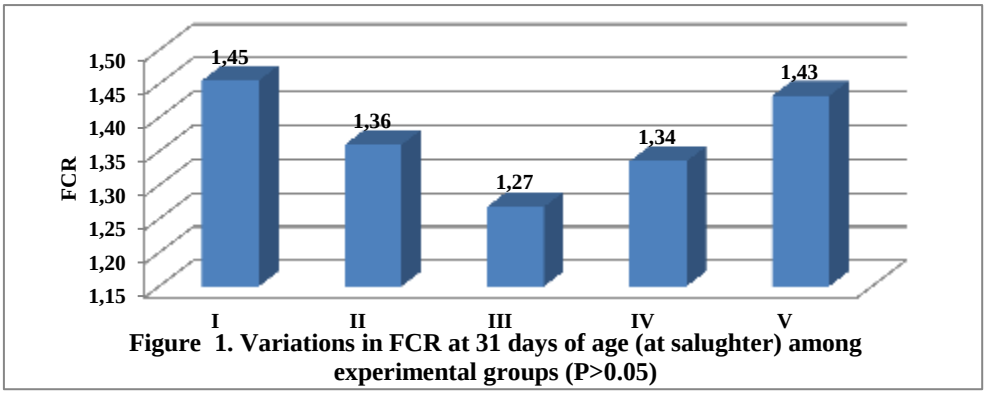

Figure 1. Variations in FCR at 31 days of age (at salughter) among experimental groups (P>0.05)

These results match with the experimental out coming of Hernandez *et.al.* (2004) and Bölükbaşi *et.al.* (2006) that both concluded that the addition of thyme and herbals did not affect significantly the performance of broiler chickens. In contradiction, Al-kassie (2009) reported significant difference between different groups and the addition of thyme. The average weight of one-day old chick was 42.02 ± 0.103g with no significant difference (P>0.05) among the five experimental groups. No significant difference was found between all groups at 19 days of age. However, in the mean values of all replicates for each group, the birds of group V recorded the highest live body weight on average 562.5g. At 31 days of age-End of growth period (slaughter), no significant difference was indicated between the five groups (P>0.05). All groups showed better average LBW (Fig. 2) compared to group I (1132.8g) that have been fed a basal diet without any additives, where group III (with the addition of peppermint) recorded the highest live body weight on average 1219.4g followed by group II (1200g), group V (1192.8g) and group IV (1171.7g).

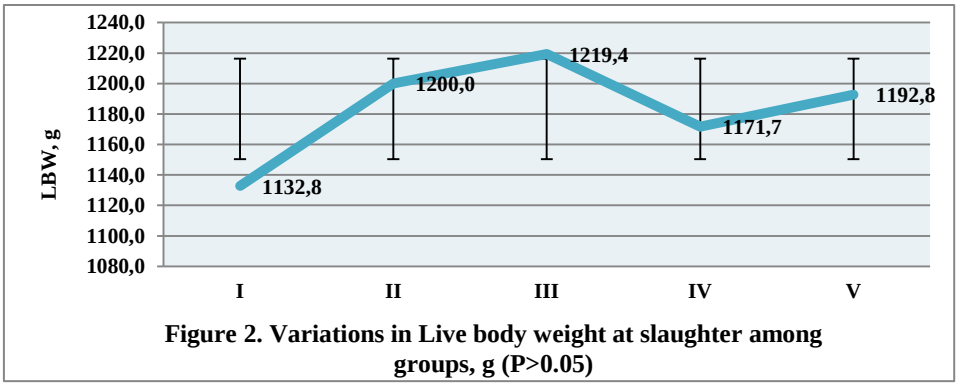

Figure 2. Variations in Live body weight at slaughter among groups, g (P>0.05)

At the end of the starter period, live body weight gain (LBWG) did not differ significantly among the average of replicate groups (P>0.05). However group V recorded the highest values of LBWG (562.5g) at 19 days of age. For the growth period, group III showed a better weight gain throughout the whole experiment (748.7g) and the control group (I) had the lowest mean values among the groups (591.2g). The net weight after slaughter (Fig. 3) without blood, feather, heads, legs and viscera did not show any significant difference between the groups. However, group II fed with an addition of antibiotics recorded the highest average 75.5 % compared to the other groups (around 72%).

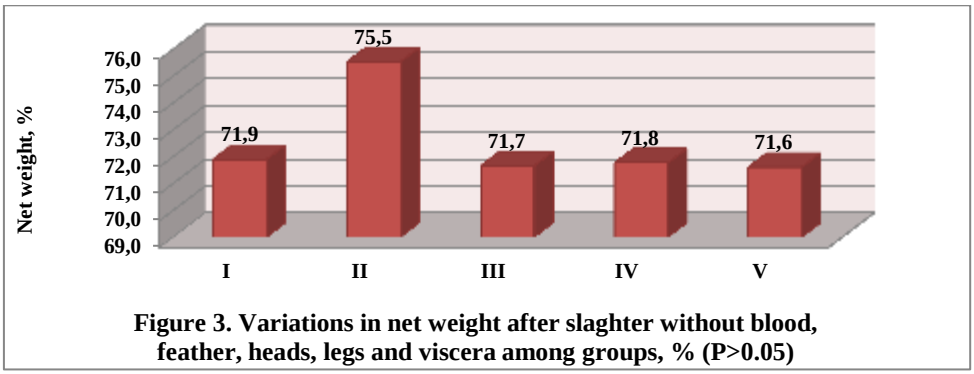

Figure 3. Variations in net weight after slaghter without blood, feather, heads, legs and viscera among groups, % (P>0.05)

The mean values of the five groups for the weight of the edible organs (liver and heart) of the slaughtered birds recorded almost similar percentage to the net weight; 3.9%, 3.9, 4.0, 3.7 and 3.9% for groups I, II, III, IV and V respectively. The variation in breast weight (left+right) after slaughter (Fig. 4) among the average of replicate-groups according to the percentage to net weight did not show significant difference between groups (22%, 21.7, 21.3, 21.2 and 21% to net weight in groups III, V, II, I, IV respectively).

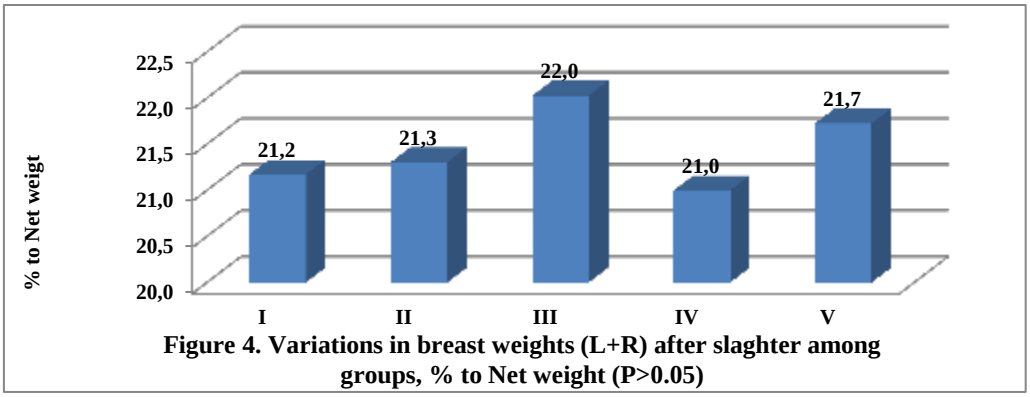

Figure 4. Variations in breast weights (L+R) after slaghter among groups, % to Net weight (P>0.05)

These outcomes differ from the results of Al-Kassie *et al*. (2010) that showed a significant difference between the average live body weight, live body weight gain, weight of edible organs and breast weight.

Conclusion

The results of this study demonstrated that all groups showed higher results compared to group I that have been fed BD without any additives. However group III recorded the highest live body weight only through the growing period. Based on the overall period of the experiment, from day 1 till slaughter age, the FCR did not indicate any significant difference between the different groups. However, group III recorded the most efficent FCR and group I had the worse. In conclusion, this experiment showed promising potential of thyme and peppermint as natural growth promoters.

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THE IMPACT OF FEEDING WITH HERBS ROSEMARY AND CHAMOMILE ON BROILER CHICKENS BODY PERFORMANCE DURING THE GROWTH PERIOD

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Abstract

Two hundred fifty day-old broilers (Ross breed) were divided into five equal treatment-groups fed five assigned diets: Control Group CG-I (antioxidants-free and antibiotics-free basic diets -BD), CG-II (BD supplemented with antioxidants and antibiotics only, group-VI (1% Rosemary dry meal added to BD), group-VII (1% Chamomile flowers dry meal added to BD) and group- VIII (1% mixture of 0.5% Chamomile and 0.5% Rosemary). Average live body weight (LBW) at 19 days of age was calculated by weighing from each group 3 batches by 3 birds each, where the average from each batch was recorded and then the total average of the 3 batches was registered. At 31 days of age, 3 batches by 3 birds from every group were weighed before and after slaughter by vein incision till complete bleeding after which the feather was manually plucked and the troops eviscerated to get carcass weight. Breasts (left and right) were cut and then heart and liver removed and weighed. The highest and the lowest Feed Intake (FI) averages ($P<0.05$) were noticed in groups VII (1020±26.5) and VI (833.3±58.6 g/bird) where birds were fed on BD with 1% herb dry meal mix, respectively. Better numerical result of Live body Weight (LBW) at slaughter was achieved in group VI (1121.1 ± 79.5 g). Better numerical results in LBWGain was obtained in groups VI followed by II, VIII, VII, and I by 719.5±60.15, 704.2±97.63, 672±2.93, 642.5±173.3 and 591.2±46.85 g, respectively. The best rate of LBWG regarding feed intake was significantly ($P<0.05$) observed at slaughter in group VI (1.25±0.05). Best % NW (net weight) to slaughter weight was achieved in treatment II (75.5±1.2%) followed by groups VII (72.3±1.6), I (71.9±3.1), VI (71.6±2.3) and VIII (68.7±8.5%). Identical results of liver + heart (%) to NW were ranging between 3.9±0.56 and 3.1±0.86%. Breast weights (%) to NW were insignificantly different ($P>0.05$) and almost the same, ranging from 21.9±0.96% to 21.2±0.64%. The results showed that 1% Chamomile and 1% Rosemary could be considered as a potential growth promoters for poultry.

Keywords: *Broiler chicken nutrition, Chamomile, Liver and heart, Rosemary, Slaughter.*

Introduction

Nowadays, the aim of the poultry industry is to maximize the health and well-being of the birds and minimize the impact of the industry on the environment. The broiler meat should be of satisfactory nutritive quality and acceptable to most consumers with respect to price and its organoleptic traits. The use of feed additives has been an important part of achieving this success since they improve the efficiency of the bird's growth and/or laying capacity, prevent disease and improve feed utilization. However, in poultry diets, these additives primarily included antimicrobials, antioxidants, emulsifiers, binders, pH control agents and enzymes. Iji *et al*. (2001), Issa and Abo Omar (2012) noted that the use of antibiotics as growth promoters is facing serious criticism. There are some important reasons that restrict the use of antibiotics such as the drug resistance in bacteria and the drug residues in meat (CAFA, 1997). To overcome the poor performance and increase susceptibility to diseases resulted from removal of antibiotics from birds diets, attempts were made to find other alternatives. Moreover, after

the European Union (Castanon, 2007) banned the use of antibiotics as growth promoters, researches were held to find alternatives that would be efficient in poultry. Herbs and their extracts showed potential feed additives and natural alternatives as replacements in growing flocks, especially under conditions of average management and quality (Cross *et al*, 2003; Lewis *et al*., 2003; Hernandez *et al.*, 2004). Odoemelam *et al*. (2013) observed that there are strong indications that herbs, spices and their products exert antioxidative, antimicrobial and growth promoting effects in livestock. Therefore, the aim of this trial is to study the impact of feeding broiler chicks basal rations free of antibiotics and antioxidants mixed with Rosemary dry meal, Chamomile flowers dry meal and their combination on body performance during growth period. The feed intake, growth rate, feed conversion ratio, carcass production, breast cuts and physical quality of broiler meat were measured.

Materials and methods

This study was carried out with the approval of an Ethical Review Process and under the Animals (Scientific Procedures) Act 1986. A total of 250 one day-old unsexed broiler chicks (Ross breed) were used for 31 days in an opened poultry house in Lebanon. The birds were housed on floor and routinely managed as any other commercial broiler flock. Chicks were randomly allotted into 5 treatments by 50 in each until slaughter (10 birds/1m^2). Chicks in 1st phase (starter period 1-19 days) were kept in an area fenced with a circular cardboard (40 cm height) and littered with wood shavings and equipped with bell drinkers and feed troughs. Water and feed were offered *ad libitum*. A continuous light program was provided by incandescent 75- Watt Tungesten pulp lamps to achieve light intensity between 5-10 lux at floor level. All diets were formulated to meet minimum nutrient requirements established by the NRC (1994). Vitamin and mineral Premix were provided to basic diet (BD). The birds were vaccinated at hatching and growing period due to a programmed schedule against Marek's and Newcastle diseases, Gumboro and Infectious Bronchitis. General health status of the birds was checked daily and number of dead chicks was recorded per pen at 19 and 31 days of age. Antibiotic-free and antioxidant-free BD in mash form based on yellow corn-soybean meal mixture (230 g CP/kg and 13.1 MJ ME/kg), was offered *ad libitum* to all chick groups in the 1st starter phase (0-19 days); whereas, grower diets (2nd phase, growth period: 20-31 days) were fed with 210 g CP/kg and 13.4 MJ ME/kg distributed as follow:

- Control diet (I) - BD antibiotics-free and antioxidants-free;
- Control diet (II) - BD with antibiotics and antioxidants supplementations;
- Group (VI) - as in control diet I supplemented with 1% Rosemary dry meal;
- Group (VII) - as in control diet I supplemented with 1% Chamomile dry flowers meal;
- Group (VIII) - as in control diet I supplemented with 1% of mixed meals (0.5% Rosemary dry meal+0.5% chamomile dry flowers meal).

Average live body weight (LBW) at 19 days of age was calculated by weighing from each group 3 batches by 3 birds each, where the average from each batch was recorded and then the total average of the 3 batches was registered. At 31 days of age, 3 batches by 3 birds from every replicate were weighed before and after slaughter by vein incision till complete bleeding after which the feather was manually plucked and the troops eviscerated to get carcass weight. Breasts (left and right) were cut and then heart and liver removed and weighed. Feed intake (FI), rate of body weight gain (LBWG) and feed conversion ratio (FCR) were calculated at the end of each feeding period (19 and 31 days of age). Statistical analyses were conducted using "Sigmastat V. 3.5" (Systat Software, 2007) to evaluate significant differences between treatments and replicate means. Significant effects were further explored using Tukey's multiple range tests to ascertain differences among treatment means. A significance level of $P<0.05$ was used. Results are illustrated as means (X) ±Standard Deviation (SD).

Results and discussion

Daily observations showed no problems in chicks health. The birds had good stature, very few leg weaknesses and showed no symptoms of diseases or feed deficiencies. The highest rate of mortality during the 1st phase was in groups II and VI (6%) followed by group I (5.9%). The lowest level of 2% was recorded in groups VII and VIII. Since all the birds were fed the same antibiotic-free and antioxidant-free basal diet, mortality rate can be attributed to management and breeding factors. Yassin *et al*. (2009) concluded that there is interrelation between 1st week mortality at broiler farms and management factors at breeder farms (like the breeder age, strain, and feed company of the breeder farms) and at hatcheries (like egg storage management, hatching management, and season). Additionally, the potential of a chick to survive the first week is directly related to the quality of the day-old broiler. The day-old chick quality depends on the genetic line of the breeders, breeder age, egg weight, egg storage conditions and duration, and incubation conditions such as temperature, humidity, gas levels, and altitude (Peebles *et al*., 1999; Vieira and Moran, 1999; Decuypere *et al*., 2001; Tona *et al*., 2004, 2005). Mortality was lower during 2nd phase (growing period) in groups II (4.2%) and I (2.1%) and higher in groups VI (6.4%), and the same as in 1st phase for groups VII and VIII by 2%. This reduction in broilers' mortalities could be explained by improvement in birds general performance and better build up of the immunity. The overall mortality (%) for both periods (1-31 days of age) averaged to 10, 12, 7.8, 4 and 4% in groups II, VI, I, VII and VIII, respectively. FI, g/bird was calculated as average for the five groups and was equal to 657.14 g/bird for the 1st phase- starter period. During the 2nd phase- growth period (20-31 days), average FI/bird of different replicates was calculated in each group resulting in almost equal ($P>0.05$) amounts of feeds (910-930 g/bird) in groups VIII, II and I. The highest and the lowest averages ($P<0.05$) of FI was noticed in group VII (1020±26.5 g/bird) and VI (833.3±58.6 g/bird) whose birds were fed basal diet with 1% Chamomile and 1% Rosemary dry meals, respectively. Feed consumption was significantly ($P<0.05$) increased (VI, VII) in the grower phase. It may be attributed to flavoring effects of 1% Rosemary (VI) and 1% Chamomile (VII) which improve the palatability of feed due to the chemical components and flavor of the chamomile flower as compared to I and II (control groups). These results are in accordance with those of Bassett (2000), Langhout (2000), Kamel (2001), Williams and Losa (2001) and Hernandez *et al*. (2004) who found that feed intake was increased with addition of essential oils derived from spices and herbs. In addition, this improvement in feed consumption may be due to the appetizing effect of active ingredient (borneol) in rosemary (Cabuk *et al*., 2003) and Chamomile (azulene, flavonoid and coumarin glycosides and fatty acids) giving anti-inflammatory, antiseptic, carminative, diaphoretic, sedative properties (Panda, 2005). Chamomile flowers inhibit the harmful intestinal microorganisms, thus counter acting excessive growth (Kolacz *et al.,* 1997). At 19 days of age (end of starter period), the variation in the average LBW was insignificant ($P>0.05$). Moreover, at 31 days of age (end of growth period), no significant differences was observed too ($P>0.05$) among replicates of each treatment where, the lowest LBW obtained was in group VII whereas, the highest average was in group I attaining the levels 1030g and 1186.7g respectively. In addition, the mean values obtained show that among all groups, better results ($P>0.05$) were achieved in birds LBW of group VI (1121.1±79.5g) followed by groups II (1200±104.1g), I (1132.8±47g), and VIII (1116.7±54.9g). The results in group VI may be due to active compounds that are presented in rosemary green meal inhibiting the excessive growth of harmful intestinal microorganisms; this may positively affect poultry health and productivity. This is in support with results obtained by Kolacz *et al*. (1997), AL-Kassie (2008), Osman *et al*. (2010), Sarker *et al*. (2010) that showed a significant improvement of LBW due to the main constituents of the herbs and their essential oils which are responsible for the bulk of the antimicrobial activity (Abaza *et al.,* 2003; Cross *et al*., 2007). It is worthy to mention that the

highest LBWG was achieved in group I whose birds were fed antobiotics-free and antioxidants-free basel diet and the lowest in group II where birds received basal diet supplemented with antibiotics and antioxidants. Data obtained on LBWG at 31 days (during the growth period) of age of replicates among all groups did not show any significant difference (P>0.05), but the highest average (816.7 g) was in group II and the lowest (444.2 g) in group VII. Better numerical results in LBWG was obtained in groups VI followed by II, VIII, VII and I by 719.5±60.15g, 704.2±97.63g, 672±2.93g, 642.5±173.3g, and 591.2±46.85g, respectively. These results agree with the obtained data achieved by Spernakova *et al*. (2007) who reported that the addition of rosemary powder at 500 mg/kg in poultry diets gave higher LBWG compared to an unsupplemented control group. At 19 days, apparent mean values of FCR/bird among all treatments had no significant diffrences (P>0.05) whereas, the least effective supplementation was obtained in group II (1.38) and the most effective treatment in VII (1.25). The best apparent mean values of FCR among treatment-groups in ascending order (P>0.05) were observed in groups I (1.22±0.08), VII (1.25±0.11), VIII (1.27±0.08), VI (1.31±0.0001) and II (1.38±0.05) where no supplementaion of neither antibiotics nor antioxidants was added to the BD. At the end of growth period (20-31 days), the best rate of body gain to feed intake was significantly (P<0.05) observed in group VI (1.13±0.07) where Rosemary was added to BD in comparison with groups I (1.58±0.08), VII (1.74±0.18) and VIII (1.54±0.08) and insignificant (P>0.05) in group II (1.30±0.14). FCR was improved significantly (P<0.05) with the addition of Rosemary dry meal if compared to FCR of the control diets. In grower phase, there was significant reduction (p<0.05) in FCR in groups VII and VIII where chamomile flower and a mixture of Rosemary and Chamomile were added. The improvement of LBWG and FCR are due to the effect of some active compounds included in chamomile flowers which had antimicrobial, antifungal, antioxidant and anti-inflammatory effects against microorganisms (Santurio *el al*., 2007). No significant (P>0.05) difference in the percentages of carcass NW in relation to LBW at slaughter among all replicates of the same treatment. Best numerical NW% was achieved in treatment II (75.5±1.2%) followed by groups VII (72.3±1.6%), I (71.9±3.1%), VI (71.6±2.3%) and VIII (68.7±8.5%) where birds were treated with a mixture of Chamomile and Rosemary meals. No significant differences (P>0.05) was obtained in the average percentage of liver+heart in relation to LBW at slaughter among all treatments. Identical results were obtained in groups I, II and VII with a very small numerical difference between groups VII and I attaining the levels of 3.9±0.56%, 3.9±0.35%, 3.9±0.39%, 3.6±0.31% and 3.1±0.86%, respectively. The results obtained about average percentage of breast (Left+Right) weight in relation to carcass NW were insignificantly different (P>0.05) and almost the same where it calibrated from 21.9±0.96% (treatment VII) and 21.8±0.9% (treatment (VIII) to 21.2±0.64, 21.3±0.73 and 21.4±1.44 % in treatments I, II and VI, respectively. These results were similarly reported by Ayad and Naji, (2007). The addition of 1% Rosemary dry meal, 1% Chamomile flower meal and 1% mix of both meals to the BD showed no significant increase in weight of breast, liver and heart compared with other treatment group. It is suggested that such promoters might be used in broiler diets, since they do not interfere negatively on the yield of the most commercialized edible cuts (Net carcass weight, breast and liver and heart).

Conclusions

The objective of the present study was to compare the effects of the supplementation of herbal natural feed additives to soybean-corn-based broiler diets on growth performance and carcass parameters. The results of trial demonstrate that the addition of 1% Rosemary and 1% Chamomile flowers meals have a potential to enhance the performance of broiler chickens. Even though most of the traits did not show significant difference in this experiment, but still

positive results were recorded compared to the control group, which can highlight that those herbs can be included in broiler feeding rations and replace antibiotics as growth promoters. It is highly recommended to use 1% Rosemary and 1% Chamomile flowers meals as a supplementation to 100 kg of basal diets in the daily feeds of broiler chickens in the growing period.

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INFLUENCE OF SEX AND TYPE OF BIRTH OF THE KIDS ON THE GESTATION LENGTH OF BULGARIAN WHITE DIARY GOATS

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Abstract

The data from the records obtained of the herd books of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan (Bulgaria) for the pregnancy and birth of 148 goats of Bulgarian White Diary were used to determine the influence of sex and the type of birth of kids on the gestation length of the same goats. Kidding was in February and March during the period 2017-2018. Very low variation in the gestation length of the Bulgarian White Dairy goats was established, depending on the parity, the birth weight of the kid, the sex and the type of birth.There was a difference in the average gestation length between goats kidded single and goats kidded twins. The average gestation length for all single born kids was 151.8 ± 0.4 days and for the twins was 150.8 ± 0.3 days. There was no significant difference between the gestation length between males and females single born kids (151.9 ± 0.6 days for males and 151.7 ± 0.7 days for females, respectively). There was a difference of 1 day between the gestation length for the male and female twins (male 150.3 ± 0.7 days and female 151.3 ± 0.6 days, respectively). Gestation length in twins with different sex continued on average 150.9 ± 0.4 days.

Keywords: *Goats, Gestation length, Sex, Type of birth.*

Introduction

The Bulgarian white dairy goat (BWD) originates from local goats improved with Saanen bucks imported from abroad. Its breeding began in 1970 and was recognized as a breed in 1992. The Bulgarian white dairy goat represents a major part of the population of goats grown in the plains and foothill regions of Bulgaria. Because of that, the goats of this breed are of interest to the research. The gestational length (GL) is a physiological variable of economic importance in the most domestic species. (Satue et al., 2011). It is a predestined characteristic for each animal species but can vary within certain limits within the breed. The influence of nutrition on foetal development during certain months of pregnancy tends to shorten or lengthen the gestation period, but the variation due to this factor is only 1.5 days (Riera, 1982). Hafez (1993) considered that the genotype of the foetus accounts for almost two thirds of the variations in gestation length of sheep; male lambs are carried longer than female lambs, spring-born lambs longer than fall-born lambs, and singles longer than twins. The gestation length for sheep and goats varies between breeds and individuals. In sheep, the early maturing breeds and the highly prolific breeds have shorter gestation periods than the slow maturing wool breeds. According to Satue et al. (2011) the management of a flock demands precise prediction of the moment of parturition. The inability to accurately predict the timing of parturition increases additionally the expenses for labour, as well as for veterinary and there is higher risk for the animals. The aim of the present study was to determine the influence of sex and type of birth of the kids on the gestation length of the Bulgarian white goat breed.

Material and Methods

The study was conducted in a herd of goats that are raised at the Research Institute of Mountain Stockbreeding and Agriculture in Troyan, Bulgaria. The records from the herd books for the pregnancy and birth in 2017 and 2018 of 148 goats of Bulgarian White Diary breed were used.

Kidding over the years was in Febuary and March. 62 of the goats kidded single, 40 male kids and 22 female kids. 86 goats gave birth to twins, 22 goats gave birth to male twins, 16 goats gave birth to female twins and 48 goats gave birth to twins with different sex. The goats which have delivered three and more kids are excluded from the data analysis because of their little number. During the winter period (pregnancy and kidding) the animals were raised at the cattle shed and fed with ration of 1,5 kg hay, 1 kg silage and 0.5 kg of concentrated fodder per head. In the summer period the animals were grazing freely and were fed with concentrate fodder 0.4 kg per head twice daily, during their milking using milking parlour. Free access to water and salt was provided. All animals were kept together to eliminate any possible pen effect and were moved to individual pens immediately after parturition. Goats were mated "by hand" and every copulation was marked in a mating notebook. The gestation length is reported from the date of mating to the date of kidding. Right after kidding the weight of the kid is measured with digital scales and written down. The gestation length of the goats is examined against the sex and the type of birth of the kid.

Data are presented as mean value and error of the mean. The results are processed by using MS Office Excel.

Results and Discussion

The gestation length of the goats is presented on the Table 1 according to sex and type of birth.

Table 1. The gestation length of the goats according to the sex and the type of birth.

	No. of observations	Gestation period (days)	Litter weight (kg)
single birth	62	151,8 ± 0,4	4±0,1
single male	40	151,9±0,6	4,1±0,1
single female	22	151,7±0,7	3,8±0,1
twins birth	86	150,8 ± 0,3	6,7±0,1
male tweens	22	150.3 ± 0.7	6,7±0,2
female twins	16	151.3 ± 0.6	6,6±0,3
twins with different sex	48	150.9 ± 0.4	6,8±0,2

Our results show that the gestation length of the goats kidded single in comparison with the goats kidded twins is longer by 1 day. If we compared the gestation length of the goats kidded male and female single kids, we would observe that there was no difference between them. However, we could observe that there was a difference between goats kidded female twins and goats kidded male twins. Goats kidded male twins had a shorter gestation length (150,3±0,7 days) than goats kidded female twins (151,3±0,6 days). The results mentioned by Karua and Banda (1990) in their study of goat crosses with Saanen bucks show that there is no significant difference in the gestation length between males and females. Gestation length in dams kidding single kids (147.14±2.86 days) was about the same as in dams kidding multiple kids (147.09±3.05 days). Similarly, the gestation length in those kidding male kids (146.94±2.7 days) were the same as those kidding female kids (147.34±2.98 days). The results therefore were in agreement with Asdell (1929) that gestation length is not affected by

litter size and sex of kid.

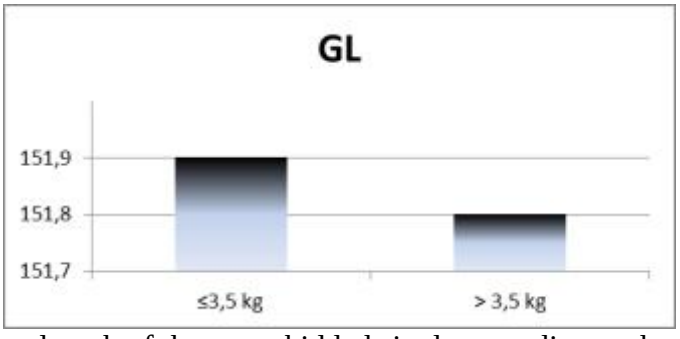

Figure 1. The gestation length of the goats kidded single according to the weight of the kids.

The gestation length of the goats kidded single with weight up to 3.5 kg and goats kidded single with weight above 3.5 kg is presented on Figure 1. As it is clear, the difference is quite negligible. This indicates that birth weight of kids does not influence on the gestation length of the goats kidded single.

Table 2. The gestation length of the goats according to the sequence of the kidding and the type of birth.

The gestation length (days)	Parity				
	I	II	III	IV	V - VII
single birth	152,2±0,8	154,2±0,7	151,6±1,6	151,6±1,1	152,2±1,1
twins birth	149,5±0,6	150,5±0,6	151,0±0,6	151,8±0,8	151,6±0,7

From the point of view of the parity (Table 2), the goats that have a second pregnancy with one kid have the longest gestation length which coincides with that found by Hoque et al. (2002) for goats of three breed groups. Goats kidded twins at the first birth had the shortest gestation length. There was no difference in gestation length between goats kidded single on the first birth and goats kidded single on the fifth birth. Our data show that with the aging (parity) of the goats kidded twins the gestation length increases and the forth kidding was 1 day longer. This trend coincides with that found by Hafez (1993) namely that the increase in the age of goats increases the gestation length. According to Asdell (1929) there was a distinct difference in the gestation length for young and older dams. This is related to the age of the dams and not to the order of the births. Gestation is shorter in the young animals than in the older according to the author.

Conclusions

It was established that the gestation length of the goats of Bulgarian White Diary breed was relatively constant value and it was observed that very low variation in the gestation length, depending on the parity, the birth weight of the kid, the sex and the type of birth.

With increasing the parity of the goats kidded twins the gestation length increased and the forth kidding was 1 day longer.

The gestation length of the goats kidded single was longer than the goats kidded twins by 1 day.

As conclusion, the results of our study can be useful for optimizing the work process in the farms.

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THE IMPACT OF DIFFERENT PROTEIN CONTENT OF POLLEN ON HONEY BEE (*Apis mellifera* L.) PRODUCTIVITY

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Abstract

The objective of the study was to investigate the impact of the protein content of pollen on the productivity of the honey bee (*Apis mellifera* L.). The protein content of the pollen influenced the productivity of the honey bees. The productivity of the bee colonies is higher, when honey bees collect pollen with higher protein content. The protein should be taken into consideration as an essential factor influencing the productivity of honey bee colonies. The pollen collected in different periods of the year has a different protein value for honey bees. The protein content in the examined samples throughout the three seasons ranged from 13.9% to 27.8%, and the average value was 20.9%. During the period when flowering plants provide pollen with protein content over 23%, honey and pollen productivity are higher than during the period when they provide pollen with protein content less than 20.6%. There is a relationship between the nutritional value of pollen and the productivity of bee colonies. The dynamic rainfall leads to increasing the moisture content of the soil and as a result it influences the flowering intensity of a great number of plants. The dynamics of the ecological factors- rain, temperature and humidity can favor or hamper the development of different plant species.

Keywords: *Apis mellifera, honey bee, impact, protein content, productivity*.

Introduction

Pollen is the source of protein, vitamins, enzymes, lipids, minerals, sugars, water and others (Kayas, 1975; Roulston & Cane, 2000) and necessary for vital processes of the honey bees (*Apis mellifera*), (Dietz, 1975). It also contains vitamin C, vitamin B complex- folic acid, riboflavin, pantothenic acid and others (Lavrehin & Pankova, 1983; Shmeleva & Nikiforova, 1989). Protein is important for the productivity of the bee colonies (Zherebkin & Mironova, 1976; Avetisyan, 1983). According to Stanley & Linskens (1974), Herbert & Shimanuki (1978) and Loper & Berdel (1980), the amount of protein ranges from 3.8% to 40.8% with the average of about 20% for the most species. Kleinschmidt & Kondos (1977) established from 7% to 37%, Radev et al. (2014) found protein content ranged from 13.88% to 25.02% and average value 19.45%. Research work conducted by Andreev (1926), Louveaux (1958), Perelson (1962) shows that the pollen of various plant species contains different amounts of protein and it makes them different in view of their nutritious value. Research work done by Roulston et al. (2000) on the protein content in the field of 377 plants from 93 families were within the range 2.5% of *Cupressus arizonica* to 61.7% for *Dodecatheon clevalandii*. According to Liolios et al. (2015) the pollen from 54 different plants was in the range from 12.8% for pollen from *Smilax* sp. to 30.1% for that of *Fallopia type* and the average of 20.8%. Differences are often observed in the protein content of the pollen that originates from the same plants due to different environments and different climate and soil (Stanley & Linskens, 1974; Bosi & Ricciardelli D'Albore, 1975; Tyurner et al. 1972). Honey bee longevity, brood rearing and honey production are all reduced when protein availability is insufficient (Crailsheim, 1990; Herbert, 2000). There is a relationship between the nutritional value of pollen and the development, reproduction and productivity of bee colonies (Radev et al. 2014).

The purpose of this study is to identify the impact of different protein content of pollen on honey bee (*Apis mellifera* L.) productivity.

Materials and methods

Bee colonies

The study was carried out using five bee colonies, made equal during 2012-2014. The experimental part of the research was carried out in an apiary located in Belozem (42.201860,25.049330), central part of south Bulgaria. The colonies were inspected, with the following taken into account: honey productivity (kg) - was found as the difference between the weight of the full combs and the empty ones after centrifugation of the honey. The total productivity of colony was determined by the total honey collected from all the extractions. Pollen productivity (kg) - was given as the amount of pollen collected with pollen traps.

Collection of pollen

Pollen traps were placed into the bee hives and the pollen pellets were harvested every 2 days. The collection of pollen started in April and continued until September. Each sample collected was with a date. Every single month was with two reporting periods (except April 2012- only one): (First) period from 1st to 15th day of the month and (Second) period from 16th to 30/31st day of the month.

Preparing the pollen samples for analysis

The collected pollen was mixed according to the accurate period. The random sample using for analysis for each period was taken using the quarter method of sampling. By this method the pollen was spread on some paper and divided it into four equal quarters, then it was removed by using two diagonally opposite quarters. The remaining material was mixed and quartered until the sample was reduced to the desired size.

Determination of protein content

Thirty-five vials with mixed pollen collected from all the colonies were analysed. For nitrogen content determination, the pollen was analysed using the Kjeldahl method, which is separated into three steps. During the first step, digestion, a quantity of 1g of pollen was placed into the tube with 20 ml of strong sulphuric acid (H_2SO_4, 95-98%) and heated for three and half hours in the presence of a catalyst, which assists the conversion of nitrogen in proteins to ammonium ions. When the digestion was complete, the samples were removed and left to cool at room temperature. After cooling, the ammonia was distilled in the presence of NaOH and collected in a solution of boric acid (H_3BO_3, 99.5-100.5%), which was then titrated against 0.1 M HCl. The crude protein content was estimated using the factor 5.60 (Rabie et al. 1983) by the volume of HCL, which was taken by an instrument and added to the pollen. In this case the following formula applied:

Protein (%) = 0.791*V/m.
Where: V = the volume (ml) of HCl taken during the titration.
M = the amount of pollen (g).

Results and discussion

The protein content of the pollen influenced the productivity of the honey bees. During the three experimental years, it was established higher honey and pollen productivity in the period of higher protein content, and lower production in the period of lower protein content (Tab. 1), which agreed with Crailsheim (1990) and Herbert (2000) who also established, that the honey production is reduced when protein availability is insufficient. Quality and quantity of pollen collected by bees affect the productivity of the hive (Kleinschmidt & Kondos, 1978).

The production seasons with higher protein content for the three years are over 23% and the production seasons with lower protein content are less than 20.6% (Tab. 2).

Table 1. Honey and Pollen production (kg)

Year	Production season with higher protein content (kg)		Production season with lower protein content (kg)		Total honey/pollen production (kg)	
	honey	Pollen	honey	Pollen	honey	Pollen
2012	110	9.2	83	5.7	193	14.9
2013	179	6.3	82	3.7	262	10
2014	161	5.4	108	1.7	270	7.1

Table 2. Protein content (%) of pollen in different periods

	2012	2013	2014
Period of high protein content	Apr-May-Jun- 23.6%	Apr-May-Sep- 23.4%	Apr-May-Sep- 24.2%
Period of low protein content	July-Aug-Sep- 16.2%	June-July-Aug- 18.6%	June-July-Aug- 20.6%

The presented results for the three experimental years (Tab. 1) according to the results shown by the protein content (Tab. 3) are statistically significant (Anova: Single factor), namely that the bees collected the most honey and pollen when the nutritional value was higher except the pollen production for 2012. For honey production for 2012/13/14 at $p \leq 0.05$, $p=0.00$. The pollen production was significant for 2013 and 2014 (at $p \leq 0.05$, $p=0.03$ and $p=0.00$), while in 2012 it was not significant (at $p \leq 0.05$, $p=0.16$), the reason is probably the ecological factors (Fig. 1, 2 and 3).

Table 3. Protein content (%) of mixed pollen in different harvesting periods

Harvesting periods of pollen	2012	2013	2014
April 1-15		21.1	27.8
April 16-30	25	23	25.5
April (average value)	**25**	**22**	**26.7**
May 1-15	24.6	23.9	24.8
May 16-31	23.4	24.4	23
May (average value)	**24**	**24.2**	**23.9**
June 1-15	23.5	15.6	22.5
June 16-30	21.5	15.7	21.6
June (average value)	**22.5**	**15.6**	**22.1**
July 1-15	17	16	19.2
July 16-31	18.3	19.9	21
July (average value)	**17.7**	**17.9**	**20.1**
August 1-15	17.1	22.8	20.7
August 16-31	13.9	21.9	18.4
August (average value)	**15.5**	**22.4**	**19.6**
September 1-15	15.1	22.6	19.9
September 16-30	16	25.1	24.2
September (average value)	**15.6**	**23.9**	**22**

During the three years the pollen was collected by using pollen traps every second day, but the yield of honey over the years was reported during the following months: 2012- May, June, July and August; 2013/2014- May, July, August and September.

It seems that in the presence of protein content in pollen over 23% the productivity of the bee colonies is higher than in presence of protein content in pollen less than 20.6%.

According to the increase of the average annual value of the protein content in the pollen during the three years (Tab. 4) it is found that the honey productivity also increases every year (Tab. 1). Between these parameters there is a positive, high and significant correlation ($r=0.98$, $p \leq 0.05$). But between the increase of the average annual value of the protein content

in the pollen during the three years (Tab. 4) it is found that the pollen productivity decreases every year (Tab. 1). Between these parameters there is a negative, high and significant correlation (r=-0.99, p≤0.05) in the pollen productivity, the reason is probably the dynamics of the ecological factors- rain, temperature and humidity (Fig. 1, 2 and 3).

Table 4. Protein content (%) of mix bee-collected pollen in different seasons

Harvesting periods of pollen	**2012**	**2013**	**2014**
Spring	24.4	23.1	25.3
Summer	18.6	18.6	20.6
Autumn	15.6	23.9	22
X for year	**19.5**	**21.9**	**22.6**

The annual dynamics of the above-mentioned factors can be observed (Fig. 1, 2 and 3). The dynamic rainfall leads to increasing the moisture content of the soil and as a result it influences the flowering intensity of a great number of plants. In 2012 (Fig. 1) there were no days with rainfall during the summer months June and August whereas in 2013 (Fig. 2) and to a great extent in 2014 (Fig. 3) there were days with rainfall. The ecological factors can favor or hamper the development of different plant species.

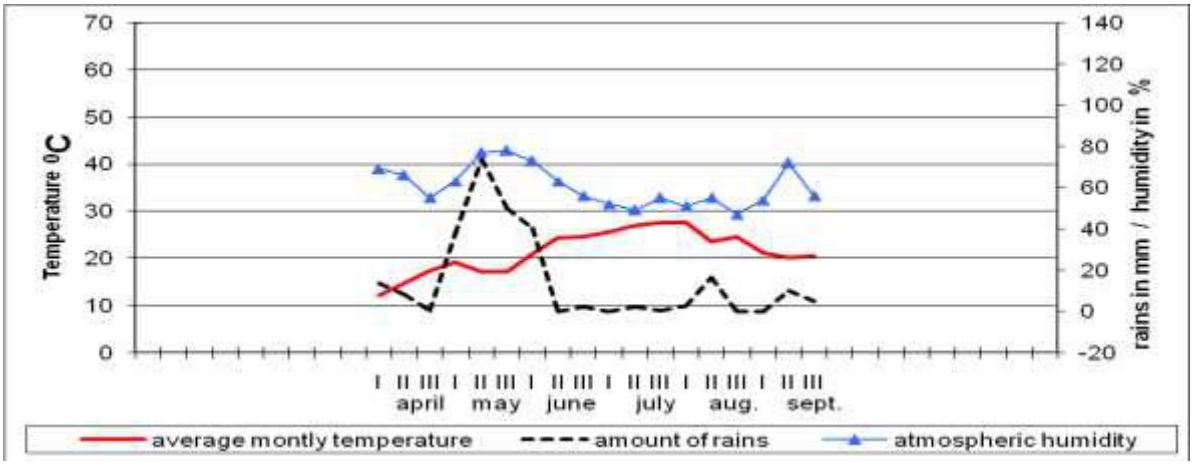

Fig. 1. Climatic characteristics of Belozem area for 2012

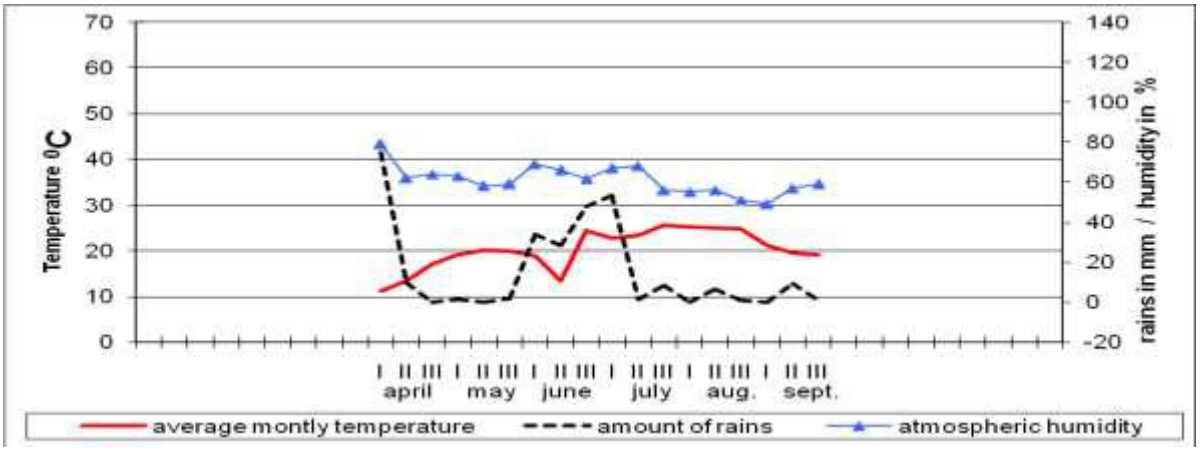

Fig. 2. Climatic characteristics of Belozem area for 2013

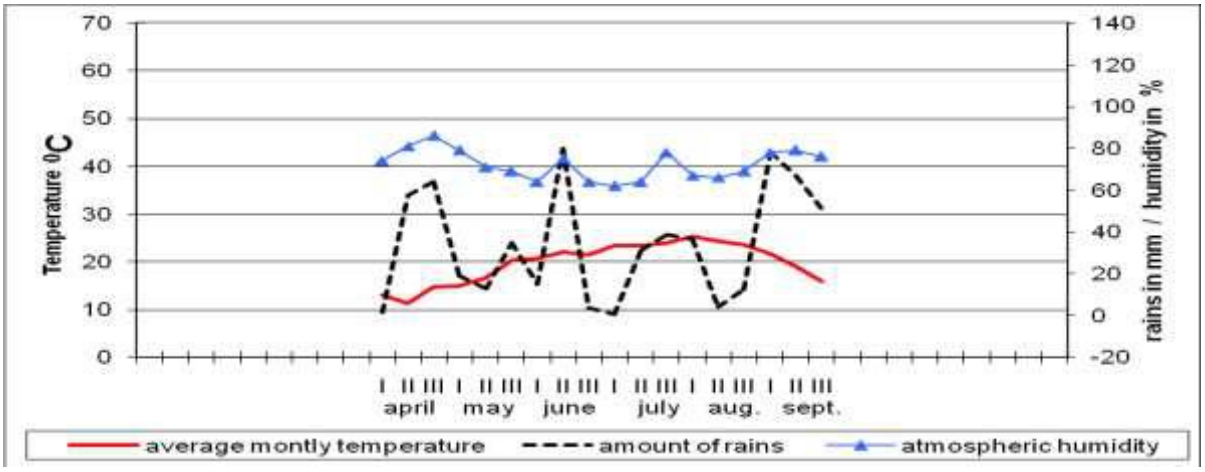

Fig. 3. Climatic characteristics of Belozem area for 2014

Conclusions

The protein content in the examined samples through the three seasons ranged from 13.9% to 27.8%, and the average value was 20.9%.

During the period when flowering plants provide pollen with protein content over 23%, honey and pollen productivity are higher than period provide pollen with protein content less than 20.6%.

There is a relationship between the nutritional value of pollen and the productivity of bee colonies.

The dynamics of the ecological factors- rain, temperature and humidity can favor or hamper the development of different plant species.

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EFFECT OF MORINGA OLEIFERA LEAVES SUPPLEMENTATION ON BEHAVIOUR AND GROWTH PERFORMANCE OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Abstract

The current study was carried out to investigate the effect of Moringa oleifera leaves supplementation on behaviour and growth performance of Nile Tilapia. Fish were classified to three groups (n=20/group, 2 replicates). First group fed on commercial diet. Second and third groups fed on 10% and 20% Moringa processed diet, respectively. Behavioural patterns, body weight, weight gain, relative growth rate, feed conversion ratio, some biochemical parameters and water quality were recorded. Results revealed that adding of Moringa by two different concentrations significantly reduced aggressive behaviour of fish in general. In addition, Moringa 20% significantly decreased body weight of fish after 10 weeks of supplementation. Moringa supplementation had no significant effect on liver enzymes, total proteins, albumins and globulins. However, fish fed with Moringa had lower serum glucose level than fish that was not fed with Moringa. Moreover, adding of Moringa in both concentrations (10 or 20%) decreased nitrite and nitrate of water in comparison with control group. Dissolved oxygen and pH of water were not affected by Moringa supplementation. It was concluded that Moringa supplementation had a favorable effect on Tilapia fish behaviour by reducing level of aggression. Also, Moringa supplementation could be used in improving water quality parameters by decreasing nitrite and nitrate levels. Generally, Moringa is nutritionally rich and can be included in fish diet at 10% levels that had no adverse effect on fish body weight.

Keywords: *Moringa oleifera, behaviour, growth performance, Nile Tilapia.*

Introduction

Fish culture is the fastest growing sector of the world's animal production by reporting an annual increase of about 10% (FAO, 1997). The Nile tilapia has its origins in Africa and is mostly farmed in tropical and sub-tropical countries. The Nile tilapia is one of the most important of the tilapia species due to its rapid growth, good survival rate in high-density populations and its disease tolerance. The tilapia species is both an omnivorous and herbivorous species, which means that it can digest both plant and animal materials. Tilapia can live in most freshwater sources, such as shallow streams, lakes, rivers and are even found living in brackish waters. The tilapia is very tolerant to poor water conditions and can endure periods with low oxygen (Francois *et al.,* 2010).

Recently, researchers have increasingly been paying attention to Moringa (Moringa oleifera Lam.). It belongs to the moringaceae family, and is considered to have its origin in the north-west region of India. Moringa oleifera (MO) can have a total dry matter (DM) yield up to 24 ton ha-1year-1 and has a crude protein (CP) content in fresh leaves varying from 193 to 246 g kg-1 DM (Sanchez *et al.,* 2006). Analytical studies have identified MO as an important source of essential nutrients; rich in protein, essential amino acids, minerals, and vitamins, with a relatively low amount of anti-nutrients (Falowo *et al.,* 2018). Moringa fresh plants have been included into the diet of different animals including fish. Moringa can be dried and used in the form of Moringa Leaf meal (MLM), 30% substitution of MLM for fish meal has been

recommended for the diet of Nile tilapia *Oreochromis niloticus* (Richter *et al.,* 2003). Furthermore, fishmeal, in the diet of *Clarias gariepinus*, can be efficiently replaced with up to 10% Moringa meal, for good growth and nutrient utilization, comparable with the fishmeal-based diet (Adewumi, 2014).

In aquaculture systems, the increasing price of feed is considered one of the most important factors that limit profitability, caused mainly by the cost of fishmeal used as a primary source of protein (McCoy, 1998). Consequently, there is a need to search for alternative protein sources for aquaculture diets. Additionally, the high cost and fluctuating quality of imported fish meal have led to the need to identify substitute protein sources for use in fish feed formulations (Olvera *et al.,* 1990).

Nowadays, plant sources have been used to replace the protein in fish meal and soybean meal, either partially or totally. Practical fish feed has been an area of focus in aquaculture nutrition research recently (Sidduraju and Becker, 2003).

This study was conducted to investigate the effect of Moringa oleifera leaves supplementation on behavioral patterns and growth performance of Nile tilapia (*Oreochromis niloticus*).

Materials and methods

The study was conducted in accordance to the regulations of Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat City, Egypt and at the period from April till August, 2014. Sixty fingerlings (average weight 18.6g) were randomly divided into three groups (n=10 fish per aquarium, 100 cm length x 30 cm width x 40cm height) with two replicates per treatment. The first group fed commercial diet. The second and third groups fed 10% and 20% MO processed diet respectively. The experiment lasted for 10 weeks. Fish were fed at a rate of 3% of their body mass. The amount of food given was adjusted every two weeks after determination of new fish weights in each group.

MO leaves were collected from a Moringa farm in Sadat city and were washed with water to remove dirt, drained properly and later shade dried for seven days. Thereafter, the leaves were ground into fine powder (Bello and Nzeh, 2013). Then this powder added to wetted commercial diet by two different concentrations (10-20% of diet) and mixing them in grind meat miller. This mixture was dried in Hot air oven (BINDER, Germany) at 50°C for twenty four hours. Two different concentrations of MO processed diets were analyzed for proximate composition according to AOAC (2000) (Table 1).

Data of behavioural patterns (feeding, foraging, elimination, resting, chafing, schooling, surfacing, chasing, mouth pushing and fin tugging) were recorded by using video camera (Sony, Japan) for 10 minutes for each aquarium two times per day at morning (9:00 -11:30 am) and afternoon (2:00 - 4:30 pm); and twice weekly. Sampled behavioural categories for all fish were scanned by one observer. The behaviour of the fish in each aquarium was recorded using instantaneous sampling method at 60 seconds intervals.

Fish were taken from each aquarium for individual weight every two weeks at 0, 2, 4, 6, 8 and 10 weeks of experiment. Fish were handled by using wetted towel to avoid stress then putted in plastic container with wetted sponge on electronic weighing balance (ACCULAB, Sartorious group, Germany). According to Lee *et al.,* (2000) the following items were calculated: weight gain= Wf - Wi ; relative growth rate= (Wf - Wi)/ $^1/_2$ (Wf + Wi) X 100 and feed conversion ratio (FCR) = [dry feed fed (g)/ weight gain (g)] where Wf= final weight; Wi= initial weight.

Table 1. Proximate composition of experimental diets:

Proximate composition	Commercial diet	10% MO processed diet	20% MO processed diet
Dry matter %	93.8	92.2	94.7
Crude protein %	28.6	30.1	30.0
Crude lipid %	5.2	4.9	4.7
Crude fiber %	6.5	7.3	7.8
Ash %	7.7	7.4	7.7
Nitrogen free extract (NFE)%	45.8	42.9	44.5
Digestible energy(Kcal/Kg)	3224	3162	3199

For collection of blood samples, fish were put in 20L worm water (25°C) with Tricaine Methanoesulfonate (0.5g/10 L, Finquel, USA) for anesthesia at the end of experiment. After that blood samples were collected from direct cardiac puncture by using 3ml sterile plastic disposable syringe in test tubes without anticoagulant. The clotted blood was centrifuged at 3000 revolution per minute (rpm) for 15 min for serum separation. Sera were frozen at -20 °C until subsequent analyses. Total protein, albumin, glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated by spectrophotometer (Light Wave II UV/Visible Diode Array Spectrophotometer, England) according to the instructions of kits.
Water quality parameters including pH, dissolved oxygen (D.O), Nitrite (NO2) and Nitrate (NO3) were measured daily for each aquarium. Data were analyzed statistically by using SAS system (2001). The proportion of fish that performed behavioural patterns per minute was calculated. All data (behavioral patterns, growth performance, biochemical parameters and water quality parameters) were analyzed with ANOVA using a generalized linear model (GLM-procedure). Results were considered statistically significant when p-values were below 0.05.

Results and discussion

In the current study, supplementation of MO leaves at either 10% or 20% had no toxic effect on Tilapia. Additionally, MO supplementation had significantly effect on Tilapia fish behaviour and performance. The observations from this study revealed that resting and surfacing behaviours increased in fish fed with 10% and 20% MO procced diets in compared with fish fed commercial diet as shown in Table (2). A finding of interest was that aggressive behaviour levels were decreased by MO supplementation in general. Moreover, fish fed with MO showed less chasing, mouth pushing and fin tugging than control group. Therefore, MO supplementation may be having a calmness effect on fish that increased resting and suppressed aggressive activities.
Unfortunately, the literatures about effect of MO leaves on fish behaviors are rare. Bakre *et al.,* (2013) found that the extract of MO leaves caused a significant decrease in grooming and locomotion and had CNS depressant effect in mice. Therefore, MO processed diets that enhanced comfort and motionless state of fish and in the same time depressed aggression activities in this study may be attributed to suppress the central nervous system activity.

Table 2. Effect of Moringa oleifera leaves supplementation on Tilapia behaviour (LSMeans):

Behaviour (%)	Control	Moringa 10%	Moringa 20%	s.e.d	*P*-value
Ingestive:					
Feeding	25.16	22.20	25.83	2.63	NS
Foraging	22.00	21.83	24.88	3.15	NS
Elimination	7.20	7.12	7.29	0.85	NS
Comfort					
Resting	28.45^{b}	33.62^{a}	30.21^{ab}	1.66	0.03
Chafing	2.61	1.95	4.83	1.20	NS
Schooling	4.06	3.87	3.00	0.63	NS

Surfacing	4.50[b]	9.83[a]	9.29[ab]	1.85	0.04
Aggressive:					
Chasing	34.73[a]	23.45[b]	28.32[b]	1.90	0.01
Mouth pushing	11.15[a]	5.76[b]	6.87[b]	1.51	0.04
Fin tugging	34.96[a]	29.83[ab]	25.83[b]	2.32	0.006

NS: non-significant. s.e.d: standard error of difference. a-b: LS Mean with different superscripts were significantly different.

MO supplementation had undesirable effect on fish growth performance at 20% concentration. However, MO 10% concentration had no adverse effect on fish body weight (Tables 3). In addition, weight gain of fish fed blank diet was higher than fish fed with 10% and 20% MO processed food (48.48, 40.78 and 40.48, P=0.012, respectively). Also fish received 10% and 20% MO processed food had less relative growth rate than fish in control group (108.74, 113.69 and 120.71, P=0.01, respectively). Feed conversion ratio did not significantly affected by 10% or 20% MO supplementations compared with control group (1.81, 1.80 and 1.54, P>0.05, respectively). Consequently, by increasing MO levels in diets the growth parameters of tilapia fish reduced. These findings may be attributed to higher crude fiber content that found in MO processed diet in compared with commercial diet as analyzed in proximate composition of experimental diets (Table 1) that may be lower the palatability of MO processed food. These results were in accordance with Adewumi (2014) and Kittisak *et al.,* (2017) who reported that high fiber content of MO diet and decreased its acceptability were the main causes of poor growth performance of fish.

Moreover, high fiber content in diets causes dilution of the nutrients reduces digestibility, resulting in growth depression, as the diets become inconsistent. Where, fiber creates bulkiness of feed in the gut, reduces feed consumption of animals and creates regular bowel movement (Adewumi, 2012). In addition, fiber depresses utilization of feed energy and essential nutrients. This energy deficit would in turn affect other biological parameters, as well as nutrient retention and thus weight loss (Adewumi, 2014).

Table 3. Effect of Moringa oleifera leaves supplementation on Tilapia body weight (g) (LSMeans):

Week	**Control**	**Moringa 10%**	**Moringa 20%**	**s.e.d**	***P*-value**
0	18.76	18.80	18.35	0.57	NS
2	25.45	23.80	24.65	0.87	NS
4	32.89	31.05	30.06	1.17	NS
6	42.30	40.31	38.16	1.70	NS
8	52.95	50.23	48.96	1.75	NS
10	64.25[a]	57.45[ab]	55.36[b]	2.19	0.023

NS: non-significant. s.e.d: standard error of difference. a-b: LS Mean with different superscripts were significantly different.

Additionally, Richter *et al.,* (2003) showed that higher substitution of MO leaf meal with fish meal had a negative impact on the growth performance because of the presence of some anti-nutrients such as phenol, tannins, phytates and saponins. Accordingly, Eusebio *et al*., (2004) found that the presence of anti-nutrients may hinder the digestibility and utilization of dietary nutrients. Phytates reduce the bioavailability of nutrients due to formation of complexes such as a phytate–mineral/protein complex. Fish are unable to use phytate-nutrient complexes because they lack the enzyme phytase (Riche *et al.,* 2001). Besides, tannins delay the digestive process by binding to digestive enzymes such as protease resulting in a decrease in proteolytic enzyme activity, leading to reduced protein digestibility (Eusebio *et al*., 2004). Tannins also reduce the absorption of essential vitamins (Francis *et al.,* 2001). Polyphenols on the other hand, form phenolic-protein-enzyme complexes, these also decrease protein digestibility and amino acid availability.

Table 4. Effect of Moringa oleifera supplementation on biochemical parameters of Tilapia (LSMeans):

Biochemical parameters	**Control**	**Moringa 10%**	**Moringa 20%**	**s.e.d**	**P-value**
AST(U/I)	31.37	13.75	15.12	6.49	NS
ALT(U/I)	5.75	3.75	2.50	1.53	NS
Glucose (mmol/L)	180.38^{a}	119.72^{ab}	99.68^{b}	28.26	0.05
TP (g/dL)	3.47	3.61	3.37	0.21	NS
Albumin (g/dL)	1.41	1.55	1.43	0.08	NS
Globulin (g/dL)	2.06	2.06	1.95	0.21	NS

NS: non significant. s.e.d: standard error of difference. a-b: LS Mean with different superscripts were significantly different.

Using of MO in diet had no significant effect on fish biochemical parameters except fish serum glucose was only reduced (Table 4). Serum glucose was less in fish fed MO 20% than fish fed commercial diet. The reason of these recordings may be due to hypoglycemic effects of MO leaves. These findings were in agreement with Amira *et al.,* (2014) who stated that aqueous extract of MO leaves possesses potent hypoglycemic effect through the normalization of elevated hepatic pyruvate carboxylase enzyme and regeneration of damaged hepatocytes and pancreatic ß cells via its antioxidant properties.

On the other hand, Mousa *et al.,* (2008) suggested that increasing in the activities of serum liver enzymes (Aspartate aminotransferase, AST, and Alanine aminotransferase, ALT) as the level of MO leaf meal increased considerably from 20% in the diet. Elevated AST and ALT activities in fish fed 30% MO leaf meal diet and above were suggested of hepatic cellular damage leading to their leakage into circulation. However, in the current study, serum liver enzymes (ALT and AST) were not affected by MO supplementation. These differences may be attributed to the usage of MO leaves by less than 30% in this study that may be have protective role to hepatic cells (Sa´nchez-Muñoz *et al.,* 2018).

Another finding of interest was that supplementation of MO had benefit effect on water nitrite and nitrate levels. Adding of MO in both concentrations (10%-20%) decreased nitrite (1.37 and 0.87 mg/L, respectively) and nitrate (29.62 and 19.30 mg/L, respectively) of water in comparison with control group (nitrite: 1.92 and nitrate: 41.62 mg/L) (P=0.004, P=0.004, respectively). However, dissolved oxygen and pH of water were not affected by MO supplementations (P>0.05). These findings were agreed with Nand *et al.,* (2012) who found that Moringa were more effective in water purification. Additionally, Suneetha and Ravindhranath, (2012) recorded that Moringa Tinctoria leaves had bio-adsorbent ability to absorb nitrate ions in water when pH was optimized.

Conclusion

It was concluded that MO supplementation had a favorable effect on Tilapia fish behaviour. Adding of MO had a calmness effect on fish as well as reducing level of aggression. Moreover, MO supplementation could be used in improving water quality parameters by decreasing nitrite and nitrate levels. Generally, MO is nutritionally rich and can be included in fish diet at low levels that had no adverse effect on fish body weight.

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IMPACT OF SOYBEAN MEAL SUBSTITUTION WITH LOCALLY PRODUCED LEGUME MEALS ON "KARAGOUNIKO" EWES BODY WEIGHT AND MILK PRODUCTION

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Abstract

The cost of feed is of great importance for small ruminant production. The present study was conducted in Greece, exploring the potential substitution of imported soybean meal by legumes (beans) meals in sheep nutrition. Eighty ewes of the "Karagouniko" breed were split in five groups and four subgroups. In the first group nutrition was based on soy legumes (beans). In the second group nutrition was based on lupine legumes. In the third group nutrition was based on pea legumes. In the fourth group nutrition was based on vetch legumes. In the fifth group nutrition was based on broad bean legumes. Subgroups within groups consisted of: a) control subgroup with null substitution of soybean meal, b) 1/3 substitution of total nitrogen by the respective legume, c) 2/3 substitution of total nitrogen by the respective legume and d) full substitution of total nitrogen by the respective legume. Body weight was measured in the beginning and at the end of the trial period. Feed consumption and milk production were recorded daily and milk quality was analyzed weekly. No statistically significant differences were found for body weight and milk production. In qualitative characteristics of milk (protein, fat and lactose content), there were significant differences between subgroups, for vetch, broad beans, lupine, peas and soy legumes (beans). Digestibility showed statistically significant differences between subgroups, for vetch, broad beans, peas and soy legumes. The results showed that substitution of soybean meal with other locally produced legumes as main protein sources is possible, without affecting body weight and milk production. Especially, soybean meal substitution of 1/3 total nitrogen with soy beans led to increased protein and fat content and lower lactose content in milk.

Keywords: *Broad beans, Vetch, Peas, Lupine, Greece.*

Introduction

Nutrition of ruminants is based mainly on soybean (*Glycine max* L.) and maize (*Zea mays* L.). For soybean, especially, there are many restrictions in European Union, because the major quantities produced are genetically modified. Organic and traditional farmers are not allowed (and are not willing) to use GMO feed for their livestock. On the other hand, soybean production is too expensive for European farmers and thus, soybean cultivation is restricted in European Union.

As alternative nutrition sources for small ruminants, other legume beans have been proposed, by many researchers (Hadjipanayiotou 2002; Christodoulou *et al.*, 2005; Lanza *et al.*, 2011), because of their high protein content. They reported that substitution of soy bean by other legumes as a protein source, in general, did not affect milk production of small ruminants (Bonomi *et al.*, 2003; Christodoulou *et al.*, 2005; Liponi *et al.*, 2007; Renna *et al.*, 2012). In case of carcass and meat quality of lambs, these protein sources showed a rather positive

impact (Lanza *et al.*, 2011; Bonano *et al.*, 2012), indicating that other legumes could totally replace soy bean in the nutrition of small ruminants, with many benefits for the farmers raising their livestock organically or traditionally. It should be mentioned that pea legumes (*Pisum sativum* L.) are the second most important legume used globally as a substitute of soy beans (Mikic *et al.*, 2009). Pea seems to be a very promising protein source with many positive effects on small ruminants' nutrition (Bonomi *et al.*, 2003; Liponi *et al.*, 2007).
In a recent study, many different protein sources were evaluated (Zagorakis *et al*., 2015). The authors depicted that all the alternative protein sources have to be treated in order to reduce their nitrogen (N) degradability, prior to their utilization for nutrition of ruminants.
The purpose of this study was to explore the potential substitution of soybean meal by other legumes (beans) meals in sheep nutrition and the impact on digestibility, milk production and quality. The tested legumes were: soy (beans), lupine (*Lupinus albus* L.), pea vetch (*Vicia sativa* L.) and broad bean (*Vicia faba* L.) legumes (beans).

Materials and methods

The study was carried out in the farm of TEI of Thessaly, in Larissa, Greece. Eighty lactating ewes of the "Karagouniko" breed were homogeneously split in five groups and four subgroups. In the first group nutrition was based on soy legumes (beans). In the second group nutrition was based on lupine legumes. In the third group nutrition was based on pea legumes. In the fourth group nutrition was based on vetch legumes, while in the fifth group nutrition was based on broad bean legumes. The general statistical design was Latin Design 4X4 in a two-factor arrangement: plant species (groups) and feed (subgroups). Subgroups within groups consisted of: a) control subgroup with null substitution of soybean meal, b) 1/3 substitution of total nitrogen by the respective legume, c) 2/3 substitution of total nitrogen by the respective legume and, d) full substitution of total nitrogen by the respective legume. In all feeds, chrome oxide was added as a marker for digestibility measurements. The periods comprised of 21 days, i.e. 14 days for adaptation to the diets (feed) and 7 days for sampling and analyses of milk and feces. Body weight was measured in the beginning and at the end of the trial period. Feed consumption and milk production were recorded daily and milk quality was analyzed weekly. Every milk sample was refrigerated (at 4 ^{o}C) and morning/afternoon milk was mixed prior to analyses for lactose, protein, fat, ash and dry matter without fat. Feces were sampled every day and a total sample of 400 g was formed prior to drying (60^{o}C for 48h). Chrome was determined using the method Schurch, *et al*. (1950). All ewes were lactating and kept in separate cells of an area of 2.5 m^2. Daily feed was given in two meals and water was available ad libitum. Records for not-consumed-feed were kept throughout the experimental period. Table 1 presents the composition of the diets, given every day at a quantity of 1 kg per ewe. Additionally, barley straw and alfalfa hay were provided every day. General linear model was used to statistically analyze data across all factors, using SPSS ver. 17, according to Steel and Torrie (1980).

Results and discussion

Problems with feed intake and consumption of the daily rations were not recorded in any experimental group, although previous studies reported that legumes used in diets have as a result a lower feed intake due to the presence of antinutritional factors (cyanogen glycosides, alkaloids, antitrypsic factors, etc.) (Liener, 1989 in Purroy *et al*., 1992). For the other parameters recorded the results were as follows:

a) Body weight:
The results showed that there were no differences between the experimental groups of dairy ewes and experimental treatments with regard to the mean body weight of the animals, on

days 1, 14 and 21, either tested on the basis of the animal or on the basis of experimental treatment. This was expected, since ewes of the experimental groups fed rations of about the same nutritional value, as per the study design. Bonanno *et al.* (2012) reported similar growth rate for lambs fed with pea beans instead of soybean concentrate, but other past studies (Purroy *et al*. 1992) showed that a lupine diet fed to lambs caused slower growth in comparison to other legumes, and more fat deposition in comparison to a soybean cake diet. Legume seeds are considered a tasty food by ruminants and therefore there are no problems of intake or consumption, even if the beans contain antinutritional factors, resulting in full consumption of the daily amount of feed provided, by all animals.

Table 1. Composition (%) of concentrated diets with the alternative legume seeds

	Control	Vetch 1/3	Vetch 2/3	Vetch 3/3	Broad beans 1/3	Broad beans 2/3	Broad beans 3/3	Lupines 1/3	Lupines 2/3	Lupines 3/3	Peas 1/3	Peas 2/3	Peas 3/3	Soy beans 1/3	Soy beans 2/3	Soy beans 3/3
Balancer	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Corn	63.6	60	56	52	60	53	46	61.6	57	54	61.6	58	51	60	56.5	54
Barley	20	20	20	20	19.1	20	19.8	20	23.9	25.5	18	13.1	12	21.9	24	24.9
Soybean meal 45%	12.5	8.7	4.5	0	1.2	5	0	8.5	4.3	0	9	5	0	8.5	4.5	0
Monocalcium phosphate	0.9	0.9	0.9	0.9	0.9	1.5	1.93	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Limestone	0.5	0.55	0.5	0.6	0.5	0.5	0.3	0.6	0.6	0.7	0.5	0.5	0.5	0.6	0.6	0.7
bypass fat powder	0	0.35	0.6	0.8	0.39	1.47	2.4	0	0	0	0	0	0.1	0	0	0
Alternative legume seeds	0	7	15	23.2	7.6	16	27	5.9	10.8	16.4	8	20	33	5.6	11	17
Magnesium oxide	0	0	0	0	0.01	0.03	0.07	0	0	0	0	0	0	0	0	0
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

b) Milk quantity:

There were no differences observed between the control group and the treatments using vetch, lupine, pea or soy beans (Data are not presented). This finding is different from the results of Bonanno *et al*., (2015), who reported higher yield for ewes fed a pea diet in comparison to soybean meal diet. Differences were statistically significant in the groups of broad beans in which, the administration of broad beans, resulted to a reduction in milk production, which was greater with the increase of broad beans content in the ration.

Table 2. Average milk production per experimental group

Group	Quantity
Control	$1.4998^{a} \pm 0.1408$
1/3 Broad beans group	$1.4004^{ab} \pm 0.1744$
2/3 Broad beans group	$1.3548^{ab} \pm 0.1786$
3/3 Broad beans group	$1.2841^{b} \pm 0.1118$

^{a,b} averages with different exponent differ statistically significantly ($p < 0.05$)

Although most researchers recommend the use of bean seeds in ruminants up to 30% of the ration, in this case our results are in agreement with Goelema *et al*. (1998, 1999), Yu *et al*., (2000, 2002), Selmi *et al.*, (2013), which notes that the bean seeds show increased degradation of starch and protein in the large intestine and only the heat treatment can reduce the degradation rate.

c) Milk quality:

As for the quality of the milk produced, there is a different picture depending on the legumes used. More specifically, in the case of the addition of vetch, the protein and fat content of the

milk increased with the increase of the seeds participation, while lactose content decreased. This may be due to the increase in the digestibility of the organic and the nitrogenous substances of the rations with the adding vetch. The digestibility of organic substance (DOM) and nitrogenous substances (ND) are presented in Table 3.

Table 3. Milk quality and digestibility per experimental group using vetch

Group	Protein	Fat	Lactose	DOM	ND
Control	$5.3354^{a} \pm 0.1498$	$6.5429^{a} \pm 0.1563$	$5.0164^{a} \pm 0.2525$	$91.5371^{a} \pm 1.8244$	$82.2486^{a} \pm 1.9764$
1/3 vetch group	$5.4000^{ab} \pm 0.1720$	$6.6054^{ab} \pm 0.1591$	$4.8600^{b} \pm 0.1003$	$92.0371^{ab} \pm 1.8243$	$83.6914^{b} \pm 1.2162$
2/3 vetch group	$5.4425^{b} \pm 0.1683$	$6.6425^{b} \pm 0.1525$	$4.8229^{b} \pm 0.1055$	$92.4794^{ab} \pm 1.8548$	$84.8028^{c} \pm 1.3683$
3/3 vetch group	$5.6461^{c} \pm 0.1114$	$6.7989^{c} \pm 0.1362$	$4.6604^{c} \pm 0.3193$	$92.8257^{b} \pm 1.6723$	$85.9143^{d} \pm 1.5203$

^{a,b,c} averages with different exponent differ statistically significantly ($p < 0.05$)

In the case that ewes fed a broad beans diet, an increase in lactose content and decrease in protein and fat content occurred, which is probably due to the increased rate of degradation.

Table 4. Milk quality and digestibility per experimental group using broad beans

Group	Protein	Fat	Lactose	DOM	ND
Control	$5.5411^{a} \pm 0.0855$	$6.7182^{a} \pm 0.0745$	$4.7400^{a} \pm 0.1971$	$92.1637^{a} \pm 1.8024$	$82.4146^{a} \pm 1.8851$
1/3 Broad beans group	$5.4789^{b} \pm 0.1179$	$6.6614^{b} \pm 0.1101$	$4.7989^{b} \pm 0.0757$	$91.9954^{b} \pm 1.7890$	$82.1554^{ab} \pm 1.1750$
2/3 Broad beans group	$5.4189^{c} \pm 0.1179$	$6.6214^{bc} \pm 0.1101$	$4.8389^{bc} \pm 0.0757$	$91.3878^{b} \pm 1.8436$	$81.5337^{b} \pm 1.3425$
3/3 Broad beans group	$5.3654^{c} \pm 0.0849$	$6.5718^{c} \pm 0.0827$	$4.9361^{c} \pm 0.1456$	$90.4015^{b} \pm 1.7678$	$80.5950^{c} \pm 1.4846$

^{a,b,c} averages with different exponent differ statistically significantly ($p < 0.05$)

Lupine beans treatments showed an increase in protein content of milk, a reduction in fat content and a decrease in lactose in comparison to the control group, possibly due to the progressive reduction of NDF rations by increasing the amount of beans.

Table 5. Milk quality and digestibility per experimental group using lupine beans

Group	Protein	Fat	Lactose	DOM	ND
Control	$5.3657^{a} \pm 0.0806$	$6.5907^{a} \pm 0.1114$	$4.9371^{a} \pm 0.1453$	92.1697 ± 1.7483	84.5444 ± 1.7483
1/3 Lupine beans group	$5.4282^{b} \pm 0.1116$	$6.5764^{a} \pm 0.0846$	$4.7650^{b} \pm 0.1488$	92.1441 ± 1.8244	84.6541 ± 1.2923
2/3 Lupine beans group	$5.5384^{c} \pm 0.0868$	$6.4318^{b} \pm 0.1214$	$4.8129^{b} \pm 0.1055$	92.0756 ± 1.8243	84.1516 ± 1.2922
3/3 Lupine beans group	$5.4986^{c} \pm 0.1161$	$6.3989^{b} \pm 0.1362$	$4.8307^{b} \pm 0.0744$	92.0371 ± 1.7818	83.9269 ± 1.7483

^{a,b} averages with different exponent differ statistically significantly ($p < 0.05$)

Similarly, when pea seeds were used, the protein content of the milk decreased and the fat and lactose content increased, with the exception of the group where the soybean meal was completely replaced by pea beans. Renna *et al* (2012) reported that incorporation of pa seeds in dairy ewes' diet did not affect significantly neither milk yield, nor milk fat, however variations were observed in milk fatty acid profile.

Table 6. Milk quality and digestibility per experimental group using pea beans

Group	Protein	Fat	Lactose	DOM	ND
Control	$5.4011^{ab} \pm 0.1686$	$6.6429^{b} \pm 0.1533$	$4.8621^{b} \pm 0.0982$	$91.5137^{a} \pm 1.8243$	$83.1746^{a} \pm 1.9764$
1/3 Pea beans group	$5.4493^{b} \pm 0.1638$	$6.5432^{a} \pm 0.1598$	$4.6654^{a} \pm 0.3078$	$92.1911^{a} \pm 1.8244$	$84.5054^{b} \pm 1.2162$
2/3 Pea beans group	$5.6261^{c} \pm 0.1114$	$6.6025^{ab} \pm 0.1138$	$4.8029^{b} \pm 0.1055$	$92.3791^{a} \pm 1.8548$	$84.6117^{b} \pm 1.3683$
3/3 Pea beans group	$5.3554^{a} \pm 0.1498$	$6.7589^{c} \pm 0.1362$	$5.0064^{c} \pm 0.2525$	$92.2568^{a} \pm 1.6723$	$84.2553^{b} \pm 1.5203$

^{a,b,c} averages with different exponent differ statistically significantly ($p < 0.05$)

Finally, the addition of soy beans to replace 1/3 of the soybean protein resulted in an increase in protein and fat content of milk and a reduction in lactose, and further replacement reduced all parameters due to the intense variations in nutritional value of the rations with the growing addition of the beans.

Table 7. Milk quality and digestibility per experimental group using soy beans

Group	Protein	Fat	Lactose	DOM	ND
Control	$5.3657^{c} \pm 0.0851$	$6.5907^{c} \pm 0.1114$	$4.9370^{c} \pm 0.1453$	$92.1141^{c} \pm 1.6908$	$82.7116^{a} \pm 1.8689$
1/3 Soy beans group	$5.4236^{d} \pm 0.1145$	$6.7521^{d} \pm 0.0837$	$4.7607^{a} \pm 0.1512$	$91.5112^{bc} \pm 1.7096$	$84.0984^{b} \pm 1.2188$
2/3 Soy beans group	$5.1907^{b} \pm 0.0851$	$6.4779^{b} \pm 0.1207$	$4.8129^{ab} \pm 0.1055$	$90.9935^{b} \pm 1.7853$	$84.6594^{b} \pm 1.3547$
3/3 Soy beans group	$5.0986^{a} \pm 0.1000$	$6.3789^{a} \pm 0.1362$	$4.8343^{b} \pm 0.0760$	$89.8144^{a} \pm 1.8026$	$84.3403^{b} \pm 1.5047$

^{a,b,c} averages with different exponent differ statistically significantly ($p < 0.05$)

Conclusions

Nutrition of ruminants is based mainly on soybean meal and maize. However, legumes constitute competitive crops in Southern Europe and low-cost alternatives for sheep nutrition. Substitution of soybean meal with locally produced legume seeds is a viable alternative for the Greek region of Thessaly and "Karagouniko" breed, as it does not affect body weight and milk production, with the exception of broad beans that caused a reduction in milk production. Furthermore, each legume diet had a different effect on milk main components, i.e. protein, lactose and fat, therefore being able to differentiate milk composition. More specifically, vetch, lupine and soy beans diets increased protein content, which is advantageous for cheese production.

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APPLICATION OF LORAWAN TECHNOLOGY IN PRECISION BEEKEEPING

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Abstract

Beekeeping is an important production branch of the agriculture and honeybees are one of the main pollinators in the world. Therefore, for the beekeepers it is crucial to have information about the state of the bee colonies. Application of information technologies in the beekeeping lead to the foundation of the Precision Beekeeping. Precision Beekeeping is based on constant data collection of bee colonies, where collected data should be transferred to a remote data centre for further data analysis. Data transmission in the field of beekeeping sometimes can be a challenging task, because beehives can be placed in rural areas without the option to get constant power supply and Internet connection. Authors of this paper chose LoRaWAN technological solution, as it allowed low energy consumption devices to communicate with Internet-connected applications over long range wireless connections for many years with only one battery. LoRaWAN network coverage and sensors were provided by Latvian company Lattelecom. Three bee colonies were equipped with LoRaWAN enabled temperature sensors. Measurements from sensors were transmitted to the LoRaWAN network gateways and servers and access to collected data was provided through the Lattelecom IoT portal web application. This research was carried out within the Horizon 2020 project SAMS "Smart Apiculture Management Services", proposing implementation of Precision Beekeeping by allowing active monitoring and remote sensing of bee colonies and beekeeping by developing appropriate ICT (Information and Communication Technologies) solutions supporting management of bee health and bee productivity.

Keywords: *Precision Beekeeping, LoRaWAN technology, Bee colony monitoring, Internet of Things.*

Introduction

In recent years fast development of Internet of Things (IoT) allows to collect data about various objects, including monitoring of living objects (Li et al. 2015). Data collection is the first stage also in the Precision Agriculture (Terry 2006) and it's branches like Precision Beekeeping (PB). Precision Beekeeping is an apiary management strategy based on the monitoring of individual bee colonies to minimise resource consumption and maximise the productivity of bees (Zacepins et al. 2015). For the data collection in Precision Beekeeping different sensors and technologies can be used (Meikle & Holst 2014). One of the issues in data collection in PB is data transfer from monitored object to the remote database or data warehouse for data storage and further analysis. As beehives usually are placed outside the urban areas, in deep forests or in rural locations, therefore connection to convenient data transmission networks (Wi-Fi, mobile networks) are limited or sometimes unavailable, as well 220V power supply for measurement devices can be unavailable. Therefore, there is a need of a new data transmission technology, which can transfer data for a long range and with low power consumption. One of the available options is to use LoRa (Long Range) technology.

LoRa stands for Long Range LPWAN (Low Power Wide Area Network) signal modulation technology proposed by Semtech (Georgiou & Raza 2017). LoRaWAN is a long-range, low-power, low-bitrate, wireless telecommunications network protocol specification, developed by

the LoRa Alliance, which uses LoRa signal modulation and is an infrastructure basis for the implementation of IoT solutions (Augustin et al. 2016). Together with long battery life, coverage is one of the most important features of the LoRaWAN solutions. Potentially communication range can achieve 10-15 km for outdoor coverage (Petäjäjärvi et al. 2016; Seye et al. 2017). Despite the potential of this technology it is not widely spread and used (Raza et al. 2016). Figure 1 below conceptually compares several data transmission technologies:

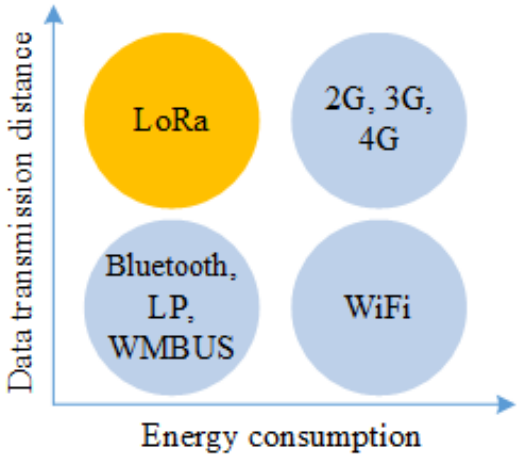

Figure 9. Comparison of data transmission technologies

Regarding agriculture sector, LoRaWAN also has huge potential. There are researches about possible implementation of that technology, for example implementation of smart irrigation (Zhao et al. 2017), soil parameter measurements (Xue-fen et al. 2017), but authors of this paper were not able to find any effort to use LoRaWAN in the beekeeping sector.

To use LoRaWAN technology it is possible to develop own infrastructure or use existing one. Many big telecommunication companies in the world provide such service, for example, Bouygues France telecommunications company has introduced LoRaWAN in 2015 and now more than 80% of the France territory is covered (http://www.objetconnecte.com/tout-savoirreseau-LoRa-bouygues). For author's research LoRaWAN infrastructure developed by Latvian company Lattelecom (https://iot.lattelecom.lv/) is used with main aim to evaluate the possibility to use it for Precision Beekeeping needs.

The Lattelecom group provides IT, telecommunication and outsourced business process solutions. Lattelecom Group is made up of five companies - SIA Lattelecom, Lattelecom BPO, Citrus Solutions and Lattelecom Technology with its subsidiary Baltic Computer Academy. The Lattelecom Group is the leading provider of electronic communications services in Latvia that offers electronic communication solutions for home, small and medium size businesses, state and municipal institutions, as well as for corporate clients.

In year 2017, Lattelecom started to develop LoRaWAN network and now is offering IoT services for municipalities and businesses for reading the utility meters, monitoring the utility infrastructure, controlling the street lights and etc. Advantages of Lattelecom network are:

- Lower cost comparing to WMBUS or 3G network;
- Low energy consumption;
- Huge amount of end devices supported by one gateway;
- Wide network coverage;
- Data transmission speed from 0.3 to 50 kbit/s.

To this moment LoRaWAN network is not covering the whole country, but is available in main cities. The capital Riga (which holds 32% of the population of Latvia, based on EUROSTAT Urban audit) has almost 99.9% network coverage (see Figure 2):

Figure 10. Lattelecom network coverage in Latvia
(source: https://www.lattelecom.lv/piedavajumi/iot/)

Concept of LoRaWAN working principle is demonstrated in Figure 3. End devices (sensors) are equipped with LoRa radio chip for a long-range data transmission. End nodes are communicating with gateways, which are connected to the Internet by the modern communication means (like fibre optics, or fast mobile internet). Those gateways are sending sensor data to the remote cloud based network server. Then developed Web application get data from the network sever to demonstrate it to the end user by the means of reports and data summaries.

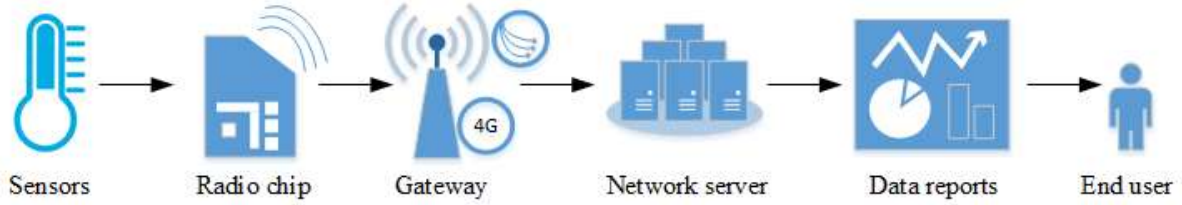

Figure 11. LoRaWAN working principle

Regarding Precision Beekeeping, there are several bee colony monitoring system architectures described (Kviesis & Zacepins 2015), in LoRaWAN scenario, each beehive (measurement node) is directly transferring the measurement data to remote network server and application using the gateway as a mediator.

Materials and methods

Based on the overall statistics beekeepers are not experienced IT specialists (not all of them), that's why installation of measurement system (measurement nodes) should not be complicated and should be done with little or no knowledge of IT. In author's case, installation of LoRa temperature sensor is trivial and user friendly – user only needs to put the sensor in the hive and mount the node to the beehive.

For authors research 3 (three) hives of *Apis mellifera mellifera* were equipped with temperature sensors for colony monitoring. Bee colonies were placed in an open environment under a hood (see Figure 4):

Figure 12. Placement of the beehives

The experiment took place at Strazdu iela 1, Jelgava, Latvia (N 56, 390, 4500 and E 23, 450, 1500). Sensors were installed on April 10, 2018. Norwegian-type hive bodies made of wood with external size 470 x 470 x 270 mm and internal size 380 x 380 x 270 mm, with a wall thickness of 45 mm, were used in the experiment. Sensors used were Adeunis Temp (see Figure 5) with references: ARF8181BA, ARF8180BA, ARF8181FA (source: https://www.adeunis.com/en/produit/temp/)).

Figure 13. Sensor Adeunis Temp

The temperature was measured every 10 minutes and data was transferred to a remote application. One temperature sensor per hive was placed above the hive body covering polyethylene foil as proposed by Stalidzans and Berzonis (Stalidzans & Berzonis 2013). Measurement units were mounted to the beehives as demonstrated in Figure 6:

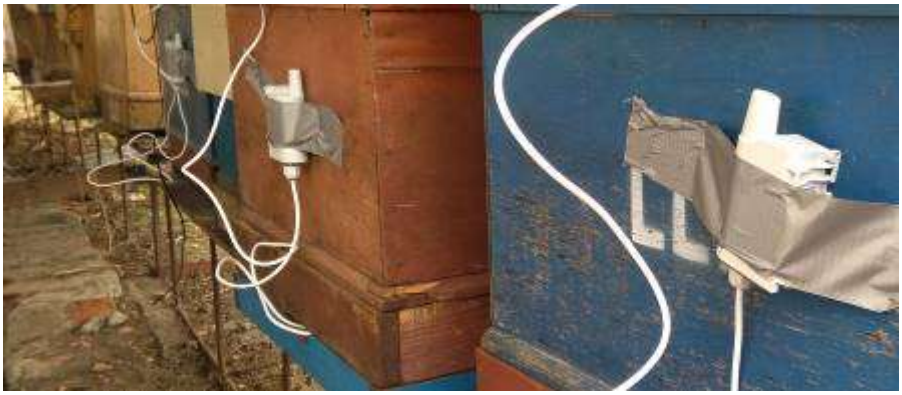

Figure 14. Beehives with mounted measurement units

Distance from the measurement point to the LoRaWAN gateway was approx. 2.4 km by the air in urban area (different obstacles, houses, trees, etc., see Fig. 7.). As a gateway Multitech MultiConnect Conduit with external antenna Taoglas OMB.868 is used.

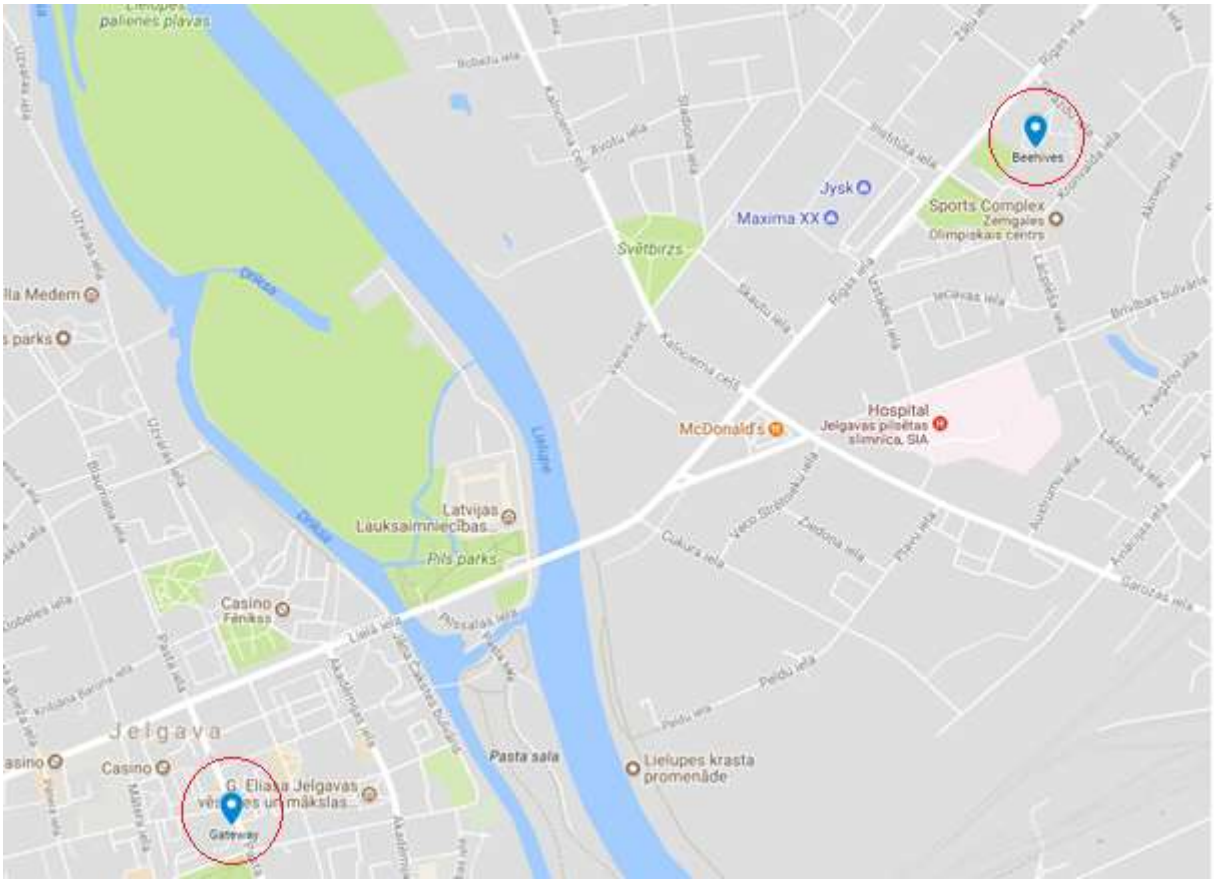

Figure 15. Location of beehives and gateway on the map

Web system for measurement visualisation

Bee colony temperature measurement data can be accessible using a Web based application (system). Web system is developed and provided by Lattelecom and is accessible by the address: https://iot.lattelecom.lv/portal/login. To get access to the measurement system administrator should register a new user and provide authorisation information (login and temporary password). After log in user can see his measurement objects (see Figure 8) and using pre-defined time periods (current month with 1 day step, last 7 days with 1 hour step,

last 24 hours with 15 min step or create custom time period) it is possible to visualise measurement data.

Figure 16. Screenshot of the web system

In addition, user can view all measurement data in table format and copy it if needed.
Usage of described above Web system is not the only option. It is possible to get access to the data warehouse and create individual system for data visualisation and analysis. This option is considered in case if more functionality is needed, for example implementing custom data analysis algorithms it is possible to define various bee colony states (death, swarming, brood rearing, etc.) and inform the beekeeper when manual inspection of bee colony is needed.

Results and Discussion

Aim of this research was to test LoRaWAN network for implementation of Precision Beekeeping. Practical experiments shows, that data transfer from the experiment beehives to the LoRa gateway was completed successfully without interruptions during the whole experiment. As distance was 2.4 km, open question is how data transfer will operate on longer distances. Within this research authors used already available infrastructure from local telecommunication company Lattelecom, but development of own infrastructure is possible too, by installing own LoRa gateways. Open question for discussion – is it economically feasible to develop own infrastructure or it is better to use existing one. This should be evaluated in each scenario and each country individually. Application of LoRa sensor for bee colony temperature monitoring is theoretically possible, but taking into account economic aspects is not so attractive, because of the measurement unit price, which is about 70 EUR per unit (2 temperature sensors per unit – out/in). However, taking into account that usually new technologies becoming more affordable after some time, authors foresee that application of LoRa sensors will be also economically feasible in the future.

Conclusions

LoRa sensors can be used for bee colony data collection for realisation of Precision Beekeeping. Advantage of LoRaWAN technology is data transmission range, which is crucial when Wi-Fi network is not available. LoRaWAN network solutions could be applicable in regions, where mobile networks have poor coverage and quality of services (e.g., Internet), for example African region, where is a good potential for Precision Beekeeping, but new technologies are not implemented so quickly.

Still Precision Beekeeping is not only limited by the temperature measurements; next important parameter is weight of the colony. Equipping scales with LoRa technology would be a good solution, because in that case amount of LoRa radio chip price will be comparable with scales price.

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LEVEL OF NATURAL RADIONUCLIDES IN ANIMAL FEED BY GAMMA –RAY SPECTROMETRY

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Abstract

The radionuclides' presence in animal feed is due both to natural radioactivity and radioactive pollution from different sources. Controls of radionuclides in animal feed will reduce the risk of radioactive hazards to animal and human health. The study was carried out in order to detect the natural radioactivity in animal feed and feed additive. Gamma spectrometer Canberra Packard with a high-purity germanium detector and Marinelli beakers (1 l capacity) were used for the samples measurement. The most prominent gamma energies observed in the spectra belonged to the naturally occurring radionuclides ^{40}K, ^{226}Ra and ^{232}Th. Other radionuclides if present occurred infrequently at low activity concentration under the measurable level. The results show that ^{40}K had the largest contribution to the specific radioactivity in all the samples. The mean activity concentration of the ^{40}K was highest in feed additive mono calcium phosphate (245.03±17.778 Bq/kg) and lowest activity concentration of the ^{40}K was measured in concentrate feed for dairy cows (23.20±2.626 Bq/kg). The average activity of the other two detected natural radionuclides in feed samples was lowest and ranged from 0.42 to 5.81 Bq/kg for ^{226}Ra and from 0.61 to 2.55 Bq/kg for ^{232}Th. The data analysis using ANOVA showed statistical significant differences in the radioactivity concentration of ^{40}K, ^{226}Ra and ^{232}Th between feed samples ($p<0.001$).

Key words: *gamma spectrometry, feeds, natural radioactivity.*

Introduction

The radioactive contamination of the animal organisms and body tissues primarily depends on the level of contamination of the food they consume, and to a lesser extent it depends on drinking water and by means of inhalation. With the use of animal feed, animals in their nutrition accumulate natural radionuclides, and to a lesser extent artificial radionuclides (^{90}Sr, ^{137}Cs, etc.). The fast industrial development and human activities in agricultural practices and livestock production significantly increase the environmental pollution, thereby leading to an increase of the concentrations of various radioactive substances such as strontium, cesium and uranium. Radionuclides have very quick migration and it is very difficult to prevent their transmission through the food chain (Petrović and Mitrović, 1994).

According Saračević (1999), the animals can be radioactively contaminated in several ways, however most commonly through nutrition or drinking water (80%).

An increasing care is dedicated to the radiation level in animal feed because ingestion through the mouth is one of the most frequent ways how radionuclides enter the living organisms. In order to increase the nutritional value of animal feed, substances containing increased levels of radionuclide activity are added, which will increase the concentration of radionuclide's in the feed. Considering that people use poultry meat and eggs as a part of their nutrition, it is necessary to monitor the levels of radioactive contamination in animal feed because it can

easily be deposited in humans through the path of radionuclides in the food chain (Hernandez et al., 2004).
Numerous researches have been performed which enable monitoring of the radioactive contamination of animal feed in order to reduce the risks that can take these quantities to humans (Carvalho et al., 2006; Casacuberta et al., 2009; Shanthi et al., 2009; Tchokossa et al., 2013).
However, in Macedonia there is a lack of data on the concentration of natural radionuclides in animal feed, namely the purpose of this paper was to assess the amount of natural radionuclides in samples of animal feed produced in Macedonia and to compare the values with already measured values of animal feed that was produced in other parts of the world. Bearing in mind that radioactive contamination in animal body tissues primarily originates from the level of contamination of the used animal feed, as well as the feeding water, a preventive measure would be to control the radioactivity of the animal feed being used, that is, if possible, it should be with a lower concentration of radioactive isotopes, which would not harm the animal organism.
Preventive measures for the reduction of the radioactive contamination of animals are also the method of land treatment which is applied for growing crops for animal feed or grazing, changes in the regime of animal management, giving binding agents or analogous products to animals and delayed slaughter of animals (Beresford and Howard, 2011).

Materials and methods

Sampling

The animal feed samples were collected on the basis of production and consumption in Macedonia. The samples were taken from several producers, and several samples were directly purchased from the market. The animal feed samples were crushed and then homogenized to a fine powder. All samples were left in hermetically sealed plastic containers with approximately 450g of sample. The containers were stored for 14 days in order to allow radium and thorium to achieve a secular balance with their (daughters) descendants.

Instrument

The samples are measured on an instrument – gamma spectrometer (Canberra Packard) with high-purity germanium detector. The measurement was performed in containers which were hermetically sealed so that ^{222}Rn produced from decay of ^{226}Ra will not result in gas leak. After the provision of time balance between the successors of ^{238}U and ^{232}Th series (21 days), these sealed samples were prepared for an analysis. The obtained spectra from the measurement were analyzed by using the program GENIE 2000. The specific activity of ^{226}Ra is calculated for energy line on 186,1 (keV) and ^{232}Th through its descendant of decay ^{228}As (second in the decayed sequence), that is, through its three gamma decay energy lines which occur on 338,4; 911,07 and 968,9 (keV).

The activities of ^{40}K were determined from its γ-line from 1460 keV. The interval of the time for calculation (counting) was 108000 seconds. The natural spectrum was recorded immediately after or before the calculation of the sample.

Activity calculation

The specific activity A (Bq/kg) is determined in accordance with the following formula by Garcia-Talavera (2003):

$$A = \frac{\frac{N}{t} - \frac{N_0}{t_0}}{\varepsilon \cdot \gamma \cdot m}$$

Where, N is clean surface of peak accumulated from a specific radionuclide in analysis of a specific sample (number of readings), N_0 is clean surface of peak accumulated from the spot of a specific radionuclide without an analysis of sample (number of readings), t is live time of accumulation of the sample spectrum (s), t_0 is live time of accumulation of the phone spectrum (s), ε is detector efficiency for a given energy (for a specific peak), γ is intensity of gamma transition in radioactive decay for a respective radionuclide (%), and m is mass of the sample (kg).

Results and discussion

The activity concentrations of ^{40}K, ^{226}Ra and ^{232}Th were assessed and they are presented in Table 1 and in Figure 1.

Table 1. Activity concentration of natural radionuclides in feed samples (Bq/kg)

Samples	n	^{40}K ($\overline{x} \pm S_{\overline{x}}$)	^{226}Ra ($\overline{x} \pm S_{\overline{x}}$)	^{232}Th ($\overline{x} \pm S_{\overline{x}}$)
Concentrate feed for pigs	5	226.13±11.911	1.81±0.269	0.61±0.086
Concentrate feed for dairy cows	5	23.20±2.626	5.81±0.982	1.99±0.281
Feed additive mono calcium phosphate	5	245.03±17.778	1.46±0.138	2.55±0.217
Maize	5	119.25±13.732	0.42±0.059	0.68±0.144
Total	20	153.40±15.542	2.37±0.411	1.46±0.164

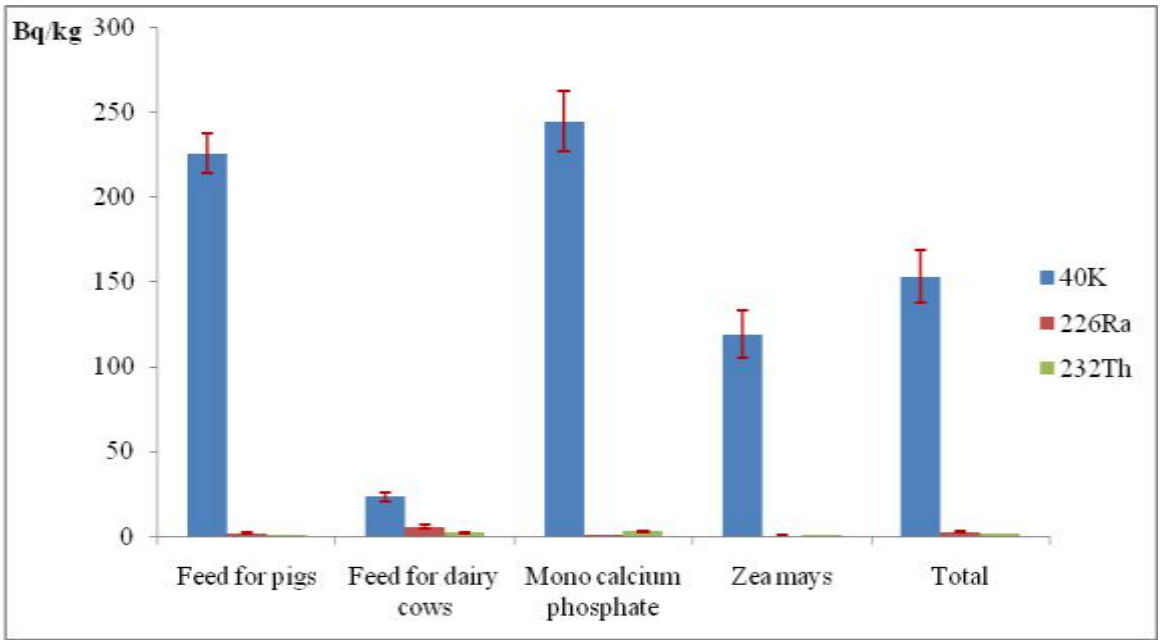

Figure 1. Activity concentration of naturally occurred radionuclides in feed samples

The results show that ^{40}K had the largest contribution to the specific radioactivity in all samples. The mean activity concentration of ^{40}K was highest in feed additive mono calcium phosphate (245.03±17.778 Bq/kg) and lowest activity concentration of ^{40}K was measured in feed concentrate for dairy cows (23.20±2.626 Bq/kg). The average activity of the other two

detected natural radionuclides in feed samples was lowest and ranged from 0.42 to 5.81 Bq/kg for ^{226}Ra and from 0.61 to 2.55 Bq/kg for ^{232}Th.

The main source of calcium and phosphorus in the concentrate for pigs is monophosphate (Ševković et al., 1991). This supplement is obtained by processing phosphorus ore, and it can also contain large amounts of uranium, thus being a potential source of radioactive contamination both for animals and people (Mitrović et al., 2011).

The results show that the measured monophosphate has a significant activity in all analyzed radionuclides. Considering the concentration of the radionuclide in the monophosphate brand, one can conclude that a large percentage of the radioactivity measured in animal feed is a result of food that contains this component. However, one cannot determine the level of radioactivity that is directly attributable to monophosphate, since the manufacturer does not specify the amount of monophosphate in nutrition. The presence of anthropogenic radionuclides was not detected, indicating that there was no contamination due to artificial radionuclides.

Conclusion

The concentrations of activity of natural radionuclides, ^{40}K, ^{226}Ra and ^{232}Th, were assessed in this study, in different samples of animal feed in the Republic of Macedonia. The results are similar with other such research. However, the values of the concentrations of the tested radionuclides are within the limits of the permitted values i.e. the transfer of such levels in animal feed and ultimately, in the human, through the radionuclide pathway, will not pose a threat when people will ultimately consume poultry meat, products and eggs of poultry fed with this feed. At the same time, systems should be established to monitor radionuclides in the main foodstuff in order to reduce human exposure to radiation through the consumption of animal products. Prevention is perhaps the best approach, and much attention needs to be paid to reduce radioactive contamination in animals and further in consumers as well. It should be emphasized that continuous monitoring of the level of natural and artificial radionuclides in animal feed is necessary in order to mitigate the amount of radioactive substances that can reach the human organism through the trophic chain.

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PRELIMINARY RESULTS ON ZINC CONCENTRATION IN WILD FISH TISSUES IN VARDAR RIVER, MACEDONIA

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Abstract

The concentrations of Zinc in fish from the Vardar river have been investigated in order to assess safety for consumers and the level of contamination. The selected tissues (skin, liver and muscle) of two fish species: European Chub (*Squalius cephalus*) and Common barbell (*Barbus barbus*) from Vardar river stream, Macedonia. Samples are collected in nine different points (Hot Spots) in total distance of 301 km and approximate 33 km between sampling sites. The effect environmental conditions and urban discharges on zinc accumulation in skin, muscles and liver were investigated. The metal analyses were performed using flame atomic absorption spectroscopy (AAS). The average of metal concentrations (micrograms per gram wet weight) in nine hot spots (HS) occurred in the following ranges: HS-1(skin 50.89503 – liver 30.8755 – muscles 8.829244) to HS-9 (skin 44.84977 - liver 43.03663 - muscles 13.8143.)The lowest levels of the zinc were detected in the muscles. The skin and liver were found to accumulate the highest amounts of Zn. In the case of organs, the highest levels were found, as follows: skin > liver > muscles. Further investigation of heavy metals is recommended, including a survey of fish consumption frequency among the local inhabitants.

Keywords: *heavy metals, zinc, fish tissue, Vardar river, Macedonia*

Introduction

Fish is an important part of the human diet, but also a good indicator of trace metal pollution in the aquatic ecosystem. Fish samples are considered as one of the most indicative factors, in freshwater systems, for the estimation of trace metals pollution potential (Rashed, 2001). Organisms retain Zn, through specific binding proteins known as metallothioneins in their liver (Allen-Gil and Martynov, 1995).

Pollution of water bodies is becoming a major cause of concern with respect to human health (Jarup, 2003). Metals in waters may be of natural origin from the rocks and soil or from human activities, including industry, domestic wastewater, agricultural discharge, mine runoff, solid waste disposal and atmospheric deposition. Metals generally enter the aquatic environment through atmospheric deposition, erosion of the geological matrix, or due to anthropogenic activities caused by industrial effluents, domestic sewage, and mining wastes. Increase in the human population has greatly contributed towards the conversion of these water bodies to impending contamination sinks (Tarvainen et al., 1997; Stephen et al., 2000).

Heavy metals are well known to be non-biodegradable and when present at high concentrations, they tend to bioaccumulate (DeForest et al, 2007). Being non-biodegradable, metals can be concentrated along the food chain, producing their toxic effects at points often far away from the source of the pollution (Fernandez et al., 2000). Heavy metals can cause a variety of ailments in humans depending on the degree of exposure. These vary from minor skin irritation to severe damages of the liver, kidney, nerve tissues and circulatory system. Similarly, although zinc (Zn) is an essential requirement for good health, excess zinc can be harmful. Zn has a multitude of biological functions in the human body. It is an important

constituent of over 100 enzymes involved in a variety of fundamental metabolic processes. It is involved in the production and function of several hormones. Zinc toxicity can occur in both acute and chronic forms. Acute adverse effects of high zinc intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches,(Jarup, 2003; Khallaf et al., 1998).

River Vardar is the longest river in the Republic of Macedonia and Republic of Greece with distance of over 388 km, with his source in the location Vrutok and stream at the Aegean sea. The Vardar river, with its tributaries, makes up a great part of the total water resource of Macedonia. These tributaries and river Vardar are directly or indirectly connected with the mining areas for Zinc (Zn) in the locations. The river is widely used for fisheries (wild fish and fish farming), sports and recreation. Very little recent information is available regarding the contamination with metals in the Vardar river fish species. The river Vardar passes near and across the biggest cities including Gostivar, Tetovo, Skopje, Veles, Negotino, Demir Kapija and Gevgelija with possibility of environmental contamination from domestic and industrial sewage Hot point spots in Fig.1.

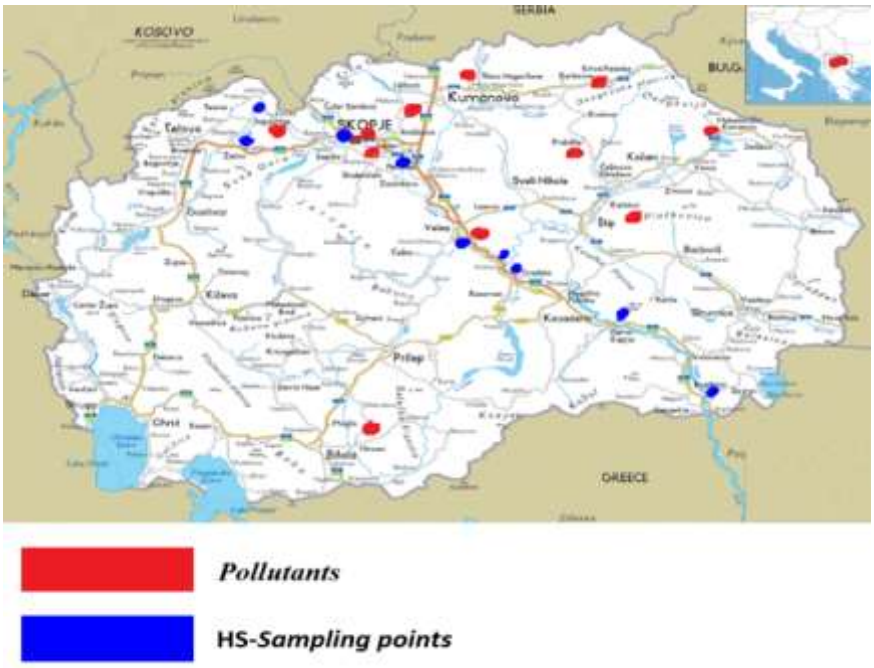

Fig.1 In the map of the Republic of Macedonia, showing hot point spots

The aim of this study is to provide information and evaluate the level of Zinc as a heavy metal in fish organs (muscle, liver and skin) from Europian Chub (*Squalius cephalus*) and Common barbel (*Barbus barbus*) fish species.

Material and methods

Samples were collected in nine (9) places with distance between them in around 33 km along the river. From each place were fishes are collected from both species with support from fishermen's.

Fish samples were transported in Pathology laboratory of the Veterinary Institute Prishtina, submitted for dissection of target organs were collected for study including skin, liver and muscle. Each sample of fish tissue has been measurement with analytical scale then set in plastic bag, marked with number which show the catching location and tissue then finally

refrigerated below the -18ºC. Total numbers of samples were sixty (60) from Europian Chub and Common barbell.

Next step was the sample and set in to the porcelain pots then the sample was heated in microwave in 105°C for 24h to drain. The next day the samples were taken out from microwave and they passed to the stove for 24h in 550°C where they were burned. After 24h the burned samples are prepare for digestion with HNO3 65%. Prepared sample after digestion are ready for reading and analyze by flame atomic absorption spectroscopy (AAS) with the Atomic absorption spectrometer produced from Thermo. All data are presented in the unit $\mu g/g^{-1}$ wet weight of a sample tissue, and are processing with comparative methods

Results and discussion

Results of this study showed that the metal concentrations accumulated in the tissue samples were in descending order of skin > liver > muscles. In the study, we found that the concentration of Zinc were different in the analyzed organs and differently in the sampling locations. The average of concentration of the Zinc in the organs (skin, liver and muscle) and the locations (nine locations) is showed in Table 1.

Table 1. Mean Heavy Metal Content ($\mu g/g^{-1}$ wet weight) in Fish skin, muscle and liver

Sampling point	Muscle	Liver	Skin
HS-1	8.82924	30.87550	50.89503
HS-2	10.25356	34.74869	36.88801
HS-3	17.63045	36.68569	59.80203
HS-4	13.39171	45.59118	41.53124
HS-5	13.16620	46.83435	42.93423
HS-6	8.74029	52.58685	40.45726
HS-7	12.19933	12.60700	46.09540
HS-8	13.17433	52.38320	39.21015
HS-9	13.81430	43.03663	44.84977

Higher Zn concentrations were found in liver tissue, while the lowest were detected in muscle tissues. This finding is in agreement with those of other studies regarding the differences between heavy-metal accumulation in fish tissues (Carpene and Vasak, 1989; Allen-Gil and Martynov,1995).

There guidelines on acceptable levels of Zn in the edible parts of fish suggested in Macedonia is according to international standards 40-100 μg/g. According to our results, there is metal contamination, but it is lower than the guidelines, in the edible part of the examined fish. The examined fish were not associated with enhanced Zn content in their muscle and were safe within the limits for human consumption.

From results we can see that we have a different Zinc concentration, and as we can see that highest level is in the HS-3 sampling site, which is the point where the river Treska is connected to the river Vardar. In the most studies of similar analyzed samples, the liver accumulate the highest concentration of Zinc in our study is shown that is the samples of skin in sampling sites HS -3 and till the HS-9, showed that fishes in the part of river Vardar from Skopje till the border with Republic of Greece accumulated higher concentration of Zinc. In previous study, increased concentrations of these hazardous substances, especially Pb and Cd, in water and sediment from the lower part of the Vardar River, influenced higher accumulations of metals in liver, gills and gonads of Gobio gobio L. (Nastova et al. 2017), concentration of Zn is not reported.

In our country the river pollution with heavy metal contaminations are in the concern because off potential thrived waste from domestic and industrial sewage, non-secured industrial landfill and mining fields. The Zn is essential elements and is carefully regulated by physiological mechanisms in most organisms (Eisler, 1988). However, they are regarded as potential hazards that can endanger both animal and human health. Knowledge of their concentrations in fish is therefore important both with respect to nature management and human consumption of fish as suggested in Amundsen et al., (1997).

Aquatic organisms have been widely used in biological monitoring and assessment of safe environmental levels of heavy metals In this study metals concentrations in the muscle of both fish species were used to investigate possible transfer of metals to human populations via fish consumption. Since accumulation of metals in the biological system is dangerous to human beings in our country, there is a need for regular or continuous monitoring of heavy metals concentrations in the aquatic environments.

In Vardar river, among all river/aquatic organisms the fish are the most interested for humans for sport activities in particular, source of food mainly for fisherman's and their families as well. Fishes are considered as indicators in river ecosystem for heavy metal contaminations with light level risk as a potential for human food consumption, because fish are in the top of aquatic food chain, heavy metal accumulation and possibly for the transfer heavy metals on the humans.

Conclusion

Our preliminary results provide information for the levels of Zn in common fish species of the Vardar river. Results will contribute to the effective monitoring of both environmental quality and the health of the organisms inhabiting the river ecosystem. According to the fish sample analyses the range of concentration with Zinc is in the lower border with the range of international standards Zn:40-100$\mu g/g^{-1}$ wet weight, and it shows that the fishes from investigated Vardar river are safe for human consumption.

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QUALITY OF PORCINE MEAT

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Abstract

The impact of the Swedish Landrace and Large Yorkshire sire breeds (SL and LW), sires within the breed, of gender and the fattening season on the variability of pork quality traits was examined in the present study. The observed measurements were the pH value (pH_{45} and pH_{24}) of the *m. longissimus dorsi* and *m. semimembranosus*, the chemical composition (water, fat, ash and protein content), the water binding capacity, the colour and thickness of the *musculus longissimus* muscle fibers. The trial was conducted at the experimental farm and slaughterhouse of the Institute for Animal Husbandry, Zemun-Belgrade (Serbia). Sires were pure breeds: Swedish Landrace (SL, n=10) and Large White (LW, n=3). The pH value was measured in 410 offspring born in the winter, summer and autumn. Samples of *m. longissimus* originate from 50 offspring (29 samples taken from the offspring of SL sires and 21 samples of LW sires). It was found that the sire breed did not influence ($P>0.05$) the pH values of the muscles tested, but that the sires within the breed influenced ($P<0.01$) the pH_2 of the *m. semimembranosus*. The birth season of offspring shows high statistically significant impact on the pH_2 of the tested muscle ($P<0.001$), while the gender of the fatteners had no influence on the tested properties ($P>0.05$). The results show that *musculus longissimus* contained on average 73.10% of water, 24.09% of protein, 1.65% fat and 1.17% ash. The influence of the sire breed ($P<0.05$) on the water content and the influence of the sire within the breed Swedish Landrace ($P<0.05$) on the protein content were determined, while the gender of fatteners showed no impact ($P>0.05$) on the chemical composition of *m. longissimus*.

Keywords: *Genotype, Sire breed, Sex, Season, Fatteners*

Introduction

Meat quality is a complex term that is used today to describe the overall meat properties. One of the most accepted definitions of meat quality was given by Hofmann (1994), who described meat quality as the sum of all sensory, nutritive, hygienic-toxicological and technological properties of meat. Andersen *et al.* (2005) defined meat quality as a complex and multivariate feature influenced by many factors in interaction, including the conditions in which it was produced. Proteins have long been regarded as the most important component of meat. However, the importance of polyunsaturated fatty acids in meat is increasingly emphasized. Essential fatty acids found in the human brain are not found in plants only in meat. For this reason, meat is irreplaceable for humans, i.e. for normal and balanced nutrition. In this regard, the lack of polyunsaturated fatty acids in the diet presents a greater problem, from the point of view of people's health, than the lack of protein. Selection can influence the content of intramuscular fatty tissue, i.e. the content of fat in meat (Dević *and* Stamenković, 2004; Mason *et al.*, 2005). Uncastrated male animals, compared to females, have a much lower percentage of fat, according to Čepin *and* Žgur (2003) in the same diet regime. Nakev *et al.* (2016) were determined the effect the season and year of slaughter have an impact on the share of meat in the carcass sides.

The selection is a powerful tool for reducing fat content, that is, increasing the share of meat in carcass sides, also, adequate animal nutrition provides the ability to reduce fat content and

fatty acid changes. For pork the appearance of changes in the color and structure of the meat is characteristic, also the ability to retain water and other properties. Čobanović *et al.* (2017) state that the examination of the presence of liver spots on the slaughter line can be an important indicator not only for the welfare of pigs on the farm, but also for the quality of the carcasses and pig meat. Today, there are growing demands on the quality of pork in the market and, therefore, it is increasingly becoming the object of the mutual interest of the pig producers and the processing industry (processing and preserving meat and meat products) in the world. Possible prediction of the quality of pork is one of the decisive and key roles in the business success of the producer, and, in relation to this, the genetic basis becomes the primary in setting the production goals (Chan *et al*., 2002).

The aim of the conducted research was to determine the effect of sire breed, sires within the breed, gender, birth season of offspring on the quality of pig meat.

Material and Methods

The trial was conducted at the experimental farm and slaughterhouse of the Institute for Animal Husbandry, Zemun-Belgrade. Sires were pure breeds: Swedish Landrace (SL) and Large White (LW). The observed properties were the pH value (pH_{45} and pH_{24}) of the *m.longissimus* and *m.semimembranosus* of three sire breed SL (n=102; n=74 and n=36 offspring) and of three sire breed LW (n=83; n=53 and n=63 offspring). The chemical composition (water, fat, ash and protein content), the water binding capacity, the colour and thickness of the *musculus longissimus* muscle fibers. The pH value was measured in 410 offspring (207 male and 203 female offspring) born in the winter, summer and autumn. Samples of *m.longissimus* originate from 50 offspring were taken between the 13th and 14th rib (29 samples taken from the offspring of four SL sires and 21 samples of three LW sires). Data was processed by applying the adequate software package "LSMLMW and MIXMDL, PC-2 VERSION" (Harvey, 1990), i.e. by using the procedure of the Least Square Method in order to determine the significance ($P<0.05$) of systematic influences on traits of meat quality. Model included: sire breed, sires within the breed, gender, birth season of offspring and carcass side mass (linear effect).

Results and Discussion

By testing the two muscles - *musculus longissimus* (ML) and *semimembranosus* (SM), identical average values (Table 1) of pH_1 for ML and SM (pH = 6.54) were determined, while the pH value of ML for 24 hours (pH_2) decreased faster than of SM (5.70: 5.77). According to Honikel (1999), who states that the pH_{45} values of the muscles of "normal" quality are greater than 6.0 and pH_{24} values range from 5.4 to 5.85, we can say that mean values obtained in the present study are within the limits indicated, i.e. that the fatteners had muscles of normal quality. The results of our study show that the pH_2 (ML and SM) varied under the influence of the birth season ($P<0.001$) and between the sires within the breed SL ($P<0.01$) and LW ($P<0.01$) and that the other included factors did not exhibit statistically significant influence ($P>0.05$). The results obtained are contrary to the studies of a group of authors who have found that the sire breed affects the variation of the pH_1 and pH_2 values (Latorre *et al*., 2003; Josell *et al*., 2003; Radović *et al*., 2009).

Table 1. The effect of sire breede and sires within breed on pH of muscle (LSMean ±S.E.)

Source of variation		pH_1- ML[3)]	pH_1-SM	pH_2- ML	pH_2-SM
μ ± S.E.		6.54 ±0.02	6.54 ±0.02	5.70 ±0.01	5.77 ±0.01
SB[1)]	Sire No.				
Swedish Landrace	1	6.50±0.04	6.48± 0.04	5.67± 0.02	5.73± 0.02[a]
	2	6.46±0.04	6.54± 0.04	5.68± 0.02	5.74± 0.02 [a]
	3	6.58±0.05	6.61± 0.05	5.73± 0.02	5.83± 0.03[b]
	Average	6.51±0.03	6.54± 0.03	5.69± 0.01	5.77± 0.01**
Large white	4	6.63±0.04	6.57± 0.04	5.70± 0.02	5.75± 0.02[a]
	5	6.52±0.05	6.51± 0.04	5.71± 0.02	5.76± 0.02[a]
	6	6.54±0.04	6.53± 0.04	5.72± 0.02	5.83± 0.02[b]
	Average	6.56 ±0.03	6.54±0.03	5.71±0.01	5.78 ±0.01**
P (sire breed)		NS	NS	NS	NS
Sex	M[2)]	6.53±0.03	6.54± 0.03	5.70± 0.01	5.78± 0.01
	Ž	6.55±0.03	6.54± 0.03	5.70± 0.01	5.77± 0.01
P		NS	NS	NS	NS
Season	Winter	6.58±0.07	6.58± 0.06	5.62± 0.03	5.67± 0.03
	Summer	6.52±0.04	6.51± 0.03	5.76± 0.02	5.85± 0.02
	Fall	6.52±0.02	6.53± 0.02	5.72± 0.01	5.81± 0.01
P		NS	NS	***	***
WCSW (b)		-0.001[NS]	0.001[NS]	-0.002*	-0.001[NS]

[1)]SB-sire breed; [2)]M- male castrated, Ž-females; WCSW (b)- linear effect of the warm carcass side weight (WCSW=81.44 kg); [3)] ML- *musculus longissimus*, SM- *musculus semimembranosus*,
P-Level of significance for factors NS - P>0.05; * - P<0.05; ** - P<0.01; *** - P<0.001

The descendants of the SL sires compared to fatteners deriving from LW sires (Table 2) had significantly (P<0.05) higher water content in ML (73.32% vs. 72.81%). The share of protein in ML differed significantly (P <0.05) between sires (23.30% vs. 24.67%).

Table 2. The effect of sire breede and sires within breed on ML chemical composition (LSMean±S.E.)

Source of variation		Water, %	Fat, %	Ash, %	protein, %
μ ± S.E.		73.06±0.10	1.67±0.08	1.17±0.01	24.10±0.12
SB[1)]	Sire No.				
Swedish Landrace	1	73.29±0.26	1.55±0.21	1.17±0.02	23.97± 0.31
	2	72.81±0.27	1.37±0.22	1.14±0.02	24.67± 0.33
	3	73.28±0.27	1.65±0.22	1.17±0.02	23.89± 0.33
	13	73.89±0.27	1.66±0.22	1.16±0.02	23.30± 0.33
	Average	73.32±0.14	1.56±0.11	1.16±0.01	23.96± 0.16*
Large white	4	72.55±0.28	1.84±0.23	1.21±0.02	24.40± 0.34
	5	72.78±0.27	1.70±0.22	1.16±0.02	24.36± 0.33
	6	73.10±0.27	1.78±0.22	1.17±0.02	23.95± 0.33
	Average	72.81±0.16	1.77±0.13	1.18±0.01	24.23± 0.19
P (sire breed)		*	NS	NS	NS
Sex	M[2)]	73.01±0.13	1.66±0.11	1.16±0.01	24.18± 0.16
	Ž	73.12±0.16	1.68±0.13	1.18±0.01	24.01± 0.19
P		NS	NS	NS	NS
WCSW (b)		-0.061**	0.006[NS]	-0.001[NS]	0.056*

[1)]SB-sire breed; [2)]M- male castrated, Ž-females; WCSW (b)- linear effect of the warm carcass side weight (WCSW=81.44 kg); P-Level of significance for factors NS - P>0.05;
* - P<0.05; ** - P<0.01; *** - P<0.001

In regard to the nutritional quality of pig meat, according to the previous studies (Ryu *and* Kim, 2005; Purslow, 2005; Kušec *et al.*, 2006), the content of water, proteins, fats and minerals range as follows (respectively): 60-75%; 18-21%; 1.5-5.9% and 0.8-1.2%. The obtained average values of the nutritional quality of ML in our study are within the stated limits except for the protein content (24.09%) which was higher than the values established by Mason *et al.* (2005) and Olsson *et al.* (2003) for the Swedish Landrace.

Table 3. The effect of sire breede and sires within breed on meat quality properties and muscle fibre thickness (LSM ±S.E.)

Source of variation		WBC[3)]	Boja mesa	MFT, μm
μ ± S.E.		57.07± 0.76	0.361± 0.010	63.80± 1.20
SB[1)]	Sire No.			
Swedish Landrace	1	57.82± 1.88	0.333± 0.025	67.74± 2.51
	2	59.97± 1.99	0.423± 0.026	62.76± 2.51
	3	52.12± 2.01	0.322± 0.027	64.18± 2.54
	13	48.38± 2.02	0.285± 0.027	-
	Average	54.58± 1.00***	0.341± 0.013**	64.90± 1.47^{NS}
Large white	4	59.29± 2.07	0.368± 0.027	64.29± 2.60
	5	60.33± 1.97	0.425± 0.026	61.41± 2.53
	6	59.08± 1.97	0.347± 0.026	62.43± 2.43
	Average	59.57± 1.17^{NS}	0.380± 0.016^{NS}	62.71± 1.59^{NS}
P (sire breed)		***	NS	NS
Sex	M[2)]	57.07± 0.98	0.370± 0.013	63.05± 1.33
	Ž	57.07± 1.15	0.351± 0.015	64.56± 1.70
P		NS	NS	NS
WCSW (b)		0.007^{NS}	0.002^{NS}	0.295^{NS}

SB-sire breed; [2)]M- male castrated, Ž-females; WCSW (b)- linear effect of the warm carcass side weight (WCSW=81.44 kg for WBC and meat colour; WCSW=80.45 za MFT);
[3)] WBC-water binding capacity, MFT-muscle fibre thickness;
P-Level of significance for factors NS - P>0.05; * - P<0.05; ** - P<0.01; *** - P<0.001

In regard to the water binding capacity (WBC), in our study (Table 3), the influence of the sire breed (P<0.01) was determined, which is in accordance with Radovic *et al.* (2009) and contrary to the research by Jukna *et al.* (2009). Our results are in agreement with Radović *et al.* (2009) who have found that the sex (gender) of fatteners did not affect the variation of WBC, but are contrary to the results of Jukna *et al.* (2009) who have determined the effect of the given factor (P<0.05). In regard to the meat colour, in our study, the variation of this property between the half-siblings of different sires of SL breed (P<0.01) was determined, which is in agreement with the research by Radović *et al.* (2009) for the influence of a sire's breed, but in contrast to the impact of sex. The obtained average value of muscle fiber thickness was in the range determined by Radović *et al.* (2009), and lower than the top value indicated by Migdal *et al.* (2005) and Makovický *et al.* (2009a, b). The thickness of the muscle fiber did not vary under the influence of the sire breed, which is contrary to the research by Radović *et al.* (2009).

Conclusions

In this study, we found that the sire breed did not influence (P>0.05) the pH values of the muscles tested, but that the sires within the breed influenced (P<0.01) the pH_2 of the *m.semimembranosus*. The birth season of offspring shows high statistically significant impact on the pH_2 of the tested muscle (P<0.001), while the gender of the fatteners had no influence on the tested properties (P>0.05). The results show that *musculus longissimus* contained on average 73.10% of water, 24.09% of protein, 1.65% fat and 1.17% ash. The influence of the

sire breed (P<0.05) on the water content and the influence of the sire within the breed - Swedish Landrace (P<0.05) on the protein content were determined, while the gender of fatteners showed no impact (P>0.05) on the chemical composition of *m. longissimus*.

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REPRODUCTIVE PROPERTIES OF COWS OF DIFFERENT ORIGIN AND REARING METHODS

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Abstract

The improvement of the production characteristics of the Simmental breed in Serbia is mainly done through purebreeding. Selection is a factor without which there can be no serious results in the improvement of the genetic basis and increase in the productivity of livestock production in general. In order to realize faster improvement of production performance, of the genetic composition and increase of number of cattle in Serbia, more and more farmers have recently decided to import cattle from countries with intensive breeding of Simmental cows, primarily from Austria and Germany, i.e. from countries where the average milk production ranges from 6500 to 7000 kg with over 4% milk fat. The aim of the study wasto examine how the import of the animals influences four reproductive traits (body weight of calves at birth, age at first calving, calving interval, duration of service period) in domestic and imported populations of Simmental cows. The present study included 954 cows, with a total of 3641 completed lactations. All cows were located in the area of Toplica district, reared by individual agricultural producers (tied system) and on farms with intensive rearing (free system). On the basis of the housing system (tied and free systems) and origin (domestic and imported) animals were divided into four groups: Group 1 (animals of domestic origin, reared by individual producers/farmers); Group 2 (imported animals, reared by individual producers/farmers); Group 3 (animals of domestic origin, reared on commercial farm) and Group 4 (imported animals, reared on commercial farm). In regard to the investigated traits, the highest values were recorded in the fourth group of observed cows, while all the reproductive properties varied very significantly ($p \leq 0.001$) under the influence of the unified factor of the housing method and origin, except for the age at first calving whose variation was not statistically significant ($p > 0.05$)

Keywords: *Reproductive properties, origin, housing, Simmental breed*.

Introduction

Reproduction in modern cattle production represents a significant scientific field and a very complex stage of production. It is an area that has often been the subject of research, but it is also an area in which many unresolved and fully undefined problems occur. As such, reproduction is an important factor in intensifying cattle production, as solving problems in this area can lead to higher production of milk and meat.

Reproduction is also significant part of cattle production in which animals are provided for production of milk and meat, as well as for the renewing of herds (Mitić *et al.*, 1987).

Research on the body weight of calves at birth, as one of the fertility traits, is done for several reasons. First of all, calves that are born heavier within a single breed have a greater predisposition for faster and more efficient growth, which is particularly important for fattening breeds. However, the high birth weight of the calves is one of the main causes of heavy calving, and mortality of calves immediately after birth. In his research, Skalicki (1983) found that the average weight of male calves of German Simmental breed was 37.39 kg in the first three calvings and 35.46 kg for female calves. Similar values, the same author states for

the Austrian Simmental cattle, where the male calves had body weight at birth of 38.48 kg and the females 36.23 kg. Nikšić *et al.* (2012) analyze the results of the biological test of the bulls of the Simmental breed in Central Serbia. On a sample of 35 bulls and 3572 calves, the average weight of calves was 44.54 kg with a range from 25 to 73 kg, with very high variation ($p \leq 0.001$) under the influence of bull-sires.

During intensive rearing in the growing period, the heifer's full and physiological maturity occurs earlier. This allows for their early fertilization and the beginning of production use, which is particularly important from an economic point of view because earlier fertilization saves costs of rearing, shortens the generation interval and increases the efficiency of selection in the unit of time. Perišić *et al.* (1998), in their researches related to the reproductive and production traits of different genotypes of the Simmental breed (genotype I animals of Domestic spotted breed, 2nd genotype German Simmental breed, genotype 3rd Slovenian Simmental animals) have concluded that the age at first calving of Domestic Spotted animals was 2.1 years, German Simmental animals 2.3 years, and Slovenian 2.24 years. Đurđević (2001) has established values for the basic parameters of fertility. In his research, he examines the genetic analysis of milk yield and the reproduction of Simmental Cows, and finds that the average age for the first fertilization is 546.66 days with a variation interval of 240 to 1170, while the age at first calving is 831.94 days. In investigation of the phenotypic and genotypic variability of the production characteristics of the first Simmental breed, Pantelić (2006) states that the average age of first calving in Serbia is 778.73 days, with a standard deviation of 86.66 days and a minimum and maximum values of 620 to 1079 days.

One of the basic parameters of reproductive efficacy is the interval between two consecutive calving. This period is called the calving interval, and it should take about 12 months for Simmental cow (Petrujkić *et al.*, 1992; Miljković, 1994). However, in our production conditions, this period often lasts much longer, and as the duration of gravidity is a biological constant, the only reason for a longer calving interval is the prolonged service period (Stančić *and* Košarčić, 2007). The calving interval lasted for an average of 412 days, while Petrović (2000) in his study of the longevity of cows shows results for the duration of service period of 130.63 days and the duration of the calving interval of 416.76 days. Examining the effect of the import of breeding animals of Simmental breed from Germany to the implementation of the breeding program in the territory of the city of Kragujevac, Kostić (2014) has obtained the following results: on average, all studied have calved for the first time at an age of 780.17 days, the second time 1171.86 days, and the third time 1552.31 days. The average service period decreased with every subsequent lactation from 100.73 days, 95.43 days to 91.13 days. Domestic cows from calved for the first time earlier in comparison – by 79.13 days, the second time by 89.51 days, and the third time by 88.62 days, and all the differences are statistically significant. Statistically significant differences are also established ($p \leq 0.01$) for the duration of the service period where cows from domestic breeding show a shorter service period of 10.19 (2nd lactation) to 15.94 days (3rd lactation).

Material and Methods

Basic data on reproductive properties, as well as the origin data of all examined cows, were collected in cooperation with the "Lazar" Blace farm, which housed a part of the animals included in this research. For animals that are grown on individual farms, data on these traits were collected in cooperation with the breeding organizations, which implement the breeding program in the area of Toplica district. In this study, differences were determined in reproductive properties of cows that were imported and housed on the farm "Lazar" and those located on farms of individual agricultural producers, also differences in these properties in the imported and animals of domestic origin on the farm, as well as in individual breeders of Simmental cows on the same area.

The total number of animals and their completed lactations was divided into four groups as follows:
Group 1: animals of domestic origin grown by individual producers (n = 1526);
Group 2: imported animals grown by individual producers (n = 234);
Group 3: animals of domestic origin grown on the farm (n = 1100);
Group 4: Imported animals grown on the farm (n = 781).

When examining the impact of the unified factor of the way of holding/housing and origin, a model with a fixed unified effect of the way of holding/housing and origin (NP) was used

$Yij = \mu + NPi + eij$

- Yij: studied trait,
- μ: population average for given trait,
- NPi: fixed unified effect of the way of holding/housing and origin (i=1,2,3,4),

eij: random error.

For statistical data processing and application of the specified model, the software SPSS Statistics for Windows, Version 23.0 was used.

Results and Discussion

Table 1 shows the statistics on the body weight of calves at birth according to the housing/holding and the origin of their mothers divided into four groups, as well as the influence of these factors on the observed trait. It can be noted that the heads of the domestic origin reared on the farm (group 3) gave calves of the lowest body weight (40.10 kg), while the imprted animals (group 4) gave the calves of the highest body weight (44.78 kg). The calves of cows of domestic origin reared by individual producers (group 1) had higher body weight value (41.01 kg) than calves originating from imported cows reared by individual producers (group 2), which amounted to 40.29 kg. The results obtained with all four groups of observed are approximate to the results obtained by Nikšić et al. (2012). The unified effect of the factors of the holding/housing and the origin of animals was statistically very significant ($p \leq 0.001$) in the observed four groups of cows for the tested trait.
If the age of cows at first calving is observed by groups as shown in Table 1, it can be seen that tit was the lowest in the domestic animals rrreared by individual producers (773.73 days), somewhat higher in the first calving heifers of domestic origin reared on the farm (774.35 days), and then in the first calving heifers originating from import and reared by individual producers (778.06 days), and the longest in the case of first calving heifers from import reared on the farm (790.72 days). The age at the first calving was not significantly statistically significant ($p > 0.05$) under the influence of the combined effect of the holding/housing and origin of animals, when observing the first calving heifers divided into four groups. The values obtained for the age at first calving were significantly lower than the value stated in the study by Perišić *et al.* (1998) in German and Slovak Simmental animals, and Đurđević (2001) in Simmental cattle in Serbia, but approximately the same values for age at the first calving are reported by Perišić *et al.* (1998), Pantelić (2006) and Kostić (2014) for cows of the Simmental breed in Serbia.

Table 1. Mean values and variability of fertility properties per groups of cows

Trait	Group	No. of calvings	$\bar{x}$	SD	SE	95% confidence interval LB	95% confidence interval UB	Min.	Max.
Body mass at birth (kg)	1	1526	41.01	3.051	0.078	40.86	41.16	20	66
	2	234	40.29	3.847	0.252	39.80	40.79	28	65
	3	1100	40.10	3.512	0.106	39.89	40.31	20	70
	4	781	44.78	3.760	0.135	44.52	45.04	27	57
Total		**3641**	**41.,50**	**3.833**	**0.064**	**41.37**	**41.62**	**20**	**70**
		F=323.030* **				**p=0.000**			
Age at first calving (days)	1	436	773.73	95.963	4.596	764.70	782.76	621	1222
	2	68	778.06	84.674	10.268	757.56	798.55	647	1110
	3	282	774.35	96.117	5.724	763.08	785.62	617	1191
	4	168	790.72	91.718	7.076	786.75	804.69	620	1118
Total		**954**	**777.21**	**94.582**	**3.062**	**771.21**	**783.22**	**617**	**1222**
		F=1.429 nz				**p=0.233**			
Duration of calving period (days)	1	1526	391.52	67.811	1.736	388.11	394.92	280	688
	2	234	394.36	65.130	4.258	385.97	402.75	297	679
	3	1100	399.50	69.566	2.098	395.39	403.62	296	679
	4	781	405.13	72.272	2.586	400.05	410.20	244	673
Total		**3641**	**397.03**	**69.337**	**1.149**	**394.78**	**399.28**	**244**	**688**
		F=7.384* **				**p=0.000**			
Duration of service period (days)	1	1526	106.58	68.063	1.742	103.17	110.00	24	455
	2	234	108.40	69.808	4.564	99.41	117.39	24	458
	3	1100	114.58	70.103	2.114	110.43	118.72	24	463
	4	781	120.15	74.606	2.670	114.91	125.39	24	457
Total		**3641**	**112.02**	**70.418**	**1.167**	**109.74**	**114.31**	**24**	**463**
		F=7.228* **				**p=0.000**			

N=number; $\bar{X}$=average; SD=standard deviation; SE=standard error; LB= lower border/limit; UB=upper border/limit; F=statistical value; p=significance ***- p≤0.001; ** - p≤0.01; * - p≤0.05; nz - p>0.05

Based on the results shown in Table 1, it can be concluded that the calving interval was longer in imported cows than cows of domestic origin. The calving interval of cows from import housed and reared on the farm was the longest and amounted to 405.13 days, while in case of imported animals grown by individual producers it was 394.36 days. The shortest interval between the two successive calvings was recorded in domestic animals reared by individual producers (391.52 days), and it was by eight days longer in animals of domestic origin on the farm (399.50 days).

The unified effect (holding/housing and origin) was statistically very significant (p≤0.001) for the observed four groups of cows for the test traits. The obtained results of the calving interval in all four groups of cows were significantly lower than the results obtained by Vasović (1991) and Petrović (2000).

From Table 1 it can be seen, that the service period as well as the calving interval lasted for a longer time in case of imported animals compared to the animals of domestic origin. The service period in imported animals lasted 120.15 days, in case of animals reared on the farm and 108.40 days in animals reared by individual breeders, while the domestic animals reared by individual producers had a service period of 106.58 days, and animals of the same origin reared on the farm - 114.58 days. The influence of the holding/housing and the origin was statistically very significant (p≤0.001) in the observed four groups of cows for the tested traits. The obtained results for the duration of service period were higher than the results reported by Vasović (1991), but also significantly higher than those indicated by Kostić (2014) in domestic cows. However, the results obtained are significantly lower in all four groups of cows observed than the results achieved by Petrović (2000).

Conclusions

Groups 2 and 4 of the observed cows showed longer intervals in traits the age at first calving, the duration of the calving interval, and the duration of the service period compared to groups 1 and 3, since these groups included imported animals. The assumption that the expression of the reproductive traits will be better in animals of domestic origin was starting point of the research. In all observed properties, the highest values were recorded in animals of the fourth group, but not significantly, which leads to the conclusion that the reproduction properties are not significantly damaged by the selection of the animals and the method of rearing. All of the observed reproductive traits varied very significantly ($p \leq 0.001$) under the influence of the unified factor of the way of holding/housing and origin, except for the age at first calving whose variation was not statistically significant ($p > 0.05$).

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THE IMPACT OF THE NUMBER OF SCOUT AND FORAGER BEES IN SPRING ON THE STRENGTH OF HONEYBEE COLONIES IN SPRING AND AUTUMN INSPECTIONS

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Abstract

The aim of this paper was to show whether the number of scout and forager bees can be an indicator of the strength of honeybee colonies. It was studied how the number of scouts and foragers in spring (March and April) influences the colony strength. Spring inspection was carried out at the beginning of April, and autumn inspection in the first decade of September. The colony strength (quantity of bees and brood) and food supplies (quantity of honey and pollen) per society were determined in each inspection. After the spring review, colonies were divided into three groups. Strong colonies had 5 frames with bees and 3 frames with brood. Medium strong colonies had 4 frames with bees and 2 to 2.5 frames with brood. Poor societies had 3 frames with bees and 2 frames with brood. The experiment was conducted in Kruševac during 2011-2012. Each group had 10 honeybee colonies that were in standard LR beehives. During March, two countings of scout and forager bees were done, and during April three countings were done. Scout bees that returned to the hive in 1 minute were counted and all forager bees that returned with the load of the pollen were counted for the same period. During the first year, the number of scouts did not reflect the strength of colonies, because the medium strong colonies had even a little more scouts and foragers than the very strong ones, while the poor were significantly behind. In the second year, scout bees did reflect the strength of colonies. The highest number of scouts (235.5) and foragers (139) was found in the strong colonies, 205.3 scouts and 107.7 foragers in the medium colonies, while poor colonies had 160 scouts and 82 foragers. In the first year, medium-strong colonies were somewhat better in autumn inspection (more bees, honey and pollen) than very strong colonies. In the second year the poor colonies reached the medium ones (higher strength and similar food supplies).

Keywords: *Honeybee colonies, colony strength, scout bees, forager bees, colony inspections*

Introduction

Frequent inspections of honey bee colonies can take a long time, especially to beekeepers who have a larger number of colonies. In addition, the frequent opening of the hives disturbs not only the colony that is being examined but also the colonies in the immediate vicinity, and sometimes the other colonies in the whole apiary. The beekeepers always wanted to be able to evaluate the strength of the colonies, but to open the hive and take out the frames as little as possible. Often this was done by looking at the floor board and what was on it, as the waste on the floor is most often grouped directly below the bees. An easier way would be to draw conclusions about the colony strength based on the number of bees that fly out or the number of bees that return to the hive. Considering that the presence of pollen in nature and its entry into the hive is very important for brood nutrition, the inclusion of the number of pollen bees would probably yield even more precise results. Farar (1936) discovered that the size of the winter honey bee population is in a positive correlation with the autumn amount of bees and

the amount of pollen. Many authors later confirmed this thesis, except for Mc Lilen (1978) who concluded that the surface under the brood was not correlated with the amount of collected or available pollen in the hive, on the day of the examination (at the daily level). This can be justified by the fact that colonies often collect more or less pollen per day, stronger colonies have more brood and have to consume larger quantities of pollen and colonies of the same strength collect different pollen at different locations (Al Tikrity *et al*., 1972). Keller *et al*. (2005) stated that the amount of collected pollen, and therefore the number of pollen in the colony, is influenced by 10 and more factors (the number of worker bees - the quantity of bees, the number of larvae - surfaces with the brood, the number of foragers (Dreller *et al*., 1999), empty space in the hive (Schmickl and Crailsheim, 2004), the removal of pollen (Lopre *et al*., 1985), surrounding vegetation, climatic factors, etc.). Eckert *et al*. (1994) artificially adjusted the amount of brood in colonies that were initially similar in terms of strength (adult bees) and food supplies (honey and pollen). A week after setting up the colonies with a high level of brood, the supply of pollen decreased. In another experiment, it has been found that colonies with more open brood have more pollen foragers, and vice versa, if the frames with open brood are replaced by frames with capped brood (from the other hive), the number of foragers decreases (Eckert *et al*., 1994). Pankiv *et al*. (1998) found that even the addition of pheromones secreted by the larvae in glass plates managed to increase the number of pollen in the colony. Several studies have attempted to establish causal relationships in the other direction, i.e. to prove that increased additions or foraging of pollen increases the area with brood and therefore the strength of the colony. Fewell and Winston (1992) found that in colonies with equal strength (bees, open and capped brood) and the amount of honey, the area with brood increased significantly by adding more pollen to the colony. The aim of this paper was to determine is there an impact of the number of scouts and the number of foragers, counted during the spring (March-April), on the colony strength in the short time period (spring inspection), as well as to discover whether this strength of the colony remains for long like this or there is a change in the later period (autumn inspection).

Material and Methods

The experiment was carried out at the apiary of the Institute for forage crops Kruševac during 2011-2012. Spring inspection was carried out at the beginning of April, and autumn inspection was in the first decade of September. In the inspections, the strength of the colonies (quantity of bees and litters) and food supplies (quantity of honey and pollen) per colony were determined. The amount of bees was determined visually based on the amount of bees that have been present on the frames. This trait is expressed in parts of the frame that are possessed by bees (1/10), and can also be expressed in percentages. By adding the amount of bees on the frames, the total quantity of bees by colony is calculated. The area under the brood was also evaluated in spring and autumn by detailed observing of each frame with an open and capped brood in the hive. The results are also expressed in parts of the frame (1/10). The area of honey and pollen were assessed in the same way and at the same time as two previous traits. After the spring inspection, the colonies were divided into three groups. Strong colonies had 5 frames with bees and 3 frames with brood. Medium strong colonies had 4 frames with bees and 2-2.5 frames with brood. Poor colonies had 3 frames with bees and 2 frames with brood. Each group had 10 honey bee colonies that were in standard LR beehives. During March, two countings of scout and forager bees were done, and during April three countings were performed. Scouts that return to the hive in 1 minute were counted and all the foragers that return with the load of the pollen were counted in the same period. At the beginning of the experiments in all colonies there were one-year-old queen bees hatched in 2010. All traits were statistically processed by the ANOVA variance analysis method on a completely random plan. Computer program STATISTICA 7.1 (StatSoft, 2006) was used for

data processing. The differences between the average values of the treatment were tested with a LSD test at a significance level of 95% ($p<0.05$).

Results and Discussion

Colonies in spring inspection had, on average, 4 frames occupied by bees and 2.3 frames with brood (Table 1). The most bees and brood were found in strong colonies and these differences were statistically significant ($p<0.05$). A somewhat different condition was determined in the spring inspection considering the amount of food, especially the amount of honey. The highest amount of honey in the first year of the spring review was found in poor colonies, slightly less in strong colonies, and the least amount was found in the medium strong colonies. Strong and medium strong colonies had almost identical amounts of pollen, and the poor ones had smaller amount of pollen, but the differences were not significant. During the counting of scouts in the first year (Table 1), the average number was 53.6 for 1 minute of counting. Medium strong colonies had the largest number of scouts, slightly smaller number was in strong colonies, and the lowest number was in poor ones. In this year, the average of 23 foragers per colony were counted. Medium strong and strong colonies were fairly uniform while the poor colonies were lagging behind considering the number of foragers. The highest percentage of pollen was found in medium strong colonies, and the differences for this trait were not statistically significant. Eckert *et al*. (1994) found that the number of pollen foragers is proportionally higher in smaller colonies than in larger ones. This finding is a compatible with the one obtained in our research and refers to medium strong and strong colonies. Jevtić (2007) found, during the pollination of alfalfa, that the percentage of pollen ranged from 27.8-40.6% in daytime countings. In the afternoon counting it has dropped considerably and ranged from 8.2 to 22.8%. Following the pollination of the red clover Jevtić *et al.*, (2013) found that the percentage of pollen in poor colonies was 27.6% and in strong ones was 29.5%. In the autumn inspection, colonies had about 8 frames filled with bees. Medium strong colonies were better than strong ones and had more bees, but they did not differ significantly, while in relation to the poor colonies, both significantly differed. Medium strong colonies had the highest amount of honey and pollen in this inspection. For the amount of honey the differences were not significant in relation to the strong colonies, and for the amount of pollen, the differences were significant (Table 1). The most brood in the autumn inspection was found in strong colonies, medium strong had a little less brood, while poor ones were far behind.

Table 1. The average number of scouts and foragers per colony and traits of colonies (occupied frames) in spring and autumn inspection in 2011

Strength	Strong	Medium strong	Poor	**Average**
Spring inspection				
Bees	4.83^{a}	3.87^{b}	3.25^{c}	**3.98**
Brood	2.67^{a}	2.31^{a}	1.88^{b}	**2.29**
Honey	3.35^{a}	2.49^{b}	3.38^{a}	**3.07**
Pollen	1.08^{ns}	1.06^{ns}	0.93^{ns}	**1.02**
Bee counting				
Scouts	56.87^{a}	57.74^{a}	46.13^{b}	**53.58**
Foragers	24.43^{a}	24.80^{a}	19.67^{b}	**22.97**
% of foragers	42.93^{ns}	43.03^{ns}	42.63^{ns}	**42.86**
Autumn inspection				
Bees	8.53^{a}	9.27^{a}	6.10^{b}	**7.97**
Brood	2.82^{a}	2.49^{a}	1.15^{b}	**2.15**
Honey	4.08^{a}	4.19^{a}	3.83^{b}	**4.03**
Pollen	0.50^{b}	0.79^{a}	0.48^{b}	**0.59**

In the second year in the spring inspection, 4.4 frames with bees were found in average, and the differences between the observed groups were significant ($p<0.05$). The identical situation (Table 2) was also determined for the second observed trait (brood size). The most brood as well as bees was found in strong colonies and the least in poor colonies. Strong companies consumed the most food, so in this inspection they had the least honey, but the most pollen. The poor colonies had the most honey, and the medium strong had the least pollen. These differences were significant ($p<0.05$). In the second year in the spring inspection, colonies had slightly more bees, brood and honey in the spring, but almost twice as low pollen compared to the previous year.

Considering the number of scouts, it was consistent with the strength determined by the inspections. The highest number of scouts was counted in strong colonies (Table 2), with 14.7% more scouts compared to medium strong and 47.6% more scouts than in the poor colonies. During the counting in the companies, the average number of foragers was 21.6. Strong colonies had 24% more foragers than medium strong and 62.8% more than poor colonies. The highest percentage of forager bees was found in strong colonies, and the lowest was in poor colonies, the differences were significant.

In the autumn inspection in the second year, poor colonies exceeded medium strong ones and had higher strength (bees and brood) while food supplies (honey and pollen) were almost identical. In this inspection, strong colonies retained their dominance for all observed traits, and the differences were statistically significant. In comparison to the previous year, the colonies had the identical amount of bees, slightly more honey (0.8 frames), but also for a whole frame less brood (56.3%) and nearly 3 times less pollen.

Table 1. The average number of scouts and foragers per colony and traits of colonies (occupied frames) in spring and autumn inspection in 2012

Strength	Strong	Medium strong	Poor	**Average**
Spring inspection				
Bees	5.55^{a}	4.32^{b}	3.24^{c}	**4.37**
Brood	2.88^{a}	2.37^{b}	1.98^{c}	**2.41**
Honey	2.95^{c}	3.22^{b}	3.56^{a}	**3.24**
Pollen	0.68^{a}	0.45^{b}	0.52^{ab}	**0.55**
Bee counting				
Scouts	47.10^{a}	41.05^{b}	31.92^{c}	**40.03**
Foragers	26.77^{a}	21.55^{b}	16.44^{c}	**21.59**
% of foragers	56.08^{a}	53.25^{b}	51.30^{c}	**53.54**
Autumn inspection				
Bees	9.43^{a}	7.00^{b}	7.48^{b}	**7.97**
Brood	1.47^{a}	1.12^{c}	1.28^{b}	**1.29**
Honey	5.25^{a}	4.57^{b}	4.58^{b}	**4.80**
Pollen	0.30^{a}	0.20^{b}	0.18^{b}	**0.23**

Conclusion

After two years of observing three groups of colonies (strong, medium strong and poor) and counting scout bees and forager bees (March-April) the following conclusions can be made:

In the first year, the number of scouts and foragers did not reflect the strength of the colonies being inspected because the medium strong companies had slightly more scouts and foragers than the strong ones, while the poor colonies were far behind. In the second year, the number of scouts and foragers did reflect the strength in the spring inspection. In the autumn inspection, in the first year, the medium strong colonies exceed strong colonies considering the amount of bees, honey and pollen. In the second year in the autumn inspection, poor

colonies exceed medium strong ones and became equal considering food supplies. Food supplies in spring inspection were as twice as large in the first year, which affected area under brood in the autumn inspection which was in the first year for 0.9 of frame higher than in the second year.

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PRODUCTION CHARACTERISTICS OF THE PIG POPULATION IN AUTONOMOUS PROVINCE OF VOJVODINA (SERBIA)

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Abstract

The paper presented the results obtained by analyzing the population of pigs under selection pressure for Vojvodina during 2017. The data were collected from main breeding organization in Novi Sad. By monitoring the number of live-born piglets, the largest number was recorded in the genotype F1 generation, which amounted to 12.93 live-born pigs, while the smallest number of live-born piglets was recorded in the genotype Pietrain with the average number of live-born piglets 9.53. The largest number of weaned piglets was recorded in the genotype F1 generation of crossing between Yorkshir and Landrac 11.54, the smallest number of weaned piglets was recorded in the genotype Pietrain 8.98 piglets. Main breeding also implemented the performance test of breeding animals. The following values from performance test were recorded by female animals analysis by genotypes: the highest life gain was recorded in the 0.614 kg genotype Pietrain, and the smallest backfat thickness and side fat thickness (8.67; 9.00 mm), while the deepest ML (musculuc longissimus dorsi) was recorded in the genotype Yorkshire 70.88 mm. Performance of male animals testing determined the following values of the tested properties: the highest life gain was recorded in the Landrace (0.700 kg) genotype, while the smallest thickness of the backfat and side fat (8.00; 8.30 mm) was recorded in the genotype of Duroc, while the deepest ML was recorded in the genotype Duroc (78 mm). Calculation of the BLUP values for the measures from the performance test recorded the following variables: for a life gain of 17.311 to 79.674; thickness of the bacon from -4.454 to 6.381; thickness of side bacon from -4.529 to 6.163; ML from -33.584 to 19.439. The obtained results show that the selection pressure should continue because of the space that exists in terms of economically important features.

Keywords: *Live-born piglets, Weaned piglets, Genotype, Performance test.*

Introduction

Pig selection is the planned and systematic selection of the best animal for breeding, which according to the production results corresponds to producers and consumers. The selection of animals of higher production abilities of a certain quality within the pig population in which the selection is performed accumulates the genes for these properties.

The annual replacement rate on commercial farms ranges between 42 and 59% (Young at al, 2009; Koketsu, 2007), which indicates that the number of culling sows will be compensated. Therefore it is necessary to provide a sufficient number of quality breeding gilts, which will be an appropriate substitute for culling sows. By ensuring an appropriate replacement, it is necessary to devote considerable attention to the upbringing of quality breeding gilts in order to match the appropriate body weight and age in the test performance.

One way of achieving higher fertility for sows is the setting up of a pyramidal organization of pig breeding in Vojvodina. A pyramidal organization set up in Scandinavian countries can be taken for example: a nucleus farm, a production farm, a commercial farm (Lotta Rydhmer, 2005).

Subject in the implementation of the main breeding program in Vojvodina are: farmers, basic breeding organizations, regional breeding organizations, main breeding organization,

organizations with special authorization, provincial secretariat for agriculture, water management and forestry, ministry of agriculture and environmental protection.
Main breeding organization plays an important role in conserving genetic resources, genetic improvement of the population, testing of breeding animals, assessment of lean meat on the line of slaughter, as well as harmonization of the selection work with the standards of the European Union. The basic breeding goals prescribed by the main breeding organization are: increasing the productivity of domestic animals, changing and improving racial structure and preventing a reduction in the number of conditions (Main breeding Program, 2014).
Selection work of the Main breeding organization is in line with the needs of the market and moves towards the repair of economically important traits such as: life growth, backfat thickness, muscle depth (ML), all in order to increase the profitability of pig production (Radović et al., 2011).
During 2017, data were collected from the Basic breeding organizations operating in the territory of the Autonomous Province of Vojvodina. The Main breeding organization analyzed and compared the obtained data with previous years in order to test the production results of different genotypes. An analysis of the breeding value of boars using BLUP plays an important role due to the increasing use of doses from artificial insemination centers. Ranking of animals by breeding value helps in choise of boars for correction certain production trait.

Materials and methods

Research was carried out on a total of 38,169 sows that were under productivity control, 621 performance tests boars, 31,959 performance tested gilts, while the progeny test tested the breeding value of boars based on the average characteristics of their offspring. The average distribution of descendants characteristics was compared with the population average, taking into account that the selected descendants for the progeny test were from multiple litters. The test we conducted was at least 16 offspring (8 male and 8 female animals) from one boar. The total number of tested boars is 1,286, while in the work will be shown 20 boars with at least 8 male and 8 female descendants. For the analysis, the data from the performance test gilts and boars were used, and the estimation of the breeding value was carried out using the BLUP (set of linear models). The genotypes that used in the analysis were the genotype Yorkshire (Y), Landrace (L), F1 generation, the Yorkshire x Landrace (YxL), F1 generation Landrace x Yorkshire (LxY), Pietrain (P) and Duroc (D).

Results and Discussion

The number of tested boars has been declining since 2014 (Table 1), which was also recorded by Trivunović et al. (2014), as well as Radović i et al. (2015). When we compare the number of tested boars in 2014 (when the highest number of tested boars in the last five years was recorded), with 2017, the drop in the number of tested animals was 40%.
In addition to the established reduction in the number of tested boars, it is encouraging that economically important features such as backfat thickness, side bacon thickness, ML and lifetime gain have a positive five-year trend. A positive five-year trend has a major role in selecting boars for parents of the next generation. When choosing boars in addition to the listed results, the test performance should be taken as an additional parameter such as food conversion, % of meat in carcass, number of tits, constitution, size and number of litter from which it originates (Yoo and Lee, 2011).

Table 1. Results of the performance test boars

Year	Number tested boars	Body weight	Life gain kg/day	Backfat thickness, mm	Side fat thickness, mm	Deepest ML, mm
2013	812	113	0.765	17.6	18.10	77.10
2014	987	112	0.585	15.92	16.38	76.69
2015	827	107	0.569	13.90	13.70	72.60
2016	724	112.74	0.603	12.81	13.02	74.82
2017	594	110.79	0.615	11.03	11.44	75.14

By monitoring the results of the performance of tested gilts (Table 2), an increase in the number of tested gilts in the last three years in Vojvodina has been observed, which is in line with the recorded by Trivunović et al. (2014) and Radović et al. (2015). By monitoring the economically important characteristics in the performance test (thickness of the back bacon, thickness of the side bacon, ML and height of life gain), a positive trend in the development of economically important traits was identified. Tummaruk et al. (2007) recorded an approximate value of backfat thickness (12.0 mm) in their researches, while they recorded a life gain of 0.539 kg / day. Analyzing the results of the performance of the test, Amaral et al. (2010) recorded the variability of the following data: life gain from 0.600 to 0.870 kg / day, backfat thickness from 10 to 23 mm in the test sample.

Table 2. Result of performance test gilts

Year	Number tested gilts	Body weight	Life gain kg/day	Backfat thickness, mm	Side fat thickness, mm	Deepest ML, mm
2013	23,270	116.90	0.520	18.50	18.40	74.20
2014	27,747	112.90	0.520	18.90	19.00	77.60
2015	25,704	111.80	0.522	15.10	15.60	74.50
2016	27,634	109.40	0.546	13.40	13.50	73.40
2017	29,724	109.60	0.558	12.20	12.00	75.00

The analysis of the control of productivity of sows in farms in Vojvodina determined the following: the largest number of live-born piglets was found at the F1 generation animal, while the smallest number of live-born piglets were recorded in the genotype Pietrain and Duroc (Table 3). No significant differences in the number of live-born piglets were observed in F1 generators. Observing the obtained results for the number of live born piglets, a certain variability of the trait was determined: the highest number of stillborn piglets was recorded in the Pietrain genotype, while the smallest number of stillborn piglets were recorded in the genus Duroc. The obtained results are not in accordance with the research carried out by Vidović et al. (2011), where they found the average number of live-born piglets for the Landrace and Yorkshire genotypes: 14.3 and 13.9 pigs, while the number of stillborn piglets was 2.4 and 2.3 piglets.

Table 3. Control of sow productivity

Genotype	Number of sows under control	Live born piglets	Stillborn piglets	Weaned piglets
Yorkshire	7,098	11.92	1.22	10.59
YxL	11,985	12.93	0.89	11.26
Landrace	10,188	11.79	1.91	10.65
LxY	8,379	12.76	0.92	11.54
Pietrain	38	9.53	3.09	8.98
Duroc	481	9.56	0.77	9.21

The results obtained by calculating the breeding value for boars can be seen in Table 4. The best estimate of the breeding value of boar 5519 was for the property of the backfat thickness (-4.08 mm). For the side fat thickness, the best breeding value was recorded at the boar 25730 (-3.92 mm). For ML the best breeding value was recorded at boar 3998 (19.94 mm), while the best breeding value for life gain was recorded at boar 2167 (102.12 g).

Table 4. Breeding value of boars for trait from performance test of gilts and boars

Boar	Backfat thickness, mm	Rank	Boar	Side fat thickness, mm	Rank	Boar	ML	Rank	Boar	Life gain, g	Rank
3998	-5.12	1	81/14	-4.58	1	3998	19.94	1	2167	102.12	1
2167	-4.67	2	3998	-4.53	2	20826	19.44	2	3998	99.32	2
52631	-4.64	3	2167	-4.41	3	11732	19.31	3	5519	98.93	3
22860	-4.56	4	2118	-4.41	4	22860	19.01	4	20826	95.49	4
9655	-4.52	5	159/14	-4.39	5	235355/17	18.89	5	2411	95.47	5
51081	-4.51	6	30054	-4.34	6	81/14	18.59	6	2249	95.47	6
2027/02	-4.51	7	694	-4.16	7	25730	18.32	7	81/14	95.3	7
20826	-4.45	8	5590	-4.12	8	845857/67	18.28	8	22860	94.66	8
1144	-4.38	9	47220	-4.11	9	2027/02	18.23	9	85784	92.04	9
187	-4.37	10	24762	-4.09	10	111496/88	18.07	10	11732	92.04	10
74450	-4.3	11	22860	-4.02	11	1144	17.67	11	2027/02	92.04	11
25730	-4.26	12	35605/800	-4.01	12	36268/802	17.62	12	47220	92.04	12
81/14	-4.22	13	845857/67	-3.99	13	30054	17.55	13	572/14	91.75	13
69515	-4.17	14	41529	-3.99	14	35605/800	17.42	14	24762	91.34	14
5944	-4.16	15	74450	-3.97	15	24762	17.34	15	1144	90.81	15
47220	-4.14	16	85784	-3.97	16	2411	17.29	16	9423	90.76	16
85784	-4.14	17	5519	-3.97	17	74450	17.22	17	34376/28	90.42	17
11732	-4.12	18	816	-3.95	18	5519	17.15	18	816	90.27	18
47220	-4.12	19	187	-3.95	19	5591	16.94	19	235355/17	89.62	19
5519	-4.08	20	25730	-3.92	20	816	16.77	20	845857/67	8946	20

Conclusion

One of the key indicators of the efficiency of pig production is the number of piglets per sow per year, the number of litters per sow per year and the number of weaned piglets per sow annually. Based on the above, it is clear that in the coming period, a higher selection pressure should be placed on the number of live born and weaned piglets on an annual basis. Also, due to the increased import of animals from abroad, it is necessary to define the criteria for importing the animals, so that the imported animals help in achieving the breeding goal in Vojvodina. The reasons for the reduced number of tested boars were expected, as the Main Breeding Organization tightened the criteria for the test of boars, increased use of boars from artificial insemination centers and increased imports of animals from abroad.

Data related to the performance test gilts are encouraging, because despite the closure of individual farms and increased imports of quality breeding offsprings, the trend of growth in the number of tested reproductive animals continues and we can consider that manufacturers

have recognized the significance of this measure. By monitoring the characteristics of the test performance, such as the thickness of the backfat and side bacon thickness, the depth of the muscle and the height of life gain, it has been determined that an appropriate selection of the abovementioned properties is carried out, and that the appropriate selection of male and female animals is made in relation to the abovementioned properties.

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THE IMPACT OF POLYMORPHISM IN THYROGLOBULIN GENE ON BEEF QUALITY

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Abstract

The objective of this study was to identify the single nucleotide polymorphism in thyroglobulin gene (TG5), determine the genetic structure of the population and analyse its effect on beef quality traits in Slovak Pinzgau cattle. A total of 56 blood samples of Pinzgau steers were used to extract genomic DNA for animal's genotyping. The SNP genotyping of all individuals was performed by using PCR-RFLP method. Across analysed individuals the allele C was more frequent (0.64) than T allele (0.36). The sufficient level of allele impact effectiveness in population indicated mainly the value of allele effective number (1.849). The obtained values of expected (0.46) and observed heterozygosity (0.5) indicated balanced proportion of homozygous and heterozygous animals that confirmed similarly the values of Wright's F_{IS} index (-0.09). In addition, the negative value of F_{IS} index showed, that the population is not affected by the increase of inbreeding. The impact of thyroglobulin gene polymorphism on beef quality was tested by using ANOVA analysis. Four carcass traits were involved in association analysis: proportion of muscle, fat, bones, and drip loss within the beef three-rib section. Despite the fact that the statistical analysis showed only non-significant effect of TG genotypes on evaluated traits, it seems to be that the T allele is favourable for all traits under consideration. The results of present study could be affected mainly by the sample size and polygenic effects associated with expression of analysed traits.

Keywords: *Association analysis, beef quality, candidate gene, polymorphism, Thyroglobulin.*

Introduction

In beef cattle breeding, the value of the animal for both the producer and the meat industry is determined by the type and carcass traits. The study of carcass quality traits is particularly important because these traits can improve economically important factors such as carcass yield and amount of meat produced per hectare. However, the rate of genetic gain in beef cattle may be reduced mainly due to the fact that the carcass traits are measured late in life. One of the way how to solve this can be the utilization of genetic markers that has been proposed in an attempt to increase the accuracy of prediction and to reduce generation interval (de Carvalho *et al*., 2012; Fonseca *et al*., 2015).

Up to now, various studies showed that the genetic modification through genomic selection in the desired direction allow to achieve permanent and continuous progress in improving quantitative and qualitative features that are economically important in meat cattle production (Van Eenennaam, 2016; Ardicli *et al*., 2017; Mehrban *et al*., 2017). It is known that the beef quality as well as carcass traits usually show low or moderate level of heritability and are under the control of polygenic inheritance (Ogawa *et al*., 2014; Liu *et al*., 2015). Thus, progress in the genetic selection process to improve beef cattle breeds depends on the accuracy of the animals breeding value estimation (Bekseitov *et al*., 2017). The permanent progress in the field of molecular genetics make it possible to identify genes controlling the economic important traits and thus increase the accuracy of estimated breeding values. Such

candidate gene approach can be used especially if the gene is located in a region that hosts a QTL or when there is prior information on gene effect on a trait (Borges *et al*., 2014).
Tyroglobulin, a product of the TG gene, is a dimeric glycoprotein hormone synthesized by thyroid epithelial cells. It is one of the largest proteins in the body and its size is 660 kDa. Thyroglobulin is secreted by the follicular lumen and together with iodine forms a precursor for thyroid hormones, triiodothyronine (T3) and tyroxin (T4), which are involved in development and metabolism regulation and also have effects on adipocyte differentiation, growth and homeostasis of fat depots (Ailhaud *et al*., 1992; Darimont *et al*., 1993; Casas *et al*., 2005). The TG gene, encoding the thyroglobulin, is located in cattle on BTA14 and contains 37 exons. One of the first studies that analysed the polymorphism of TG gene was study of Barendse *et al*. (1997). They identified a C/T single nucleotide polymorphism (SNP) in the 5' untranslated region of the TG gene (named as TG5), which were later associated in cattle with marbling and included in commercial genotyping panels (Barendse *et al*., 1997; Casas *et al*., 2005). In addition, the TG gene is considered as functional candidate gene of post-weaning average daily gain, birth weight, and weaning weight traits (Zhang *et al*., 2015).
The aim of this study was to determine the genetic structure of population based on analysis of SNP TG5 in gene encoding thyroglobulin and to analyse its impact on beef quality traits in Slovak Pinzgau cattle.

Material and methods

A total of 56 genomic DNA samples of Slovak Pinzgau steers were included in this study. The genomic DNA was extracted from blood samples by using salting-out method following the protocol proposed by Miller *et al*. (1988). After genomic DNA extraction, the concentration and purity of each DNA sample were tested by the spectrophotometry measurement using optical density at wave length of 260 nm. To identify the TG5 polymorphism the PCR-RFLP (restriction fragment length polymorphism) method was used. Specific target segment of DNA with length 545 bp was amplified by PCR (polymerase chain reaction) using oligonucleotide primers proposed by Barendse *et al*. (1997). In the next step, the products of PCR reaction were digested using restriction enzyme *Psu*I (Fermentas) (at 37°C for 3 hours). Both the products of PCR reaction and restriction fragments were visualised by horizontal electrophoresis in 2.5 % agarose gel (200 V for 15 min) stained with fluorescent nucleic acid dye GelRed (Biotium).
The genetic structure of analysed population were determined based on the calculation of allelic and genotypic frequencies using Genalex v6.1 (Peakall and Smouse, 2012). To estimate the impact of factors such as inbreeding, artificial selection, migration or genetic drift on the population genetic structure the differences between observed and expected genotypic frequencies were tested by Chi-square test (χ^2). Moreover, several basic genetic diversity parameters (effective allele number, polymorphic information index, and molecular equivalent of pedigree based inbreeding coefficient expressed by Wright's fixation index F_{IS}) were calculated using Genalex v6.1 (Peakall and Smouse, 2012) in order to estimate the level of genetic diversity within analysed population.
To assess the impact of TG5 polymorphism on beef quality traits the GLM (one-way ANOVA) procedure adopted in SAS 9.3 was used. Four carcass traits were involved in association analysis: proportion of muscle, fat, bones, and drip loss within the beef three-rib section. Those parameters of carcass value (proportion of fat and bones) and quality of beef meat (drip loss) were measured according to Honikel (1998).

Results and discussion

The PCR-RFLP analysis revealed the presence of all three genotypes of TG5 polymorphism in analysed population of Slovak Pinzgau steers. The homozygous genotype CC was detected

based on three fragments with length 278 bp, 195bp, and 72 bp, whereas homozygous TT genotype was represented by two fragments with length 437 bp and 72 bp. The heterozygous animals was identified based on four fragments (473 bp, 278 bp, 195 bp, and 72 bp). The heterozygous CT animals were most frequent in the analysed population (50 %). The lowest frequency was found for homozygous TT animals (10.71 %), whereas the CC genotype was found in 39.29 % of animals. The C allele was in population predominant (0.643) compared to T allele (0.357). The predominance of C allele was similarly found for example in Charolais (Thaller *et al*., 2003; Casas *et al*., 2006), Holstain (Thaller *et al*., 2003) and Angus cattle (Barendse *et al*., 2004; Casas *et al*., 2006). Relatively higher frequency of T allele compared to our results was reported for simmental cattle (Rincker *et al*., 2006) and Wagyu (Van Eenennaam *et al*., 2007).

In terms of genetic diversity the high proportion of heterozygous animals in population was reflected in sufficient level of both observed (H_o=0.50) and expected heterozygosity (H_e=0.46). The balanced proportion of heterozygous and homozygous animals confirmed similarly the negative value of F_{IS} index (-0.09) that also signalized only non-significant impact of increase in inbreeding on analysed population. The expected Hardy-Weinberg equilibrium in population (based on previous parameters) confirmed also the Chi-square test that indicated only non-significant differences between observed and expected genotype frequencies (P>0.05). The effectiveness of allele impact of locus in population was expressed by two parameters: the effective allele number (N_a) and polymorphic information content (PIC). Both of this parameters indicates sufficient level of allele effectiveness in population (Na=1.849, PIC=0.354). The obtained value of PIC indicated for analysed SNP medium level of polymorphism.

Across analysed individuals the proportion of muscle within the beef three-rib section at level 60.62 ± 6.71 % was detected. The average proportion of fat was 17.38 ± 6.65 % and proportion of bones within the beef three-rib section was 21.99 ± 2.46 %. The average proportion of drip loss that referred to the quality of beef meat was found at level 2.38 ± 1.82 %. Table 1 summarizes the average values of each analysed trait with respect to the specific TG5 genotype. The obtained results indicated that the differences between the TG5 genotypes was very low. Due to this and the fact that the statistical analysis showed only non-significant effect of TG5 polymorphism on the variability of analysed traits, it can be conclude that the obtained results have only informative characters. However, compared to previous studies it seems to be that the T allele is favourable for all of analysed traits.

Table 1 Average values of analysed traits depending on the TG5 genotypes

Genotype	% of muscle	% of fat	% of bones	% of drip loss
CC	61.31±5.55	16.95±5.68	21.74±2.65	2.46±1.97
CT	59.00±7.65	18.64±7.66	22.36±2.53	2.27±1.83
TT	65.66±1.27	13.11±1.56	21.23±0.84	2.63±1.38

Conclusion

Despite the fact that our study did not confirm the role of TG gene in genetic control of carcass traits, such analyses of SNPs in genes affecting the economically important traits are still very useable for identification of genetic markers that can be applied in gene assisted selection of cattle. The results of present study could be affected mainly by the sample size and polygenic effects associated with expression of analysed traits. To obtain more reliable results in the future is therefore necessary to increase the sample size (including breeds with different genetic composition) as well as number of loci in the panel of candidate genes.

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EFFECT OF REPLACING CORN AND SOYA BEANS BY WHITE SORGHUM AND HORSE BEANS ON MILK PERFORMANCES OF SICILO- SARDE SHEEP IN TUNISIA

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Abstract

Twenty Sicilo-Sarde ewes of local sheep were divided into two homogenous groups regarding age (5.3 vs 5.7), live weight (33.83 ± 5.63Kg vs 33.95 ± 5.58 Kg) and the rank of lactation were used to evaluate the effect of replacing corn and soya beans by white sorghum and horse beans on milk produced and quality of milk (pH, density, freezing point, fat content, protein content, lactose and fat solids). Animals were logged in two big boxes and received 1.5 kg DM / ewe / day of oat hay. Two concentrates (i) 500 g/ewe/day of concentrate that included corn (43.3%), barley (25%), soybean meal (17.7%) and mineral and vitamin supplement (4%) for the control group (RM) and (ii) a second concentrate that included white sorghum (66%), horse beans (30%) and mineral and vitamin supplement (4%) for the experimental group (RS) were used. The result showed that the diet did not affect the daily milk yield. The pH of milk was higher ($p< 0.05$) in ewes of RS group than in those of RM group. The density was affected by the diet. The freezing point was not affected. Fat content, protein content, mineral content and lactose were not affected by diet. However Solid Not Fat was affected by the diet. It is possible to replace corn and soybean by white sorghum and horse beans in the sicilo-sarde sheep.

Keywords: *Sicilo-Sarde ewes. local feed resources. milk yield and quality.*

Introduction

The ovine breeding can affect the social and economic status of people who live in arid and semi-arid regions. The development of ruminant livestock uses different sciences (nutrition. reproduction. genetics. health) need to conduct in parallel. In an integrated manner in a culture system. Sicilo-Sarde is the main dairy ovine in Tunisia. This sheep breed only thrives in the north of the country. The average milk yield of this breed is 72 l for a milking duration of 124 days on average and following a breastfeeding period of 114 days (Djemmali et al., 1995). The feeding of sheep is based on fodder with low nutritional value and little diversified and concentrate that is made from imported raw materials (corn and soybeans). Thus the increase in the price of the latter two. We must look for local primary materials to feed our livestock by covering their needs. The nutritional imbalance of the rations available to these animals, the low forage potential of these areas and the drought conditions in semi-arid to arid regions are considered the main factors hindering the development of livestock systems and threatening the sustainability of these (Ben Salem and Smith, 2008, Ben Salem, 2011).
The main objective of this study is to determine the effect of replacing corn and soya beans by white sorghum and horse beans on milk performances of sicilo- sarde sheep during lactation.

Materials and methods

Animals and management

The study was carried at the experimental station of the Higher School of Agriculture of Mateur (Tunisia) on 2010. A total of 20 ewes Sicilo-Sarde were divided into two groups

homogenous groups regarding for age (5.3 vs 5.7), live weight (33.83 ± 5.63Kg vs 33.95 ± 5.58 Kg) and the rank of lactation. Animals were logged in two similar big boxes and received 1.5 kg DM / ewe / day of oat hay. Ration was complemented by one of two concentrates: (i) 500 g/ewe/day of concentrate that included corn (43.3%). barley (25%). soybean meal (17.7%) and mineral and vitamin supplement (4%) for the control group (RM) and (ii) a second concentrate (500 g/ewe/day) that included white sorghum (66%). horse beans (30%) and mineral and vitamin supplement (4%) for the experimental group (RS) were used. Diets were iso-energetic. The daily meal was offered in two times in the morning 8h and at 14h. They were 2 week of adaptation. The repartition of control (RM) and experimental (RS) groups are presented in Table 1.

Table 1. Repartition of group.

Group	Live weight (kg)	Age (years)	Rank of lactation
Control: RM	33.83±5.63	5.3±1.25	4.3±1.25
Experimental : RS	33.95±5.58	5.7±1.15	4.6±0.96

Feed intake and Milk Production and physico-chemical parameters

The feed intake were calculated by subtracting refusal from distributed amounts. During the whole experimental period, the ewes were milked by hand 2 times per day (10 am and 3.30 pm) for daily milk yield. Every Tuesday, a physico-chemical analyses were determined for the ewe milk. The differents parameters (fat content, SNF, density, protein, mineral, lactose and freezing point) were determined by using Lactoscan (Milkotronic LTD, Serial n° 4696, Hungary). The pH was measured by pH-meter.

Results and discussions

Composition of diets

The chemical compositions of different meals are shown in table 2.

Table 2. Chemical composition of the meal (% DM basis)

Nutrient	Concentrate		hay
	C	S	
Dry matter	94.7	94.7	92
Organicmatter	91.0	88.3	92.1
Crude fibre	12.7	3.7	35.6
Crude protein	16.3	14.65	4.9

C: 10% barley; 43.3% corn; 25% wheat bran; 17.7% soybean meal and 4% CMV; S: 66% white sorghum; 30% horse bean and 4% of CMV;

The results of this analysis show that the hay is low in CP (4.9%) and rich in crude fibre (35.6%) confirming the results reported by several studies (Mahouachi and Khaldi. 1987). The CP and crude fibre concentrations in the diets containing corn were greater than diet containing sorghum.

Feed intakes and Milk Production

The mean feed intake was 1334 g DM/d for RS and 1306 g DM/d for RM. Feed intake of animals was not affected by diet. Mean daily and total milk yield was showed on table 3. Ewes of RS group produced more milk than those of control group (Fig 1). Rouissi et al (2008) and Selmi et al. (2010) reported similar findings. This can be explained by the significant degradability of horse beans proteins relative to soybean protein (Rouissi et al, 2006).

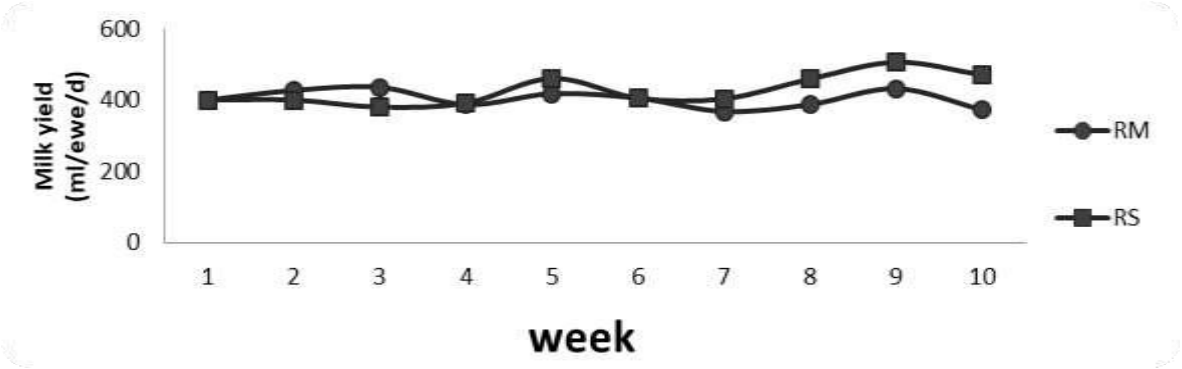

Fig 1. Weekly milk production of ewes

Table 3. Milk yield

	RM	**RS**	***Pr***
Milk yield/ewe (L)	28.22±17.15	29.94±29.72	*NS*
Milk yield/ewe (ml/d)	403.26±24.51	427.62±42.46	*NS*

NS : Non Significatif.

Milk physico-chemical parameters
pH, density and freezing point

Table 4 showed that the group RS had higher pH values than the group RM (6.61 vs 6.57). Diet affected pH significantly (P<0.001). The same result reported by Rouissi and al (2006a). the density of RS (1036.747) is higher than that of RM (1035.769). Density was affected by diet. These results agree with those reported by Assenat (1985) and Selmi et al. (2010). Freezing point was not significantly affected by diet. While the freezing point was -0,583 °C et de -0,602 °C respectively for RM and RS. The current results agree with the findings obtained by Rouissi et al (2008).

Table 4. Effects of diet on physical quality of milk of sicilo-sarde

	RM	***RS***	***Effet régime***
pH	6.57^{a}±0.040	6.61^{b}±0.049	*
Density	1035.769^{a}±0.639	1036.747^{b}±0.656	*
Freezing point, °C	-0.583±0.049	-0.602±0.053	*NS*

Means with different superscripts (a, b) within each column are significantly different (P<0.05); * = P < 0.05; NS : Non *Significatif.*

Milk fat, protein,SNF, mineral and lactose content

As shown in table 5, milk fat content was higher for RS group (7.588% vs 7.219%). Rouissi et al (2008) and Selmi et al (2010) found the same result. The milk fat content obtained in this trial is higher than that reported by Hammami et al (2008) who found an average of 5.34 to 5.83%. Milk fat content is generally negatively correlated to energy balance of ewes (Bocquier and Caja, 2001). Milk fat was significantly affected (p<0.01) with the diet. Protein is the most important fraction of milk. In fact, the protein Milk of RS is higher than the group RM (6.047% *vs* 5.865%). Diet did not affected (P>0.05) milk protein. This can be explained by the fact that the protein content of milk is positively correlated with the energy balance of

the diet. The energy supply stimulates the synthesis of microbial proteins in the rumen (Bocquier and Caja, 2001) while the diets are iso energetic (0.98 and 0.99 UFL / Kg MS respectively for RM and RS). The solid not fat was 11.372% for RS vs 11.043% for RM. The data in table 5 indicated that SNF was significantly affected by the diet. Rouissi et al (2008) observed no effect of diet on solid not fat. Table 5 showed that there was a tendency for mineral content to increase in RS group in comparison to RM group (1.026% for RS vs 0.995% for RM). However, this difference was not statistically significant between this to groups. Lactose is a sugar composed of galactose and glucose. The data represented in table 5 showed the effect of diet on lactose content. These data indicated that the lactose content is not significantly different (P> 0.05) depending on the diet (Table 5). However, lactose content of the ewes' milk in the experimental group (RS) is higher than that of the ewes in the control group (RM) (4.276% for RS and 4.159% for RM). Rouissi et al (2008) previously observed similar results.

Table 5. Effects of diet on chemical quality of milk of sicilo-sarde

	RM	***RS***	***Pr***
Fat (%)	7.21±0.41	7.58±0.69	NS
Protein (%)	5.86±0.54	6.04±0.57	NS
SNF (%)	11.04^{a}±0.18	11.37^{b}±0.21	*
Mineral (%)	0.99±0.044	1.02±0.048	NS
Lactose (%)	4.15±0.558	4.27±0.554	NS

Means with different superscripts (a, b) within each column are significantly different (P<0.05); * = P < 0.05; NS : Non *Significatif.*

Conclusion

The current study revealed that the effect of replacing corn and soya beans by white sorghum and horse beans on milk performances of sicilo- sarde sheep can improve some parameters le pH, density, and SNF but without modification significantly other parameters.

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RELATIONSHIP BETWEEN MILK PRODUCTION AND LAMB GROWTH IN SUCKLING PERIOD OF SICILO-SARDE DAIRY SHEEP IN TUNISIA

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Abstract

Sicilo-Sarde is the only dairy sheep breed in the North of Africa that is mainly raised in northern Tunisia. This micro-sector contributes, through a better identification of "terroir" products, to rural development. Its milk production (MP) remains low as a result of mixed milk-meat production system with suckling period exceeding two months. Furthermore, MP in the beginning of lactation is important for both total MP and lamb growth. Dairy ewes produce about one quarter of total MP during the first month of lactation. This work aimed to estimate the suckling MP (SMP) using the method of weighing lambs before and after suckling and to determine the relationship between ewe MP and lamb growth. Ten Sicilo-Sarde ewes aged 3.90 ± 0.50 years and nursing single male lambs were used to estimate SMP during the two first months of lactation. Results obtained indicated that SMP increased with suckling duration. The means of SMP were 36.18±6.6, 57.28±10.2 and 74.80±12.2 kg respectively for week 4, 6 and 8 of suckling period. Lambs weight at 28 days (10.85±1.83 kg) and Average Daily Gain between 10 to 28 days ($ADG_{10\text{-}28;}$ 222±46 g/d) were highly correlated with SMP of week 4, 6 and 8 ($0.82 \leq r \leq 0.85$; $P<0.01$). The MP regression equations on lamb's growth traits before weaning showed that $ADG_{10\text{-}28}$ was a good trait for estimating SMP. In conclusion, Sicilo-Sarde ewe has an interesting dairy potential during suckling period that may be estimated referring to lamb growth at the first month of age.

Keywords: *Sicilo-Sarde, Suckling milk production, Lambs growth, Correlation, Regression.*

Introduction

In Tunisia, dairy sheep farming is mainly represented by the Sicilo-Sarde, only dairy breed in the North of Africa (Aloulou et *al*. 2018). Several studies have shown that the milk production level of Sicilo-Sarde ewe remains low (50-120 l/lactation: Mohamed, 2008; Meraï et *al*. 2014). This production level is lower than that of Sarda and Comisana breeds which are at the origin of this breed (158 kg per 200 lactation days and 132 kg per 150 lactation days, respectively; Haenlein, 2007). The lower milk production could be associated to a longer suckling period that generally exceeds the first three months of lactation (Djemali et *al*. 2009; Meraï et *al*. 2014). In addition, sheep dairy farming has kept a traditional management, essentially characterized by extensive feeding, hand milking and artisanal making cheese.

Milk production in early lactation period is an important factor determining for both total milk production of dairy ewe and growth rate of lambs until weaning. In fact, McDonald et *al*. (1995) reported that dairy ewes yielded about 38 and 30% of total milk production respectively during the first and second months of lactation. Also, McKusick et *al*. (2001) had reported that dairy ewes produced about 25% of total milk production in the first month of lactation. On the other hand, approximately 6 litres of milk is required to produce 1 kg of lamb body weight (Boyazoglu, 1963; Pulina et *al*., 2006).

The sources of variation in milk production level of Sicilo-Sarde during the milking period have been widely studied (Rouissi et *al*. 2008, Djemali et *al*. 2009; Meraï et *al*. 2014) and

results obtained indicate that milk production can be improved by appropriate breeding management including genetics, reproduction, animal health and nutrition techniques. However, little informations on milk production level in suckling period were available (Khaldi, 1987, Mohamed et *al*., 2008). Thus, a better evaluation of the real dairy potential of the Sicilo-Sarde ewe appears to be necessary through, an update of the knowledge of milk production level on the suckling period. Therefore, our study aims to estimate milk production level of the Sicilo-Sarde ewe during the suckling period. The second objective is to determine a relationship between suckling milk production and lamb growth before weaning.

Materials and methods

The study was conducted during spring season of 2017 at the farm of the Cooperative Unit of Agricultural Production of Methline (Bizerte), situated in the northeast of Tunisia. Ten Sicilo-Sarde ewes in second and third lactation and nursing single male lambs were used to estimate suckling milk production (SMP) on the first two months of lactation. Ewes were reared indoor and received 1.5 and 0.7 kg of oat hay and concentrate per ewe, respectively at two times daily (7 h and 16 h). Also, lambs received during the experimental period oat hay and concentrate *ad libitum*.

The measurement of SMP started on week 2 (at day 12th after lambing) to week 8th of lactation (at day 56th after lambing) and was carried out weekly during the suckling period by the weighing–suckling–weighing method. At the control day, ewes and lambs were housed in two adjacent plots of 20 m² and 12 m² respectively. The separation between the animals was done by a roasting that avoiding the access of the lambs to their mothers during the separation and keeping the visual, olfactory and auditory contact between them. The lambs were separated from their mothers at 6h for 3 hours and the first suckling was made at 9h. Lambs were weighed just before suckling using an electronic balance and allowed to suckle their mothers and then re-weighed again. The amount of milk sucked by lamb is defined as the difference between the weight of the lamb before and after suckling. In total, during the 24 hours, seven measurements of SMP were made at the rate of one measurement every 3 hours except a night measurement at six-hour interval (9 h, 12 h, 15 h, 18 h, 21 h, 3 h and 6 h). The daily SMP is equal to the sum of the quantities of milk suckled by lamb during the 24 hours. Total SMP during suckling period was calculated using the Fleishman method by the following equation:

$$\mathbf{SMP = MP1 * D1 + \sum_{i=1}^{7} \left(\frac{MPi + MP(i+1)}{2} \right) * (D(i+1) - Di) + MP8 * D8}$$

Where:

SMP: Total suckling milk production (kg); MP1: Milk production measured at the first test day; D1: Age of lamb at the first test day; MPn: Milk production measured at the nth test day (n vary from 1 to 8); D(i+1)-Di: Interval between two successive test days; MP8: milk production measured at the eighth test day; D8: Interval between last test days and weaning age.

Weight controls were used to calculate weights at 10 and 28 days (W_{10} and W_{28}, respectively) and average daily gain (ADG_{10-28}). Regression equations between dairy and growth traits were studied to establish a relationship to predict ewe SMP from lamb growth performances.

Data were analyzed with Statistical Analysis Systems (SAS, version 9.1). Pearson correlations coefficients were determined between SMP and growth performances and considered significant when $P \leq 0.05$.

Results and discussion

Results of ewe suckling milk production (SMP) and lamb growth performances are shown in Table 1. SMP increased as lactation progressed. The means of total SMP were 36.18±6.60, 57.28±10.16 and 74.80±12.15 kg respectively for week 4, 6 and 8 of suckling period. This indicated an increase of +52 and +23% in the production of milk marketed when lambs were weaned at four and six weeks, respectively, compared with weaning lambs at week eight of age. Similar findings were obtained by Khaldi (1987) and Mohamed et *al*. (2008) for the same breed. However, SMP obtained at week 6 of suckling period was greater than 49.28 kg reported by Khaldi (1987) and lower than the result (64.6 kg) of Mohamed et *al*. (2008) for ewes nursing single lamb and suckled for 42 and 45 days respectively. Thus, these differences among trials could be associated with a difference in rearing conditions (flock, month and year of lambing, parity, etc.; Djemali et *al*., 1995; Moujahed et *al*., 2009; Meraï et *al*., 2014) and probably to the method used to determine SMP. The daily SMP for ewes nursing single lamb were 1.29, 1.36 and 1.34 kg/day respectively for week 4, 6 and 8 of suckling period. These results underline an important dairy potential for this breed compared to previous studies that have shown a low level of production that does not above 100 liter per lactation (Djemali et *al*., 1995; Mohamed, 2008). This finding indicates may be that the transitive period between suckling and exclusive milking, usually accompanied by a decrease in milk production ranging from 30 to 40% (Gargouri et *al*.,1993; Marnet and Negrão, 2000), was not well mastered. Otherwise, the partial milking of ewes for a week just before weaning is recommended to avoid the milk production drop at weaning (Marnet and Negrão, 2000; McKusick et *al*., 2001). For growth performances, lambs weighted 6.87±1.16 and 10.85±1.83 kg respectively at 10 and 28 days of age. As a result lambs realized an average daily gain (ADG) of 222 g/d during the first four week of age. The relatively high value of the $ADG_{10\text{-}28}$, an excellent indicator of the dairy ability of the ewe, is mainly related to the high level of average daily milk production recorded during the suckling period that ranged from 1.29 to 1.36 kg/d. The mean of $ADG_{10\text{-}28}$ was higher than 200 and 137-140 g/d reported respectively by Khaldi (1987) and Rouissi et *al*. (2008) for single male lambs suckled by multiparous ewes.

Table 1. Ewe total milk production of week 4, 6 and 8 on suckling period and lamb growth during the first four week of age of Sicilo-Sarde dairy sheep breed

Variable	Mean	Standard deviation
Suckling Milk Production		
SMP_{w4} (kg)	36.18	6.60
SMP_{w6} (kg)	57.28	10.16
SMP_{w8} (kg)	74.80	12.15
Growth Performances		
W_{10} (kg)	6.87	1.16
W_{28} (kg)	10.85	1.83
$ADG_{10\text{-}28}$ (g/d)	222	46

SMP, suckling milk production; W, weight; w, week; ADG, Average daily gain

Pearson Correlation coefficients between traits of SMP during the suckling period and lamb growth performances at the first four week of lactation are reported in Table 2. The highest correlations were found between SMP_{w6} and SMP_{w8} ($r=0.99$, $P<0.001$) and SMP_{w4} and SMP_{w6} ($r=0.94$, $P<0.001$). Lambs weight at week 4 and $ADG_{10\text{-}28}$ were highly correlated with all SMP of week 4, 6 and 8 ($0.82 \leq r \leq 0.88$; $P<0.01$). Also, Benyoucef and Ayachi (1991) reported higher correlation between SMP and lambs growth at the first month of lactation ($r=0.83$) for Hamra ewe non-dairy sheep breed. However, the correlation coefficients between

weight at ten days and all SMP were little important and ranged from 0.57 to 0.64. Only this between W_{10} and SMP at week 8 was not significant (P>0.05).

Table 2. Pearson correlation coefficients between ewe suckling milk production and lamb growth during the first month of age in the Sicilo-Sarde dairy sheep breed

	SMP_{w6}	**SMP_{w8}**	**W_{10}**	**W_{28}**	**ADG_{10-28}**
SMP_{w4}	0.94 (***)	0.91 (***)	0.64 (*)	0.80 (***)	0.85 (**)
SMP_{w6}	-	0.99 (***)	0.64 (*)	0.79 (**)	0.82 (**)
SMP_{w8}		-	0.57 (ns)	0.74 (*)	0.88 (**)
W_{10}			-	0.94 (***)	0.65 (*)
W_{28}				-	0.87 (***)

SMP, suckling milk production; W, weight; w, week; ADG, Average daily gain; ns: no significant, P> 0.05; *: P<0.05; **: P<0.0l; ***: P<0.00l

The estimating milk production at the beginning of lactation, especially during the first month of suckling by some techniques (milking, weighing of lambs, etc.) can disrupt the animals (ewes and lambs) and consequently affect the accuracy of results. Regression equations were determined to establish a relationship between SMP and lamb growth before weaning (Table 3). The regression analysis indicated that growth traits at the first week were the best criteria to predict the SMP (R^2= 70.8%). That is in agreement with the higher correlations coefficients between SMP and ADG_{10-28} determined previously (r≥0.82; P<0.01).

Table 3. Regression Coefficients of the ewe daily suckling milk productions (SMP_{w4}, $_{w6}$ and $_{w8}$) on first month lambs growth performances

	Constants	**ADG_{10-28}**	**W_{w4}**	**W_{w2}**	**R^2 (%)**
SMP_{w4}					
1	8.94708	0.12251	-	-	69.4
2	6.27231	0.09272	0.08569	-	66.7
3	13.25032	-1.12073	-6.63835	6.71083	70.8
SMP_{w6}					
1	17.00677	0.18117	-	-	63.2
2	11.99914	0.12540	0.16042	-	60.4
3	17.48193	-0.82804	-5.21591	5.36595	56.3
SMP_{w8}					
1	26.88830	0.21553	-	-	62.4
2	24.28833	0.18657	0.08329	-	57.5
3	31.11728	-1.00096	-6.49655	6.56691	53.1

SMP, suckling milk production; W, weight; w, week; ADG, Average daily gain

Conclusion

This paper presents the results concerning the milk production of Sicilo-Sarde ewe during the suckling period. The milk production level of ewe and the lambs' growth performances obtained are acceptable and indicate an interesting dairy potential of this breed that may be not exteriorized. Thus, more appropriate breeding management, especially in relation to weaning age, earlier start of the milking period, and mastered of the transitive phase suckling/weaning/milking should be takes for a better exploit of the real vocation of this breed.

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DIAGNOSIS OF TECHNICAL AND HYGIENIC MILKING CONDITIONS IN DAIRY SHEEP FARMS (TUNISIA)

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Abstract

Milking of Sicilo-Sarde dairy sheep breed in Tunisia remains always manual by calling qualified milkers who have become more and more rare. The emergence of some flocks using mechanical milking is strongly encouraged, but its use is still inefficient or inadequate. This exploratory study aimed to evaluate the technical and hygienic milking conditions in the two dairy sheep areas in the North of Tunisia. A sample of 955 Sicilo-Sarde ewes in mid and late lactation belonging to seven flocks (four public farms, three of which use machine milking in pot, and three private farms) was used to evaluate the milking conditions. The diagnosis of the milking equipment had revealed a relatively satisfactory situation of the milking machines working. The pulsation rate was between 80 and 105 cpm. Also, the average milking cadence observed for mechanical milking was relatively low (65 ewes/milker/hour), but it was acceptable for manual milking (50 ewes/milker/hour). However, farms with mechanical milking did not have a milking hallway that disturbed ewe circulation and contention during milking. For hygienic milking practices, udder preparation and teat disinfection after milking were not carried out in all farms. These results indicate that a great improvement in hygienic milking conditions must be made in dairy sheep farms. The study of the comparative impacts of mechanical milking of Sicilo-Sarda ewe on mammary health, teat integrity and milking comfort is needed to identify the appropriate settings for a best development of this system in Tunisia.

Keywords: *Sicilo-Sarde, Machine milking, Manual milking, Technical conditions, Hygienic practices*.

Introduction

The milk production and cheese making of sheep represent a millenary and traditional activity in the Mediterranean countries (Barillet, 1985). The Sicilo-Sarde breed is the only dairy ewe in the North African region, constitutes the basis of dairy sheep farming in Tunisia. This breed represents a micro-sector concentrated around two large production areas in the regions of Beja and Bizerte, where the potential of forage production is abundant (Djemali et *al.*, 1995; Rouissi et *al*., 2001; Mâatoug et *al*., 2015; Aloulou et *al*., 2018). Milking sheep is essentially manual and done twice daily with an interval of 12 hours. The once daily milking is adopted in late lactation, when the milk production level decreases significantly. Mechanical potting is recently adopted by some farmers who had a flock with large number of animals. Milk production is exclusively used for cheese processing. The quality of the milk as well as the cheese products is closely related to the milking conditions. The knowledge of hygienic and sanitary conditions of the ewes as well as the technical conditions of the milking have demonstrated specific relations with the milk production, and also with the improvement of the working conditions of the milker. The importance of respect the hygienic practice measures at the farm level, during milking is discussed. In this perspective, the present investigation proposes to evaluate technical and hygienic milking conditions in dairy sheep farms of the Sicilo-Sarde breed in the regions of Beja and Bizerte.

Materials and methods

Technical and hygienic diagnosis of milking conditions and breeding practices were carried out during a follow-up at milking in seven Sicilo-Sarde farms distributed as follows: five in the region of Beja: Cooperative Unit for Agricultural Production (UCPA) Gnadil, UCPA Essemen and three private farms Nour, Ben Youssef1 and Ben Youssef2) and two in the region of Bizerte: UCPA Methline and Livestock and Pastures Office (OEP) Fretissa. Four and three farms practice manual and mechanical milking pot, respectively. The follow-up was performed on a sample of 955 Sicilo-Sarde ewes in the middle and at the end of lactation.

The diagnosis of the milking equipment focuses on the general state of the milking machine and to assess the pulsation rate and the vacuum level. As for hygienic practices, the follow-up is based on cleanliness of equipment, milker and milking place for both manual and mechanical milking as well as the udder preparation and teat disinfection after milking. Also, global conditions of the milking were carried out (tranquillity, speed, completeness, hygienic ...). Various incidents observed during milking were also noted. These incidents may be related to the ewe, the milking machine, the milking place and the milker. Finally, the Practical Effectiveness of Milkers (PEM; also called milking cadence) was determined based on the following expression: PEM (ewes/milker/hour) = number of ewes milked x 60 min / total milking time (min) / number of milkers.

Total Duration of milking (TDM) was also determined.

Results and discussion

Technical and hygienic characteristics of milking machines followed

All the dairy sheep farms followed used the classical fixed milking trolley equipped with two Milking Clusters (MC) an one milking pot (MP), named Mechanical Milking Pot (MMP: 2MC + 1MT). The Technological and hygienic characteristics of followed milking machines are shown in Table 1. The diagnosis of the milking machines used revealed a satisfactory state of maintenance and running. Furthermore, a large variability of vacuum levels and pulsation rate was observed. The vacuum level is 40 and 60 kPa respectively in the region of Beja and Bizerte (Table 2). These values were different from those reported by Dubeuf (1999). This author had reported that the vacuum level for pot milking were between 33 and 36 kPa in France, 39 and 46 kPa in Italy and less than 52 kPa in Greece. Otherwise, the pulsation rate varied between 80 and 105 cpm (Table 2). These results were lower than recommended value (120 to 180 cpm; Dubeuf, 1999). For milking sheep, increased pulsation rate reduces vacuum fluctuations and thus has a better efficiency. This also makes it possible to work with a lower vacuum level, and consequently limit the risks of aggression on the udder. The choice of high pulsation rate seems limited by the performance of the pulsators (and therefore their costs). For pneumatic pulsators generally equipping milking trolley, it is advisable to choose settings between 80 and 120 cpm. This makes it necessary to choose a relatively high level of vacuum, which may be at the origin of an increase in somatic cell counts that contributing to the elevation of the risk of mastitis. The genetic improvement of the milking ability of dairy breeds is highly sought to apply a drop in vacuum levels, as practiced in France. Indeed, the morphological characteristics (udder depth, position and length of the teats ...) and physiological characteristics (ease and milking rate ...) of the udders influenced generally a particular parameters especially the vacuum level and pulsation rate. However, the material itself may differ from one country to another or between farms, which requires specific settings. To this end, the establishment of references on the aptitude of milking ewes for mechanical milking and the proposal of technical elements for the selection and adjustment of milking equipment may lead to the development of a technical referential for dairy sheep profession in Tunisia.

Table 1. Technical and hygienic situations of milking equipment on the farms followed

Characteristics	UCPA Gnadil	UCPA Essemen	UCPA Methline
Milking system	MMP1 & MMP2 (2MC + 1MP)	MMP1 (2MC + 1MP)	MMP1 (2MC + 1MP)
Graduation Manometer	kPa	kPa	bar
Position of the needle at a standstill	Superior to Zero	Superior to Zero	Zero
Advancement of the needle in motion	Regular	Irregular	Regular
General assembly of the installation	Correct	Correct	Correct
General state of the installation	Average	Average	Good
State teat cups	Average	Satisfactory	Good
State Piping	Average	Average	Good
State Seals	Average	Average	Good

MMP, Mechanical Milking Pot; MC, Milking Clusters; MP, Milking Pot

Table 2. Functioning parameters identified at the milking machines followed

Farm		UCPA Gnadil		UCPA Essemen	UCPA Methline
Milking system		MMP1 (2MC + 1MP)	MMP2 (2MC + 1MP)	MMP1 (2MC + 1MP)	MMP1 (2MC + 1MP)
Vacuum level (kPa)		40	40	40	60
Pulsation rate (cpm)	**MT1**	105	102	80	80
	MT2	105	102	80	80

MMP, Mechanical Milking Pot; MC, Milking Clusters; MP, Milking Pot; MT, Milking Trolley

As for the cleanliness of the milking equipment, cleaning is carried out after each milking and is considered satisfactory. The hygienic cleaning conditions are similar in both UCPA Gnadil and UCPA Methline. In these two farms, the cleaning is done only by water mixed with bleach, which is not enough to ensure the elimination of all microbes and dirt. The use of a product with acid detergent alternating with another alkaline is recommended for ensuring good cleaning efficiency (Chambre d'Agriculture de Bretagne, 2010). At UCPA Essemen, cleaning is only done using an alkaline detergent. This also affects the quality of cleaning, because the absence of use of an acid detergent causes the accumulation of calcium and magnesium salts (Chambre d'Agriculture de Bretagne, 2010).

For the four farms using manual milking, the place and equipment were almost similar. Manual milking is done in the sheepfold and the material used is rudimentary. The milker is satisfied with a bucket, where he collects milk and a container equipped with a filter, when he pours the milk when the bucket is full. It should be noted that the milkers neglect completely the recommended operations during milking (cleaning the teats before milking, elimination of the first milk jets, disinfecting the teats after milking ...) that can negatively affect udder health and deteriorates also the milk quality. The smooth running of the manual milking is conditioned by the respect of the ambient conditions (light and sound). These conditions are generally respected in farms using manual milking. Ewes were usually docile. However, primiparous are more agitated. The respect of a certain routine (milking time, same milker ...) is necessary to ensure a quiet milking. In a general way, except the hygienic quality of the milking which has not been not regularly respected, all the other conditions (calm, fast and complete treatment) were respected in all the farms followed.

Technical and hygienic characteristics of mechanical milking

For all the farms using milking machines, hygienic conditions were not respected. In fact, both teat cleaning before the milking and the follow-up of the order of ewes milking were not respected. At the UCPA Gnadil, the first milk jets were eliminated on ground. Also, the over milking operation was carried out only in this farm. According to Portolano et *al.* (1999), over milking is an operation to avoid in mechanical milking, because it affects negatively the udder conformation and could damage teats. Teat disinfection after milking is abandoned on all farms. However, milking was described as complete and rapid in all farms. In addition, milking is considered calm with the exception of UCPA Gnadil farm. At this farm, it should be noted: more noise (including noise from the milking machines used) thus affecting the overall sounds cape during milking; ewes were more agitated and nervous. Thus, this situation is not comfortable for the milkers.

The average practical effectiveness of milkers (PEM) in manual milking was 50 ewes/milker/hour (Table 3). This average is higher than that reported by Marion-Brilli (2014), estimated at 40 ewes/milker/hour. However, there is a difference between farms, the minimum value of the PEM is recorded on the Nour farm (43 ewes/milker/hour). This can be explained by the high number of milked ewes (303 head). For farms using mechanical milking, we also noted the absence of a milking hallway which making the organization of ewes difficult and complicated. In addition, the ewes were more agitated and nervous, which affected the quality of milking.

Table 3. Total duration of milking (min) of ewes and practical efficiency of milkers (ewes/milker/ hour) in all farms followed according to the milking system used

Farm	Number of ewes (head)		Number of milkers	TDM	PEM
	Present	Milked			
UCPA Gnadil (1) (3)	307	184	2	**152**	67
UCPA Essemen (1) (3)	216	124	1	**125**	74
UCPA Methline (2) (3)	443	155	1	**175**	53
Average mechanical milking					**65**
Nour (1) (4)	319	303	2	**213**	43
OEP Fretissa (2) (3)	234	90	1	**98**	55
Ben Youssef1 (1) (4)	66	66	1	**69**	57
Ben Youssef2 (1) (4)	33	33	1	**45**	44
Average manual milking					**50**

(1) Beja; (2) Bizerte; (3) Organized Sector; (4) Private Sector; PEM, Practical Effectiveness of Milkers; TDM, Total Duration of milking.

The higher value of PEM was recorded at Ben Youssef1 farm (57 ewes/milker/hour). This result is due to a better organization of the milking as a result of the presence of a milking hallway which facilitating the ewe circulation and their contention during milking. In addition, the milker of this farm is qualified and the size of the flock was low making thus the milking less tiring. Overall, according to the literature, the practical efficiency of milkers varied mainly with breed (milkability), milk yield and lactation stage. In this context, Delmas (1984) found that the Lacaune ewe is difficult to milk by hand (20 ewes milked / hour). Le Du (1986) reported that for dairy sheep breeds that are easy to milk, more than 60 ewes were hand milked per hour. For mechanical milking, the higher PEM was obtained at the UCPA Essemen and UCPA Gnadil, respectively, 74 and 67 ewes/milker/ hour. While, the lower PEM value was recorded at UCPA Methline (53 ewes/milker/ hour). These results can be explained by the good organization of the milking in both UCPA farms in the region of Beja with a sufficient number of workers to facilitate the circulation and contention of ewes during

milking. However, on the UCPA Methline, the milker takes care alone of all tasks during milking which making it longer and more exhausting. The good performances recorded at the UCPA Essemen are also due, in part, to the existence of a single milker, which is not the case at UCPA Gnadil. According to Piedhault et *al*. (2005), the use of a single milker is recommended to reduce the milking time.

During the following of milking process, there were several incidents that affected the total milking time, and consequently the PEM. In fact, there is no sorting of ewes by batch: all the dry, lactating, sick, healthy, aborted ewes, selected for the reform and antennas enter the place of milking. Thus, the passage of all the animals increases the TDM. In addition, all local of machine milking pot are devoid of hallway that disturbed ewe circulation and contention during milking (Photos 1 and 2). It is also noted that the volume of the pot-milker is not adapted to the milk production of the flocks. The milkers are then strained to milk into a large pot each time when the full pot-milker. Comparing the two milking systems, we found that the PEM is generally more important in farms using mechanical milking. Therefore, to improve milking conditions, in terms of time and rapidity, it is advisable to adopt mechanical milking especially for farms with large herds of animals. According to Marion-Brilli (2014), PEM in the milking parlor can reach 400 ewes/milker/hour (milking rate was about ten times more than that in manual milking).

Photo 1. Place reserved for milking ewes in the UCPA Gnadil (Beja, Tunisia)

Photo 2. Equipment for milking ewes in the UCPA Gnadil (Beja, Tunisia)

Conclusion

This work evaluated the technical and hygienic conditions of milking in the seven dairy farms of Sicilo-Sarde. These results indicate a lack of hygienic conditions during milking. Similarly, milkers do not follow the order of milking ewes, which can promote the spread of mastitis in the herd, including a mammoth sheep to a healthy one. In addition, the average of milking cadence for manual milking was adequate, while this for mechanical milking was well below the recommended milking parlor, but it is far better than that recorded in manual milking. The gradual abandonment of manual milking of ewes in favour of the generalization of mechanical milking and perhaps the gradual transition to larger and more profitable structures with equipment adapted for farms having large herds are in great demand in Tunisia for the revival of dairy micro-sector and the assurance of its sustainability, thus contributing to rural development.

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HATCHABILITY OF BRONZ TURKEY EGGS AFFECTED BY BREEDER AGE AND RELATIVE HUMIDITY

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Abstract

The present study was carried out to determine the effects of breeder age and relative humidity levels on egg weight loss during incubation, hatchability of total eggs and hatchability of fertile eggs in eggs of Bronz turkeys. The eggs were collected from a Bronz turkey breeder flock at 35 (young) and 66 (old) weeks of age, and were incubated in 55-60% and 61-65% humidity during the first 24 days of incubation period. After that period, the eggs were incubated under hatching conditions during hatching period. The effect of incubation humidity and breeder age on hatchability of fertile eggs, hatchability of total eggs and egg weight loss was significant ($P<0.01$). The eggs obtained from young flock showed a higher hatchability of fertile eggs and total eggs, and a lower egg weight loss during incubation when incubated at a relative humidity of 55-60%. On the other hand, similar results were observed for the eggs obtained from old flock and when incubated at a relative humidity of 61-65%.

Keywords: *Bronz turkey, Incubation humidity, Hatching characteristics, Breeder age*

Introduction

The turkey (*Meleagris gallopavo*) is one of the most important of commercial poultry species, especially in western countries, and during last decades it has gained importance as an alternative poultry production, especially to meet animal protein demand. The essential goal of turkey production is to obtain maximum hatchability and with high quality one day old poults (Anandh *et al.*, 2012). In poultry production, hatchability is an important economic parameter and affected by some factors, for example breeder age, egg quality, nutrition of breeder flock, egg handling and storage and incubation conditions e.g. incubation temperature, humidity and turning conditions (Weis *et al.*, 2011; Ipek et al., 2014; Ipek and Sözcü, 2015). Breeder hen age is an important factor that has effects on egg quality, fertility and hatchability (O'Sullivan *et al.*, 1991; İpek and Şahan, 2001; Peebles *et al.*, 2001; Nangsuay *et al.*, 2011; İpek and Sözcü, 2015). As breeders get older, egg weight shows an increment and causes a decline in hatchability (Elibol *et al.*, 2000) and increment early and late term embryonic mortalities (Elibol and Brake, 2003; Tona *et al.*, 2004; Joseph and Moran, 2005). During incubation, relative humidity also affects some important parameters such as egg weight loss, hatchability and chick hatching weight (Yahav, 2000). It is reported that a lower humidity level during incubation period results with excessive egg water loss, and subsequently embryo dehydration and moreover embryonic deaths (Reinhart and Hurnik, 1984). Also, it may affect embryo development by fluid deficit in amniotic or allontoic sacs, therefore it causes small and dehydrated chicks (van der Pol *et al.*, 2013). Furthermore, if the relative humidity is too high, there could be a shortening of incubation period and an increment in number of wet chicks with residual albumen (Taylor, 1999; Decuypere *et al.*, 2002; Tona *et al.*, 2003). The present study was carried out to determine the effects of breeder age (young and old) and relative humidity levels (standard and high) on egg weight loss during incubation, hatchability of total eggs and hatchability of fertile eggs in eggs of Bronz turkeys.

Material and Methods

The research was performed at the Research and Experimental Farm of the Department of Animal Science in Uludağ University in Turkey. A total of 480 hatching eggs (75-80 g) were collected from two Bronz turkey breeder flocks at different ages as 35 weeks (Y: young flock) and 66 weeks (O: old flock) of age. All eggs were numbered and weighed with ± 0.1 precision before incubation. Then the eggs were incubated in two fully automated, ventilated, programmable incubators at 37.5◦C and two different humidity levels as 55-60% (S: standard humidity) RH and 61-65% RH (H: high humidity) during the first 24 d of incubation. The eggs from each age group and humidity groups were randomly placed into incubator trays consisting of 40 eggs (n = 3 trays/humidity/breeder age).

During incubation period, the egg weight loss was determined between days 0-6, 7-12, 13-18, 19-24 and 0-24. On d 25 of incubation, eggs were transferred to a hatcher. The hatcher was operated at 37.0°C temperature and a 72% RH during hatching period. After the completing of hatching process, all of the hatched poults were pulled out according to standard hatchery procedures. The poults were classified into two categories as saleable poults (clean, dry, and without deformities) and culls (splayed legs, unhealed navels, and so on; Molenaar *et al.*, 2011). The percentages of saleable and cull poult were determined as a percentage of fertile eggs (Molenaar *et al.*, 2011). Unhatched eggs were opened to macroscopically determine fertility and embryonic mortality (early-term, mid-term and late-term embryonic mortalities). Mortality and fertility were calculated as the percentage of total eggs at set to fertile eggs.

Data were subjected to two-way analysis of variance (SAS Institute Inc., Cary, NC), utilizing ANOVA procedures for balanced data. The model included breeder age and relative humidity levels and interaction between them as main effects. Analysis for percentage data was conducted after an arcsine transformation of the data. Significant differences among treatment means were determined by Duncan's multiple range test. Only significant means of breeder age × relative humidity interactions were given in results. Data are presented as means ± SE. Differences were considered significant at $P < 0.05$.

Results and Discussion

The effects of breeder age and relative humidity on hatchability parameters are presented in Table 1. The initial egg weight was similar among experimental groups as 79.7 g in Y × S, 77.1 g in Y × H, 78.1 g in O × S and 76.2 g in O × H, respectively. Early, mid and late term embryonic mortalities were found to be the highest with values of 6.9%, 3.9% and 9.8%, respectively in eggs obtained from old flock and incubated at standard humidity level ($P<0.01$). The highest percentage of cull poult was found to be the highest (7.4%) in the same group. On the other hand, hatchability of fertile and total eggs were the highest in eggs obtained from young flock and incubated at standard humidity levels (87.4% and 75.0%, respectively, $P<0.01$). A higher poult hatching weight were observed in Y × S (55.8 g), Y × H (56.4 g) and O × H (56.9 g) compared to the O × S (52.1 g).

The results showed that the embryonic mortalities and hatchability were affected by both breeder hen age and relative humidity levels in bronz turkeys. The observed changes in these parameters could be related with eggshell conductance and the conductance constant that could be changed according to breeder hen age (Christensen and Nestor, 1994). Especially late term embryonic mortalities could be associated with these factors that might be combined with the relative humidity levels during incubation period. These findings are supported by previous results of Fairchild *et al.* (2002).

Table 1. The effects of breeder age and relative humidity on hatchability parameters

Parameters	Early-TEM (%)	Mid-TEM (%)	Late-TEM (%)	Percentage of cull poult (%)	HOFE (%)	HOTE (%)	PHW (g)
BA × RH	**	**	**	**	**	**	**
Y × S	3.9±0.5^{c}	1.9±0.3^{c}	6.8±0.8^{d}	2.2±0.8^{d}	87.4±0.5^{a}	75.0±0.6^{a}	55.8±1.5^{a}
Y × H	4.9±0.7^{b}	2.9±0.5^{b}	7.7±0.7^{c}	5.8±0.9^{b}	84.6±0.6^{b}	71.7±0.9^{b}	56.4±1.8^{a}
O × S	6.9±0.7^{a}	3.9±0.6^{a}	9.8±0.8^{a}	7.4±0.5^{a}	79.4±0.5^{d}	67.5±0.7^{d}	52.1±2.4^{b}
O × H	5.0±0.5^{b}	2.9±0.4^{b}	8.9±0.6^{b}	4.8±0.8^{c}	83.2±0.7^{c}	70.0±0.8^{c}	56.9±2.1^{a}

$^{a-d}$ Means ±SEM in column that possess different superscripts differ significantly (*P<0.01;P < 0.05*)
*P<0.05, **P<0.01, NS: not significant.
Early-, Mid-, Late-TEM: Early-, Mid-, Late-Term Embryonic Mortalities; HOFE: Hatchability of fertile eggs, HOTE: Hatchability of total eggs, PHW: Poult hatching weight
BA: breeder age; Y: 35 wks of age; O: 66 wks of age
RH: relative humidity; S: 55-60%; H: 61-65%

The effects of breeder age and relative humidity on egg weight loss during incubation are presented in Table 2. As seen in table 2, the highest means for egg weight loss were observed in the eggs obtained from old flock and incubated at standard humidity level during incubation period (P<0.05). It is accepted as optimum that 12-14% egg weight loss during this period in broiler and turkey eggs (Rahn *et al.*, 1981). In the study, egg weight loss between 0-24 days changed from 10.3% to 13.9% in the experimental groups.

Table 2. The effects of breeder age and relative humidity on egg weight loss (%) during incubation

Incubation days	1-6	7-12	13-18	19-24	0-24
BA × RH	*	*	*	*	**
Y × S	2.9±0.2^{b}	3.0±0.1^{b}	3.1±0.2^{b}	3.2±0.1^{b}	12.2±0.2^{b}
Y × H	2.6±0.1^{c}	2.4±0.1^{d}	2.7±0.1^{c}	2.6±0.1^{d}	10.3±0.5^{d}
O × S	3.2±0.2^{a}	3.5±0.2^{a}	3.5±0.2^{a}	3.7±0.1^{a}	13.9±0.6^{a}
O × H	2.1±0.1^{d}	2.7±0.02^{c}	3.2±0.2^{b}	2.9±0.1^{c}	11.1±04^{c}

$^{a-d}$ Means ±SEM in column that possess different superscripts differ significantly (*P<0.01;P < 0.05*)
*P<0.05, **P<0.01, NS: not significant.
BA: breeder age; Y: 35 wks of age; O: 66 wks of age
RH: relative humidity; S: 55-60%; H: 61-65%

It is known that the egg weight loss is one of the most important factors that affect embryonic death (Tiwary and Maeda, 2005). In the eggs obtained from old flock and incubated standard humidity level, observed higher embryonic mortalities could be related with a higher percentage of egg weight loss during incubation in this group.

Conclusions

In conclusion, egg weight loss during incubation, embryo's survival rate and hatchability are affected by breeder hen age and relative humidity level during incubation period in bronz turkey eggs. It can be offered that, for a maximum hatchability with a high quality poult production, the relative humidity levels should be manipulated according to breeder hen's age. A relative humidity level of 55-60% for the eggs obtained from younger flock and a relative humidity level of 61-65% for the eggs from older flocks should be recommended for a better hatchability.

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EFFECT OF THE BROILER GPS LINE AND AGE ON EGG WEIGHT LOSS, HATCHABILITY AND CHICK YIELDS

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Abstract

The present study was conducted to investigate the influence of the grandparent stock male - female line broiler (MF-GPS) and age of hen on egg weight, egg weight loss, hatchability, hatched chick weight and chick yields. Furthermore, the study was also conducted to determine the level of correlations between GPS male and female line and age of hen for the investigated parameters. A total of 1503 hatched egg from separate hatches were obtained by MF- GPS at 27-33 (I), 34-45 (II) and 46-64 (III) weeks of age. The data of the study obtained from a private commercial grandparent stock breeder hatchery. The eggs were incubated in an incubator at 37.5°C temperature and 84% relative humidity until 18th day of incubation. Then the eggs were transferred to a hatcher at 36.6°C temperature and 87% relative humidity until the chicks hatch. In the study, the weights of eggs and chicks were found to be similar for the male (M-GPS) and female (F-GPS) lines (P>0.05). The egg weight loss during incubation and hatchability were found to be significantly higher in F-GPS (P=0.001), while the chick yield was higher in M-GPS (P=0.001). The breeder age affected the egg weight, egg weight loss during incubation, hatchability, chick weight and chick yield (P=0.001). The interactions between the lines and the ages were significant for the hatchability (P=0.001), chick weight and chick yield (P=0.031 and P=0.017). Significant positive correlations were found between the age and egg weight (r = 0.837; P=0.001), egg weight loss (r = 0.161; P=0.001), chick weight (r = 0.783; P=0.001) and chick yield (r = 0.057; P=0.028), and for MF-GPS as well. The GPS breeder line and age of hen affected hatchability, chick weight and chick yields.

Keywords: *Grandparent stock broiler breeder line, age, egg weight loss, hatchability, chick yield.*

Introduction

Today, hatcheries with modern equipment have sufficient level of possibilities, knowledge and experience in terms of pre-incubation and incubation factors that affect the output. But the ongoing development and competition in the poultry sector requires poultry farmers to move to more technical practices in order to reach the highest level of efficiency. From this point of view, obtaining better quality of output and chick quality in hatcheries is gaining importance.

The age of hen and genotype affects hatchability, quality of chick and also growth performance of hens (Tona et al., 2004). In addition that egg weight effects incubation periods, incubation results, hatched chick weight and growth performance of birds (Witt de and Schwalbach, 2004). The weight of chicks affected by genotype, egg nutrient composition, egg weight, egg incubation conditions, egg weight loss during incubation (Wilson, 1991).

As we know egg consist albumen (60-63%), yolk (28-29%) and shell (9-11%) (Altan, 2015). The egg composition varies depend on genotype and age of hens (Vieira and Moran, 1999). During the incubation period it is necessary for succesful hatch that the weight loss in the egg is average 10-12% (Tazawa, 1980). O'Dea et al. (2004) found that weight loss and conductance of egg accreted with breeder age. Thus, egg weight accreted with breeder age. The larger eggs shell was become thinner and fractured and also more porous, it is provides more water loss and gas exchange from hen eggs (Shafey, 2002). The low egg shell quality has been caused low hatchability (Narushin and Romanov, 2002). In the hatchery this factor

should be noted, when incubated different weighed eggs to ensure ideal moisture loss for incubation (Ulmer-Franco, et al. 2010).
Chick weight is in general used for hatched chick quality evaluation (Decuypere et al., 2002). There is a correlation between egg weight and hatched chick weight in broiler chickens (Abiola et al., 2008). However, the breeder age affect chick weight and chick yield (Iqbal et al., 2016). Thus, egg weight increased with beeder age (Roque and Soares, 1994) and also chick weight (O'Dea et al., 2004). It is important for growth performance of broiler, there is a relationship between hatched chick weight and growth for broilers (Jiang and Yang, 2007).
In commercial hatcheries, eggs are obtained from different genotype and age of hens and they incubated standard incubation conditions. The number of high-quality breeder chicks obtained from GPS breeder eggs is important for hatchery. This study was aimed to investigate the influence of the grandparent stock male-female line broiler and age on egg weight, egg weight loss in incubation, hatchability, hatched chick weight and chick yields. Furthermore, the study was also conducted to determine the level of correlations between GPS male and female lines of breeders and breeder age for the investigated parameters.

Material and Methods

The data of the study obtained from a private commercial the grandparent stock hatchery in Marmara Region of Turkey. A total of 1503 hatched egg individuals were obtained by the grandparent stock male-female line broiler breeder (MF-GPS) at 27-33 (I), 34-45 (II) and 46-64 (III) weeks of age. A total of 563 separate hatches were obtained from Male Line (M-GPS) and 940 obtained from Female Line (F-GPS). The number of hatches used for different age groups respectively 164, 666 and 673 for I, II and III wks of age.
Eggs in each age group were stored for 3 to 16 days under 55% relative humidity and 18 °C. All eggs were weighed before setting into incubator. Eggs were incubated under 37.5 °C temperature and 84% relative humidity. The eggs were turned through an angle of ±45°automatically six times a day until 18^{th} day of incubation. At 18^{th} day of incubation, all eggs were weighed for determining the egg weight loss. Then eggs were transferred hatcher incubated under 36.6°C temperature and 87% relative humidity until chicks hatch. At hatch all the hatched chicks were counted and weighed. The hatchability and chick yield determined. The chick yield calculated with the formula hatched chick weight divided by egg weight at the time of setting into incubator multiplied by 100 (Iqbal et al. 2016; 2017).
Prior to statistical analysis, analyses data of egg weight loss, hatchability and chick yield were conducted after square root of arc sine transformation. The GPS line of broiler breeder and age of breeder were the main effects. The data were analysed with using a general linear model two way ANOVA (Minitab, 2013). Significant means were separated using Duncan multiple range test. Differences were considered significant at $P \leq 0.05$. The correlations between MF- GPS line and age for egg weight, egg weight loss, hatchability, chick weight, chick yield were analysed with Pearson correlation analysis (Minitab, 2013).

Results and Discussion

The effects of the grand parent stock male - female line broiler breeder and breeder age on egg weight, egg weight loss, hatchability, hatched chick weight and chick yield were given in Table 1. In the study egg weight was found to be similar for both male and female line grandparent stock of broiler (P>0.05). However, in their study Wolanski et al. (2007) suggested that strains had an impact on egg weight. In the study egg weight loss during incubation and hatchability were found higher in F-GPS broiler breeder, however egg weight loss was found low in M-GPS broiler breeder (P=0.001). That might be result of low rate egg production which attributed to the thicker egg shell in M-GPS lines.

In the study the hatched chick weight was found similar both GPS male and female lines of broiler breeders (P>0.05); despite that chick yield was found higher in M-GPS (P=0.001). The difference of hatched chicks weight of different genotype hens was not only related with egg weight differences, but also reflected genetic differences (Hardin, 1972). In their study (Wolanski et al., 2007) found that large male line had heavy eggs and chick hatch weight. Also different female lines produced different weight eggs, however, their hatch weight of chicks did not different. The chick yield of male line 70.4% and it was higher than the chick yields of commercial strains (Wolanski et al., 2007).

Tona et al. (2004), Ulmer-Franco et al. (2010), Alsobayel et al. (2013) found that hatched chick weight rising with increasing age of broiler breeders. Accordance with this findings in the study egg weight and hatched chick weight increased with breeder age (P=0.001).

The eggshell conductance was found low in young breeders eggs (Christensen et al., 2005). Accordance with them, in the study lowest egg weight loss during incubation was found in 27-33 wks of age group (P=0.001). The egg weight loss in incubation risen with parental age in duck eggs thus larger eggs have greater amount of water (El-Hanoun, 2012). However, Ulmer-Franco et al. (2010) found that young breeders' eggs had a higher percentage of weight loss than old breeders' eggs.

The united effects of low ventilation and over heat production of large eggs might be resulted increase embryo temperature and also increase embryonic mortality (Deeming, 1996) in old age breeders. Thus, in the study lowest hatchability was found in 46-64 wks of age group (P=0.001). Accordance to our results Traldi et al. (2011) reported that hatchability of eggs obtained from older breeders was 8.3% lower than eggs of younger breeders. Tona et al. (2001) found that hatchability was low in young and old aged flocks. In contrast to our findings Ulmer-Franco et al. (2010) reported that breeder age did not affect hatchability of fertile eggs.

The chick yield was interval 62 to 76% (Wilson, 1991), but the ideal chick yield is 67-68% (Aviagen, 2011). The high chick yield is an indicator of several factors that eggs were incubated at low temperature and high humidity, or incubation time short, or eggs stored long periods, or eggs obtained from very young or old breeders (Aviagen, 2011). In the study the chick yield at 34-45 wks of age group (69.99%) was found lower than that of at 27-33 wks of age (70.58%) and 46-64 wks of age (70.55%)(P=0.001). Thus Iqbal et al. (2016) found that chick yield was not influenced by egg size. But, breeder age affect chick weight and chick yield. However, Traldi et al. (2011) reported that the composition of the eggs obtained from young and adult breeders was different, but chick yield was found similar, varying between 67 and 70%.

In the study interactions between the grandparent stock male or female line broiler breeder and breeder age were found significant for hatchability (P=0.001), chick weight and chick yield (P=0.031 and P=0.017). The lowest hatchability and highest hatched chick weight was found in 46-64 wks of age group of MF-GPS of broiler breeders (P=0.001 and P=0.031). Hocking and Bernard (2000) found that breeder age not affected hatchability of male broiler breeders, but hatchability was lower in 27 to 29 wks age and 55 to 57 wks of age female broiler breeders than 35 to 37 wks of age ones. In the study the lowest chick yield was found in 34-45 wks of age group of F-GPS (P=0.017). However, there were no any significant interactions for egg weight and egg weight loss during incubation (P>0.05).

Table 1. The effects of male or female line grandparent stock broiler breeder and age on egg weight, egg weight loss, hatchability, chick weight and chick yield

	EW, g	EWL, %	H	CW, g	CY, %
Line					
M-GPS	62.88 ± 0.10	10.81 ± 0.05	65.77 ± 0.53	44.40 ± 0.09	70.62 ± 0.10
F-GPS	63.17 ± 0.08	11.29 ± 0.04	68.26 ± 0.43	44.28 ± 0.08	70.13 ± 0.08
P	*0.060*	*0.001*	*0.001*	*0.294*	*0.001*
Breeder Age					
I	57.38 ± 0.16^{c}	10.66 ± 0.08^{b}	74.04 ± 0.84^{a}	40.48 ± 0.15^{c}	70.58 ± 0.16^{a}
II	63.82 ± 0.08^{b}	11.22 ± 0.04^{a}	73.17 ± 0.42^{a}	44.67 ± 0.07^{b}	69.99 ± 0.08^{b}
III	67.86 ± 0.08^{a}	11.27 ± 0.04^{a}	53.84 ± 0.42^{b}	47.87 ± 0.07^{a}	70.55 ± 0.08^{a}
P	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*
Line X Age					
M X I	57.12 ± 0.25	10.54 ± 0.12	71.04 ± 1.28^{b}	40.31 ± 0.22^{d}	70.61 ± 0.25^{ab}
M X II	63.75 ± 0.13	10.96 ± 0.06	71.27 ± 0.67^{b}	44.92 ± 0.12^{b}	70.48 ± 0.13^{ab}
M X III	67.76 ± 0.13	10.93 ± 0.06	55.01 ± 0.67^{c}	47.97 ± 0.12^{a}	70.79 ± 0.13^{a}
F X I	57.64 ± 0.21	10.78 ± 0.10	77.03 ± 1.08^{a}	40.64 ± 0.19^{d}	70.55 ± 0.21^{ab}
F X II	63.90 ± 0.10	11.48 ± 0.05	75.07 ± 0.52^{a}	44.41 ± 0.09^{c}	69.52 ± 0.10^{c}
F X III	67.97 ± 0.10	11.60 ± 0.05	52.67 ± 0.51^{c}	47.78 ± 0.09^{a}	70.31 ± 0.10^{b}
P	*0.593*	*0.069*	*0.001*	*0.031*	*0.017*

^{a,b,c,d} within a row, values with different superscript letters differ significantly (P<0.05).
EW: Egg Weight, EWL: Egg Weight Loss, H: Hatchability, CW: Chick Weight, CY: Chick Yield
M-GPS: Male Line Grand Parent Stock, F-GPS: Female Line Grand Parent Stock;
I: 27-33 wks of age, II: 34-45 wks of age, III: 46-64 wks of age

The fertility, hatchability and chick weight are related heritable traits that vary between varity and or individuals of breeds (King'ori, 2011). The correlations between the grandparent stock lines and ages of broiler for investigated parameters were given in Table 2. In the study significant positive correlations were found between breeder line and egg weight (r = 0.051; *P*=0.050), egg weight loss (r = 0.267; *P*=0.001) and significant negative correlation for chick yield (r= -0.149; *P*=0.001) for MF-GPS.

Table 2. The correlations between line and age for egg weight, egg weight loss, hatchability, chick weight and chick yield of male or female line grandparent stock broiler breeder

	MF-GPS		MF-GPS		M-GPS		F-GPS	
	Line	***P***	**Age**	***P***	**Age**	***P***	**Age**	***P***
EW	0.051	*0.050*	0.837	*0.001*	0.819	*0.001*	0.850	*0.001*
EWL	0.267	*0.001*	0.161	*0.001*	0.088	*0.037*	0.203	*0.001*
H	0.031	*0.223*	-0.623	*0.001*	-0.555	*0.001*	-0.664	*0.001*
CW	-0.023	*0.370*	0.783	*0.001*	0.757	*0.001*	0.804	*0.001*
CY	-0.149	*0.001*	0.057	*0.028*	0.049	*0.243*	0.070	*0.033*

M-GPS: Male Line Grandparent stock, F-GPS: Female Line Grandparent stock
EW: Egg Weight, EWL: Egg Weight Loss, H: Hatchability, CW: Chick Weight, CY: Chick Yield

Egg weight is an important factor that affect hatched chick weight (Silversides and Scott, 2001). In the study significant positive correlations were found between breeder age and egg weight (*P*=0.001), egg weight loss (*P*=0.001 and *P*=0.037), chick weight (*P*=0.001) and significant negative correlation for hatchability (*P*=0.001) for MF-GPS, M-GPS and F-GPS. Thus, there was a strong correlation between egg weight and hatched chick weight (r = 0.90 by Bray and Iton, 1962), (r = 0.87 by Wolanski et al., 2007). There were relationships

between breeder age and egg weight (Tona et al., 2001) such that older hens lays bigger eggs (Ulmer-Franco et al. 2010) and heavier chicks (Christensen et al., 2002). But in their field's report Tona et al. (2001) indicated that there was no any significant relationship between breeder age and egg weight loss, however, there was a relationship between breeder age and hatchability. In the study there were significant positive correlations between age and chick yield for MF-GPS and F-GPS (P=0.028 and P=0.033), but except for M-GPS (P>0.05).

Conclusions

In the study eggs were obtained from different age of male and female line GPS broiler but incubated same standard incubation conditions. The results of current study indicate that the genetic stock lines affected egg weight loss, hatchability and chick yield. All investigated parameters were affected by age of chicken. In addition, the interaction between line and age on hatchability, chick weight and chick yield was found to be significant. From this point, our research provided basic information that could be useful for GPS hatcheries. Thus different line and age breeders' eggs need different incubation conditions for ensure ideal egg weight loss during incubation, so producers should be take this into consideration for better hatchability and chick yield.

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LEGUME GRAINS IN DAIRY COWS FEED

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Abstract

Holstein-Friesian Black-and-White cows were grouped into four treatments groups according to the analogue principle (n=4×5). Lactating dairy cows were included in the trial in the initial lactation phase with the average milk yield of 30.00 kg per day, fat content 4.10% and 3.20% protein content in milk. The analyses of the chemical composition of legume grains showed, that crude protein was higher in fodder beans than in peas, respectively 29.97% and 25.04% of dry matter. The undegraded intake protein (UIP) content also was higher in fodder beans than in peas, respectively 40.51% and 39.69% of crude protein. The highest content of starch was in peas - 48.54%, beans - 43.29% but the lowest in soybean meal - 7.62% of dry matter. Even though the daily milk yields decreased for all the cow groups during the experiment, which was normal during the lactation period, yet the milk yield decreases for the trial groups. The milk yield decreases for the experimental groups (1st and 2nd) were smaller – 0.8 kg, 1.3 kg, respectively, compared with the initial stage of the experiment ($P< 0.05$). In contrast, group 4 showed a significant decrease in the average daily energy corrected milk yield (4.9 kg), compared with the initial stage of the trial. Compared with the control group, none of the dietary interventions showed significant ($P>0.05$) deviations. The fat content of milk slightly increased, on average, by 0.04% in the 3rd and 1st groups and by 0.01% in the 2nd group, compared with the control group ($P<0.05$). The protein content of milk increased in all the experimental groups. The protein content of milk increased on average by 0.31% in the 1st group, 0.17% in the 2nd group and 0.27% in the 3rd group, compared with the control group ($P>0.05$).

Keywords: *Beans, peas, chemical composition, dairy cows, milk production and quality.*

Introduction

With many feed ingredients available to producers, protein is still a sought after nutrient, not necessarily due to a lack of availability, but the fluctuating, yet increasing costs associated with protein. One potential solution may be the botanically named *Vicia faba*, common name "faba bean". This bean is also known to have various other common names such as "horse bean", "field bean" and "broad bean". The use of alternative plant proteins in place of the soybean meal protein in diets for farmed animals aims to reduce the extra-EU soybean import and partially substitute the GMO in the food chain. Among the possible alternatives, the faba beans and peas appears interesting for dairy cow diet. The common reason for livestock producers to plant faba beans is to obtain a protein source that is homegrown and that can easily be processed on-farm, as they contain little oil. They also do not contain anti-nutritional enzymes and therefore do not need roasting. Faba beans are related to lima beans and contain approximately 30% protein (Volpelli *et al*., 2010; Heeg, 2016). Field peas are highly digestible and highly fermentable in the rumen, but have a slower starch and protein fermentation rate than several other common feeds. Therefore, fodder beans and peas be used as an important forage legume to enhance feed values for dairy ruminants, especially of importance to today's high yielding dairy cows (Jensen, 2002; Ipharraguerre and Clark, 2005). The European Union policy on the protection and enhancement of biodiversity on agricultural holdings has contributed to an increase in the area sown with legumes in Latvia.

Areas under nitrogen-fixing crops, including fodder beans or field beans and peas, are eligible for financial support regarding environment-friendly agricultural practices; consequently, the total area under field beans in Latvia in the period 2010-2016 increased approximately 11 times (from 2.8 thousand hectares to 31.4 thousand hectares) and that under peas – 7 times (from 1.2 thousand hectares to 8.9 thousand hectares). The sown areas of pulses continue to grow. In 2016, the total area of pulses increased by 32.1%, of which the area of field beans rose by 5.4 thousand hectares or 20.9% (Agriculture of Latvia, 2017). This means that the growing area under faba beans and peas in Latvia can supply agricultural animals with the necessary amount of protein as well as increase the proportion of domestic protein rich feedstuffs in the consumption of feed and reduce the production cost of livestock products, i.e. contribute to higher efficiency (Proskina and Cerina, 2017).
The objective of the present paper is to investigate the legume grains in dairy cows' feed.

Material and Methods

Trials were carried out on the farm "Upites", Allazu Parish, Allazmuiza Municipality. For the trial, four analogue (according to yield, lactation phase, live weight, fat content and protein content) treatments groups of 20 animals of Holstein-Friesian Black-and-White cows were used in the study. Feeding trial was carried out from December 2015 to February 2016, *i.e.* for 90 days. The experimental design is reported in Table 1.

Table 1. General scheme of the trial.

Group of cows	Number of cows	Feeding programme
1st trial	5	Basic feed + 10-12% *Pisum sativum*'Bruno' + 10 - 12 % *Vicia faba minora*'Lielplatone'
2nd trial	5	Basic feed + 20-24% *Pisum sativum*'Bruno'
3rdtrial	5	Basic feed + 20-24% *Vicia faba minora* 'Lielplatone'
4th control	5	Basic feed with soybean cake

The average live weight of cows in all groups were 650 kg the mean age was 3.0 lactations. The cows were in the initial phase of lactation were included in the experiment with the average yield of 30.00 kg per day, fat content 4.10% and protein content 3.20% ($P > 0.05$). The cows were kept under the tied-housing system, milked twice a day at an interval of 12 hours are kept on the same farm, under equal feeding, housing and exploitation condition. During the trial, the dairy cows received the basic feed ration which consisted, calculation per cow per day of: 40 kg silage (grass plus legume), 3 kg hay (grass plus legume), 4 kg fodder (grains), 4 kg complementary, 0.15 kg mineral additive. In the experimental period, the cows of all groups were fed the same diet except that the diet of the cows in the control group was supplemented with 1 kg soybean meal that of the 1s trial group with 1.82 kg that of the peas plus beans, that of the 2nd group with 1.9 kg peas and that of the 3rd trial group with 1.7 kg beans (Table 2).

Table 2. Dairy cows feeding during the trials.

Feedstuffs	Amount, kg	1st trial	2nd trial	3rd trial	4th control
Silage	40	40	40	40	40
Hay	3	3	3	3	3
Concentrated feed	4	4	4	4	4
Complementary feed	4	4	4	4	4
Peas plus beans	-	1.82	-	-	-
Peas	-	-	1.9	-	-
Beans	-	-	-	1.7	-
Soybean meals	-	-	-	-	1
Mineral additive	0.15	0.15	0.15	0.15	0.15
Feed ration contains:					
Dry matter, kg	-	21.60	21.70	21.50	20.80
Crude protein, g	-	3266	3261	3276	3258
NEL, MJ	-	142.7	143.8	139	137.20
Calcium, g	-	153	157	155	162
Phosphorus, g	-	82.0	83.0	85.0	82.0

The feeding ration varied according to each cows' milk yield and physiological state, and was corrected monthly depending on the results of control milk yield, dry period and state of health. When elaborating the feeding rations, we took into consideration the following: amount of feedstuffs, dry matter (DM), net energy for lactation (NEL, MJ), amount of crude protein, calcium and phosphorus (Nutrient Requirements…, 2001).

Each cows' individual milk yield and whole groups average, content of milk fat and protein was recorded, basing on the control milk yield sheets. Control of the milk yield in cows' groups was performed each day, individual milk yields were controled once a month.

A full value chemical analyses were conducted by the accredited Scientific Laboratory of the Agronomical Analyses under the Latvia University of Agriculture. Chemical analyses of the feed samples was carried out in accordance with the ISO 6498: 1998 standards, dry matter - Feed Analyses met.2.2.1.1: 1993, crude protein - LVS EN ISO 5983-2: 2009, starch - LVS EN ISO 10520:2001, crude fat - ISO 6492:1999, digestible undegraded intake protein (UIP) - calculation method, crude fibre - ISO 5498: 1981, neutral detergent fiber (NDF%) - LVS EN ISO 16472: 2006, acid detergent fiber (ADF%), net energy for lactation (NEL, MJ kg^{-1}) – LVS EN ISO 13906: 2008, crude ash - ISO 5984:2002/Cor 1:2005, calcium - LVS EN ISO 6869: 2002, phosphorus - ISO 6491: 1998 and digestibility (De Boever *et al*., 1988). Milk quality analyzes were performed determining the milk fat and protein content, the method ISO 9622-2013 (E) / IDF 141: 2013 (E).

The biometric data were analysed by a Mann-Whitney test at the significance level $\alpha = 0.05$ to identify differences in comparison with the control group (Montgomery, 2012). All statistical analyses were performed using SPSS for Windows version 20.0.

Results and Discussion

The chemical composition of fodder beans, peas and soybean meal are presented in Table 3. As can be seen from data, the content of dry matter in peas "Bruno" and fodder beans "Lielplatone" was high, 90.78% and 90.44% respectively but in soybean meal - 87.41%. Crude protein content was the highest in soybean meal - 50.61% and beans - 29.97%, while in peas - 25.04% of dry matter. Soybean meal had significantly high content of UIP - 73.99% of crude protein.

Table 3. The chemical composition of fodder beans, peas and soybean meal, on dry matter basis, % .

Item	Beans (*Vicia faba minor*) "Lielplatone"	Peas (*Pisum sativum*) "Bruno"	Soybean meal
Dry matter, %	90.44	90.78	87.41
Crude protein, %	29.97	25.04	50.61
UIP of crude protein, %	40.51	39.69	73.99
Crude fat, %	1.09	1.22	1.60
Starch, %	43.29	48.54	7.62
Crude fiber, %	9.5	7.21	3.57
NDF, %	15.79	20.58	13.98
ADF, %	11.36	9.59	6.91
NEL, MJ kg^{-1}	7.7	7.84	7.89
Crude ash, %	3.61	3.28	1.76
Ca, %	0.12	0.07	0.42
P, %	0.66	0.43	0.71
Digestibility, %	80.00	81.40	81.80

After summarizing reference data, undegraded intake protein (UIP) content in soybean meal in the rumen fluctuates within the range of 75 to 78 per cent (Singh *et al*., 2012). Relatively high percentage of UIP was also in fodder beans - 40.51%, whereas in peas it was 39.69% of the crude protein. According to Batterham and Egan (1986), the amount of UIP can make up 46% of crude protein content.

The analysis of productivity indices proved that the feeding of legumes grains showed, that the average milk production in all groups of cows during the trial was trend decreasing in Table 4. In 3 recording months' the daily milk yield decreased for all the cow groups. The greatest decrease in cow productivity was observed for the control group – by 3.98 kg of milk but a smaller decrease was observed for the 3 rd group – by 0.26 kg, of milk compared with the before the trial ($P < 0.05$).

Table 4. Cow productivity during the trials, on average, kg.

Group of cows	Before the trial	Beginning of the trial	Middle in the trial	End of the trial	Comparison between before and end values
1st trial	22.68	23.52	22.24	20.46	-2.22
2nd trial	23.48	21.58	21.76	21.38	-2.10
3rdtrial	20.74	19.70	20.28	20.48	-0.26
4th control	24.62	24.92	21.96	20.64	-3.98
p–value (relative to control)					
Group of cows	Before the trial	Beginning of the trial	Middle in the trial	End of the trial	*P*-value (ralative to values at the before the trial)
1st trial	0.465	0.600	0.917	0.917	0.225
2nd trial	0.917	0.347	0.917	0.754	0.138
3rdtrial	0.251	0.251	0.917	0.916	0.893
4th control	-	-	-	-	0.043 ^{S}

^{S} significant differences ($P < 0.05$)

The influence of the content of legume grains on milk quality in groups of cows is shown in Table 5 and 6. As the cows' productivity decreased during the trial and lactation period, the fat and protein contents of milk increased for the 1st, 2nd and 3rd groups, compared with 4th the control group of cows. The fat content of milk slightly increased, on average, by 0.04% for the 3rd and 1st group and by 0.01% for the 2nd group, compared with the control group, and from 0.33% to 0.37% ($P < 0.05$) compared with the before the trial. The fat content of milk decreased by 0.36% for the control group, compared with the before the trial.

Table 5. Fat content in milk during the trials on average, %.

Group of cows	Before the trial	Beginning of the trial	Middle in the trial	End of the trial	Comparison between before and end values
1st trial	4.6	4.7	4.7	4.9	0.3
2nd trial	4.7	4.6	4.5	4.9	0.2
3rdtrial	4.6	4.8	4.8	4.9	0.4
4th control	5.3	4.9	4.4	4.9	-0.4
p–value (relative to control)					
Group of cows	Before the trial	Beginning of the trial	Middle in the trial	End of the trial	*P*-value (ralative to values at the before the trial)
1st trial	0.602	0.465	0.465	1.000	0.345
2nd trial	0.465	0.251	0.917	0.917	0.686
3rdtrial	0.465	0.465	0.602	0.754	0.138
4th control	-	-	-	-	0.500

The protein content of milk increased in the 1st group (0.31%, on average) in the 2nd group (0.17% ,on average), but 3rd group (0.27%, on average) compared with the control group, and by 0.60%, 0.40% and 0.40%, respectively, compared with the before the trial ($P > 0.05$).

Table 6. Protein content in milk during the trials on average, %.

Group of cows	Before the trial	Beginning of the trial	Middle in the trial	End of the trial	Comparison between before and end values
1st trial	3.1	3.4	3.3	3.7	0.6
2nd trial	3.2	3.4	3.2	3.5	0.4
3rdtrial	3.2	3.3	3.2	3.6	0.4
4th control	3.1	3.1	2.9	3.3	0.3
p–value (relative to control)					
Group of cows	Before the trial	Beginning of the trial	Middle in the trial	End of the trial	*P*-value (ralative to values at the before the trial)
1st trial	0.917	0.251	0.117	0.074	0.043 S
2nd trial	0.754	0.117	0.173	0.599	0.043 S
3rdtrial	0.60	0.35	0.120	0.300	0.043 S
4th control	-	-	-	-	0.225

S significant differences ($P < 0.05$)

It is known that the cow farming technology, including cow feeding, can considerably affect milk yield. After examining the effects of different amounts of dietary legumes on productivity of dairy cows, Vander *et al*., (2008) demonstrated no significant changes when soybean flour is partially replaced by peas (150 g kg^{-1}). A similar finding was made by Tufarelli *et al*., (2012) who reported that replacing soybeans with faba beans (345 g kg^{-1}) in the diet for highly productive dairy cows (the average milk yield of 35 kg a day) did not influence the cows' productivity. According to Mordenti *et al*. (2007) pointed out that faba beans and peas (200 g kg^{-1}) incorporated into the dairy cow diet increased milk fat content but decreased milk yield.

Conclusions

Obtained results of chemical composition of legume grains the content of dry matter in peas and field beans was high, 90.78% and 90.44% respectively. Crude protein content was the highest in soybean meal - 50.61% and fodder beans - 29.97%, while in peas - 25.04% of dry matter. The highest content of starch was in peas - 48.54%, beans - 43.29% but the lowest in soybean meal - 7.62% of dry matter.

During the trial, the cow productivity indicators decreased for all the groups, which was normal during the lactation period, yet the daily milk yield decreases for the trial groups (diets comprising peas and beans) were smaller – 2.22 kg, 2.10 kg and 0.26 kg, respectively, compared with before the trial and the control group.

The fat content in milk from the 3rd group and the 1st group slightly increased, on average, by 0.04%, while that from the 2nd group – by 0.01%, compared with the 4th group. The protein content in milk increased by 0.31% for the 1st group, 0.17% for the 2nd group and 0.27% for the 3rd group, compared with the control group ($P < 0.05$).

The research results proved that the use of legumes as domestic feedstuffs for the purpose of raising the nutritional value of the feed and balancing protein in the feed ration for dairy cows is important and promising, as the legumes help better maintain the milk yield level during the lactation period and enhance the milk quality indicators.

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HAPLOTYPE DIVERSITY IN GENES RESPONSIBLE FOR DROUGHT STRESS RESPONSE IN MAIZE

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Abstract

Great efforts have been made over the past several decades for development of improved cultivars adapted to different agro-ecological areas due to the on-going climatic changes. In order to increase further the selection gain and to accelerate breeding processes in maize, a profound knowledge is required regarding genes and genomic regions encoding for agronomically important traits. In this context, the use of haplotypes could improve selection of quantitative traits with a low heritability due to strong environmental influence. Twenty temperate drought tolerant (15) and sensitive (5) maize inbred lines from Maize Research Institute Zemun Polje (MRIZP) were subjected to SNP genotyping. Additionally, 17 maize (13) and teosinte (4) genotypes were selected for comparison from Panzea database to represent the functional diversity of maize. For SNP identification direct PCR sequencing of eight abiotic responsive candidate genes was done. A small number (3 to 8) of distinct and highly diverse haplotypes were observed in all eight (8) marker genes. Haplotype analysis based on the SNPs revealed the highest haplotype diversity in *MYBR96* (0.817) and the lowest in *MYB8* (0.3235) gene. Network analysis showed a linear relationship between haplotypes for some genes, while for the rest of genes the network graphs reflected more complex relationships between a large numbers of haplotypes. The deployment of the identified haplotypes could be a powerful complementary tool to improve accuracy and efficiency of modern breeding strategies such as marker assisted selection and genomic selection for developing drought tolerant maize genotypes.

Key words: *maize, drought, SNPs, haplotypes.*

Introduction

Global crop production is challenged by severe climatic changes like drought (Lesk *et al.*, 2016) and food demands of exponentially growing world population. It has been predicted that yields of important commodity crops need to be increased by almost 40% by the middle of 21st century (Tester and Langridge, 2010). Achieving sustainable yields of commodity crops in inconsistent environments represents a demanding task for the breeders. However, cutting-edge advances in genome analysis technologies provide high resolution molecular information which can perfect advanced quantitative genetic approaches. Low-cost genotyping tools that can capture sequence variation are now available for all agronomically important plant species (Huang and Han, 2014) and they provide an effective means for crop genetic research studies (Ganal *et al.*, 2012), such as providing a basis for genomic selection (GS) or prediction of hybrid performance.

High-throughput single nucleotide polymorphism (SNP) marker arrays or SNPs detected by DNA sequencing are the genotyping markers of choice for crop genomic research. However, their major limitation is that they provide only bi-allelic information at a locus, hence their information content compared to multi-allelic markers is low, limiting the resolution at which SNP–trait relationships can be delineated. An effective approach to surpass the biallelic limitations of SNPs is to construct haplotypes based on linkage disequilibrium (LD), one of

the most important features influencing genetic analysis of crop genomes (Qian *et al*., 2017). A haplotype is described as „two or more SNP alleles that tend to be inherited as a unit" (Bernardo, 2010). Marker-assissted selection (MAS), which has succesfully been used for mono- or oligogenic inherited traits, failed for highly quantitative traits with a low heritability due to strong environmental influence. MAS strategies proved inadequate due to statistical overestimation of QTL linked markers or complex genetic architectures for most important traits (Bernardo, 2008). That is why the concept of GS with densely spaced genome-wide markers is presently being adopted for many crop breeding programs. Genomic selection methods apply the concept that the breeding value of an individual which has not been phenotyped can be estimated purely on the basis of its genome wide marker profile. In the context of both MAS and GS, the use of haplotypes could be a powerful complementary tool to improve their accuracy and efficiency. Because of their increased information content compared to bi-allelic SNP markers, fitting haplotypes with statistically significant trait associations to phenotypes as fixed effects in GS models could further improve prediction accuracies. The use of haplotype-assisted GS should more accurately depict the complex relationships between genotypic information and phenotypes than single SNPs alone are able to do; hence, this approach could ultimately help to further increase selection gain per unit of time (Qian *et al*., 2017).

The aim of the research presented herein was to identify SNP mutations in drought candidate responsive genes in 20 drought tolerant and susceptible maize inbred lines from Maize Research Institute Zemun Polje (MRIZP) and based on the SNP information identify conserved haplotypes. Also, the nucleotide diversity in these genes between the inbreds from MRIZP collection and Panzea database was analysed.

Material and methods

Twenty temperate drought tolerant (15) and sensitive (5) maize inbred lines from MRIZP were subjected to SNP genotyping. Four sensitive and two tolerant inbreds are commercial lines used as parental lines for development in MRIZP breeding programs, while the remaining 14 are from MRIZP genebank. Additionally, a total of 17 foreign maize genotypes including tropical maize inbreds (5), teosinte lines (4), African inbred M37W and temperate maize inbreds (7) were selected from Panzea database to represent the functional diversity of maize. Most of them (without teosinte lines) have been used in crosses to develop the Nested Association Mapping (NAM) population consisting of 302 lines designed to capture the diversity of maize and to preserve historic linkage disequilibrium.

For SNP identification in 20 inbred lines from MRI direct PCR sequencing approach was applied on eight abiotic responsive candidate genes - *MYBR96* (MYB-related-transcription factor 96), *SDG110a* (histone-lysine N-methyltransferase, H3 lysine-36 and H4 lysine-20 specific), *IDP507* (protein kinase G11A), *MYB67* (myb domain protein 67) *MYB8, PCO089553b (PSF2), GA20OX1* and *DHN1*. PCR products were subjected to double stranded sequencing on ABI 3130xl platform. The raw sequencing data were base called and assembled in contigs using phred (Ewing *et al*., 1998) and phrap (Green, 1996) with default parameters. The contigs were aligned and analysed for SNP detection using BioLign software (Hall, 2001). Nucleotide sequences were converted into amino acid sequences and were compared with protein sequences (http://www.ncbi.nlm.nih.gov; http://www.panzea.org/) of the corresponding genes in maize to verify if the identified SNPs were able to produce functional mutations with amino acid changing. The sequence information is given in forward direction.

Conserved haplotypes, representing DNA sequences containing identical allelic variants at all polymorphic sites at particular locus, but originating from separate individuals, were identified visually or by application of NETWORK 5.0 software. Haplotype networks for the

whole set of 35 maize lines but separately for each gene were drawn using the same software. The network figures show the number of haplotypes observed for each gene and the SNP position which separates each haplotype from each other. Haplotype frequency is depicted by circles. Circle sizes correspond to the frequency of the corresponding haplotypes. The bigger the circle, the more genotypes are represented by that haplotype. The color in the circles corresponds to the affiliation of genotypes to the relevant collection.

Results and Discussion

Haplotype analysis of MRIZP and Panzea collections based on the SNPs from the small window size, i.e. gene window (all markers within a gene) is presented in Fig. 1. Number of identified SNPs and haplotypes, as well as haplotype diversity, for each gene is given in Table 1. The highest haplotype diversity was found in *MYBR96* (0.817) and the lowest in *MYB8* (0.3235) in the whole set of maize inbred lines (Table 1). Comparison analysis between both collections showed slightly higher level of haplotype diversity in *MYB8* (0.4902) and *MYBR96* (0.817) in MRIZP collection consisting of only temperate inbreds. The level of haplotype diversity in the rest of genes was higher in Panzea collection represented by an extremely diverse set of temperate, tropical and teosinte lines. Removal of tropical inbreds and teosinte lines led to some reduction in the level of haplotype diversity in Panzea collection, but it still remained higher in comparison to the MRIZP collection in genes *DHN1*, *IDP 507*, *MYB67* and *GA20OX1* (data not shown).

Table 1. Haplotype diversity of eight abiotic responsive candidate genes in MRIZP and PANZEA maize collections

genes	Collection (inbred lines)					
	MRIZP			PANZEA		
	SNP number	haplotype number	haplotype diversity	SNP number	haplotype number	haplotype diversity
DHN1	7	3	0.3856	18	7	0.8088
IDP507	5	3	0.3922	5	4	0.6544
MYB67	5	4	0.3987	14	7	0.7206
MYB8	4	6	0.4902	2	3	**0.3235**
MYBR96	13	6	**0.817**	16	6	0.8015
PCO089553B	3	3	0.6013	4	4	0.6397
GA20OX1	3	3	0.3856	7	7	0.6618
SDG110a	10	6	0.6993	4	5	0.7721

In this study a small number of 3 to 8 distinct and highly diverse haplotypes were observed in all eight genes in the set of 35 genotypes from both collections. These results are in accordance with those observed by Ching *et al*. (2002) for 36 elite US maize inbreds, but lower than the identified 14 haplotypes in the RTCS (rootles concerning crown and seminal roots) in 73 Chinese elite inbreds from five temperate heterotic groups and some tropic germplasm.

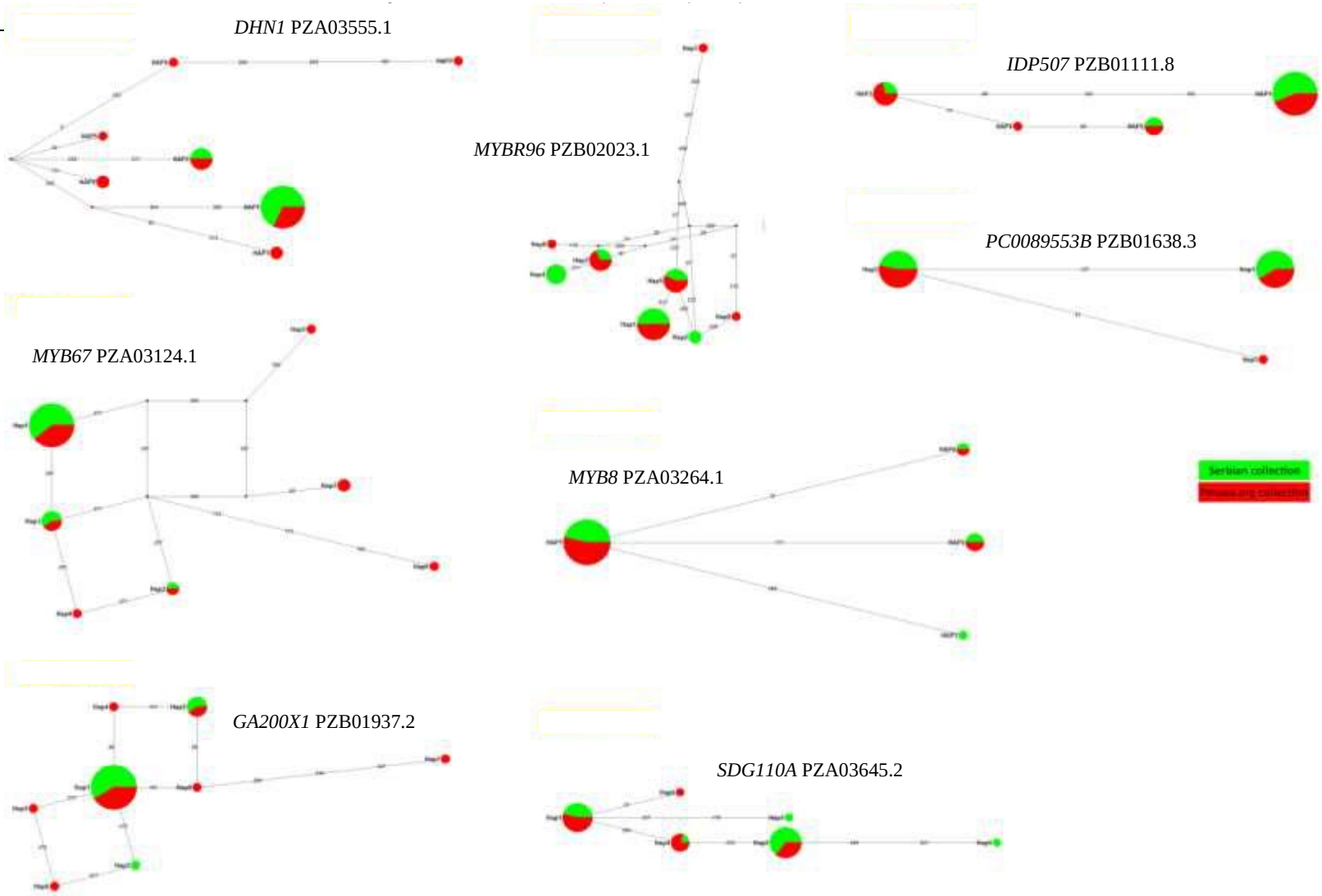

Fig. 1. Haplotype network of the eight marker genes developed on affiliation of maize genotypes to MRIZP and Panzea collections. Each circle represents a haplotype and is labeled accordingly. The two color codes, green and red in the circles represents the genotypes belonging to the two collections, MRIZP and Panzea respectively.

Network analysis showed a linear relationship between haplotypes for some genes such as *IDP507, PCO089553b, MYB8* and *SDG110a*, while for *DHN1, MYB67, MYBR96* and *GA20OX1* the network graphs reflected more complex relationships between a large number of haplotypes. This was more definitely pronounced in *MYBR96* gene where the presence of eight haplotypes was identified in the set of 35 maize genotypes. The haplotype network of *MYBR96* gene showed the presence of four major haplotypes (Hap1, Hap3, Hap4 and Hap5), which are connected to four minor ones by 1-2 SNPs. It is interesting to note, that one of the major haplotypes Hap4 is composed only of five temperate inbreds from MRIZP elite mini core collection for drought tolerance. In these lines a unique mutation (A→G transition) was observed in the acidic Ser/Thr – rich area located downstream from the SANT domain (SWI3, ADA2, N-Cor, TFIIIB DNA binding domain). This transition leads to amino acid change from Tryptophan to Alanine at the corresponding position of the protein and despite the fact that it does not affect the binding site of *MYBR96* transcription factor, this replacement might possibly reflect the spatial conformation through changing of its functional activity. To our knowledge the observed mutation is the first one reported for this position of the *MYBR96* gene as compared to the 44 other inbred lines representing the functional diversity of maize from the Panzea database (http://www.panzea.org/) (Assenov *et al*., 2013).

The simplest haplotype network consisting of only three haplotypes was observed for the marker gene *PCO0089553B (PSF2)*, one of the core genes that encode proteins which have an important role in DNA replication machinery in plants. The two major haplotypes include equal number of genotypes and are split by the marker SNP PZB01638.3 (A/T) in position 107, which is located in intron1 of the gene. This marker SNP was associated with different phenotypic traits (anthesis-silking interval, grain yield and selection index of drought tolerance) under water stress conditions at four environments in China (Hao *et al*., 2011). However, in our analysis no obvious differentiation of genotypes according to their stress response was observed.

Conclusion

Our study showed unbalanced distribution of the maize inbred lines in the analyzed haplotypes, most of which consisted of only teosinte and/or CML germplasm. The largest haplotypes consisted of lines from both MRIZP and Panzea collections. The analyzed haplotypes of the selected eight abiotic responsive genes did not comprise lines according to their level of tolerance to the drought stress, except Hap4 in *MYBR96* which was composed exclusively of five MRIZP drought tolerant lines with a unique mutation and it could be used in breeding programs for developing drought tolerant genotypes. A more profound analysis of genes expressed under drought stress in search for informative SNPs and haplotypes valuable for marker assisted or genome selection is underway.

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RURAL DEVELOPMENT AND AGRO-ECONOMY

EFFECT OF MICROCLIMATIC FACTORS ON THE THICKNESS OF THE WOOL FIBRES IN DUBSKA AND PIVSKA PRAMENKA AND ITS USE IN THE TEXTILE INDUSTRY

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Abstract

Thickness of wool fibres in various breeds of Pramenka sheep (Dubska and Pivska) varied; it was examined during different periods, spring and autumn, and it came from different locations – mountain Vlašić in Bosnia and Herzegovina (BandH) and Pivska mountain in Montenegro. Air temperature and the degree of insolation have a major significance on the quality of wool fibres in both Pramenka breeds. Seasonal changes, average temperature and insolation together with the age of animals and the region of the body from which samples were taken, affect the quality and the use value of wool fibres. Wool as a raw material is exposed to the activity of various insults – mechanical, thermal and chemical. The quality of wool products depends on physical and technological characteristics of the wool. The textile industry requires high-evenness of the wool primarily to reduce the sorting costs, which also increases the value of wool. Respectively, the data represents a contribution to the study of the fineness of wool fibres and their use in industry, the use for technical purposes and especially to the importance of the wool as a strategic ecological raw material.

Key words: *Dubska, Pivska Pramenka, wool fibres, micro-climate*

Introduction

In some areas the wool is the most important product of sheep, and sheep farming focuses strategically on breeds of sheep intended for production of wool, meat, milk and skin. Wool was the first textile product used by humans in Old Egypt, Palestine, Ancient Greece and Rome, Ovčarstvo (2007). Our research was a comparative study involving the Dubska and Pivska indigenous Pramenka breeds, Bosnia and Herzegovina (BandH) and Montenegro, from the aspects of the quality of wool fibres. Climate factors (air temperature and degree of insolation) had a significant impact on the quality of the wool fibres in both breeds. Comparison of the mean values of the thickness of wool fibres in May and November between Dubska and Pivska Pramenka in relation to average air temperature and degree of insolation shows certain differences.

The examinations showed that thickness of wool fibres is smaller, i.e. the fibres are thinner and finer in higher temperatures and in areas with a greater number of sunny days-hours, regardless of the Pramenka breed, the region of the body, the season – spring or autumn. In our studies, we determined an important relationship between the thickness of the wool fibres, average temperature and insolation, and taking into account also the studies by Pilling et al., (2011). This data represents a contribution to the study of the fineness of wool fibres and their use in the demanding textile industry, other industrial branches and their use for technical purposes. The significance of the wool as a strategic ecological raw material is emphasized.

Materials and methods

The samples for microscopic examinations of the skin and fibres of Dubska and Pivska Pramenka were taken during 2015. from Vlasic mountain in Bosnia and Herzegovina and Pivska mountain in Montenegro. The wool fibres for microscopic examination were taken from various parts of the body -- shoulder, rump, root of the tail-- by cutting the strands next to the skin. The samples from both locations and from different periods of time were placed in marked plastic bags until it was time to carry out microscopic examination. All impurities were cleaned in advance of microscopic examination. The samples were washed using neutral soap; they were rinsed with water, and then rinsed again with distilled water. To achieve transparency, the samples were immersed in H_2O_2-hydrogen peroxide for 24 hours, and then in xylol for 48 hours. Again, the samples were rinsed with distilled water, after which they were left to dry. The prepared samples were placed on glass slides, embedded into glycerol droplets and examined under the microscope. Measuring of thickness of the wool fibres from different parts of the body (rump, shoulder, tail root) and from different locations and periods of time -- spring-May and autumn-November – was carried out with binocular light microscope Motic 120M, under magnification of 200 and 400 times. The program that was used for measuring was Motic Image Plus 2.0ML.

Data on the average air temperature and hours of insolation was received from the Federal Hydrometeorological Institute of Bosnia and Herzegovina on 15th of October 2015, as well as the official data from the Institute's web page www.fhmzbih, and the Institute for Hydrometeorology and Seismology of Montenegro on 28th of October, 2015.

Results and discussion

Sheep farming sector in Bosnia and Herzegovina (BandH) is gradually restored – according to the FAO data, the number of sheep in 2012 was 1,515,000. The Pramenka breed makes the highest percentage of sheep population in Bosnia and Herzegovina (BandH),FAO (2012) and the wool fibres of this breed are classified between D and E. They are coarse fibres and the rest (20%) are fine fibres. (The wool in (BandH). Wool coverage of the sheep's body is also a characteristic of the breed. Coarse wool sheep, including the Pramenka sheep, are poorly covered on the stomach, legs and head and they bring smaller yields of wool per head compared to Merino breeds in which the entire body is covered with wool and some even have skin folds that significantly increase the size of the skin and therefore the amount of the wool produced Mioč et al. (2006).

In our region, Palian (1956) studied the impact of micro-climate factors, air temperature and natural, ecological and genetic factors on the types of Pramenka.

The most coarse fibres are found on the upper legs, lower back and around the root of the tail. The thinnest and the thickest fibres are on both sides of the trunk,Sheep farming (2006).

The organized purchase and wool processing would solve the ecological problem, which, we can say, is escalating, and BiH cannot become an EU member unless it resolves this issue. Working in this segment of sheep farming, we stressed the importance of wool, as a useful raw material in sheep farming in BiH, rather than waste. (Programme for Development of Agriculture in Central Bosnia Canton (2009-2015).

Comparison of the mean values of thickness of the wool fibres in the spring – month of May between Dubska and Pivska Pramenka and comparison of the average air temperature and degree of insolation show differences. It should be noted that the average air temperature in May at Babanovac Vlašić was 9.4°C, and the degree of insolation was 173.8 hours. During the same period, meteorological station at Zabljak Montenegro measured the average air temperature of 8.9°C, and the insolation was 162,5 hours. The mean value of the thickness of wool fibres in Dubska Pramenka from Vlašić mountain was generally smaller compared to the Pivska Pramenka during the same period, May 2014, regardless of the age -- three years and

above, and regardless of the region of the body that was examined. Evenness of the fleece in narrow terms represents the evenness of the wool's fineness on certain parts of the body. Fleece that is more even in terms of fineness and other properties is better for use and has a greater value, Sheep farming (2006). The indicators in this study say that the mean value of the thickness of the wool fibres from the shoulder of Dubska Pramenka was 38.14μ, and 39.14μ in Pivska Pramenka. The mean values of the thickness of the wool fibres from the rump was 43,70μ for Dubska Pramenka and 47,14μ for Pivska Pramenka. The mean value of the thickness of wool fibres from the root of the tail was 41.01μ for Dubska Pramenka and 43.60μ for Pivska Pramenka (Table 1).

Table 1.: Mean values of thickness of wool fibres of Dubska and Pivska Pramenka – month MAY

WOOL FIBERS (MAY) μm		
Region of the body	Dubska Pramenka	Pivska Pramenka
Shoulder	38,14	39,14
Rump	43,70	47,14
Tail root	41,01	43,60

Similar studies, those referring to seasonal changes: insolation and average air temperature were carried out also by Nikolić (1947).

It should be emphasized that in addition to a large supply and competitiveness of synthetic fibres, the wool has a strong and irreplaceable role in the textile industry; it is similar with fur and skin.

In Montenegro, wool and skin in the last 10-15 years have lost their economic significance. Promotion and affirmation of wool crafts and exports of raw unwashed wool have contributed to its more significant role in the past 2-3 years. We emphasize that the skin is placed successfully also as an exporting raw material.

Throughout a year, sheep require proper and high-quality diet, adequate ambient – airy and dry environment and sheep barns with covered floors, Mitić (1984). Wool becomes shorter and thinner under poor diet conditions. Sheep kept in poor zoo-hygienic conditions yield low-quality wool. Sheep pens and barns without sufficient litter, wet, muddy, tight and poorly aired environments are the biggest damaging factors to the wool and its health Mioč et al. (2000,2006).

The wool fibres are primarily qualitative parameters in production of wool and in determining its usability in further processing, however, they also represent quantitative parameters of fleece of sheep. The fibres are of uneven quality, both in terms of chemical and technical characteristics. These characteristics depend on the race i.e. breed, diet, breeding conditions, hormonal status, zoo-hygienic and climate conditions and so on, Savić et al. (2007). In his studies, Gutić et al. (2006) also emphasized the impact of the climate factors on the quality and amount of wool. Stressing that fleece is an important protection of the body against negative impacts of climate factors. Protection against severe weather conditions, moisture, increased circulation of air, wind, draft, emphasizing that the fleece plays a role in preventing heat drain and higher energy consumption in sheep.

In November 2014 – autumn, the research studies involved the same breeds of Pramenka and the same locations. The average monthly air temperature on mountain Vlasic during this period (November 2013) was 3.0 °C, and the degree of insolation was 57.9 hours. During the

same research period, but in November 2014, meteorological station Zabljak measured the average monthly air temperature of 4.9°C, and the degree of insolation was 100.9 hours.
The wool fibres, the mean value of the thickness in November for Dubska Pramenka were as follows: 65,00μ-shoulder and 56,40μ-rump. The mean values for Pivska Pramenka were shoulder:rump, 48,93μ:52,54μ. The root of the tail showed the following values of the studied parameter 57,74μ for Dubska Pramenka and 53,99μ for Pivska Pramenka (Table 2).

Table 2.:Mean values of thickness of wool fibres in Dubska and Pivska Pramenka – month NOVEMBER

WOOL FIBRES (MAY) μm		
Region of the body	Dubska Pramenka	Pivska Pramenka
Shoulder	38,14	39,14
Rump	43,70	47,14
Tail root	41,01	43,60

During both research periods we came to know that the air temperature and degree of insolation affected the thickness of the wool fibres, which were thinner and finer in case when the average air temperature and degree of insolation were higher. Our results are similar to the results of Adžić (1997). The results of our research support the breeding goals for the meat-wool production in sheep breeding in Federation of Bosnia and Herzegovina (2016).
The results of the study by Wickham and McDonald (1982) show that the sheep bred in warm and dry climate have somewhat finer wool than the sheep of the same breed bred in wetter and colder areas and at the higher latitude. The wool in younger sheep is finer than the wool in the old sheep. In almost all breeds, wool is most coarse in sheep age 5-6 years. Inadequate diet may affect the quality of the wool, especially expressed in the last quarter of gravidity and during the maximum lactation.

Conclusions

Mean values of thickness of the wool fibres in Dubska Pramenka in May were smaller compared against the Pivska Pramenka during the same period, regardless of age and region of the body.
In November, the average air temperature in Zabljak was higher than 4.9°C and so was the degree of insolation, 100.9 hours in regard to the mountain Vlašić, which affected the thickness of the wool fibres in Pivska Pramenka, as they were thinner and finer compared to the Dubska Pramenka.
Our studies show that thickness of the wool fibres varied, i.e. the wool fibres are thinner and finer in higher temperatures and in areas with a greater number of sun hours regardless of the breed, age, region of the body i.e. season.
Wool as raw material cannot be classified as most suitable for use in the textile industry because of uneven and lower quality. It can be used for production of other wool products.
Our research studies can also be applicative, and inadequate disposal of wool in the environment can be significantly reduced through a continual education of farmers, proper shearing and adequate assortment of the wool. This will increase the use of wool as an important ecological raw material, its classification for the use in various industrial branches (even in ecological civil engineering), Zametica et al. (2016).

Our research has provided a major contribution toward treating wool as an ecologically important raw material, rather than waste.

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THE INFLUENCE OF THE SPACE UTILIZATION ON THE FINANCIAL RESULT OF PRODUCTION IN GREENHOUSES

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Abstract

Vegetable production in the protected area belongs to highly intensive agricultural production, and it is followed by appropriate results of production. It brings a number of advantages as opposed to the production of vegetables in the open area and is therefore very attractive for an agricultural producer. In this paper, the production of vegetables in a protected space without heating in the territory of the city of Bijeljina (Bosnia and Herzegovina) has been analyzed, as well as the specificities and possibilities for improving the business results. The results of the research indicate that the most common way of using protected area is to cultivate one line during the year, while on two farms two crops were grown in the protected area. The highest average gross margin was achieved in the production of tomatoes in the observed model. The t-test affirmed that there is a statistically significant difference in the average gross margin between the protected space with one production line and the protected space with two production lines.

Key words: *Protected area, Gross margin, Vegetables*

Introduction

Vegetable production is characterized by high labor inputs and production assets per unit of production area. However, due to the fact that the demand for this type of agricultural products is rapidly growing, there is an increase in the area under it. The reasons for the increase in demand are found in the nutritional value of most vegetables products, which makes this type of foods irreplaceable in human nutrition. Thanks to the positive aspects that the consumption of vegetables has for the human body, numerous studies recommend 400 grams of the aforementioned in human nutrition daily (Bodiroga 2015). The high intensity of vegetable production is accompanied by high production results. Thus, the production of vegetables in the open area can provide five to eight times higher value of production, while the production of vegetables in greenhouses has 190-250 times higher value than wheat (Vlahović et al., 2010). A large number of vegetable crops have high requirements for light and temperature, and as such can not be cultivated in the open field out of the season. The problem can be solved by cultivating them in different forms of protected area where this problem can be partially or completely eliminated. All the benefits of vegetable production are further enhanced by the cultivation of vegetables in a protected area (Hadelan et al., 2015). With the production in the protected area significantly higher yields compared to the open field production are accomplished and thus more favorable financial results are achieved. Vegetable crops are directly under the influence of a variety of variables such as weather variations (temperature, humidity, carbon dioxide concentration, etc.), nutrition, biotic factors and cultivation (Davioglu and Acioglu, 2017). The financial result will depend on a number of aforementioned factors, but also factors such as the type of protected space, production technology, space utilization during the year, and the like. Therefore, agricultural producers must use a production strategy that will optimize production and profitability, and economic evaluation is often used to assess the overall performance of the production system

(Saeid Mohamed et al, 2017). In this paper we are going to present the characteristics of greenhouse production in the area of Bijeljina (Bosnia and Herzegovina), economic results, as well as the differences that can be derived from the different use of protected space. The aim of the paper was to determine whether significant changes in business results can be achieved by changing the way of utilization of the protected area.

Material and the Methods

For the needs of the survey, agricultural producers from the area of Bijeljina, who are engaged in production in a protected area, were surveyed. Combining open and closed questions, all the necessary information for the defined goal of the research were sought. Households were selected randomly. Research was done in 2015. year and involved 20 farms. The minimum area that the farms should have in order to participate in the survey is 500 m² of protected space. In the survey the results of production in the protected area were observed, while the other lines on the farms were not analyzed. The financial result in production for individual lines was determined using the calculation of variable costs (Direct costings). The obtained data were processed by methods of descriptive statistical analysis. The method of testing hypotheses on the difference of arithmetic mean by using t-test is used for determining the significance of the difference between the gross margin in greenhouses with one and two realized lines annually as well as by the method of testing hypotheses on the difference of arithmetic mean by using t-test. For the purpose of statistical analysis the statistical software SPSS was used.

Results and Discussion

Nowadays, in the production of vegetables in a protected area, traditional methods of cultivation can be distinguished, which are applied in simple objects without modern irrigation systems and conditions control conditions, and modern, mostly computerized modes with complete control of plant growth and development. (Djurovka and Martinovic, 2009) The results of the research indicate that in the analyzed area there is a traditional method of cultivation with several different construction solutions used for production in the protected area. These are mostly low, semi-high, and high tunnels, but also modern greenhouses of various constructions.

High tunnels as well as modern greenhouses are the most common form of protected space in the observed sample. The production capacities of the farms, when it comes to the protected area, are given in the following table.

Table 1. Production capacity of observed holdings (protected area)

Indicators	$\bar{X}$	X_{min}	X_{max}	CV
Area under greenhouses (m²)	5033,00	500,00	40 000,00	173,42

*Source: Research by the author

From the table it can be noticed that the average area under greenhouses was 5033 m². There were high variations in surfaces under protected space, which can be concluded by observing data on minimum and maximum surfaces as well as the value of the coefficient of variation (CV).

All researched farms produce vegetables in conditions without heating. By carefully combining the crops in conditions without heating, it is possible to use the protected area completely during the year. Some of the ways to use the protected area without heating in the conditions of the continental climate, including the city of Bijeljina, is given in the following table.

Table 2. Possible combinations of crops in a protected area without heating

	Place in crop rotation	**Combination 1**	**Combination 2**	**Combination 3**	**Combination 4**
Vegetable species	Previous crops	Salad	Green onions	Spinach	Salad
	Main crops	Peppers	Tomato	Cucumber	Cucumber
	Post-crops	Green onions	Spinach	Green beans	Spinach

*Source: Bodiroga (2015) quoting Lazić et al. (2001), Kurtovic, (2008)

The results of the survey show that the households do not fully exploit the protected area and that one production line is most often realized during the year (peppers, cucumbers or tomatoes). The maximum number of production units on each farm was present two line during the year, in a different combination.

Production results on farms were determined using calculation based on variable costs (Direct costing method). Variable costs are costs whose value changes with the change in the volume of production, or the degree of utilization of the capacity of the means of production. (Sredojevic, 2015) The original name, the direct costing method does not express quite precisely its essence, since the term "direct" here implies variable costs, while in classical analytical calculations this term is used for costs that directly and in whole relate to individual lines of production but can also have the character of fixed costs. (Andric, 1998)

By applying this type of calculations, it is possible to draw conclusions as to whether revenues from individual lines can cover the costs incurred for obtaining them, while fixed costs are omitted and considered as non-evasive costs and are present when there is no production on the holding.

The basic descriptive gross margin indicators obtained as the difference between the generated income and the variable costs of individual lines are given in the following table.

Table 3. Gross margin at different lines in the protected area (KM / 100 m²)

Production line	**Average**	**Maximum**	**Minimum**	**CV %**
Tomato	973,76	1436,43	581,66	27,39
Bell pepper	522,11	783,40	223,20	37,92
Kapia type pepper	504,38	800,00	190,50	43,50
Cucumber	592,83	1536,66	224,35	51,02
Salad	100,00	100,00	100,00	-------

*Source: Research by the author

The highest average gross margin was achieved with tomatoes, while in individual cases cucumber had the highest gross margin. Salad was present only in one agricultural holding. According to the data on the realized gross margin for all surveyed farms, the average gross margin is calculated for farms which realize only one production line during the year, as well as with farms that realize two production lines during the year.

For households with one line growing in the greenhouse, the average gross margin is 742,65KM, while for farms with two production lines, growing in the same space, the value is 984,91KM. In order for the information on the difference in the gross margin of the farms in the observed sample to be applied to the entire population of farms from the observed area, the t-test was applied. The condition for applying the t-test is that both sets have equal variances, that is, their variances have to be homogeneous. (Lovric et al., 2006) Using the SPSS statistical software, the Levene test was applied in order to test the homogeneity of the population and the equivalence of the variances of the sets.

The results of the Levene test indicate that there is only few reasons to doubt the homogeneity of the population of variances, which can be concluded on the basis of the data in the following table. Since the conditions for application are fulfilled, assuming the normality of the population comes from the zero hypothesis, which reads as follows: $H_0 : \mu_1 = \mu_2$ The t-test was applied, the results of which are given in the following table.

Table 4. Output results of Levene's and t-test

Independent sample test									
Type of test	Levene's Test for Equality of Variances		t-test equality of arithmetic meanings						
Indicators	F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	1,15	0,29	-2,16	38	0,038	-242,26	112,44	-469,88	-14,65

*Source: Research by the author

From the table it can be seen that in the t-test the calculated "p" value (sig) is less than the risk of error α, so the zero hypothesis is rejected and we can say that there is a statistically significant difference between the arithmetic average of the populations. The limits of confidence interval for the arithmetic average of the population with 95% of the safety indicate that the average gross margin for greenhouses with two production lines may be higher than the average gross margin of greenhouses with one line max. 469.68 KM, and a minimum of 14.65 KM observed at the level of the entire population.

Conclusion

A number of benefits from vegetable consumption contribute to the growth of demand for this type of product on the market. Production in a protected area allows obtaining vegetables in those parts of the year when it can not be produced in the open area, which positively affects

their price and thus the financial result of production. In the analyzed area, production in the protected area is characterized by the cultivation of the most common one and maximum two production lines during the year.

The highest average gross margin observed on individual lines was recorded in tomatoes. Taking into account all lines in the sample, the average gross margin in a protected area, where only one production line is realized during the year, was 742.65KM, while in the protected area with two production lines it reached the amount of 984.91KM. Using the t test, it is concluded that there is a statistically significant difference between gross margins in these protected areas.

Finally, it can be concluded that by introducing an additional production line during the year, retaining existing technology at the same level, agricultural holdings achieve significantly more favorable business results.

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FINANCIAL MANAGEMENT AND CONTROL, RISK MANAGEMENT AND DISCHARGING IRREGULARITIES IN THE AGRICULTURAL SECTOR IN BOSNIA AND HERZEGOVINA

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Abstract

Financial management and control are a comprehensive system of internal controls that are established and for which the managers of institutions are responsible, and by which, by managing risks, ensure that the budgetary and other resources will be used correctly, ethically, economically and efficiently in achieving the goals. Within the overall economy and after more than two decades after the war, Bosnia and Herzegovina agriculture is still burdened with numerous problems and does not show the vision of its own social development. By adopting several laws and establishing institutions, it gradually builds an agricultural framework, but it still remains with numerous problems due to undefined measures of support to domestic production. The aim of this study is to protect the means of loss, abuse and damage in accordance with laws and other regulations. This system includes all business transactions, and in particular those related to revenues, expenditures, assets and liability protection. The internal control system is carried out by the responsible persons of the institution and the employees of the institution. Experiences from the practice show that the key to establishing financial management and control is a timely and permanent incorporation of all knowledge and solutions, the activation of all employees in raising awareness for financial management and control. These findings were achieved through the method of direct implementation of projects on the market (local self-government, budget and extra budgetary users, state-owned trading companies and local self-government units) through financial management and control projects, strategic plan, asset register and risk management. Today, it is clear that 80% of the problems arise from 20% of the causes – that is why employees should be educated so that they can recognize them and act effectively on them.

Keywords: *mission, vision, goals, processes.*

Introduction

In BiH, the gross value added (GVA) of agriculture (together with forestry and fishing) varied in absolute terms (1.6-1.8 billion BAM) in the past period, but in general it is growing. Nevertheless, in relative terms the sector is declining (from 8.1% in 2006 to 6.2% in 2015), due to the faster growth of GVA of other, non-agricultural sectors. In doing so, agriculture has a higher significance for the Republic of Srpska than for the Federation of BiH and the Brčko District of BiH (Agency for Statistics of BiH). In the Federation of Bosnia and Herzegovina, the GVA of agriculture ranged between 707 (2006) and 857 million BAM (2015), and its share in the GDP of the Federation of BiH declined, and in 2015 it amounted 4.6%. In the Republic of Srpska, GVA of agriculture in 2015 (857 million BAM) is almost identical to its value in 2016 (897 million BAM), but in some years it exceeded 900 million BAM. The share of agriculture in the GDP of the Republic of Srpska is decreasing, and in 2015 it was 9.3%. In the Brčko District of BiH, the GVA of agriculture is increasing in absolute terms (in 2006, 52 million BAM, and in 2015 66 million BAM), and in relative terms, its share in GDP of the Brčko District of BiH declined from 14% in 2006 to 10% in 2015.

What is generally necessary for agricultural producers to generate an incentive, both at ministerial and local government levels, is to register with the one who grants incentive funds.

Therefore, in the case of the Republic of Srpska, it is necessary to register with the Ministry of Forestry, Agriculture and Water Management and update the registration every year, and then submit a request in accordance with the rules on incentives.The big problem in BiH is the diversity of farmers, that is, insufficient association, resulting in a bad organization. Only rarely sell their products through associations or cooperatives and jointly procure raw materials. All this reduces competitiveness in both the domestic and foreign markets.

Basic shortages in organizing the agricultural sector development of Bosnia and Herzegovina

In order to solve the lack of reliable information, statistical and administrative data systems for efficient management and support to the sector, it is necessary to upgrade all existing systems of services and support of the sector, at all levels, in accordance with the constitutional competences. The EU has developed standardized approaches, methods and processes to collect such information and systems that are needed to support it. It is necessary to strengthen information on agriculture, statistical and administrative systems in BiH in order to support the implementation of agricultural policies and their monitoring, and use the results for the assessment of the impact of the policy, and on that basis, make adjustments in certain measures to make them as efficient as possible. This measure will support the establishment of the basic elements of a harmonized agricultural information and administrative system in order to support the sector, and it refers to the development of improved registers of agricultural producers according to EU standards at Entity level and in the Brčko District of BiH, which will represent a platform for the eligibility for producers for future financial support (Strategic Plan for Rural Development of Bosnia and Herzegovina (2018-2021)).

Experiences from other countries show that investing in public goods - such as research and advisory services, market infrastructure, and natural resource management - yields much higher returns than subsidies, and in particular direct subsidies for production (the Agricultural Sector Policy Study in Bosnia Herzegovina, Study on trade and integration policies). At this point, however, 60 percent of BiH's agricultural consumption is focused on subsidizing production, while only a small portion of the already limited budget is allocated to services.

In order to eliminate irregularities in the agricultural sector, or any public sector the users of public funds establish financial management and control, which is implemented by policies, procedures, processes and activities with the task of providing reasonable assurance that these goals would be achieved through:

1) Business in accordance with regulations, internal acts and contracts;
2) The reality and integrity of financial and business reports;
3) Economical, efficient and effective use of funds;
4) Protection of assets and data (information).

The term and concept of internal financial control in the public sector (IFCP) has been elaborated by the European Commission in helping to understand and implement well-developed and effective control systems during the process of accession to the European Union. The goal of the system is to ensure that public funds (national and EU funds) are effectively managed and controlled (Krpan and Rukavina, 2013).

Legislative framework for financial management and control

As Bosnia and Herzegovina is divided into two entities, the legislative documents were issued at different times:

* Handbook for Financial Management and Control in the Institutions of Bosnia and Herzegovina (Ministry of Finance and Treasury of Bosnia and Herzegovina, 2014).
* Rulebook on Implementation of Financial Management and Control in the Public Sector in the Federation of Bosnia and Herzegovina (Official Gazette of the Federation of BiH, 2017).
* Law on Financial Management and Control in the Public Sector in the Federation of Bosnia and Herzegovina (Official Gazette of the Federation of BiH, 2018).
* Law on Internal Financial Control System in the Public Sector of the Republic of Srpska (Official Gazette of the Republic of Srpska, 2016).
*Instructions on the method of establishing and implementing a financial management and control system (Official Gazette of the Republic of Srpska, 2017).
*Decree on the contents of the report and the manner of reporting on the system of financial management and control (Official Gazette of the Republic of Srpska, 2017).
*Amendments to the Rulebook on the contents of the report and the manner of reporting on the financial management and control system.

Financial management and control are carried out in accordance with international standards of internal control (Article 6) of the Law using related elements:

1) Control environment,
2) Risk management,
3) Control activities,
4) Information and communication,
5) Monitoring and evaluation of the system.

Mission, vision and objectives of the work organization in the implementation of financial management and control

The mission as a term is the basic reason for the existence of a particular organization. The mission defines focus and purpose and is presented to all members of the organization, independent of the position and gives guidance in making key decisions. Since the mission is the main reason for the existence of the organization, and it consists of the scope of activities, values and goals need to be achieved, it can be said that a mission of a public sector organization consists of providing the best services to the public by efficient, effective and economical management of the public or budgetary funds.

Example of mission definition:
The mission of a public agricultural house-holding is the efficient and responsible management of jobs, through efficient and transparent work of the household in accordance with the interests and needs of the development of the domestic product and ensuring the conditions for the continuous economic and social development of the household.
The vision statement shows the direction and describes what the organization wants to achieve in the long-term, i.e. in the period from 15 to 20 years. The vision statement should be ambitious enough and realistic. The vision statement is a written document, and it is necessary to consider and approve it before it is published.
An example of defining a vision:
We want to be an efficient and transparent house holding that will, through the provision of quality and available products, enjoy the trust of stakeholders: citizens, subjects in social activities, business entities, and business partners.
The organization sets goals that provide general guidance on what needs to be done, and which are specific enough to directly relate to the organization.
They determine the clear direction of the movements and actions of the taxpayers in order to achieve the vision and mission by achieving each of them.
An example of defining goals:
- *increase participation in the domestic market,*
- *increase exports,*
- *become a recognizable brand,*
- *placing new products,*
- *sustainable development*
- *development of modern, efficient and transparent production,*
- *development of an entrepreneurial initiative based on the development of new technologies,*
- *spatial planning of the households and environmental protection,*
- *construction of communal and transport infrastructure,*
- *increase the quality of the product.*

Strategy

As one of the most important segments of financial management and control, the strategy should include: introducing a taxpayer, defining a mission and a vision from the Mission and Vision Statement on the vision, on the mission, the analysis of the situation - SWOT, defining general objectives, defining specific goals, ways of achieving goals, performance indicators, tracking and evaluation, risk management. In a strategic document or a strategic plan document, the first part refers to general introductory information. Here is included the description of the scope of the organization as well as the description of changes in organizational classification. The organization records data on agencies, institutions and other users within its jurisdiction. According to the above, defining missions and visions greatly assist in strategic planning. A well-established vision is the starting point for setting strategic goals. Therefore, the vision in strategic planning must include understanding of the problems and the existing situation, information on all existing data, as well as the perspectives of interest groups. Since from a good and well-defined vision, and also the mission, strategic goals for achieving the above will be created. The basic goal of strategic planning is to clearly identify the nature and character of the organization and sectors, the scope that it represents and manage its development in the future. Through the process of strategic planning, the units of local self-government (regional) define their goals, priorities and strategies, and define measures for assessing the success for achieving these goals (Krpan and Rukavina, 2015).

Analysis of the situation - SWOT ANALYSIS

The most widespread approach is the SWOT analysis, by which the organization identifies its advantages and weaknesses from the internal environment, and the opportunities and threats that come from the environment and can influence the work of the organization (Krpan and Rukavina, 2013).

Strengths	**Weaknesses**
• What are our strengths and quality? • What other people consider our advantages? • What advantages do we have compared to others?	• What are our weaknesses? • What do we miss? • What is done, and can be improved? • What should we develop better? • What should we avoid?
Opportunities	**Threats**
• Where are the good opportunities? • What are the interesting trends that you are aware of? • What extreme situations provide new development opportunities? • Good opportunities can come from changes in technologies, markets, changes in social practices, population profiles, and local changes.	What are the threats from the environment? What should you pay attention to? What do we have to consider? What extreme negative effects can adversely affect the work of the organization?

An example of SWOT analysis for agricultural holdings in the Republic of Srpska

STRENGTHS	**WEAKNESSES**
* Natural Resources * Human Resources * Geographical position * Agricultural potential * Developed water infrastructure * Manufacturing industry * Traffic connections * Favourable climate * Good resources for agriculture	* Outdated technology * Lack of skilled workforce * Unordered proprietary relationships that hinder adherence * Insufficient co-ordination of educational programs and needs in the labour market * Insufficient efficiency of local government (administration, courts) * Technical notion of infrastructure projects to bid for EU funds
OPPORTUNITIES	**THREATS**
* stimulative programs for small entrepreneurs * incentives for agricultural production * developing a favourable entrepreneurial climate * establishment of agricultural cooperatives and other interest associations * transfer of knowledge and technology * development of rural population * EU funds and financing of development programs and projects (in key development areas)	* Unfavourable business environment * Non-acceptance of project proposals at the national level * Non-inclusion in national programs * Globalization * Migration * Natural disasters * An expensive and inefficient state * Global economic crisis * Politically unstable environment

Conclusion

Effective elimination of irregularities in the agricultural sector and the achievement of development goals must begin with the creation and improvement of the necessary institutional and legal frameworks for the implementation of strategic objectives and measures, that is, respecting the legislative framework for the development of the sector as a whole. First of all, it is necessary to strengthen the institutional capacities, gradually introduce measures of agrarian policy, which have been implemented in European countries for a long time, strengthen cooperation and coordination of all interest groups and cooperation with countries in the region. It is clear that without a well-established legislative framework, processes and procedures, in practice, an effective control system that will guarantee the elimination of irregularities and progress in the development of agriculture will not be possible. Creating an information basis for the programming and monitoring of agrarian and rural production implies, on the one hand, institutional entrusting responsibility for this process to structures within certain public institutions or by contracting these services with other institutions and organizations. On the other hand, this implies the establishment of an adequate information system in agriculture, the basis of which would be the agricultural census, which implies further improvement and maintenance in accordance with the constitutional competences. In order to achieve these goals, it is necessary to strengthen the institutional capacities, and to improve capacities for the implementation of official controls and audit of official controls. It is of great importance to introduce consulting services that will contribute to business planning, management, productivity improvement, market linkages, efficiency, risk reduction, creation, planning and project management. In the process of planning, if we do not think about potential events that could create difficulties in achieving goals and do not consider possible measures to mitigate them, in the future, these events will be a problem for us and we will often be asked for a quick reaction, which is most often focused on the consequences. Managing the risks we are trying to solve the cause of the possible problem. To spot key risks and take appropriate control activities in a timely manner is to avoid those financial effects that will necessarily arise in order to solve problems. Therefore, when setting up the goals, business organization, making business decisions, etc. the starting point is to look at potential events that can create difficulties in achievement of goals.

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THE WINE TOURISM IN BULGARIA: AN OVERVIEW AND KEY CHALLANGES

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Abstract

Rural wine tourism is a way to diversify the mass-tourism product and bring more prosperity to disadvantaged rural areas. It is a very recent concept in Bulgaria but it is foreseen to grow in the future. However, there is a lack of research-based publications on wine tourism in the country in general and its potential role in sustainable rural development. The aim of this research is to explore the state of wine tourism in Bulgaria and to present the key challenges the country is facing. A threefold approach was used to collect information for this study: (I) literature review; (II) semi-structured interviews; and (III) winery visits (observation). Eleven wineries were visited and fourteen interviews with stakeholders were conducted. Relevant stakeholders to rural wine tourism development were identified as wineries, NGOs and associations, government and tourism businesses. The main challenges facing the development of the wine tourism industry are a lack of regional competitiveness, public awareness and recognition, a lack of regional and local infrastructure (roads and signage), service delivery skills, capacity and proactive attitude in wineries, a lack of tourism strategies (national, regional and local level), planning processes and governance structure, a lack of service quality assurance mechanisms and a lack of available market research. There is a strong potential for developing rural wine tourism in Bulgaria. The presence of wineries, cultural heritage attractions, nature, food and accommodation options, as well as the defined wine regions have all laid the foundation for it.

Keywords: *Agritourism***,** *Bulgaria Rural Tourism, Rural development, Tourism development Wine tourism.*

Introduction

Wine tourism is often regarded as a subset of food and wine tourism, also called culinary or gastronomy tourism. Food and wine tourism is a niche tourism product, also called special interest tourism type. In the 1970s, food and wine tourism started to develop in a more organized manner. Wine tourism was defined by Hall & Macionis (1998) as "*visitation to vineyards, wineries, wine festivals and wine shows, for which grape wine tasting and/or experiencing the attributes of a grape wine region are the prime motivating factors for visitors"*. Another definition is "*experiential tourism occurring within wine regions provides unique experiences which include wine, gastronomy, culture, the arts, education and travel*" (Dowling 1998; Saayman & van der Merwe, 2014). It must be recognized that wine tourism also involves destination planning and marketing strategy (Getz, Dowling, Carlsen & Anderson, 1999) this recognition manifests itself in the Western Ausralian Wine Strategy (2000), which defines wine tourism as*:"…travel for the purpose of experiencing wineries and wine regions and their links to lifestyle. Wine tourism encompasses both service provision and destination marketing."* According to Okech (2016), wine tourism is considered as a form of agritourism and by being seen as a distinct niche market that is gaining popularity in wine producing regions. This type of tourism demonstrates a symbiosis between tourism and agriculture that provides potential for their mutual benefit (Carmichael, 2005). The wine industry, mainly a primary industry, involves the cultivation of grapes and other fruits as raw material for wines and liquors, the making of the wine or liquor and the actual selling of the beverage products. On the other hand, the tourism industry is a tertiary industry oriented

toward providing services related to travel for recreation, leisure, sensory experience - taste, smell and sight which in combination with the destination can act as a "pull factor" (Kivela, 2006). Besides, wine tourism requires the involvement of wine producers (the wine industry), tourism agencies (the destination) and consumers. Wine tourism is both a concept and a strategy that can develop the tourism appeal of a destination. It has the potential to be a strategy that allows for better marketing opportunities for wineries, for wine regions and ultimately, for the country. It allows the wineries and other actors involved to reap financial benefits through leveraging tourism opportunities. Integrated rural development in disadvantaged rural regions is a high priority for Bulgaria. Wine production can help rural areas redesign their economies for both wine making and the hospitality associated with fine dining (Getz &Brown, 2006). In the past, rural communities played a significant role in the economic development of Bulgaria. Agriculture was a significant economic sector. Yet, in the last 20 years, there has been a shift to a service-based economy. In 2010, the gross value added to the national economy was distributed by 31.4% for the industrial sector, 5.4 % for the agrarian sector (agriculture, forestry, hunting and fisheries) and 63.2% for the services sector (Ministry of Agriculture and Food, 2011). Rural areas have suffered with declining population mainly due to urbanization and younger generations seeking better opportunities abroad. The migration of the population, abandonment of the agricultural cooperative system and lack of investment has resulted in many abandoned and dilapidated farm and residential buildings as well as an outdated and insufficient rural infrastructure. The objective of this paper is to explore the wine tourism development in Bulgaria and to describe the challenges for wine tourism implementation.

Materials and Methods

This paper is based on qualitative research method carried out in the summer of 2015 in Bulgaria. A threefold approach was used to collect information - *literature review* - articles on tourism related subject, books by leading experts in the filed of tourism planning and policy, wine and food tourism; sustainable tourism; government online portals were used such as the Ministry of Agriculture and Food (MAF) and the Ministry of Energy and Economy (MEE). Online sources relating to the Bulgarian wine sector were used such as the Executive Agency on Vine and Wine and the National Vine and Wine Chamber. *Winery visits* (*observation)* were made to eleven wineries in the southern and southeastern wine regions of Bulgaria. Their size can be classified according to wine production, winery capacity or size of vineyards namely small wineries; medium-sized wineries; and large wineries. A typical visit would involve a guided tour of the wine making facilities and a wine tasting session during which the semi-structured interview would take place. The visits were an opportunity to understand the winery visitor's experience. Fourteen *semi-structured interviews* were conducted with tour operators (three), wineries (eight) and with NGOs / private trade associations (three). Interviews with winery representatives were conducted onsite at the winery in connection with a visit. Interviews with tour operators took place at their respective offices in Sofia and Plovdiv. Interviews with NGOs and private trade organizations took place either in their respective offices or in an informal setting in a café. A participant information sheet introducing the research topic and explaining the purpose of the research was made available to interviewees. Confidentiality or anonymity was explicitly not guaranteed. The reception of the information sheet is considered informed consent of the interviewee to participate in the research. The objective of this paper is to explore wine tourism development in Bulgaria as a country with long tradition in wine making. Besides, the challenges for implemention this type of tourism business are also analysed.

Results and Discussions

Winery visitors, be they foreign or domestic, can be segmented into different categories. If asked *who visits Bulgarian wineries for tourism purposes*, the answer is: I) tour-operator organized groups; II) local residents and their friends/families; III) nearby hotel & restaurant guests; IV) wine lovers and connoisseurs; V) wine merchants, buyers or distributors; VI) industry colleagues and VII) individual walk-ins. Additionally, it is not uncommon that wineries host events on a request basis ranging from industry events, corporate events, art events, harvest related ceremonies and staff parties. An interest was expressed in the potential of the corporate segment that not only utilizes wineries as a venue to entertain clients or for internal events but is also keen to purchase large quantities of wine. According to interviewed tour operators, there is low to medium demand for visitation to wineries, wine tastings and wine tours. An increase in interest in tourism rooted in culture, heritage and food has been noticed in recent years both in Bulgaria. The country faces the challenge that it is still an unknown destination even for cultural heritage tourism and most certainly for special interest tourism such as wine tourism. The wineries interviewed reported various collaborative steps taken to seek wine tourism business such as entering into formal agreements with tour operators to bring groups in and informally working with local hotels and restaurants to promote wine tours to existing guests. While some wineries had received some regular business through their collaboration with tour operators, others expressed that very few booking requests were coming from tour operators. Wineries often attributed the lack of interest from tour operators to the remote location of the winery under the assumption that there is no interest to visit a remote winery especially if the region has nothing additional to offer such as cultural heritage sites, tourist attractions, activities, restaurants and hotels.

A lot of emphasis was placed on the location of the winery. The conclusion drawn by many wineries was that the success of wine tourism depended on the proximity of the winery to major tourist attractions and/or a city inferring that the more remote and the more isolated the winery is, the less attractive it is to visit. The appeal of the region was considered a major factor in the success of wine tourism and other forms of rural activities as well as the variety of choices available in the region. There was a wide agreement that good quality wine is a prerequisite for a thriving wine tourism industry. Most interviewed wineries were willing and to a certain extent are ready to fully embrace wine tourism, while some wineries choose to focus on their core competency that is wine making. Unwillingness to engage in wine tourism was attributed to management decisions to focus on producing quality wines and gaining global recognition age and nature. Genuine desire to succeed in wine tourism was more evident among newer, boutique wineries typically opened post year 2007. Boutique wineries were more committed to wine tourism development; tourism was considered an integral part of their business plan even prior to construction of the winery resulting in wineries that were built in a more visitor-friendly manner. In terms of being ready to welcome tourists into the winery and to provide other services, it is clear that some wineries are more ready than others. A fundamental prerequisite is space to accommodate tourists especially for wine tastings that often require a dedicated wine tasting room. Other prerequisites are clean and well-maintained equipment, fixtures and furniture in areas presented to the public and available trained personnel to take the lead on tours and wine tastings. While some wineries have a way to go before getting fundamental prerequisites in place, others are ready to welcome guests. It is important to acknowledge that wineries have historically been focused mainly on the production and selling of wine – not on entertaining visitors. The wine industry is currently heavily focused on producing high quality wines, regaining consumer trust, venturing into new export markets and building the reputation of Bulgarian wines. Wineries have not historically entertained paying customers visiting for leisure purposes. Wine tourism entails a change in the existing paradigm of the wine industry. Two of the wineries visited have taken a

serious interest in tourism and invested in additional facilities to facilitate more tourism. These wineries have added a hotel, spa and restaurant to their facilities essentially creating a complex like product with accommodation, dining facilities, swimming pool, wine retail, and additional activities (e.g. horseback riding) as well as offering regular wine tours. Other wineries are currently planning to or have already started to add additional facilities to their wineries such as hotels and restaurants. Several wineries expressed the intent to host visitors in a serious manner but only after all visitor facilities were completed while others ascertained that given ample lead time for instance for incoming groups, it was not a problem to shape up the winery and acquire required services such as tour guides, refreshments, music or folklore entertainment through local partnerships.

Key wine tourism challenges

Wine tourism in Bulgaria faces issues that need to be addressed. There are challenges that are both demand-side and supply-side related as well as internal and external to the winery. 1. *Lack of regional competitivenss*. Bulgaria has not yet mastered the task of effectively organizing and promoting the wine regions. It was frequently mentioned that the wineries within the region need to be better at cooperating instead of competing against each other over visitors and attention. 2. *Awareness and recognition*. A common sentiment among wineries was that Bulgaria has to fully master production of high quality wines first and ensure that the quality is acknowledged globally. While there was a wide agreement that good quality wine is a prerequisite for a thriving wine tourism industry, a contradicting point was raised that some wineries are better at making quality wines while others are better in tourism. 3. *Regional and local infrastructure: roads and signage*. The roads are highly trafficked, strategic ones are poorly maintained to the point where they pose a risk to drives and deter potential visitors. Directional road signs are also either not present at all or poorly placed. Another issue is lack of English signage making it difficult to get around as a tourist. Winery signs were also not always strategically placed. *4. Service skills, capacity and education.* Due to the lack of experience with wine tourism, there is currently a lack of service culture in wineries. Employees lack training in customer service and hospitality. Wine tours are often delegated to the oenologist on duty who is required to take time away from daily duties to give wine tours. 5. *Governance, strategies and planning.* A common concern was the lack of national and regional strategies on not only the development of wine tourism but also other rural tourism forms and how they can fit in with broader regional goals. There has not been much support from any tiers of government apart from municipalities occasionally using wineries for entertainment purposes. 6. *Service quality assurance.* Bulgaria has a reputation for varying quality not only in the wines but also in the tourism sector partially due to very low prices and sometimes wildly fluctuating prices in tourism. Another aspect is that wine tourism is so new to the country that there is still a need for the concept to mature and the quality of the tourism product to be realized. 7. *Market research and marketing efforts***.** There is a tendency for local entrepreneurs and potential business owners to act without understanding the characteristics of the market. Short-term focus impedes thinking about long-term sustainability of businesses. Furthermore, tourist expectations are not understood by people who have limited experience in the tourism industry such as wineries. There is not one entity that has taken on the responsibility to market wine tourism in one or more regions. Not the State Agency for Tourism nor the National Vine and Wine Chamber.

Conclusion

There has been no study on wine tourism in Bulgaria and this one lays the foundation for future research in this field. The results reveal that wine tourism is in very early stage of development. Efforts have been made by several actors in recent years to develop wine

tourism albeit in a non-coordinated or cooperative way. The findings of the research describe the category of tourists visiting the wineries as well as willingness of wineries to involive into tourism business. We found out that boutique wineries are more open to welcome tourists on their premises not only for a wine tour and tasting but to host events on a request, art workshops or harvest pinics than the large-scale wineries. Furthermore, special attention is paid to the key challenges that the wine tourism industry in Bulgaria is facing. Such an example is the lack of global and national awareness that Bulgaria in fact does have a budding wine tourism industry as well as interesting wineries and wine regions contributes to sluggish demand. Tourism is a service sector where training and professional approach to the guest is very important for the satisfaction of the tourist product offered. The study reveal the need for education and training to the staff employed in the wineries. Moreover, quality assurance mechanism for wine tourism should be designed in order to sell tourism product effectively. The state has not been proactive in supporting the development of wine tourism. National tourism policy does not reflect a commitment to rural tourism or gastronomic tourism. There is hardly any cooperation between the MAF, MEE (State Agency for Tourism) and Ministry of Rural Development that all have an interest in both alternative tourism development and rural development. It is clear that wine tourism alone cannot ensure socio-economic development and revive rural areas. However, in cooperation with other stakeholders, wine tourism can act as a catalyst to reach goals in rural development aspirations and national tourism product aspirations.

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APPLICATION OF AGRICULTURAL RISK MANAGEMENT POLICY IN BULGARIA

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Abstract

Up-to-date challenges to agriculture and sustainability have led to a practical need to minimize the risks of farming activities. Emerging uncertainties, risks, and crises related to the natural environment, technology, economic and political environment as well as globalization are new challenges addressed to risk management in the agrarian sector. Important for effective risk management is its understanding by all actors in the chain of the types of risks. Some of the risks faced by agriculture are critical and require a constant search for solutions for its management. The main aim of the study is to analyze the application of risk management policy in Bulgaria. The hypothesis is that policies related to agrarian risk management will reduce the negative consequences of its occurrence and impact. The paper is divided into several parts. The first part includes a theoretical background of sources and types of risk in agriculture. The second part relates to review and assessment of the legislative framework and the existing instruments for risk management in agriculture. The analytical part is based on a survey of risk management policy in Bulgaria. The collected empirical material is a part of scientific project "Integrated risk management approach in the agricultural sector". According to the results, general conclusions and policy recommendations are made.

Keywords: *agricultural risk, risk policy management, Bulgarian agriculture.*

Introduction

Agricultural risk may vary from independent (e.g. localized hail or farmer disease) to highly correlate (e.g. market price or widespread drought) losses. Risk management in agriculture is particularly challenging, as many risks are strongly associated, which results in simultaneous damage to whole communities. The risk is the likelihood of loss or non-receipt the income that is intended to be realized through the use of capital investments in the farm (Nedelcheva, 2012). Some authors (Fleisher, 1990) expose the connection between the access to different forms of insurance and subsidies paid by the government, and as a side effect reducing the risk of agricultural activities. The author considers that by using the measurement of agriculture policy farmers would apply risk friendly practices as insurances. Very often the studies of the literature directed to risk management states that the mechanism as subsidies in crop insurance has resulted in more plantings and extensive margin (Clark, Fleming, Skees, 2003). The authors' findings state that by using risk reduction mechanisms, the farmers would be more willing to implement activities with higher risk, as well to reflect in changing technologies, type of plants, even operational areas by using different land. These practices can cause as well negative environmental impact on the environment, because it is possible higher intensity or use more prepares to reduce the risk factors in plant sector.

Risk analysis consists of its identification and evaluation. Risk identification classifies several different risk categories that agricultural holdings may encounter in production, financial, personal, price, institutional, natural, political, and others. Risk assessment can be done through a variety of methods, from more elementary subjective methods such as completing a map of risk assessment indicators to more complex methods involving different quantitative

calculations (Boehlje, Lins, 1998). Risk assessment can be done in two ways: potential and probability. The risk potential is the magnitude of the negative consequences for the holding that would occur as a result of an event, and the likelihood is the chance of a potential event occurring (Detre, Briggeman, Boehlje & Gray, 2006).
Risk management analysis and connected policy should be proposed at levels as individual component (raw material and material supply, farm, processing, transportation, distribution, region, sub-sector, food chain). As well the policy should be directed at macro level (Bashev, 2005, 2012). In the theory can be traced out the statement that farmers do not have the qualification and statistical skills to calculate adequately the risk of their activities (Nedelcheva, 2012).

Material and methods

In the paper are used several methodological approaches. First part of the article is based on research of theoretical review and is used system and historical approach of analyzing the authors' view dedicated to risk management policy. The second part includes official data of applied policies related to agrarian risk management in Bulgaria. The risks were classified in two categories: 1) plant and 2) livestock sectors. According to collected literature framework and Bulgarian policy was set up an extended structure interviews in a questionnaire aimed to evaluate the risk management policy in Bulgarian agriculture. The sample includes farmers from different subsector and the aim was to obtain a sample close to product structure distribution of Bulgarian agricultural sector. The survey was held in 2018, under scientific research project NID, NI 16/2018, Integrated risk management approach in the agricultural sector.
Respondents have described the occurred risk event in last 5 years and the impact on their activity and evaluate the most common risk factors of their activities. The used scale is an ordinal Likert scale of 1 to 5, where 1 is low influence/impact to 5 strong influence/impact. Last part of the questionnaire were open questions where interviewees shared the good practices which they apply in their business activities connected to reduction of risk impact.

Results and Discussion

Analysis and procedures of policies related to agrarian risk management in Bulgaria

Ministry of Agriculture and Food is responsible for risk management policy in Bulgaria. A special structure has been set up at the Ministry of Agriculture and Food -the Food Chain Risk Assessment Center. Their role is to assess the most significant risks in agriculture and provide mechanisms to prevent their negative impact on the sector. On the basis of the assessment carried out by the assessment center, an annual plan is prepared for the prevention of risks in agriculture with measures to influence risk management. According to their classifications, some of the risks by sectors are shown in table 1.

Table 1. Classification of agricultural risks in Bulgaria

Crop	**Livestock**	**Forestry**
Risks:	Risks:	Risks:
▪ Hail ▪ Natural disasters ▪ Fires of the roots ▪ Thunderstorms ▪ Frost ▪ Storms ▪ Earthquakes ▪ Late spring leaning	▪ Fires ▪ Natural disasters ▪ Accidents ▪ Contagious diseases ▪ Non-infectious diseases ▪ Forced destruction ▪ Slaughter by necessity ▪ Earthquakes	▪ Fires ▪ Floods ▪ Landslides ▪ Crushed forest ▪ roads ▪ Broken / torn dam walls ▪ Hail

▪ Early autumn trimming ▪ Frost and pull ▪ of crops (cereals) ▪ Diseases and diseases invasions (pests)	▪ Parasitic diseases	▪ Storms ▪ Thunderstorms ▪ Frost ▪ Oscillation ▪ Snowmobiles, avalanches ▪ Earthquakes

*Source: MAF, 2018

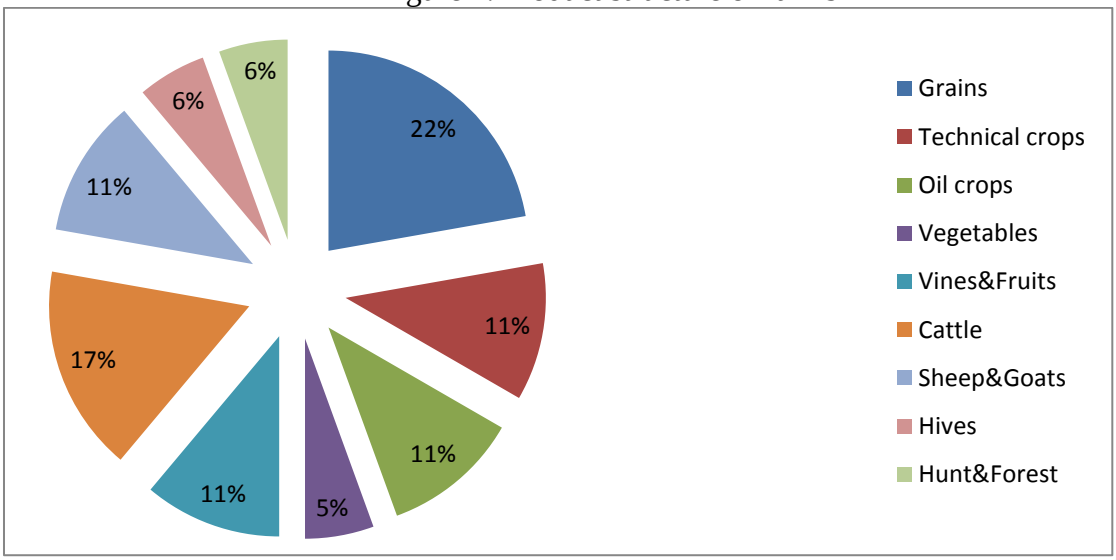
Once the risks have been identified and their likelihood and impact assessed, an appropriate response should be considered. Taking measures and actions to respond to identified and assessed risks is a very important stage in risk management. The government structure as well prepares different types of risk response (Ministry of Agriculture and Food, 2018). The following response options are possible: limitation, transfer, tolerance and ending of risk. When selecting appropriate actions/responses, account is taken of the requirement that their costs do not exceed the expected benefits. This choice also depends on the assessment of the level of residual risks the organization can accept without taking further action. Any major risk management activity needs to be documented to ensure traceability of the entire process. The documentation shall include an appropriate description of the identified risks, the appropriate response/action chosen and the personnel responsible for carrying out these actions within specified deadlines.

A risk-register is used to document the results. Ensuring the effectiveness of the risk management process requires regular and continuous systematic monitoring / monitoring at each stage and reporting periodically the identified risks and action is taken to reduce them. To carry out systematic monitoring, the risk-register shall be reviewed at least once a year.

Survey results

The surveyed farm owners have a different product structure. The distribution is shown in Figure 1. The sample includes average representatives (most common) of each sub agrarian sector presented in Bulgaria.

Figure 1. Product structure of farms



*Source: Author's elaboration based on the questionnaire survey results.

Of the sample, 11% of the farmers did not experience a risky event, financial risk accrues in 44 % of the farmers, risk of lack of personnel in crucial period- 50%, loses of actives- 40%, financial risk- 44%, other risks (22%).
External risks occurred in the past five years in the surveyed farms of plant sub sectors are presented in table 2, and livestock breeding in table 3. From table 2 and 3 can be made conclusion that the biggest share of the participants in the survey had experience risk events, which affected their activities.

Table 2. Occurred risk events last 5 years in crop sectors in Bulgaria

Hail	50%
Natural disasters	11%
Fires of the roots	20%
Thunderstorms	0%
Frost	6%
Storms	0%
Earthquakes	0%
Late spring leaning	6%
Early autumn trimming	11%
Frost and pull of crops (cereals)	44%
Diseases and diseases invasions (pests)	26%

*Source: Author's elaboration based on the questionnaire survey results.

Table 3. Occurred risk events last 5 years in livestock sectors in Bulgaria

Fires	0%
Natural disasters	6%
Accidents	11%
Contagious diseases	16%
Non-infectious diseases	40%
Forced destruction	22%
Slaughter by necessity	22%
Earthquakes	0%
Parasitic diseases	0%

*Source: Author's elaboration based on the questionnaire survey results.

In plant sectors hail is the biggest risk which has occurred, followed by frost and pull of crops. In livestock sectors non-infectious diseases occurred to 22% of the studied farms.
Of these, 67% state that their activity is risky. After the main risks were identified, the respondents assessed the weight of the factors from low risk to high risk. The results are presented in Table 4.

Table 4. Risk factors in agriculture. Self-evaluation

Reason	**Score 5 high risk to 1 low risk**
Perpetual of production	5,00
Manipulation by the middleman, lack of a mechanism of the price market.	5,00
High quality requirements for milk, /crop production diseases high mortality, animals stealing and others.	4,75
Unstable prices of fodder, veterinary services, the reseller.	4,50
Production volumes set out in the business plan and strong dependence on nature and markets.	4,50
Uncompetitive compared with colleagues from other European countries due to different subsidies received.	4,25
Often a too-close farm that self-sustains the entire cycle with its own production (fodder-stock-breeding). When there is a risk in the chain, it affects the entire farm.	4,20
Lack of qualified personnel.	4,00
Uncertainty in agriculture prevents long-term goals from being set up.	3,50

*Source: Author's elaboration based on the questionnaire survey results.

The maximum score is given to the perpetual of the production and problematic markets operating in the country. On the second place as a reason of high risk activity are pointed high quality requirement of the production. As well farmers have the opinion that high dependence of the nature make their activities with high risk. With relatively low estimation are inabilities of long term planning due to environment, lack of personnel and too close farm that self-sustains the entire cycle with its own production.

Part of the questions was dedicated to reveal the opinion of the farmer opinions connected with their own good practices in risk management. The main practices can be summarized as follows:

- Using innovative technologies which can reduce the risk from the activity;
- Closing the chain of production cycle- produce-end market;
- Farm contracting which can be used for a credit line;
- Contracts with producers of inputs and outputs;
- Diversification of the production;
- Insurances;
- Implementing better and sustainable breeds/seeds;
- Using a robotic technic;
- etc.

Some of the questions which respondents gave opinion are related to evaluation of current Bulgarian risk policy in agricultural sector. All of the respondents share the opinion that the policy is insufficient in the area of the risk management. Of them 75% are pointing that the biggest issue is connected with very complicated applying procedure , 50% of them do not know the measures which they can use to reduce the impact of risk events.

44% of the farmers applied for risk management measure to CWRS, all of them received compensatory payment. Farmers received up to 80% of the expenditures which they did, but their statement that this is not sufficient payment, as well as payment were delaying. Another conclusion from the interviews is the lack of coverage of some very specific risks, which are facing some of the sectors, as bee hives.

The proposed policy recommendation can be summarized as follows:

- Part of payment of SAPS to be connected with risk events by change of the Ordinance of SAPS;
- According to the market risk, the government to implement mechanism which will keep minimum price of the products as a milk;
- Each agricultural sector to have specific strategy;
- Help to farmers to find a proper market with decent condition for selling the animals;
- The payments for small farms to differ from these of big farms; The effect of risk to be calculated as a share of the farm;
- Training courses for farmers on the following topics: -risk management according to agricultural sector;
- possible risk policy measurements in Bulgaria;
- Etc.

Conclusions

The agrarian sector is important, dynamic and its nature can be defined as a high-risk potential event to occur. Farmers involved in the survey identify the risk of their activity and outline several different risk categories, such as production, financial, personal, price, institutional, natural, political, etc.

In Bulgaria is existing risk policy management but at this stage the farmers do not know for possible measure and mechanism. The results of the survey confirm the literature review finding that risk management policy should be proposed at levels as individual component (raw material and material supply, farm, processing, transportation, distribution, region, sub-sector, food chain).

Agriculture is evaluated as sector with a high level of risk and policy-makers should adjust the current policy due to minimize the effect of occurring and helping the agricultural sector to become sustainable one.

Acknowledgment

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SUSTAINABILITY OF UNDP PROJECTS IN EGYPT: A CASE STUDY IN SIWA OASIS (SIWA ENVIRONMENTAL AMELIORATION PROJECT)

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Abstract

The main objective of this research paper was to evaluate the effect of one of the international projects in Egypt, in order to know the benefit of such projects and to discover the reasons of their success or failure from different point of views. Siwa Environmental Amelioration Project (SEAP) was chosen as one of the famous projects in Siwa Oasis. It worked there for two phases for 6 years in order to set sustainable development activities in the oasis through loan activities in the agricultural field, in order to raise the quality of life for the oasis people and set a perfect example for the capabilities and the good effect that such projects can do. Ven diagram and pairwise ranking matrix were used to identify the linkage between different actors involved in the project and the problems importance according to their point of view , The results showed that faced the project a lot of local obstacles faced the project and didn't help SEAP in achieving its goals. Besides, the other local NGO partner (SCDEC) was not qualified enough to take over the projects after SEAP left. Even with the success of organic agriculture production as an innovation, the problem of marketing already killed the idea, and small farmers couldn't cope with it, and it ended up by cancelling the activity.

Keywords: *sustainable agriculture, innovation.*

Introduction

According to the Egyptian definition, Siwa Oasis is considered to be the city and district of Siwa. It located in the western desert, 800 km from Cairo the capital, and 300 km from Matrouh city, the capital of the Governorate, which it is administratively, follows. Siwa was isolated for a very long time since there was no direct road to drive there.

Through all this period of time(800 years according to different studies) Siwan people were managing their land and ground water sources within ten tribes through their own special social contract and a special tribes council that arrange most of their life issues socially and economically.

Although Siwa is located in Matrouh governorate, but it would had taken 18 hours to reach it from Matrouh city and almost one day from Cairo.

In the early 80s a road was structured by the petroleum companies in order to connect Siwa Oasis with Matrouh city governorate. By that time the road was the innovation that connected Siwa to the world and helped in delivering many medical and technical services. Since that time tourism started to be a basic source of income for Siwan community, this road even gave the chance for Siwan youth to join university education.

Study problem

No doubt that many international organizations were interested to study and help to develop this isolated community in order to improve those people's life and help in developing their sustainable resources management, UNDP (United Nation Development Program) and EIECP (Egyptian Italian Environmental Cooperation Program) were the first international organizations that started projects aiming to develop the Siwan community through improving the quality of life, that's why the current study aimed to evaluate one of the famous projects

out there SEAP and its impact and sustainability with its positive or negative impact on Siwan community.

Study goals: Evaluation of SEAP in achieving its goals and Evaluating the benefits of such projects for the Siwan community

Materials and methods

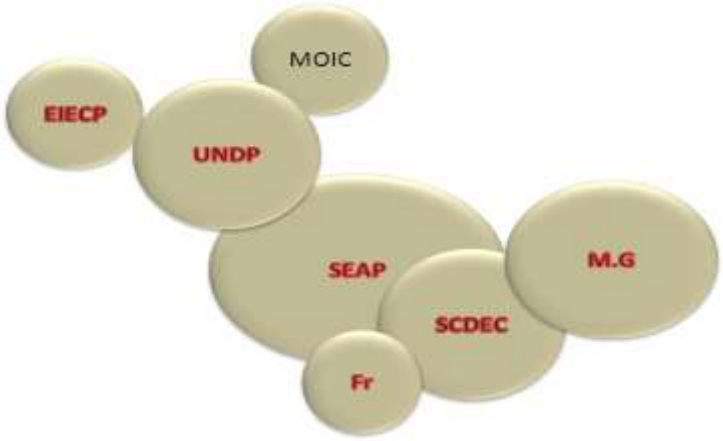
A long field visit was prepared from 1-10/4 /2016
Personal interviews were done to the key persons and informants. Group discussion technique took place with the farmers' organic agricultural group (16 people), as well as secondary SEAP' data.
Some of SEAGA (Socio Economic and Gender Analysis) tools were used according to the field study and community special traditions:
Ven diagram and Pair-wise analysis
- A ven diagram was prepared with SEAP management unit in order to know the linkage between different actors involved in the project. The importance of each actor is represented by a circle sized according to its importance, while the level of overlapping between them shows the degree of contact.
-A pair wise analysis was done three times. One with each of SEAP team, SCDEC management unit, and thirdly with the farmers as a way to identify the main problems faced and how each sees SEAP success or failure from their different point of view.

Results and discussion

UNDP with the cooperation of EIECP started in 1998 applying SEAP first phase for three years. This project aimed at developing Siwan community through giving micro credits to each Sheikh of a tribe who was responsible to recycle the loans inside his own tribe among the farmers, but by the end of the project a big dilemma started to arise since most of the loans were not given back.
SEAP project discussed with the tribes the idea of establishing an association responsible for recycling those loans, as a way to solve the problem of not paying back the loans.
The association was established in 2001 by 31 members with a management council of 13 members representing the 10 tribes (one member from each tribe) and three villages (one member from each village).The council management members' changes every six years.
In 2004 the second phase of SEAP was agreed with different perspectives between EIECP and MOIC (Ministry of international cooperation) under the supervision of UNDP
The project had three main goals

1. Turning Siwa into an international natural park.
2. Achieving sustainable agriculture development through
    a. Achieving organic agriculture system
    b. Improving local animal production
3. Using SDEC organization as a local partner to take over the activities after they will leave.

The following ven diagram in (fig 1) was used to show the linkage between different actors in SEAP, the area of the circles shows the degree of importance while the level of over lapping shows the degree of contact.



(fig 1) Ven diagram showing the linkage&
The degree of contact between actors in SEAP.

Key:
MOIC: Ministry of International Cooperation
EIECP: Egyptian Italian Environmental Cooperation Program
UNDP: United Nation Development Program
SEAP: Siwa Environmental Amelioration Project
SCDEC: Siwa Community Development and Environment Conservation
M.G : Matrouh governorate
Fr: Farmers

Meeting and group discussion results:

1. The international natural park was not applied because suddenly and with no reason the governor of Matrouh decided to be an obstacle in front of SEAP goals, he negotiated for more than one year the possibility of cutting part of the project fund for achieving a medical natural ecology which was impossible because it was not mentioned in the agreement signed before.
   That's illustrating why UNDP stopped negotiation and transferred the budget of that part to another place, leaving SEAP only with a small budget for the agriculture issues.
2. According to the agreement signed in 2003, SEAP was obliged to use consultants only from national research centers, SEAP Management suffered a lot because they were unqualified consultants and miss leaded them.
3. The project was left with a small fund to work on the second and third objectives only, according to the socio economic studies done by the specialist, SEAP management decided to start organic agriculture production by establishing a demonstration organic farm, 16 Fadden given to 16 youth fresh graduate Siwan, they were chosen carefully according to special application and within the tribes applied by SCDEC and supervised by SEAP.
   Consultants taught them about organic agriculture steps and compost preparations, drip irrigation system was installed and all infrastructures were settled dawn by SEAP and given to farmers as a loan should be paid back to SCDEC to recycle it and transfer the new innovation to other farmers
   The final highly coast product could not be sold in local markets or even in organic markets whether in Alexandria or Cairo due to the large distance and the small production for the16 feddan

Unfortunately farms removed the drip irrigation system and went back to surface irrigation system and now they are cultivating natural products for local markets and loans were never paid back.

4. The animal production specialist after two weeks of studying the Siwan conditions delivered special cows to the association in order to deliver to small farmers as a loan but the cows died directly after arriving due to high temperature and ticks. The contract with the research center for delivering the animals was canceled, SEAP management decide to build an animal demonstration farm for goats and sheep to produce animals given as loans for small farmers and women and to be managed by the association even after SEAP will leave.
   After the project was finished the association refused to take over the demonstration farm and sadly it was closed and loans were never paid back
5. Pairwise ranking matrix was used to show the most important problems

Matrix (fig 2) represent SEAP management point of view and was prepared through personal interviews.

Pairwise ranking matrix according to SEAP management		
Problem	Time preferred	rank
Matrouh governer	4	1
Inexperience specialists	3	2
Unqualified association members	2	3
Cutting fund	1	4
Farmers adopting new innovation	0	5

It was clear from SEAP management that Matrouh governor was the strongest obstacle against achieving the project goals, and on the other hand the project suffered to use certain specialists from certain place and didn't had the flexibility to choose.

Matrix (fig 3) represent SCDEC management point of view and was prepared through personal interviews

Pairwise ranking matrix according to SCDEC management		
Problems	Times prefered	ranking
Goals of SEAP were not clear	4	1
Shortage of trained people	3	2
Shortage of funding	2	3
Farmers connection	1	4
Tribes council	0	5

SCDEC is local NGO created in 2001 with the help of SEAP first phase, the association is considered to be the formal way of the tribes' council to be presented and take over responsibilities to manage the Siwan resources and to support supervise the projects applied in siwa since that the agreement of the association management council mean the agreement of the tribes

SCDEC under the tribes control failed to take over and manage the sustainable agriculture projects, we have to mention that SCDEC reputation among other projects started to fail dawn since that a lot of projects now are not interested in SCDEC to be their local NGO partner, and of course there is no other NGO in Siwa

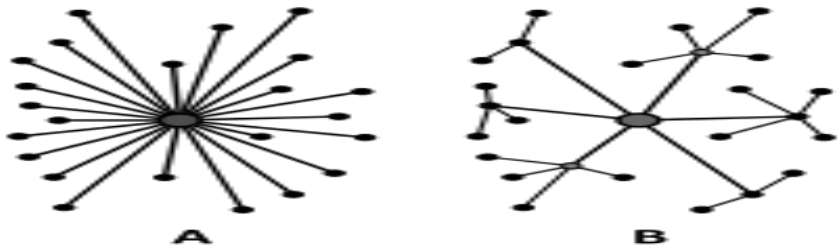
Ranking matrix (fig 4) represents the farmers' point of view

Pairwise ranking matrix according to farmers point of view		
Problems	Times prefered	ranking
Marketing problems	4	1
High costs of infra structure	3	2
Bad comunication with SCDEC	2	3
Organic certification problems	1	4
Water problems	0	5

It was clear from the group discussions with the farmers group that they faced a lot of problems in marketing especially with the high infrastructure cost which should be given back to SCDEC as a loan in order to recycle the experience and transfer the innovation to other farmers.
Since the final products was highly costly with no suitable market and no good price, they failed to continue after SEAP ended and turned to traditional agriculture products, even when they tried to build another association for organic farmers in siwa nobody supported them.

Conclusion

1. SEAP failed to achieve sustainable agriculture development in Siwa due to Matrouh governor and miss leading specialists' recommendations.
2. SCDEC as an only association in the Oasis failed to take over and manage the assets and activities left by SEAP and shut them all down.
3. The farmers couldn't cope with the organic agriculture process with no support within from tribes or Association.
4. Decentralization is the process of redistributing or dispersing functions, powers, people or things away from a central location or authority(free decionory.com, 2013), I was clear that there is a huge problem in the management system that allowed the governor of Matrouh to be an obstacle. On the other hand decentralization should be the system that international organizations apply when they are offering or designing a plan or projects to help the undeveloped countries.



(fig 5): Graphical comparison of centralized and decentralized system.

The word "centralization" came into use in France in 1794 as the post-French Revolution French Directory leadership created a new government structure. The word "decentralization" came into usage in the 1820s (Vivien A. Schmidt,2007)

Leopold Kohr, author of the 1957 book The Breakdown of Nations—known for its statement "Whenever something is wrong, something is too big"—was a major influence on E.F. Schumacher, author of the
1973 bestseller Small is Beautiful:Economics As If People Mattered(Dr. Leopold Kohr,1994).

According to a 1999 united nation development program report:

"A large number of developing and transitional countries have embarked on some form of decentralization programs. This trend is coupled with a growing interest in the role of civil society and the private sector as partners to governments in seeking new ways of service delivery...Decentralization of governance and the strengthening of local governing capacity is in part also a function of broader societal trends. These include, for example, the growing distrust of government generally, the spectacular demise of some of the most centralized regimes in the world (especially the Soviet Union) and the emerging separatist demands that seem to routinely pop up in one or another part of the world. The movement toward local accountability and greater control over one's destiny is, however, not solely the result of the negative attitude towards central government. Rather, these developments, as we have already noted, are principally being driven by a strong desire for greater participation of citizens and private sector organizations in governance ("Decentralization: A Sampling of Definitions", 1999,)

So I guess we are not trying to approach a new idea or a new system of work. This is weird really why international organizations don't apply this system in order to apply the needed projects according to the people needs especially in Egypt.

On the other hand the decentralization system is not working in Egypt in a good way because such projects usually are applied and approved according to certain agreements done between Egypt and other countries under the umbrella of UNDP and in the end it is just waist of money.

5. Community participation could be the first step of solving the centralization problem through community development associations, and NGO,S .

   The people should feel the power of social participation and the affect of their enrolment in decision making. It is unbelievable that a community Like Siwa with ten trips only works through one organization, another youth organizations should take place to gain the trust of international organizations.

Recommendations

1. Egyptian government should start a survey in such locations to build a check list for the most important project needed by local people, so that the interested international organizations can choose from them easily.
2. Since there is a signed agreement it's important that the governor should not be an obstacle for such projects, laws should be updated.
3. Siwa need badly another new association with youth educated people in order to regain the trust of international organizations again.

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CLIMATE EFFECT ON RURAL TOURISM DEVELOPMENT, CASE STUDY OF GUILAN PROVINCE (IRAN)

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Abstract

One strategy that has been recently considered by many countries in the world, even applied and implemented in some leading to positive results and outcomes, is tourism development in potential rural areas. Rural tourism entails diverse activities conducted by tourists in rural areas. In other words, rural tourism constitutes of all the activities that the tourists might be able to undertake in a rural area with respect to its natural attractions, culture, rural traditional context, art and handicrafts, and customs which might be classified as agricultural tourism, green tourism, farm tourism, and food and hunting Agritourism. Rural tourism encourages economic growth, diverse and sustainable livelihood, commercial and industrial productivity, promotion of income opportunities in the form of multifaceted activities, creation of new markets for agricultural crop and forms the cornerstone of developing a local productive economy. In the geographical span of Iran, Guilan province has a significant and valuable tourism status. Its geographical features and unique economy of Guilan along with ancient, historical, religious and cultural monuments make this region of Iran unique and transform it to a massive hub of tourism in Iran. The main goal of this research is planning to adapt the existing context based on climate potentials in this province to develop rural tourism industry in Guilan province. This is an applied research using descriptive-analytical methodology. In this paper the province climate is determined using climate classification method and TCI climate tourism method climate zoning is inducted. Then the issue of using climate determinants to develop rural tourism economy is addressed and potentials and new ideas are identified and strategies andrequired solutions to develop rural tourism in this region are introduced.

Keywords:*Climate, Climate parameters, Rural tourism, Guilan province.*

Introduction

Many contemporary planners perceive rural tourism as a key and crucial activity in rural development and considered it from various perspectives; economists consider tourism industry as "invisible export". This industry is a vast scope activity that has important economic, social, cultural and environmental impacts. Therefore, planning for rural tourism development requires a clear understanding of the impacts and consequences perceived by the host society which ultimately leads to sustainable development in the region [5]. Rural tourism has been expanding markedly since the 1950s. Given the attractiveness of the economic consequences of tourism development, early studies during the 1960s focused more on the positive economic effects of this phenomenon. But in the 1970s, the economic consequences of tourism development have been evaluated by researchers with a wider perspective [3]. In the course of this period, there was mostly a negative approach to tourism development; so that researchers often emphasized its negative effects [2].The benefits and positive economic effects of tourism are divided into two categories of direct and indirect benefits. Direct benefits include: new employment opportunities, currency injection, improving people's living standards, sale of services and income generation, economic mobility, recruitment of young work-force; and indirect benefits include: construction boom, crafts boom, horticulture flourishing, fishing, development of services and consumable goods,

infrastructure development and improvement [10].Of course, tourism effects are not always positive and lack of monitoring on the use of tourist environments might result in destruction or cause recession in some sectors of the economy [4]. Besides the economic effects, tourism has social and cultural implications. Social impacts result from various types of tourists travelling to the region. Presence of tourists in rural areas leads to communication with local communities with significant mutual impact on cultural and social development, literacy promotion, and establishment of health care centers, promotion of women's role in often traditional societies, and promotion of social relations in remote communities [11]. Cultural impacts include changes in life style, architecture, the art and customs of the host community [7].

Material and Methods

This research is based on the applied purpose and in terms of its descriptive-analytical method, describes the climate classification of a region's climate. In this method, the climatic profile is calculated from the following equation and in this formula, P is the mean annual precipitation in millimeters and T is the annual temperature in degrees centigrade. According to the De Marton Formula, each region is classified into six types of climates, as shown in Table 1. [1, 8]. Accordingly, and the current statistics on climate zonation of the entire province were carried out (figure1)

Table (1): De Marton climatic classification

Climate	De Matron Drought Ratio (I)
Arid	>10
Semi-arid	10 – 19.9
Mediterranean	20 – 23.9
Semi-humid	24-27.9
Humid	28 – 34.9
Very Humid	35 – 54.9
Very very humid	<55

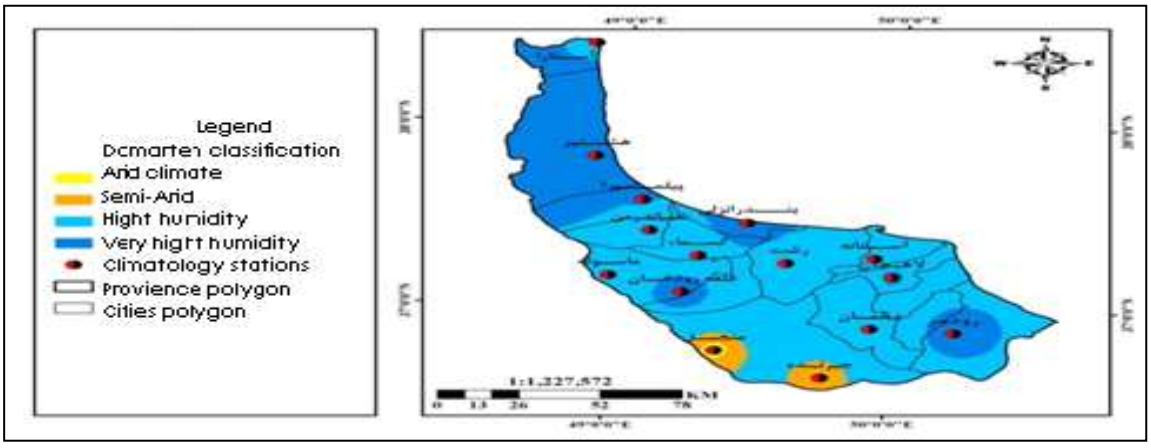

Figure (1): Climate zoning map of Guilan province by De marton method

Results and discussion

- Preparation, extraction and climatic data of synoptic stations of the study area,
- Use of climatic components in the region including temperature, precipitation, relative humidity, wind, sunny hours, number of cloudy days,

- Using EXCEL, GIS, SPSS software to map different geographic and climatic maps in the area,
- Use of different climatic indexes such as TCI to determine the appropriate climatic index of the area,
- Climatic zoning in different regions of the province using different climatic methods.

Guilan province is one of the northern provinces of the country with an area of 14711 km2. The Alborz mountain range with an average height of 3000 m is drawn like a wall in the west and south of Guilan, and this area, other than the Manjil valley, has no other dirt way to the Iranian plateau. The shortest distance of the mountain from the Caspian Sea (in the Hawiq section of the city of Talesh) is about 3 km and its maximum distance from the sea (in Imam Zadeh Hashem, Rashte-Qazvin road) is about 50 km. This province is limited to the Caspian Sea and the independent countries of Central Asia from the north, from the west to Ardebil province, south to Zanjan province and Qazvin, and east to Mazandaran province.
Based on the latest national divisions, Guilan province has 15 counties, 52 towns and 43 districts.

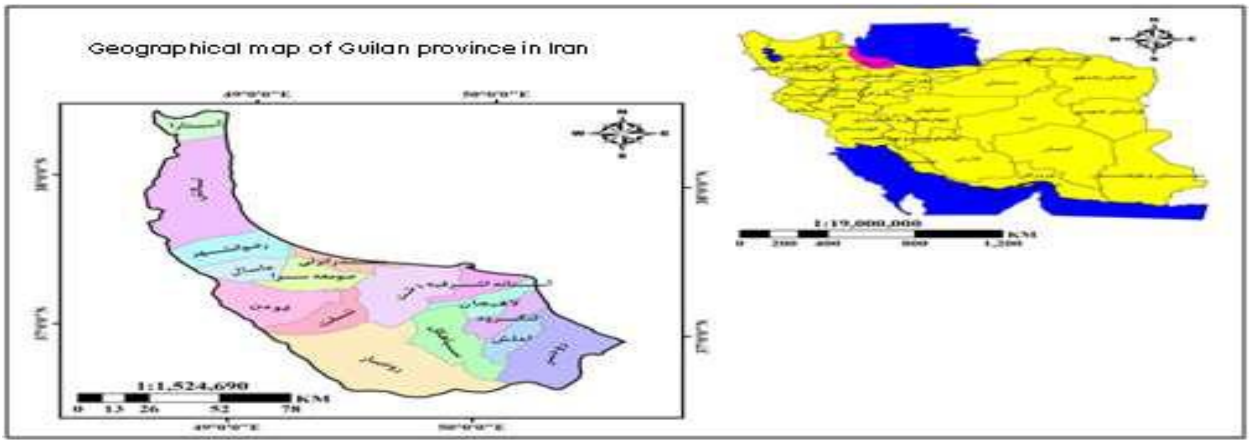

Figure (2): Geographical map of Guilan province in Iran

-Guilan Province Climate Zoning

The results of De Marcton climatic coefficient estimation in Guilan province are shown in Fig. 2. Figures 3 and 4 show the isothermal map of Guilan province using the De Marton method in two statistical periods of 1985-2000 and 2000-2015; Also, the isohyet map of Guilan province using the De Marton method in the same statistical period is presented in Figures 5 .

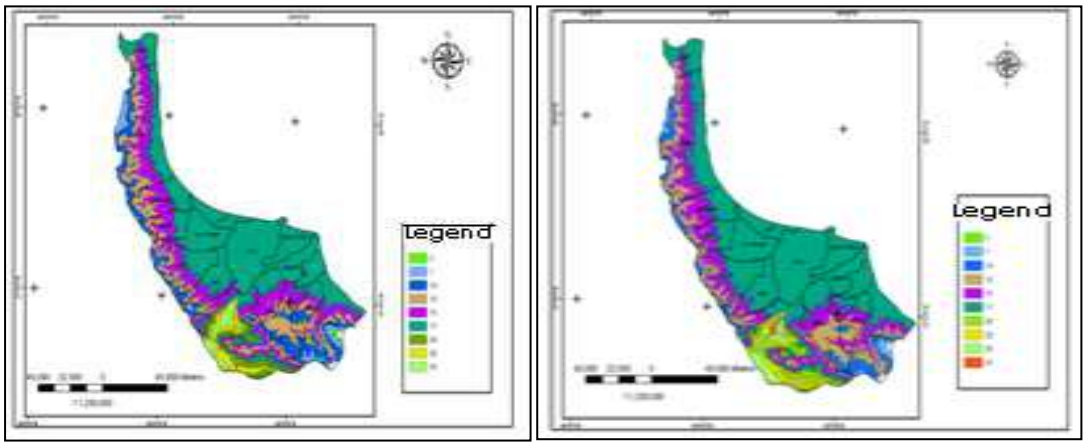

Figure (3): Isothermal map of Guilan province, De Marton method, 1985-2000 and 2000-2015

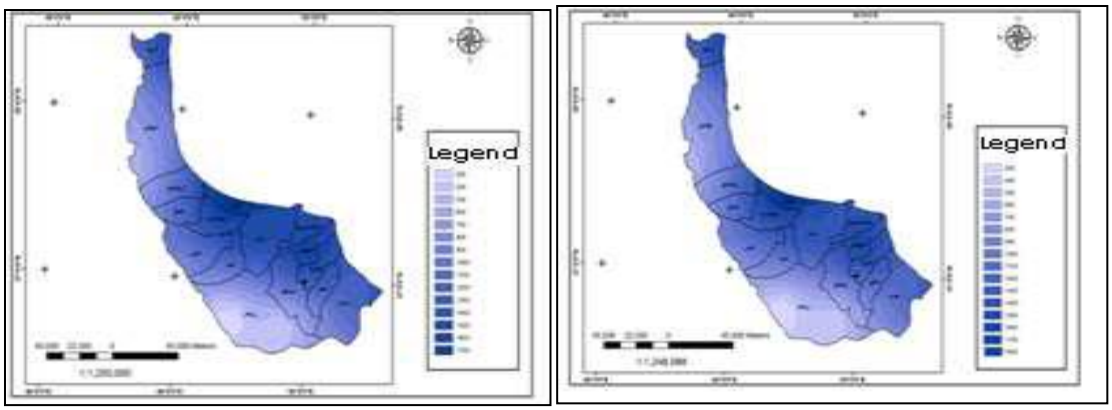

Figure (4): Isohyets map of Guilan province, De Martonmethod, 1985-2000 and 2000-2015

-TCI Climate Zoning

Figure 5 demonstrates the tourism climate status in the stations of Guilan province for the twelve months of the year. In April, the climate improves relatively. In this time of the year, the whole province shares relatively similar climate comfort index except for a number of regions in the central parts with an index of 70-79 with good conditions; other parts of the province with a range of 100-80 indexes fall within excellent and very good climatic groups.

Finally, it can be concluded that spring, late summer (early September), early autumn and March are the best time for tourism in terms of climate comfort conditions in Gilan province, and due to the climatic variation in the province and slight weather difference in the northern stations compared to the southern stations; in a general overview, the whole Guilan region is well-suited for tourism.

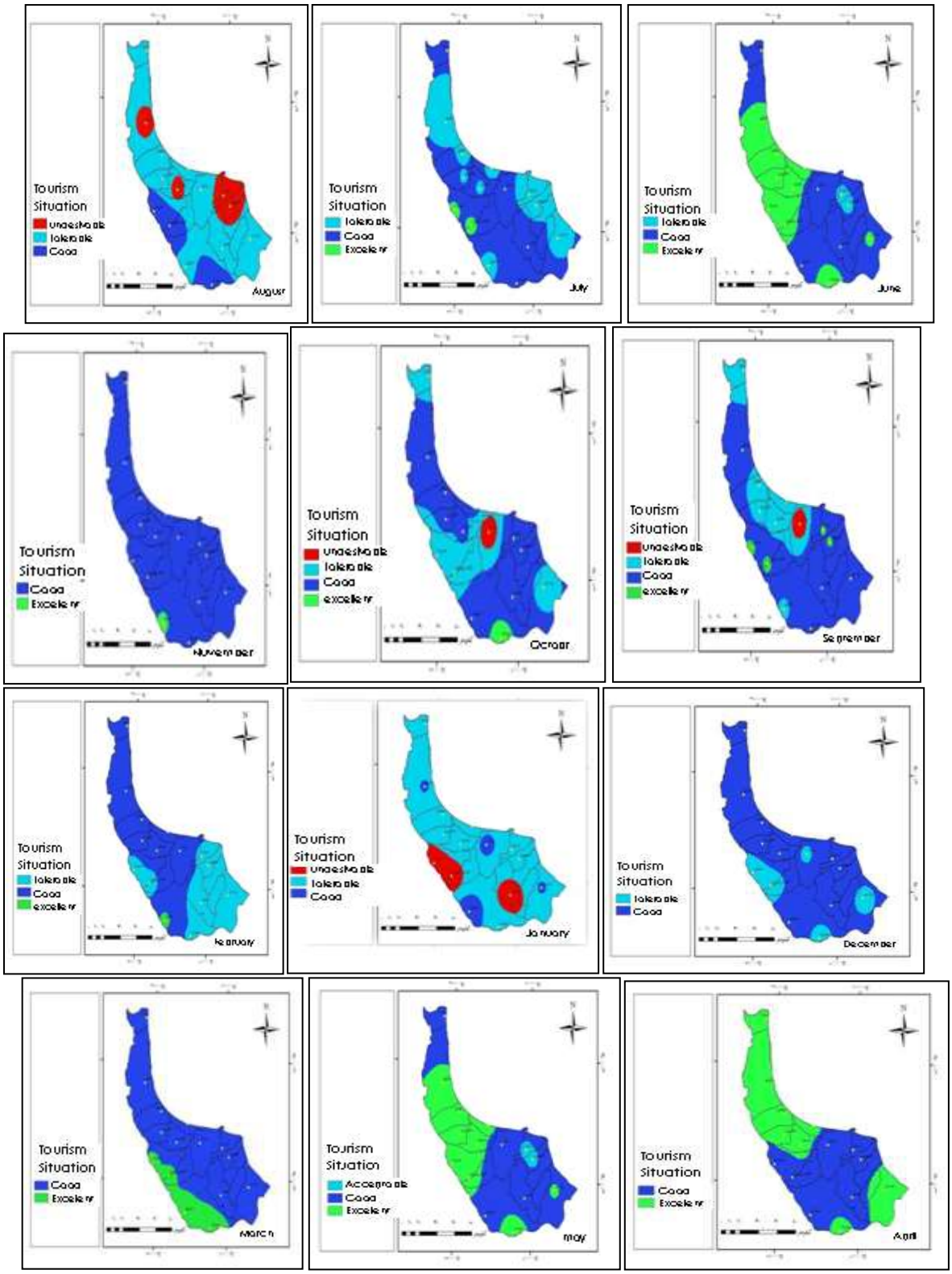

Figure (5): The climate zonation of Guilan province is based on the index TCI during the months of the year

Key Climatic Elements of Tourism in Guilan:

The differentiation level and combination of climatic elements and factors resulting from geographic variation create different climatic zones, each of which can have a particular impact [12]. Iran has been subjected to sub-tidal pressure in terms of cluster division in a dry and semi-arid region. Nonetheless different climatic conditions can be observed in Iran and the province of Gilan is no exception.

According to the studies, elements such as temperature, precipitation, humidity and wind are effective elements in tourism of the province.

In terms of temperature, according to the climate comfort estimations, spring (May and June) is the perfect; summer (July and August) is inappropriate; September is suitable; the winter and autumn (November and December) are also inappropriate for tourism.

In terms of precipitation, autumn (October and November) is unfavorable and the remaining months and seasons are favorable.

In terms of humidity, spring is favorable and the other seasons are relatively unfavorable.

New Ideas:

[1] Provision of indoor glass halls with suitable tourist facilities (tourism incubator):

The incubator literally is the device that provides the heat needed to convert eggs to chicks and supports a premature infant towards required growth and development. The question is that how this definition of incubator relates to the trade and style of tourism. An incubator or development center constitutes of one or more buildings that accommodate proper research units and facilities and benefits support services. Each incubator typically has a common space, services and advanced facilities for the benefit of creative and innovative ideas, appropriate welfare and business services, institutions and advertising companies. This idea might be implemented in Guilan province as a tourism incubator. It is with due consideration that it is this Caspian region (with moderate & humid climate) has the highest precipitation and is the greenest region of Iran where rainfall & humidity is observed in most seasons, and there are two mountainous and plain areas in this coast.

It is recommended to construct halls in special areas with required potentials and facilities to accommodate full and proper facilities for tourists to see the snow in the winter, rain in the fall, and to protect them from the summer heat and humidity where they can benefit favorable and required services. This shall contribute to tourist attraction, income generation, region introduction and creation of new ideas.

[2] Use of natural materials for climatic integration in indigenous, urban and rural communities:

Native dwellings in the north of the country with their distinct appearance, adapted from the nature and geography of the region, respond to the needs of people whose economic activities and bio-culture are different from other parts of Iran. Based on such assumption, use of structural techniques and climate-friendly designs in Gilan will improve the living conditions and decrease the amount of environmental degradation and reduce energy consumption. It is to say that the use of natural roofing material of rhubarb (rice stems), the use of wood of various native species with high resistance to humidity, as dwelling pillars and structure, use of stones in the foundation to strengthen the infrastructure, indicate that the nature provides construction materials and teaches the native communities the building techniques. Also, use of natural roofing material is suitable for temperature adjustment in buildings (the temperature is 5 degrees warmer in summer and 5 degrees cooler in winter under tiled ceilings). It also prevents burning of straws by native communities and its subsequent environmental. Use of indigenous roof structures in touristic complexes, hotels and urban buildings is vital to tourist attraction and conservation of indigenous architecture and environmental considerations.

[3] Recommendation, forecast and change of summer holidays:
May is the best and most beautiful month in Guilan province with a high potential of tourist attraction. However, the official holidays do not correspond to the season. An executive proposal is to consider summer holidays for public institutions in this season and move 10 days of summer holidays into this month and consider a Reading Week Holiday for university students. Such provisions will make it possible to benefit proper temperature, humidity, precipitation and natural scenery to attract tourists.

[4] Use of elevated railroad modern technology:
It is recommended to use modern transportation and road facilities in touristic areas such as Anzali wetland which has natural and unique attraction but suffers road facilities and buffer zones. In mountainous regions and in the foothills, road construction is difficult due to rainfall and humidity and landslide; and it will result in farm and agricultural land destruction; and construction is difficult in foothills due to gradient and forest lands.
Use of motor boats in Anzali wetland causes noise and environmental pollution and construction of roads is not cost-effective and will result in degradation; hence it is recommended to employ a more effective means which is elevated railroad for tourist transportation.

[5] Province's zoning in terms of climate role in tourism development:
Based on the geographic and climate data of Guilan province, several zones are identified. These zones vary in their altitude, topography, climate, vegetation, and crop types. Guilan province consists of the southwest lands of the Caspian Sea; Talesh and Alborz mountains; and three geographic regions: plains, foothills and mountains. The plain area (zone) ranges from 24 meters to 100 meters, accounting for about two-thirds of the province's land, and the largest population of the province resides in these areas. The second zone is at an altitude of 100 to 500 meters and is a subtropical zone, and the third zone is above 500 meters known as the mountainous zone.
For tourism planning purposes, while identifying these three zones, it is suggested to provide coastal and maritime tourism in zone one, which has warm and humid coastal or plain climates.
For the second zone, which is a forested area with a high tide, it is recommended to consider leisure or picnic tourism (short-term tourism).And for the third zone, with mountainous and semi-Mediterranean climate, bungalow tourism (summer travel of the inhabitants of the region).

[6] Optimal use of natural scenery (coastline):
Considering the climatic nature of Iran (dry and semi-arid), the province of Gilan, located next to the Caspian Sea, its unique cover, specific climatic factors, unique Caspian coastlines, different ecosystems (forests, animals), significant climate difference between the plains and the mountains and foothills; large variation in adiabatic law in mountainous areas of the province (Adiabatic decline in the climatology including temperature drop against increasing altitude; that is, a drop of 6 ° C per each 100 meters), whereas the difference in this province is 10 The 12 ° C per each 100 meter elevation makes it unique amongst other provinces.
Considering the existence of 680 km of suitable coastline and 300 rivers running from the mountains to the sea (due to melting snow and successive precipitation), these lands have a special ecosystem. Considering the possibility and potential of proper development in these lands in the tourism sector, it is recommended to take measures to equip the coastline and

construct tourist towns instead of employing destructive technologies and construction of high-end hotels to maintain the natural scenery of the coastline.

Conclusion

The tourism climate conditions at the stations of Guilan province for various months indicate that spring, late summer (early September), early fall and March are the best times in terms of comfort conditions for the arrival of tourists to Gilan province, and due to the diversity The climate in the province and the somewhat different weather of the northern stations of the province with the southern provinces of the province shows that in a general look, all areas of Gilan are considered suitable for tourism.

In the winter, the southern half and the warm semi-northern seasons are suitable for tourism activities.

The country lacks tourism industry goal and policy recognition; some of which are as follows:

[1] Lack of coordination in various decision making and planning authorities.

[2] Pessimism of authorities and local communities against tourism industry.

[3] Lack of technical and experienced experts in various segments of the tourism industry.

[4] Lack of sufficient and quality training institutions.

[5] Negative propaganda of foreign media in an effort to present and unfavorable image of the country to isolate the Islamic Iran.

[6] Lack of required facilities for the import of the industry's essential equipment & items.

[7] Limited accommodation facilities and low maintenance and renovation of such facilities and low urban and intercity hosting facilities.

[8] Lack of relevant statistical inputs and data.

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TERRITORY, GOVERNANCE, LOCAL COMMUNITIES AND TERRITORIAL MANAGEMENT CONTRACTS: CASE STUDY IN SARDINIA ISLAND, ITALY

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Abstract

The authors, through a vision toward new paradigms, joining tradition and innovation, the approach of the Smart Communities and Smart Territories, the bottom-up new model of Territory Governance as the Territorial Management Contracts -TMC's, design a framework of model that combine the territory risk management with solution development of driving and sharing by the local populations. The proactive involvement of population in the TMC's must be managed by the economic instruments. A model to measuring the benefits recognized by the contractors it's proposed crossing the approach of DPSIR- Drivers-Pressures-States-Impacts-Responses, oriented at the Payment of the Ecosystem Services-PES, with the techniques based in the Willing to Accept-WTA such as simplification of the contingent valuation or choice experiments. The authors demonstrate that the TMC's are concrete way to commit the Local Community the ability to design their own future in a Sustainable Strategy approach. The authors present, in this way, their own large experience in the research, than in projects, teaching and training, in this innovative approach that is strictly linked with the targets of the Sustainability Age. They show also the practical case of the *"S'Ortu de Tzviriu"* in Sardinia Island where the Local Community, driving by the QEDORA Association, is ingoing trough the contracting phase, with the Municipality of San Gavino Monreale, to achieve and set up a TMC *Multifunctional Park o Happiness* of *"S'Ortu de Tzviriu".*The implementation of the case study show that the TMC's are shared and democratic repeatable models that could be adopt in all areas of the world.

Key words: *Sustainable Development, Risk Management, Preventive Management, Territorial Contracts Management, Ecosystem Services.*

Introduction

General Framework

The many domestic and international disastrous events, had in the last years and till in the current weeks, proved to us reflect the dramatic situation that is Italy but also in the prevalence of the areas of all countries.

In the face of a disaster, as the limits of politicians and technicians, at a different level, the management begins with the citizens who are forced to solve the problems solely by themselves and/or only with the presence of many courageous volunteers.

This further drama repeats the tremendous difficulty with which states never give up illuminating examples of real-ready preventive and emergency response capabilities. The unimaginable capacity of people's reaction (with their own means, with their desperation, with their own sense of common good, with real application of subsidiarity and bottom-up approach), shows that they have the skills in managing emergencies but in particularly they have awareness of the changing the approach in preventive management. This highlights that people are primarily capable to preventing and make proactive action on the territory that is the only real modality to limit these calamitous events. The financial crisis, which is still struggling in the management of public accounts, not only in Italy but around the world, suggest that the Public Authorities do not have and will not, perhaps for decades, the ability to

activate direct intervention policies in the proper and effective management of the territories. It is time then to shift the attention and methods of managing the territory, in the process of slimming the public presence, by counting the self-management capacity of Local Communities. These have always given and give wonderful examples (see all the Italian system of the Territorial Collective Assets which, among other things, affects 1,800,000 hectares according to the latest Census data in 2010) when it comes to reacting to the emergency but also to the proper prevention.

The territory at this height should be considered as a common good for which the responsibilities of preserving productive capacity, of its physical, historical, cultural, identity and integrity can, with precise rules covering all Common Goods *(Ostrom,1990,1993),* be commended to Local Communities and actors in the area (first of all between them the farmers). As everyone knows, the European Union itself is implementing, with the collaboration of the various States of the Union and the various Local Governments, the Development Programs, with various instruments covering the multiplicity of sectors and European society as a whole the period 2014-2020.

The Unique Strategic Framework emanates the various Funds (ERDF, EAFRD, ESF, etc.), which put the focus of the various strategies on economic mechanisms that encourage active participation (to the objectives of various policies) of citizens and communities, guaranteed to operate with "good practices" for achieving those goals.

This analysis leads directly to the possible use, within this framework, of tools that, through the Mechanisms of Participated Programming, the Public and Private Management Contracts for the approach of Preventing Territory Management

Territorial Contract Managements and Ecosystem Services

The idea of the Territorial Management Contracts-CGT has been conceived and analyzed since some years *(Ciani & Oth., 2012)* consists in establishing, within a well-defined regulation context, a contract between rural operators (particularly farmers) and the Institutions. Premises that promote all the necessary actions for environmental and territorial protection (drainage, cleaning of ditches, construction of dams and bridle, soil consolidation, planting and management of tree boards, management of the undergrowth, etc.) both on their properties and those bordering on each individual or group of rural operators. The actors adhering to a TMC's are engaged in carrying out the activities described above, in compliance with precise technical parameters, for which they should receive a tangible direct and/or indirect benefit that may be related to the area concerned, to the degree of risk of the area, and the monetized benefits rendered in terms of conservation/creation of ecosystem services *(Reed M.S. & Oth, 2017).* The TMC's must be "strictly localized", promoted and implemented on the basis of the specific characteristics of the territory to which they refer. The case *"S'ortu de Tziviriu"* in San Gavino Monreale represents a concrete reality on which to implement an Experimental Pilot Project of the TMC's.

The study case

The *S'Ortu de Tziviriu* project was born in August 2016 with an active citizenship action driven by the *Association of Social Promotion Qedora* which involved the inhabitants of the neighborhood adjoining the urban garden, through the realization of participatory planning meetings.

From these meetings the project took shape, which involves the construction of the first food forest of Sardinia on public area, through a continuous, periodic and structured participatory planning.

S'Ortu de Tziviriu is located in Sardinia, in San Gavino Monreale (SU) in an area owned by the ~~Municipality.~~This area was completely abandoned and was sometimes used as an illegal

landfill, constituting a significant environmental risk rather than a heritage available to citizens. The surface directly interested by the *S'Ortu de Tziviriu* is around 0.5 ha. The total area of Municipality is 8,750 ha. In territorial management level this is linked with the Pardu stream for about 5,500 meters of length where 200 meters border the *S'Ortu de Tziviriu*. The area bordering of the small river is in total lack of maintenance and very close to the historic center and civilian homes. In 2014 was affected by flooding as a result of exceptional rains occurred in Sardinia. In the current year the water level of the channel has reached the high limits.

S'Ortu de Tziviriu represents a green space for socialization, responsibility, attention to public affairs as well as for pleasure and encounter in a natural environment. On this structural and social basis, the idea of implementing the Territorial Management Contract-TMC is being set up as the *Multifunctional Park of Happiness S'Ortu de Tziviriu*.

Targets, Materials and Methods

The paper have the goal to verify in a concrete case if the theoretical approach at the governance of territory trough the Territorial Management Contract-TMC's could be implemented as the best practice to achieve, in the Sustainable Development Strategy *(Sachs, 2015; U.N., 2015)* the implementation of Smart Territories and Smart Communities*(E.U. Commission, 2012; Ciani & Oth., 2016)*

The materials used are two different types. First at all the research was directed to achieve an updating framework of reference concerning a global vision on modern and innovative territory governance. After this, consequently at the previous activities of the Qedora Social Promotion Association, was established a *Territorial Social Inclusive Laboratory-Te.S.I.La,* with three days activities in December last 2017.

The core of the activity was arranged involving S'Ortu de Tziviriu Operators and Citizen Community, with an Exercise Training for Participatory Evaluation of Ecosystem Services *(E.U., 2014)*.

The purpose of this work is the economic evaluation of the ecosystem services of S'Ortu de Tzviriu, or of all the benefits that this sit on man *(E.U., 2013).* The evaluation aims to improve the management and conservation strategies of the naturalistic and territorial common good, in accordance with the value that the citizens perceives for them *(Italian Parliament, 2015).*

For the determination of the ecosystem services value was adopted the criteria in the approach of the Willing to Pay-WTP or Willing to Accept-WTA and simulate, in a way of the of the revealed preferences of the consumers *(Reed, 2017)*

In this way was also experimented an original contribution of the research. The criterion where the benefit that citizens can assign to the ecosystem services and they conserver or create, is equal to the value that they accept as payment at the hours of work award in the context of participation in the TMC. The substitute labor market is used in this case for the purposes of evaluating the ecosystem services. For the simulation of the substitute market was create original questionnaire that is divided in three parts; the first is an introduction at the environmental and territorial question; the second is a description of the ecosystem services and their value; the third with the demand how, about the number hours of work that each participant is available to award at the TMC**,** and how much he can accept as payment for their same work. With the simple weighted arithmetic average was calculated the WTA payment of working hours by the participating Community to the experiment.

Results and discussion

The Territorial Social Inclusion Laboratory-Te.S.I.La of San Gavino Monreale has had the participation of various community stakeholders and among them 25 accepted and signed their own filled questionnaire. The following data emerged from the analysis of the same. In reference to the willingness to offer their hours for week for the 52 weeks for year, it was an average of two hours a day but for 1.6 times a week. The average remuneration expected and acceptable for one's work hour is on average 1.4 Euro. Overall it emerges that the value of one's annual work offered for activities related to the Territorial Management Contract of S'Ortu de Tziviriu is 226 Euro per year. On the basis of the methodological prerequisites adopted, it therefore emerges that the value of ecosystem services that citizens estimate that they are maintained or created for their activity within the TCM of Multifunctional Park of Happiness S'Ortu de Tziviriu is equal to € 226 per year. According to this value creation, the benefit that the TMC should assign them, through the public intervention, that could be by direct payment or indirect benefit (lowering taxes, supplying services, etc.). As a final act of the activities of the three days there was a final Conference at the end of which the participants prepared a Declaration of San Gavino that in the final part committed the Qedora Association and the Municipal Administration to sign the Declaration itself and to stipulate a Memorandum of Understanding for the implementation of the Territorial Management Contract Project for the creation of the Multifunctional Happiness Park of S'Ortu de Tziviriu in San Gavino Monreale. On May 20, 2018 the Memorandum was signed and a working group headed by the Qedora Association will have to deliver the Project Proposal within 90 days. Concerning the the concrete implementation and perspectives of the Project was prepared the SWOT analysis (Fig. 1).

Strengths	**Weakness**
Leading of the Qedora Association High sense of territorial identity Historical Linkage People Land Awareness of the problems of the area Availability for Active Participation Propensity to the Valorization of the Territory Awareness of the value of Natural Capital Local Administration Availability Previous experience of administrative bartering Territorial Value of San Gavino	Human Resources in the Municipality Not Broad Band Not adequate friendly dissemination of ICT Weak Territorial Policy at the Regional level Loss of good agricultural practices Tradition that prevails on Innovation Financial Resources National Policy and Role of Local Community Job supply / demand system Work safety regulation
Opportunity	**Threats**
Transforming San Gavino in a Smart Territory Transforming San Gavino in a Smart Community Growing of the resilience of the City of San Gavino Improvement of the identity of the territory Improvement of the risk territorial management Improvement of the image of the City Improvement of the family budgeting Improvement of the quality of life in area Increasing of the small business network in the area Development spirit and commitment	Termination of interest of the people Competition Failure of the local participation Not activation of the Local Community Failure of the Pilot Project Gap between policy and not enough financial resources Loss of the traditional behavior and identity Gradually broken of the social cohesion in the Villages. Failure or delay of the Broad Band Failure of transparency in the Management of the TMC

***Source:** Author s' elaboration based on the obtained result.

Figure 1._SWOT Analysis of the Territorial Contract Management of Multifunctional Park of Happiness of S'Ortu de Tziviriu in San Gavino Monreale

Conclusions

The territory at this height must be considered a common good for which the responsibility of preserving the productive capacity of their physical, historical, cultural, identity integrity can, be referred to the Local Communities (first of all farmers).

It is way it's time to capture the New Paradigms and New Approaches that the Territory has to offer. Among the New Paradigms we can include, in addition to the four related at the Sustainable Development strategy, those as following:

- *Territory is a living entity consequently at the vision as book to be able to read;*
- *Territory is the reservoir of resources to be put into the cycle of a sustainable production system of goods and ecosystem services;*
- *Territory is an intelligent cognitive system that educates and learning with a smart use of the ICT;*
- *Territory is specificity, rarity and specialty but social inclusive*
- *Territory is "TRADI-OVATION" (intended as an acronym for "Territory, Rural Areas, through Development, Innovation, Organization, Valorization, user friendly Technology, ICT sharing, Online Networking)(Ciani, 2012, 2015). In this sense is the key word and the main theme around which any innovative process can be structured to give effective credibility and to erase the increasing disillusion that follows major international meetings, in order to move from words to actions and create the foundations for the New Model of Management and Promotion of the all Territories.*
- *Territory send us the option to Overcoming of the Labour Systemic Prison by the Free Time and the Recovering of the Environmental Space*

As everyone knows, the European Union itself in 2014 began a process to develop, with the cooperation of the various States of the Union and of the various Local Governments, Development Programs, as a whole, for the period 2014-2020.This analysis leads us directly to use as possible, within this framework, tools that provide, through the mechanisms of Participatory Planning, Management Agreements between the Public and the Private to the preventive management of the territory. On these principles underpinning the strong assurance that the Territorial Management Contracts-TMC's can truly guarantee a new season at the Country for a preservation of its territory, to be a fundamental part of people improvement the economic, environmental and social status. Territorial Management Contracts-TMC it's a proposal with a vision of the General Contract/General Program between the Local Communities and Public Administrations within which the various aspects (rivers, lakes, landscapes, forests, etc.) can and must be taking into account. If this is not the case, we will soon be in a position to launch, especially in the most innovative ways of innovation, such a set of contract hypothesis that will only lead to conflicts between the various sectors/contracts. The model of organization, evaluation of benefits to assign at the participant at the action and the choice of the better alternative of TMC's, from the theoretical point of view, in the paper have achieved strong and solid inductive and deductive consistency. The case study of the TMC of *Multifunctional Park of Happiness S'Ortu de Tziviriu* say us that we can do and for that we must do. Some concrete cases, in the other different areas, appear suitable to implement with possible others experimental Pilot Projects. The idea of TMC's is therefore to transform the weakness of every area due to high risk, into an intelligent, inclusive, sustainable, transparent, proactive, concrete opportunity. To achieve for all rural areas of the world the character of Smart Communities and Smart Territories. To abandon the Gross National Product Approach Toward the Gross National Happiness Strategy.

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AGRICULTURAL AND PISCICULTURAL ACTIVITIES IN THE BAHOUAKAHA LOWLAND PREPARED FOR THE FAILURE OF THE TINE HYDRO-AGRICULTURAL LACUSTRINE SYSTEM (IVORY COAST)

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Abstract

Lakes of hydro-agricultural dams are technical innovations developed by the Ivorian State in the countryside of the north of the country in the 1970s. The presence of these water reservoirs has helped to boost agricultural production and to begin an extension of aquaculture activities through the construction of various fish farms. Today, however, the dam of Tiné in the sub-prefecture of Bahouakaha, like many of these works, is in a poor state of operation due to a lack of regular maintenance of its facilities. This reality negatively impacts the agricultural activities practiced in this lowlands of an area of more than 200 hectares. The objective of this study is to examine the consequences of the failure of this lake development on the cultural and halio-aquaculture activities that depend on it. The methodological approach is based on an exploitation of the documentation relating to tributary activities of fluvio-lacustrine environments. Semi-structured interviews and direct observation of the facts were also used during the field surveys. It appears that the dysfunction of the lake supply system has led to the closure of a state-built fish farm in the lowlands. Currently, a water leak due to the damage of the stop valve, at the monk tower of the dam, affects rice and vegetable productions. This situation undermines the livelihoods of user communities and threatens the food security of local populations.

Keywords: *Ivory Coast, lacustrine system, agricultural activities, fish farming, failure.*

Introduction

In its development policy for the northern part of its territory, the Ivorian State has provided it with several small artificial reservoirs for agro-pastoral activities and drinking water supply. These dam lakes are concentrated in the current regions of Bagoué, Poro and Tchologo with a high density in the department of Korhogo (37% of the reservoirs). The Northeast and Northwest have only 9% and 1% (Anonymous, 1992). Thus, although "unequally distributed throughout this geographical zone" (Da Costa et al., 2004), these hydraulic developments constitute "technical innovations" (Akindès, 2007) that have favoured the increase in agro-pastoral production and fish extension. For Le Guen (2004), the hydro-agricultural dam is adapted to this zone because it favours the cultivation of floodable land all year round, intensive rice production for self-sufficiency and substantial income for the Senoufo indigenous populations. Consequently, it is "indisputable that the agricultural and pastoral policies developed at the beginning of the 1970s" by the realization of these works "engaged the "Northerners" in new dynamics of exploitation of the terroirs" (Le Guen, *op.cit*.).

It is in this perspective that the lake of the "*mixed use*" dam (Silué, 2012) of Tiné was created. Its creation was followed by the creation of a fish farm and the development of lowland for cereal and market garden crops. Despite its capital role in the agricultural system of hundreds of rural actors, this water infrastructure suffers from the dilapidated state of its installations whose various failures threaten the sustainability of activities; hence the following concern: how does the lack of maintenance of the Tiné hydro-agricultural dam affect agricultural, halio-aquaculture and pastoral activities?

This article examines the consequences of the failures of the Tiné hydro-agricultural dam lake on rice, vegetable, fish and pastoral activities in this zone characterized by a long annual dry season. The identification of the impact of the failures of the Tiné lake system on the activities of the operators is thus presented as a decision-making aid for regular maintenance of its installations. The methodological approach for achieving the objective of this study is as follows.

Material and methods

The Tiné hydro-agricultural dam was created in 1974 by SODERIZ (Company for Rice Development) on the Kô river, a tributary of the Solomougou. It is located in the department of Korhogo, a dozen kilometers from the city (capital of the Poro region, Ivory Coast). It generated a 45 hectare lake used for irrigation of rice and market garden plots, livestock watering and halio-aquacultural production (Silué, 2012; Traoré, 1996). A lowland of more than 200 hectares developed downstream of the structure extends over the terroirs of about twenty villages over a distance of about 12 kilometers. It is exploited by several hundred rice farmers and market gardeners. The study was based on documentary research, field observation and semi-structured interviews with "key informants" (Gumuchian and Marois, 2000), involved in the operation and management of the two spatial entities analyzed (the hydro-agricultural dam and the developed lowland). Secondary data collection was done through the use of literature on activities subject to fluvio-lacustrine environments and permitted to refine the study variables. The field survey was conducted in Pégnankaha and Bahouakaha, two rural localities in the dam's area of influence. The first community is adjacent to the lake and the second, further downstream from the water reservoir, contains part of the developed lowland. It is the headquarters of the Wowêdjo cooperative, the only rice cooperative with 404 members in 12 villages, operating in the Tiné lowland. In Pégnankaha, the village chief, two notables and the former manager in charge of the stewardship of the community fish farm during its operation were interviewed. In Bahouakaha, talks were held with the cooperative's managers and three market gardeners. Discussions focused, among other things, on the types of relationships of populations to dams and lowlands, the management system put in place, the type of problems related to their management, the reasons for the dysfunctions observed, the types of activities dependent on the lake, agricultural calendars, the quantities produced per season, the purpose of production, revenues and their modes of use. Field observations, begun in January 2017 as part of a research program on "Water and socio-economic development in the Poro region", permitted to monitor the spatial dynamics of agricultural activities around the lake and in the lowland. Regular bi-monthly visits to the study sites helped to understand the evolution of the situation at different periods of the agricultural production cycles and to observe the usual cultivation practices of the stakeholders. This approach was particularly useful to apprehend the changes that occurred in the developed perimeter, between the normal operating period of the dam and the one characterized since January 2018 by the water shortage in the reservoir following the damage to the shut-off valve of the monk's tower. The processing of the data collected has led to the following results.

Results and discussion

When it was created in 1974, five main activities were directly dependent on the Tiné water reservoir: rice growing, market gardening, livestock farming, aquaculture and fishing. The first two activities are mainly practiced in the lowlands, on plots of about 35 m^2. If, for socio-cultural reasons, rice cultivation is only the business of men, market gardening brings together actors of both sexes. In addition, rice and vegetable crops are mainly produced for marketing. Only small quantities (about one fifth for rice and less for vegetable products) of production are self-consumed by farmers and their families. This observation is confirmed by Fromageot (2008) who notes that since the 1990s, the production of fresh vegetables for the cities of the Ivorian coast has spread to the Senoufo savannahs on the Ivorian-Burkinabe border. Moreover, in terms of the number of farmers, market gardening is the main activity practiced in the lowlands because almost all households are engaged in it.

The waters of the lake provide water for the herds of village cattle. Aquaculture was present there until 1983 through the construction by the State, a little more than two kilometers downstream from the dam, of a fish farm with 14 ponds as part of the extension of this activity in the region.

The development of lake fishing has benefited from the introduction, by the Tropical Forest Technical Centre (CTFT), of several dozen juveniles of *Lates niloticus* from a trial to control the tilapia population in ponds (Kouassi et al., 2007).

This diversity of activities led Silué (2012) to qualify this hydraulic development as a "multiple-use" dam compared to four other "single-use" types. These, also present in this northern region of Ivory Coast, are intended either for the irrigation of sugar cane and rice crops, or for livestock watering or drinking water supply (Silué, *op.cit*.).

The first dysfunctions of the Tiné lake system occurred in 1978, 4 years after the dam was put into service. They concerned the water supply canal of the fish farm. This structure, a little more than two kilometers long, was filled under the combined effect of erosion (due to agricultural overexploitation of the land upstream of the canal) and a lack of maintenance, resulting in the cessation of water supply, as well as farm activities in 1983. There are no figures on the production and marketing of fish from this fish farm during its years of operation. However, it is certain that the issues of promoting the activity among local populations, the spatial dissemination of farming techniques, and the production of fish for self-consumption and sale on the local market, which guided its realization, like other farms of this type built at that time (Anonymous, 1999; Gosse, 1984), could not be reached.

Despite this first setback, the dam facilities underwent only one maintenance period in 2000, during the four decades of operation. The durability of a hydraulic structure being a function of the design and construction work but also of maintenance (Lassailly-Jacob, 1984), the control stem of the shut-off valve failed due to lack of maintenance in January 2018. This mechanical failure caused the reservoir to dry out completely following a continuous flow of water from the lake in about fifteen days. The consequences of this new failure were almost immediate and perceptible at various levels: disruption of crop calendars, loss of rice and market gardening production and income, cessation of fishing activities and migration of fishermen, modification of the village livestock watering route.

The variety of rice grown in the managed lowland of Tiné is *Wita 9*, an improved seed whose production cycle is 3 months with an average yield of 7.5 tons per hectare. To do this, the irrigation of the plots depends essentially on the proper functioning of Lake Tiné, which is filled during the single winter season (May-August). The annual practice of the activity is organized in two seasonal cycles (first cycle in yellow; second cycle in green) spread over the two respective semesters of the year (Table 1).

Table 1. Annual crop calendar of the Wowêdjo rice cooperative

	Period (month)												
Main activities	J	F	M	A	M	J	J	A	S	O	N	D	Observations
Soil preparation													
Nurseries													
Transplanting													
Weeding													
Cover fertilizer													
Harvest													Two Harvests
Climatic seasons	Dry S				Rainy S				Dry S				Two Seasons

Sources : Field Investigations, 2017-2018 ; Silué, 2012.

In the usual operation of the dam, the availability and regulation of water distribution make it possible to irrigate all the 170 hectares of plots cultivated by rice farmers. However, the use of water for rice production is subordinate to the climatic seasons. Thus, compared to the first cycle, production in the second cycle (July-December) is less dependent on lake water. The first growing cycle takes place in 2/3 of its period from January to June, in the dry climatic season. It is, therefore, intrinsically linked to the water retained in the dam basin during the previous rainy season. On the other hand, the second cultural cycle depends much less on it. Indeed, it starts in the middle of the rainy season and is half covered by the latter whose rainfall peaks are observed in August. This situation allows a rationing of the lake, the plots also benefiting from rainwater during this period.

The vegetable production calendar (tomato, cucumber, cabbage, zucchini, salad, aubergine, etc.) is also divided into two cultivation cycles based on the two semesters of the year. The total area of the lowland used for market gardening varies from 40 hectares in the dry season to about 15 hectares in the rainy season. This reduction in cultivated area is due to the fact that during the second cycle, upland crops such as cotton, maize and groundnuts are favoured by the actors to the detriment of vegetable crops.

In addition, market gardeners are produced on plots that are not used for rice cultivation and therefore not covered by the irrigation canals of the rice plots. As a result, they are watered daily from hundreds of shallow (1.5 m) wells set up on the farms. The permanence of the lake water upstream favours the availability of well water throughout the year.

From the above, it appears that the loss of all the water from the dam due to the failure of the shut-off valve is detrimental to the rice and market gardening activities practiced in the lowlands and thus to the stakeholders. Indeed, during the first half of 2018, the irrigation of the rice plots, essential at the beginning of the first cycle, could not be done for lack of water. The soil shriveled by the long annual dry season could not be prepared for sowing as well as nurseries; hence the lack of production, thus the absence of income for farmers. For example, in 2017, with normal operation of the dam facilities, the total rice production of the Wowêdjo cooperative for all two annual cycles was 1,445 tons (850 tons in the first cycle and 680 tons in the second cycle). For the first harvest, the price per kilogram of paddy rice fixed by the State to producers ranged from 150 FCFA (0.23 €) to 175 FCFA (0.27 €). On this basis, the average market value of the Wowêdjo cooperative's first cycle production was 114 750 000 FCFA (174 935, 25 €). In the second cycle, this value was 85 000 000 FCFA (129 581,66 €); the price per kilogram having been fixed at 125 FCFA (0,19 €). As the shut-off valve has not been repaired so far, significant revenues are at stake for the hundreds of operators. Moreover, this reality largely conditions the possibilities of execution of the second cycle of the year 2018, by the hope of an abundance and regularity of rains during the annual winter season. According to the farmers, this eventuality remains hypothetical, all the more so as the rainy season only partly covers this second crop calendar and only one of the three months of the

growing cycle of the cultivated rice variety. This result shows that the lack of maintenance of hydraulic equipment is, like the lack of control of irrigation techniques by producers, one of the obstacles to achieving intensive production with biannual cultivation cycles (FAO, 2005).
The impact on market gardening activities was equally significant in the first production cycle. In the absence of lake water, the long eight-month dry season caused the watering wells to dry up. Faced with this constraint, most operators have had to deepen their wells as a means of resilience. This solution, however, proved derisory as the dry season continued. Thus, the difficulties experienced by the unavailability of water in the watering wells of crops led to the stopping of farms or poor production during the first crop cycle. However, the second cycle could be better if winter rains are sufficient to allow efficient groundwater recharge in the lowlands and permanent water availability in wells.
Usually, five artisanal Bozo fishermen, of Malian nationality, exploit the fishery resources of Lake Tiné. These professional fishermen market their production on the "fish market" (Koudou *et al*., 2015; Kouassi *et al*., 2007) in the town of Korhogo. The absence of water in the lake led to the interruption of their activities and their departure towards the Tongon gold mine reservoir located about sixty kilometers from Korhogo, their place of residence.
Since its impoundment, the Tiné dam has been the main daily watering site for cattle herds in the riverside villages. Its accidental drying up, in the middle of the dry season, forces the shepherds to modify their grazing paths towards other more distant aquatic spaces, notably those of Sédiogo (5 km), Sinématiali (12 km) and Lokpoho (18 km), already occupied by other actors. This situation is likely to give rise to the risk of conflicts with other breeders or farmers whose farms are established on these new routes or border these water points.

Conclusion

The consequences related to the failure of the Tiné hydro-agricultural dam facilities are significant and diverse. These include disruption of cropping schedules, loss of production, loss of farmers' rice and market gardening income, cessation of fishing activities, migration of fishermen and modification of livestock watering routes in riverside villages. This situation shows that the sustainability of the valorization of this water infrastructure through the permanence of agricultural, halio-aquacultural and pastoral activities can only be possible through regular maintenance actions of its installations.

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MODELING FOR IMPROVED WATER HARVESTING AND INVESTMENT IN RANGELANDS AREA IN JORDAN

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Abstract

A national environmental socio-economic project was conducted In October 2017 in a rural village named Era in the north western part of Jordan . Era is considered as an agricultural and rage land area. It has good soil fertility, suitable environmental conditions in addition to relatively high rain water precipitation, which encouraged local community to work in different farming activities either planting or grazing. Last years, due to climate change and biodiversity retardation; rain fall in the area became low which reduce the amount that can be available for plant growing. Local farmers adopted traditional agricultural practices gain low income retain. This leads to shifting the farming work to wives and youth while men are usually go to their formal work in the city. It was of great important to target women and youth in training project deals with new agricultural techniques overcome the effect of climate change. A pilot cooperative project was established by the effort of local community, charity association, university consultant and funded by UNDP-Jordan. The project dealing with training 200 individuals of local communities' beneficiaries from which 70% of them are women. The project set ups for rangelands and drought management system with several practices; rainwater harvesting, soil quality analysis, area productivity improvements, adopting nil tillage or zero tillage, introduction of new planting technology; as hydroponic and aquaponics. The training of the concerned participants tailored to ensure that those trainees will be able to transfer the knowledge and skills gained to others in the community or even other interested in the near areas. The projects ensure providing the area with demonstrative pilot project which is considered as a field school for others.

Keywords: *Rage land, Biodiversity, Pilot project, Hydroponic, Water harvesting, Local communities, Rio convention*

Introduction

Jordan is one of the middle east country with a total land area of approximately 90,000 km^2. Most of the land is desert (El-Naser et al. 2014) and the country is landlocked except for a 30 km strip of coastline along the Gulf of Aqaba. It is weather characterized as arid to semi-arid with about 200 ml of average rain fall, forests covering less than 1.5% of the country's land area. Given its relative size and position in the heart of the middle east, Jordan is particularly vulnerable to external political and economic influences (Risk, 2013). Era is a rural village of about 5 km^2 area within the territory of Balqa Governorate in Jordan. It rises approximately 580-620 meters above sea level so it considered as semi Ghour area according to Jordan geographical mapping (figure 1). It is characterized by its moderate atmosphere in all seasons of the year with about 500 ml of precipitations during winter season. It is mediated between the capital city Amman and the central agricultural area of Jordan valley with a population density of about 985 individuals/km^2. The mountains surrounding Era are covered by Aleppo pine and oak trees, which have a unique aesthetic dimension and are visited by tourists in the spring. Several herb plants, including medicinal herbs used in natural therapy are present as native plants, in addition to the spread of black Iris known in Jordan as national flower which reflect high biodiversity in the area. In domestic area olive trees are dominated in addition to

various seasonal traditional crops such as wheat, barley, lentils, chickpeas, and rainfed vegetables such as zucchini, okra, onion, garlic and potatoes. A long time ago, local community work in various agricultural activities, either agriculture or grazing. Climatic variability and frequent droughts in addition to high rates of population growth further intensify the pressures on natural resources throughout the area. The most of Era men left the village to work in governmental jobs which leads to rural area immigration. The farming works in Era is mainly family work, making wives and youth participate in different farm and agricultural activities. The combined effect of these natural and anthropogenic factors has often led to the utilization of unsustainable farming practices, resulting in the continuous degradation of agricultural soils and desertification of previously productive lands. Since this area near Al-Balqa Applied University which has a strategy to play a critical rule in technology transfer and rural area development. A cooperative work between Al-Berah charity association (31°59'07.8"N 35°41'28.9"E) which is located in Era area and a researcher from Al-Balqa Applied university funded by UNDP Jordan as a modeling project for mainstreaming Rio convention provisions into national sectoral policies. The project was deals with establishing rain water harvesting system and invest the harvested water in planting. On a selected area of about one hectare the model was established and training section were applied. The main core of the project was to train local community people targeting women and youth on using harvested rainwater in hydroponic system under greenhouse conditions as one of new environmental socio-economic novel technology in rural area (Haddad, M. *et al.*, 2009) and adopting zero tillage in open field area with introduction of new foraging crops to the area. That help justifies the use of water and increase the efficiency of its use. This is a first project in Era area deals with comprehensive agronomic-environmental-economic nature, which will, therefore, be of high relevance for the agriculture sector in the rural area in Jordan.

Figure 1. Era area in which Al-Berah charity association is located 31°59'07.8"N 35°41'28.9"E.

Materials and Methods

Pilot Plant Setup: The pilot plant is located in Al-Berah association within one-hectare area. About 10 km west-southern corner of Al-Balqa applied university campus. The pilot consists of a rain water harvesting tank that is collecting the rain water from over 300 m^2 surface area of Al-Berah association building. The total capacity of the tank is about 16 m^3 of water. The collected water were oriented for the planting area, which contains a model of greenhouse hosting the hydroponic system, and an open area for bica planting *Vicia sativa*. The green house model is 9 m wide, 36 m long, and 5.5m high, made of galvanized steel pipe frame and

covered with plastic layer. Hydroponic system was established in the green house by building concrete ponds inside the greenhouse (Figure 2).

Figure 2. Green house preperation for hydroponic system (a) Ponds are made in double lines inside the greenhouse with four section per unit leading to 8 separated ponds which is filled with irrigation solution as flooding and air pump is used for aeration (b)

Ponds are coated with anti-corrosion layer, with length of 27m, width of 3 m, and height of 35 cm. The ponds are made in double lines inside the greenhouse with four section per unit leading to 8 separated ponds which is filled with irrigation solution as flooding and air pump is used for aeration (Figure 3). The open reuse area consists of an area extending for 1 hectare which is used annually for parley plantation by local farmer, he used to incorporate it with tractor then plant it with barely seeds as rain fed area. As a part of the project this area was not cultivated and planted with *V. sative* seeds as alternative method and crop for such a pastoral area.

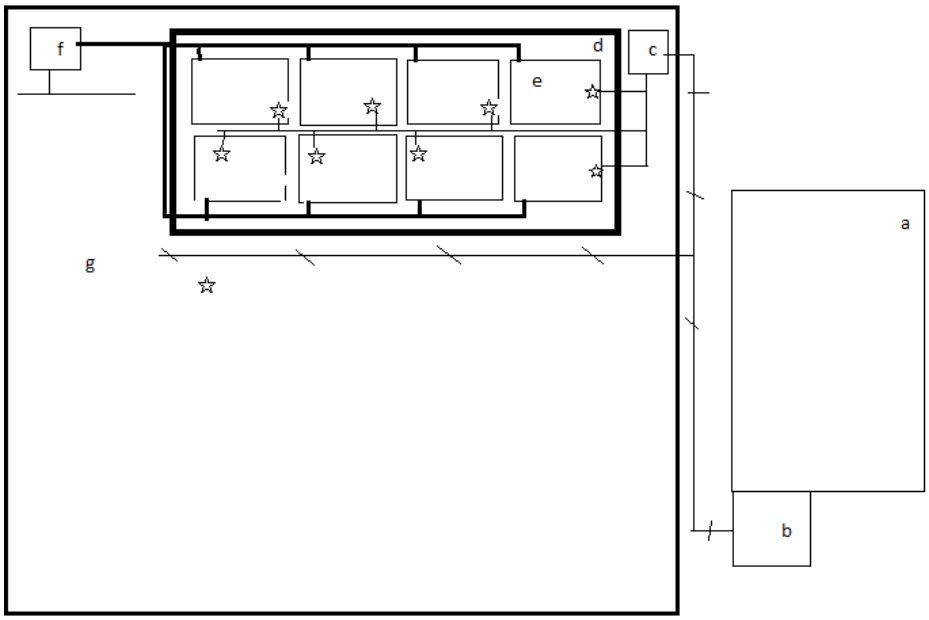

Figure 3. Schematic of Pilot Plant rain water harvesting tank, green house with Hydroponic system with horizontal channels covered with floating polystyrene sheath, which is the carrier for plastic cups filled with 1:1

perlite: petmoss containing vegetables or herpes transplants. The channels are without media allowing moist and wetting atmosphere for the roots of the plants, each channels has a pump for recycling of hydroponic solution and aeration. a: Al-Berah association building as water harvesting space b- water harvesting tank, c: hydroponic solution preparing tank, d: greenhouse e: Hydroponic channels, f: filtration and drainage tank g: open field area. Stars represent bumps for recirculation of water and aeration.

Used Plants: In the hydroponic system most of the plants was vegetables but with introducing new crops to Era area such as celery, red cabbage, and iceberg lettuce. For the open field, we also introduced new foraging crop *V. sativa* which is from legumenacy to improve soil fertility in addition to its high value as feeding crop.

Capacities developed for the conservation of rural area environment: Enhance awareness and understanding of social actors think critically on how to contribute to the sustainable development process and national development priorities to faces challenges with water and resource scarcity shortage of arable land and with accelerating desertification, soil degradation, and deforestation by engaging large numbers of local communities including youth, women, academia, and the private sector in capacity building and training sections for 6 months' period such as:

1- Water harvesting system design.

2- Adopting new agricultural methodology: zero tillage, below soil drip water irrigation and Hydroponic.

3-Chosing proper crop according to its requirements and its revenues.

4-Agricultural crop waste management.

5- Training workshop on integrated resource management in the training model.

A total number of about 200 individuals of local communities most of them are women were getting this training courses (figure 4). Each course has its own pre and post evaluation form to assist the needs and evaluate the output of the course. Monitoring and evaluating plan was conducted every two months of the project to improve the activities when required for more goals achievements. Practical training was applied for each training course under the field conditions.

Figure 4. Field visit and training on hydroponic concept for middle girl school students at Era area

Results and discussion

1. This project was its first one of its kind in Era village, it needs a condense announcement and encourage information to motivate the local community in participating in its different activities. The most important way for spreading the information about the project and its important was by using social media channels as Facebook, Wats up and Al-berah Association website. After one month of introducing the project the participating of local communities' members starts to elevated and their interest in activities became higher. Some

of the participant start adopting new activities in their farms and home gardens as water harvesting, hydroponic and recycling concepts. As a first season of the project, the total collected water in the harvesting tank was about 90 m^3. This water was used in hydroponic system which is succeeds in production of almost all the transplanted crops: celery, red cabbage, and iceberg lettuce (figure 2). Due to short production period in comparison with traditional method there was three planting rounds within 6 months of project period. We use the fourth round of planting native herbs such as thyme *Thymus vulgaris*, sage *Salvia officinalis*. The quality also of the products was very high since we did not use chemicals as pesticides or herbicides, it is considered as organic plantation (Ferguson *et al.,* 2014). Currently irrigated agriculture consumes over 60% of Jordan water budget, however severe competition between sectors for water is only expected to increase (El-Naser, Telfah, & Kilani, 2014). To maximize the efficiency of used water we are working now with consultant from Al-Balqa Applied University to connect the system with a filtration unit, which can improve the quality of the drainage water and facilitate its reusing in open field irrigation (Kumar *et al*., 2014). For field plantation, the land give good growth of vicia but it needs more time to study the difference between feeding animal on barely and that fed on vicia as main source.

2. Jordan has identified water and a number of factors including habitat degradation, pollution, and intensive use of agrochemicals threatens energy as the priority issues for development, the country's biodiversity. Furthermore, agricultural land, forests, and other protected areas are being lost to the growing urbanization and request for the inadequate resources. According to the UN Country Assessment, (UN, 2011) Given the challenges facing the country, Jordan has confirmed its commitment to improving governance and strengthening sustainable management of natural resources and the environment. The goal of this project is to deliver environmental benefits through more holistic and effective management of the natural environment to meet national socio-economic priorities. Growth and development are inclusive and sustainable, incorporating productive capacities that create employment and livelihoods for the poor. Key government and non-government actors have capacities to undertake gender-sensitive management of natural resources in a climate-resilient manner in targeted governorates. Operationalize national green economy action plan in a gender sensitive and inclusive manner. Integrate disaster risk reduction and climate change adaptation into strategies and operational plans at national and local levels. Best practices and innovative approaches for its activities include awareness-raising and education, capacity building, public policy advocacy, and business networking. Improving knowledge management, education, and awareness-raising are crucial elements in the sustainable development. Technical training and technology transfer, using research for policy-making; academia, private sector, and civil society integration to identify potential innovative to solve critical socio-economical environmental problems. Local community empowerment; emphasize their demonstration and build the capacity of local governments and communities to operationalize mainstreamed sector development plans. Horezental mainstreaming for environmental social activity will be the base point to be completed by vertical mainstreaming to rech sustainable development (Nunan, *et al.,* 2012) Resource mobilization assess long-term opportunities to mobilize resources to replicate and sustain project outcomes.

Acknowledgment

This work was a result of extensive work of Al-Berah Association with local community, the association tackle the social responsibilities for adopting the manipulation of such a project in Era village and use all its social communication to encourage local community individuals to participate in project activities. The genesis of this work would not have been possible without the dynamic and effective financial and technical support of UNDP-Jordan

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FINANCING AND LENDING TO A FARM IN LATVIA

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Abstract

Financing a business encompasses all the activities necessary to provide the enterprise with finance. This represents the process of acquiring and spending funds and making investments, as well as the cash flow. Farms could use both internal and external sources of finance. The research aim was to identify the sources and amounts of finance that ensure the successful operation of farms. The research was conducted on a medium-size commercial farm "Saulaini". The farm had 190 hectares of agricultural land. The key kind of its business was milk production. The farm "Saulaini" had access to and used both kinds of finance. Since the establishment of the farm in 2001, the total investment made in the business has amounted to EUR 317.6 thousand, of which 37% was internal finance. The farm attracted external finance (approximately EUR 200 thousand) – a loan and EU funding – in order to purchase machinery. The production costs of the farm "Saulaini" exceeded its revenues and the farm made losses. However, EU support payments improved the financial performance of the farm. In 2016, the farm received EU financial support in the amount of EUR 46.7 thousand. Effective financial management on the farm contributed to its high liquidity ratio and high rate of return on capital.

Keywords: *financing, lending, farm, sources of finance, EU support payments, Latvia.*

Introduction

After developing a successful business idea, financing the business is the next most important step – the way how to acquire and invest funds in order that the enterprise can expand its economic activity.

At present, there are quite a lot of opportunities for acquiring finance to start up a new business as well as to develop existing enterprises. The key is to assess one's own abilities, then to seek creditors and investors and analyse whether it is possible to acquire national or European Union financial assistance.

Enterprise finance is a system of a legal entity's financial relations. The enterprise makes financial relations with capital owners, creditors, enterprise employees, national institutions, investors and business partners.

Financial relations have a common feature – a cash flow –, which is some kind of circulatory system for the enterprise, and the viability of the enterprise depends on the cash flow. The key task of financing an enterprise is to make a rational choice of sources of finance to finance its expenditures and achieve the desired revenue level. One of the key reasons why a well-operating enterprise needs additional finance is the expansion of its activity, requiring additional current and fixed assets.

Materials and Methods

The present research performed a review of the specific literature and used information from scientific databases (Science Direct). The research analysed the legal framework – European Union regulations and laws and Cabinet regulations of the Republic of Latvia – and acquired quantitative data from the Central Statistical Bureau (CSB) of Latvia.

To get insight into the ways of financing agricultural holdings and the amounts involved, the research employed the single case study method that allows performing in-depth examinations

of modern phenomena in real life contexts (Yin, 2013). B.Flyvbjerg (2006) believes that progress in various science disciplines has been achieved owing to particularly skilfully selected cases and an analysis of the cases. An examination of the CSB data revealed that the number of small holdings with a utilised agricultural area (UAA) of less than 50 ha decreased fast in Latvia, while the number of holdings with an UAA in the range of 100-200 ha strongly tended to increase. In the period 2010-2016, the number of such farms rose by 17%. One of the factors that affected the increase was the availability of funds for business expansion. The research performed an in-depth analysis of the amount and sources of lending to and financing for the farm "Saulaini", as the size and scope of its economic activity represented a medium commercial farm in Latvia. The farm "Saulaini" was established in 2001. The farm managed 190 hectares of agricultural land. The key kind of business of the farm was dairy farming (which was the key kind of livestock farming in Latvia), which accounted for 90% of the farm's revenue in 2016. Of the total area of the farm, 148 ha of agricultural land were used for feed production, while 42 ha were cropped with cereals and legumes.

Results and Discussion

Financing is determined by two kinds of principles – horizontal and vertical. The horizontal principle states that long-term investment has to be financed by own capital and borrowed long-term capital. However, the vertical principle prescribes that the key factors determining a ratio between own and borrowed capital are turnover stability and return on equity (Rurāne, 2005).

The sources of finance for enterprises are divided into internal and external.

Internal sources of finance are initially controlled by the management of the enterprise, the funds do not have to be acquired, as they are generated from economic activity. The internal sources of finance are as follows:

- enterprise revenues from sales;
- depreciation deductions;
- profits.

Compared with the other kinds of financing, internal financing (self-financing) has the following advantages: no cost of borrowing; no certain term of financing; an opportunity to repay a loan ahead of the schedule and reduce the amount of interest paid; own capital grows progressively; the financial security and creditworthiness of the enterprise are higher due to own capital (Saksonova, 2010).

External sources of finance are not controlled by the management of the enterprise up to the moment the funds are acquired.

The external sources of finance are as follows:

- equity financing;
- lending – all the kinds of it (incl. leasing, overdrafts etc.).

The key difference from internal financing is that the enterprise will have to pay for the borrowed capital from an external source of finance: in case of a loan – interest to a bank, while in case of equity financing – dividends to shareholders.

Agricultural enterprises in Latvia use several kinds of external financing for their development – European Union investment projects offered by the Rural Support Service (RSS), JSC Development Financial Institution ALTUM loans, loans for farmers granted by various credit institutions, as well as leases granted by machinery dealers. ALTUM is a government-owned financial institution that provides government support to some target groups in the form of financial instruments (loans, guarantees, investments in venture capital funds etc.). ALTUM offers the following programmes for farmers:

- land purchase loan programme;
- loans for purchasing current assets;

- SME growth loans;
- micro-credit programme;
- agricultural and rural development loan guarantee programme.

The data on the sources of finance used by the farm "Saulaini" in Table 1 reveal that the farm used both internal and external sources of finance. Internal financing accounted for the largest proportion of financing, even though depreciation deductions and retained profits were affected by the external financing acquired in previous years.

Table 1 Sources of finance used by the farm "Saulaini" in the period 2013-2016, EUR

Indicator	**2013**	**2014**	**2015**	**2016**
Total financing	**414060**	**456072**	**495901**	**381139**
Internal financing				
Revenues from sales	62776	63685	54743	51360
Depreciation deductions	173837	182721	197885	49044
Retained profits	46837	63997	126966	123841
Total	***283450***	***310403***	***379594***	***224245***
As a percentage of total	***68.46***	***68.06***	***76.55***	***58.84***
External financing				
Loans	0	80280	41624	60869
EU and national support payments	64539	65389	67890	69633
EU project investments	66071	0	6793	26392
Total	***130610***	***145669***	***116307***	***156894***
As a percentage of total	***31.54***	***31.94***	***23.45***	***41.16***

Source: authors' calculations based on farm "Saulaini" data

The farm used mainly internal sources of finance. In 2016, this trend began slightly changing, and the sources of both kinds of finance became equally significant.

As shown in Table 1, *European Union direct payments and national support payments* were of great importance for agricultural holdings in Latvia; the payments ensured the solvency and viability of most of the holdings. Unlike loans, this funding does not have to be paid back.

The economic performance data of the farm "Saulaini" (Table 2) indicate that the farm's production costs exceeded the revenues in the period of analysis. For this reason, support payments for both land management and agricultural products were very necessary to purchase machinery and modernise technologies. The farm could not be able to be solvent without the support payments, and its cash flow would be negative. Most of the support payments (approximately 90%) were composed of area-based payments and national decoupled and extra payments, as well as various national compensations and payments for complying with financial discipline.

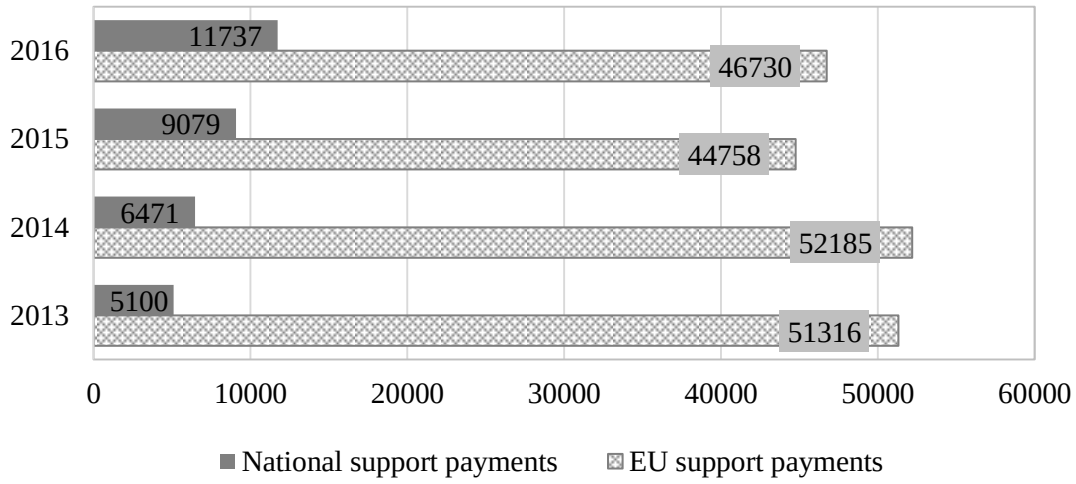
Table 2 Economic performance of the farm "Saulaini" in the period 2013-2016, EUR

Indicators	**2013**	**2014**	**2015**	**2016**
Net turnover	62776	63685	54743	51360
Production cost of sold products	71862	64901	84881	81301
Gross profit or loss	-9086	-1216	-30138	-29941
EU and national support payments	64539	65389	67890	69633
Reporting period profit	40845	77332	28387	52523

Source: authors' calculations based on farm "Saulaini" data

Area-based or direct payments represent annual national and EU financial assistance paid to farmers or forestland owners. Any natural or legal person is entitled to national and EU financial assistance that is paid for every hectare of agricultural land or forestland managed. The legislation, however, imposes obligations on the applicant, as well as makes the applicant assume responsibility for providing correct information to the Rural Support Service (RSS).
Since Latvia joined the European Union, the farm "Saulaini" has received both EU area-based payments and various national support payments.

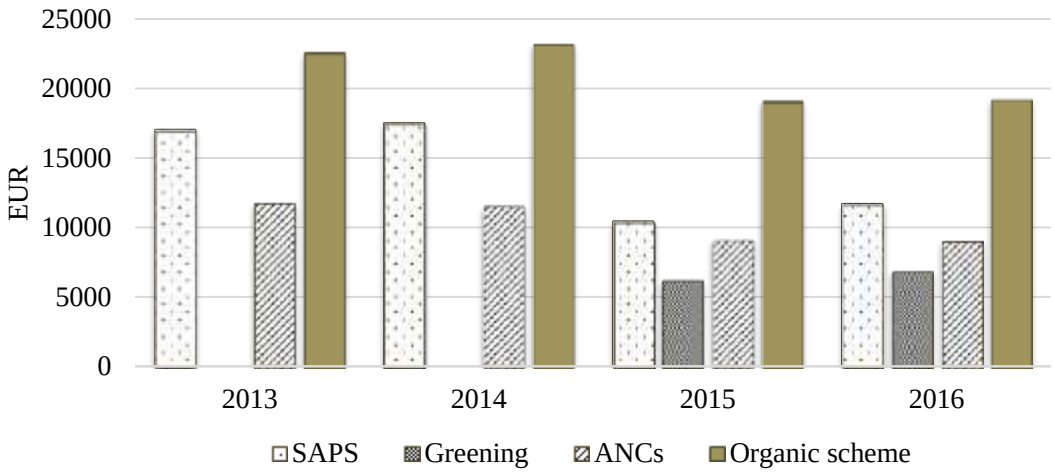
Figure 1**.** Amounts of European Union and national support received by the farm "Saulaini" in the period 2013-2016, EUR



Source: authors' construction

As shown in Figure 1, the amounts of EU area-based payments were considerably larger than those of national support payments, with small decreases in 2016 and 2016, which were determined by a decrease in the farm's land area by 10 ha. However, the amount of national support payments increased more than twofold in the period of analysis.
The farm "Saulaini" received three kinds of area-based payments: single area payments, organic farming payments and payments for areas with natural or other specific constraints. Since 2015, single area payments have been divided into two parts – the first part remained the same, whereas the other one was transformed into a new payment with a new name – the payment for agricultural practices beneficial to climate change and the environment or the green payment.

Figure 2. Breakdown of EU area-based support payments received by the farm "Saulaini" by kind of scheme in the period 2013-2016, EUR



SAPS – the Single Area Payment Scheme;
Greening – the Green Direct Payment Scheme for climate- and environment-friendly agricultural practices;
ANCs – compensatory payments for other areas with considerable natural constraints;
Organic scheme – payments for the preservation of organic farming practices and techniques.

Source: authors' construction based on farm "Saulaini" data

After the required amount of capital is identified, the farm invests it in its assets, which is a prerequisite for the expansion of the farm's business.
The need for investment is determined by a number of reasons, of which three main ones could be distinguished:
renewal of the enterprise's material and technical resources;
increase in output;
commencement of new kinds of economic activity (Leonoviča, 2005).
Using both internal and external sources of finance, the farm "Saulaini" has built up considerable material and technical resources by renovating its farm buildings and purchasing machinery. The amount of investment totalled EUR 317 thou. during the entire period of the farm.

Table 3 Breakdown of investment in farm buildings, constructions and machinery by the farm "Saulaini" by source of finance

Source of finance	**Investment, EUR**	**Breakdown of investment, %**
Internal financing	57 715	18.2
Mixed financing (farm's co-financing)	80 021	25.2
External financing	179 823	56.6
Total	**317 559**	100.0

Source: authors' calculations based on farm "Saulaini" data

An analysis of the farm's sources of finance revealed that the proportion of external financing in particular was the highest with 56.6%. The external financing was comprised of both bank

and ALTUM (development financial institution owned by the government) loans and considerable EAFRD (European Agricultural Fund for Rural Development) funding. The farm's internal financing would not be enough to make investments and build up its material and technical resources. However, as the amount of fixed assets increased, production costs in the form of depreciation deductions also increased, which made a significant effect on the economic performance of the farm but did not affect its cash flow.

Conclusion

Corporate finances is a system of relations of a business entity, which is formed by means of finance. The sources of finances for agricultural holdings as any enterprise are divided into internal and external. The largest proportion of internal finances of agricultural holdings is composed of depreciation and retained profits, yet their sizes depend on the external finances borrowed in previous years. In Latvia, external finances – EU and national support payments – are important for agricultural holdings to be operational. A very significant source of finances is European Union direct payments and national support payments. Unlike loans, this kind of finances does not have to be paid back.

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GASTRONOMIC TOURISM IN LATVIA: FEATURES AND OPPORTUNITIES FOR DEVELOPMENT

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Abstract

As world trends show and according to the Guidelines for Tourism Development in Latvia for 2014–2020, the tourism industry represents one of the opportunities for economic growth in the country and one of the priorities of the services sector, as it makes a considerable contribution to the gross domestic product of the country and is a significant source of export revenues. The objectives of the tourism industry of Latvia are to reduce the effect of seasonality, increase the number of multi-day visitors and to contribute to the profitability of tourism products and annual increases in exports of tourism products. The research aim is to identify the most characteristic features of gastronomic tourism and to examine development opportunities for gastronomic tourism in Latvia. Specific research tasks: 1) to characterise the position and role of gastronomic tourism as well as to identify the characteristic features of it; 2) to assess the characteristic features of gastronomic tourism and the potential development of gastronomic tourism in Latvia. *Research methods used:* monographic, graphic, data grouping, analysis, synthesis. The research concluded that the most characteristic features of gastronomic tourism in Latvia were spatial stillness, regionality, the individuality of a gastronomic tourism product, the effect of seasonality, the focus on domestic tourism and the concentration of tourism activities in historical regions and ethnographic and industrial centres. To effectively contribute to the development of gastronomic tourism in Latvia, it is required to make a number of enhancements to strengthen regional gastronomic tourism, which would link the cultural and historical heritage and gastronomic tourism.

Keywords: *gastronomic tourism; kinds of gastronomic tourism, development.*

Introduction

In Latvia, just like elsewhere in the world, food is an integral component of culture, with large potential for tourism. Gastronomy as one of the mirrors of local culture has become an important resource in the tourism industry, where the leading role is played by the local culture and traditions. In Latvia, the supply of gastronomic tourism products begins representing high-quality, interesting and unique traditions of cooking, serving and eating food, which is a successful prerequisite for the expansion of gastronomic tourism in Latvia. To purposefully develop and contribute to the development of gastronomic tourism in Latvia, it is required to identify the resources and typical features that characterise and represent gastronomic tourism in Latvia as well as to ascertain development opportunities for this kind of tourism in Latvia. The Sustainable Development Strategy of Latvia until 2030 states that one of the cornerstones of sustainable national development is the use of the potential of cultural heritage for the development of creative tourism. Creative tourism is one of the ways for Latvia to use the potential of its tangible and intangible cultural heritage and cultural landscape. It is an opportunity to support the economy of local communities, to promote the preservation of the rural cultural environment, the development of craftsmanship and creative industries, particularly in rural territories. However, tourists have an opportunity to feel the culture, environment and traditions of the relevant community in an authentic location and even for a moment to have the feeling of being a local inhabitant.

The Guidelines for Tourism Development in Latvia for 2014–2020 address not only the role of tourism in national development but also highlight the significant effect of cultural tourism on the strategy for this industry. If we adhere to the definition that cultural tourism is a kind of tourism and the key purpose of travel by tourists is to get familiarised with the culture and cultural heritage of one's own nation or other nations, a number of authors stress both the cultural heritage and the diverse dimensions of cultural heritage: the tourism dimension, the dimension of utilisation of cultural heritage objects, the dimension of cultural experiences and the dimension of consumption of local products (Richards G.,2007). Modern tourists become more and more educated and experienced, and they are interested not only in visiting foreign countries and the most interesting sightseeing places there but also in getting familiarised with the local culture. One of the elements of a culture is its cuisine – national and traditional dishes and beverages and the ways of preparing and enjoying them. One of the kinds of tourism – gastronomic tourism – has emerged and developed within this element. The key purpose of travellers is to enjoy high-quality dishes and beverages of national cuisine. Gastronomic tourism activities usually occur in historical and ethnographic areas where local cultural traditions are kept alive or in areas where the art of cooking is advanced. The World Tourism Organisation states that gastronomic tourism is based on experimental taste experiences, learning from other cultures and knowledge of cooking dishes and qualities and features of the dishes. Culinary tourists partly or fully plan their travel being subordinated to learning and enjoying the local cuisine and other activities related to the peculiarities of the local cuisine and culinary heritage (World Tourism Organisation, 2012). Other authors, however, stress the association of a region's opportunities for development with gastronomic tourism that involves many opportunities for entrepreneurship and business, e.g. restaurants and hotels, cooking art schools, dish and beverage festivals and farmers' markets (Yeoman et al., 2015). The role of tourists themselves is essential, as they contribute to the development of the particular region. Therefore, it is important to make tourists in particular go to a tourism object or place in order to become gastronomic tourists and consume gastronomic tourism products – local food and dishes (Hall et al., 2007). Some authors, e.g. Kiralova and Hamarneh (Kiralova, Hamarneh, 2017), significantly complement this economic benefit by asserting that food tourism is a local phenomenon making a positive effect on the economy, employment and local cultural heritage.

Thus, research on gastronomic tourism in particular and its characteristic features would be one of the ways how the regions of Latvia could promote their tourism development strategies.

Targets, Materials and Methods

The research aim is to identify the most characteristic features of gastronomic tourism and to examine development opportunities for gastronomic tourism in Latvia. Specific research tasks: 1) to characterise the position and role of gastronomic tourism as well as to identify the characteristic features of it; 2) to assess the characteristic features of gastronomic tourism and the potential development of gastronomic tourism in Latvia.

The research used mostly information sources and research papers on the classifications and development of gastronomic tourism. The legal documents, strategies and policy documents of the Republic of Latvia were employed to identify the situation in the country.

Research methods used: monographic, graphic, data grouping, analysis and synthesis.

Results and Discussion

Role, position and characteristic features of gastronomic tourism.

An analysis of the most important trends in gastronomic tourism in the world allows concluding that one of the threats to it is globalisation that enables tourists to enjoy worldwide popular dishes in any country. Resistance movements increasingly emerge in the world owing

to globalisation, e.g. the slow food movement that views food not only from the perspective of nutritional value but also in the context of lifestyle. In recent years, the development of gastronomic tourism as regional tourism occurred owing to culinary media, social networks as well as the interest of tourists themselves in it. Travel and food has become inseparable owing to various television shows that stress the importance of tasting local and authentic dishes when traveling; culinary television shows tell their viewers about and show how to cook national dishes. Examining the behaviours of consumers, i.e. tourists, it is important to comprehend what motivates individuals to travel. Most often, the following five motivators are emphasised: physical, emotional, personal, personal development as well as social status expression and cultural motivators (Swarbrooke, Horner, 2008). All the mentioned motivators could be attributed to gastronomic tourism: the physical motivator is hunger; the emotional motivator represents all the emotions related to enjoying a dish – wellbeing, pleasure, surprise, adventure; the personal motivator is a chance to meet friends and family during the meal; the personal development motivator represents knowledge about dishes and new skills; the social status expression motivator relates to enjoying exclusive or special dishes; and the cultural motivator contributes to becoming familiarised with a new culture and nation through dishes.

However, the role of dishes in motivating tourists, according to Hjalager and Richards (2002), involves four categories of motivators: physical, cultural, interpersonal, social status expression and prestige motivators.

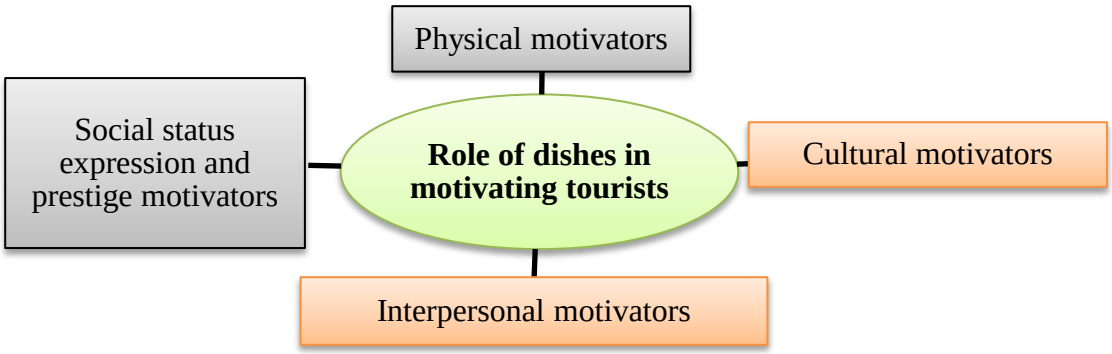

Fig. 1. Role of dishes in motivating tourists

Source: authors' construction based on Hjalager, Richards, 2002

If focusing less on the physical motivator, the cultural motivator in particular shapes gastronomic tourism, as it is an essential factor affecting the development of gastronomy and tourism. National dishes represent one of the intangible values of national culture; therefore, tasting local dishes also involves enjoying a new culture if the dishes are authentic, historical or specific to the particular culture and location. The interpersonal motivator explicitly represents a social function that takes the form of an important dimension in gastronomic tourism. Active communication and an opportunity to learn local traditions in cooking and serving dishes as well as a story about the dishes are an important tourism motivator as well.

Food tourism as a kind of special interest tourism is divided into several segments, and food tourists are defined according to some typical features: the extent of their interest in it and the importance of dishes as a motivator of travel.

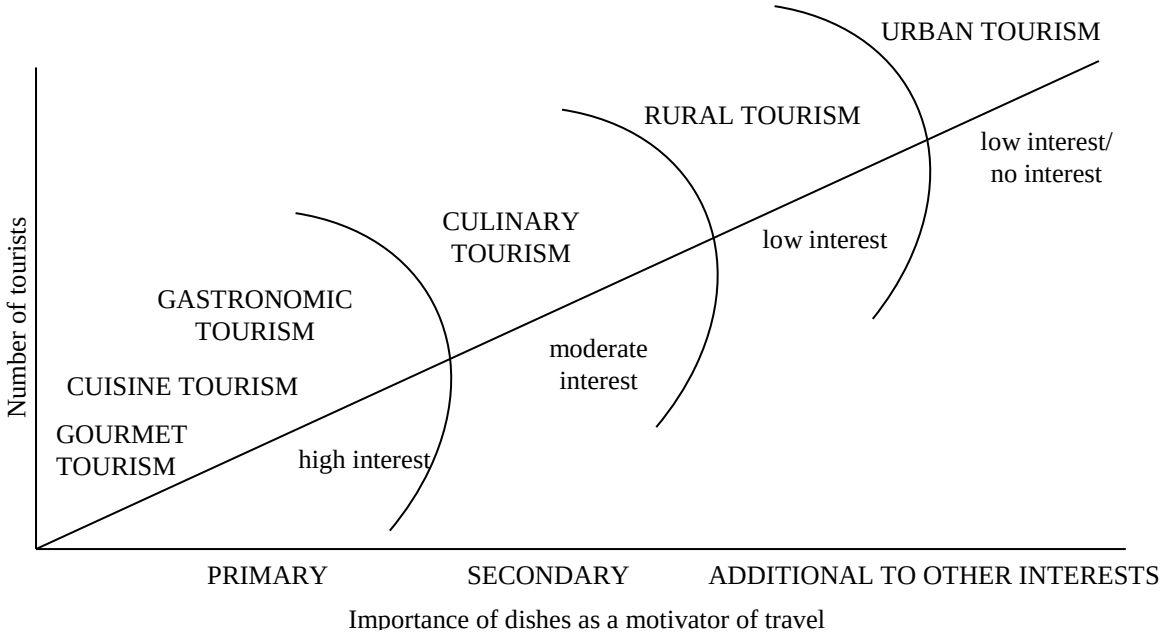

Fig. 2. Division of food tourism as a kind of special interest tourism

Source: authors' construction based on Hall et al., 2007

Food tourism is specific to the following kinds of tourism: rural and urban tourism, culinary tourism, gastronomic tourism, cuisine tourism and gourmet tourism. The interest of rural/urban tourists in food tourism is low. Food tourism as a motivation for travel is only an extra interest, which is not primary. Culinary tourists are moderately interested in food tourism, and often it is a secondary motivation for travel. Gastronomic and cuisine tourists are highly interested in food tourism, and the food as a motivation for travel is key to both kinds of tourism and play a decisive role in the choice of a destination. Gourmet tourists have the greatest interest in food tourism, as the determinant and primary motivation for travel is their interest in food. Figure 1 shows a causal association – the greater the interest in food, the smaller number of individuals are involved. For example, in rural/urban tourism, which is a popular kind of tourism, the number of tourists interested in food tourism is relatively large, while in gourmet tourism, in which the interest in food is primary and key, the number of interested tourists is small.

Characteristic features of gastronomic tourism in Latvia and the potential development of it.

According to a research study carried out in one of the regions of Latvia (Kurzeme), there were five key trends that promoted national dishes and contributed to growth in gastronomic tourism in the region:

1) increase in the standard of living (as incomes increase, consumers more often spend their money on dining outside their homes; therefore, domestic gastronomic tourism among regions emerge);
2) demographic and household changes (the concentration of households around major cities and the capital city creates opportunities to develop domestic gastronomic tourism);
3) rejection of fast food restaurants (tourists increasingly reject fast food; instead, they prefer local foods that reflect local national traditions);
4) increase in the number of multi-cultured consumers (globalisation, growth in tourism in the Baltic region increase the demand for local gastronomic tourism services along with rural and urban tourism services);
5) influence of celebrity chefs and the media (magazines, books, online magazines, television shows and the popularity and visibility of culinary shows).

An essential role in the growth of gastronomic tourism is played by the tourism target markets set by the Tourism Marketing Strategy of Latvia, which requires accurately drawing up guidelines for gastronomic and cultural tourism and examining the motivations of the tourists.

Table 1 Foreign tourism target markets set by the Tourism Marketing Strategy of Latvia

	High-priority target markets	**Priority target markets**	**Secondary target markets**	**Prospective target markets**
Countries	Lithuania, Estonia, Germany, Russia, Sweden, Finland	Norway, Denmark, Great Britain, Italy, Spain, Netherlands	Other European countries	USA, Japan, China, India

Source: Tourism Marketing Strategy of Latvia

The strategy has set Lithuania, Estonia, Germany, Russia, Sweden and Finland as high-priority foreign target markets based on the fact that most tourists arrive from these countries, as well as they stay longer at accommodation establishments in Latvia. Gastronomic tourism services are mainly focused on this target audience.

Norway, Great Britain, Italy, Denmark, Spain and the Netherlands have been set as priority target markets based on the number of incoming foreign tourists, easiness to get to Latvia as well as a suitable supply of tourism services in Latvia. All the other European countries have been set as secondary target markets where minimum marketing activities are carried out. The USA, Japan, China and India are regarded as prospective target markets; at present, there is no strong cooperation with the countries, yet in the future they could represent potential for tourism.

The Tourism Marketing Strategy of Latvia as well as several informal discussions with tourism industry professionals have suggested certain approaches to be employed to motivate tourists to visit Latvia as a destination of gastronomic tourism or make the tourists stay longer, which could be achieved by means of specific activities. The activities are as follows:

1) offering dishes as a tourist attraction (focus on cities);
2) making foods an integral component of a tourist product (special tourist routes; focus on rural tourism);
3) tasting dishes as part of tourism experiences (gourmet tourism and special activities, such as "*Taste Latvia"*);
4) stressing the role of food in the cultural heritage (cuisine tourism and rural tourism);
5) making a link between tourism and food production (synergy between tourism and entrepreneurship, with a focus on the regions of Latvia; the promotion of craftsmanship and home production).

Although tourism in Latvia tends to grow dynamically, the opportunities for gastronomic tourism could be enhanced. A series of activities have been carried out and strategic guidelines have been designed at the regional level, yet some activities are at the initial stage, e.g. developing a common tourism brand, carrying out marketing activities aimed at the target audience in particular, strengthening the role of regional tourism information centres and developing initiatives with rural entrepreneurs.

Conclusions

1. Gastronomic tourism is not only one of the kinds of tourism; it also encompasses a broad system of components: accommodation, agriculture, transport, regional food producers, rural tourism and catering enterprises.
2. The development of gastronomic tourism is shaped, on the one hand, by tourists for whom the role of dishes and the related motivation are important, while on the other hand, by a number of economic and regional factors: the standard of living, the number of multicultural consumers, cooperation with food producers and other factors.
3. At present in Latvia, gastronomic tourism is mainly based on domestic demand, yet marketing activities have been carried out to attract foreign gastronomic tourists to Latvia.
4. Further activities that allow the regions of Latvia to accurately design strategies for promoting gastronomic tourism based on the geographic location as well as the overall situation in the region should be identified and designed as well.

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STRUCTURAL ANALYSIS OF SOCIO-ECONOMIC DISPARITIES IN ECONOMIC DEVELOPMENT OF THE REPUBLIC OF MOLDOVA

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Abstract

The concept of regional development is in fact an European idea, one of the basic principles of the EU's functioning and a main condition for administrative reform in the Member States, candidates or associate countries. In Moldova, the real development of the economy takes place asymmetrically from a regional point of view. The regional disparities in the country are due to the uneven distribution of production factors, urbanization levels, quality of technological infrastructure and institutional. The origin of such factors may result in the creation of a potential gap within the country and in the perpetuation of existing social and economic imbalances, affecting as result the economic growth. When statistical aspects and regional accounts started to be developed, the analysis of the regional dynamics in the study focused more on the identification of the convergence or divergence processes, of the concentration poles at different sectors level as services, investments, industry and agriculture. The aim of this research is to identify the existing socio-economic disparities in regions development and to analyze its impact on Moldova's economic development. Different research methods will be used in the study such as: systemic analysis, functional approaches, retrospective analysis (historical approach), diachronic analysis (analysis of changes in the structure of object over time). Methods of scientific knowledge, such as comparison method, graphical analysis and logical thinking methods, statistical analysis will allow to carry on a systemic approach of the researched problem, to identify regularities and connections in regional development and to draw conclusions and recommendations on the researched aspect.

Keywords: *agriculture, economic development, regions, regional policy Republic of Moldova.*

Introduction

Regional disparities in terms of economic development represent a challenge in the modern world. The uneven concentration of financial assets generates major inequalities in living standards between different territories. Moldova is not an exception, registering an enormous concentration of the economic potential in large cities (Chisinau, Balti). There are present major discrepancies in the standard of living between large cities and smaller localities, but also between urban and rural localities within the regions. The policies promoted in the area of Moldova's regional development start from the notification of local, inter and intra-regional disparities, stipulating the ways to minimize these disparities as an objective of the country's regional development policies. Regional development has a national and a European dimension. In the case of the development of national regional policies, the socio-economic differences between different regions require to promote concepts and programs that address the issue of regional development. Regional development has also been linked to industrial policy. In recent years, the national dimension of regional policies has dimmed, but the European dimension in this area has become more dynamic. The first regional programs at European level were initiated within the E.U., after being joined by countries that, due to a weaker level of development, caused significant disparities.

The main objectives of regional development are: achieving balanced and sustainable socio-economic development in Moldova; reducing the imbalance in socio-economic development levels between and within regions; strengthening financial, institutional and human opportunities for the socio-economic development of the regions; support the activity of local public administration authorities and local authorities, oriented towards the socio-economic development of localities and coordination of their interaction with national, sectoral and regional development strategies and programs.

Material and Methods

The given research aims to analyze the structure of the economic disparities in the development of Moldova in terms of the following criteria: the share of allocations for regional development as a share of Gross Domestic Product (GDP), the contribution of development regions to the Regional Gross Domestic Product (RGDP), the contribution of development regions in the value of agriculture, forestry and fishing as part of the RGDP. The social dimension of regional development was based on the analysis of the disparities in the quality of education, health, consumption and social relations, linked to the components of structuring or conditioning of regional differences, accessibility to services, infrastructure and employment. The data of the National Bureau of Statistics (NBS) of the Republic of Moldova to determine and analyze the indicators were used. In order to achieve the research objectives the following research methods were applied: the analytical method; method of systemic analysis; descriptive method; the synthesis method; the logical framework method used to analyze the indicators; the method of quantitative and qualitative analysis, which was applied to the analysis and interpretation of the indicators

Results and Discussion

Moldova has entered the process of transition to a market economy with significant regional imbalances caused by current production structure, being particular the predominance of the agri-food sector, the dependence of the social and economic development of small towns on a limited number of large industrial holdings. Thus, stimulating economic growth outside the largest cities, especially in rural areas that have suffered from the loss of traditional agri-food supply markets, has been one of the main challenges for the Moldovan authorities since independence.

Sustainable regional development is an imperative assumed by Moldova and confirmed by the Government through its firm commitment to equitable distribution of development opportunities across the country. This is confirmed both at the legislative level by Law No.438-XVI of 28 December 2006 on regional development adopted at the end of 2006, with subsequent amendments approved during 2010 and at the level of strategic planning, with regional development taking place in the National Development Strategies (2010-2012, 2013-2015, 2016-2020).

In order for a sector to develop efficiently, it must benefit from a strong financial support. Thus, a regional development financing system was developed within the framework of the implementation of the National Regional Development Strategy (NRDS) 2010-2012. In fact, this system or mechanism has demonstrated relative viability, flexibility and sustainability. However, this mode of financing is exclusively focused on the National Regional Development Fund (NRDF), the resources of which are insufficient compared to the needs on this dimension. For example, according to the State Budget Law from 2016, about 200 million Moldavian Lei (MDL) were allocated for NRDF, which represents almost 0.8% of its revenues or about 0.3% of GDP. It is, in fact, a small sum of what is to be done under the new NDSR project for 2016-2020, including rallying to the priority priorities for regional development (Morcotolo, 2014)

Compared to other European countries, Moldova is distinguished by a very pronounced economic polarization, so that half of its GDP is made in the capital city, which concentrates only a quarter of the country's population. The most visible evidence of this economic polarization is the concentration of economic activity, the best human resources and the best infrastructure in the largest city. The other regions in the country are even less active economically. This economic and geographical structure is clearly confirmed by a number of indicators: migration, poverty, the number and density of companies, the number of employees, the value of sales, the evolution of budgets, etc.
According to recent NBS data, one of the biggest regional discrepancy is an excessive concentration of economy in Chisinau, followed by a large difference in the Balti municipality (Table 1).

Table 1. Moldova's Gross Domestic Product by development regions, MDL

Regions	2013	2014	2015
Chisinau municipality	56.038,0	62.869.976,0	71.535.731,0
Center	15.964,9	17.973,6	19.933,0
North	18.381,9	20.519,0	21.818,7
South	9.001,7	9.704,2	10.073,7
UTA Gagauzia	2.834,8	3.064,6	3.210,1

*Source: based on data from National Bureau of Statistics, 2015-2017

The emphasis of these two cities is largely focused on structurally differentiating the factors of production that exist in these regions and the rest of the country. The indicators in the agricultural and industrial sectors are above the values registered in the service sector, which highlights the fact that the latter is concentrated in the Chisinau municipality and has a small presence in the other development regions. Thus, for Moldova two major aspects of the regional economic development are characteristic. The whole economy (with the exception of Chisinau municipality) would be specific agricultural land, while for Chisinau and partially Balti - more focused on services and manufacturing. However, the lack of statistical data and relevant analyzes has affected the good policy implementation in the field and has been reduced to a more short-term process without addressing in detail the main development factors and issues.
Besides the uneven distribution of the sectors in the regional profile, the regional economic discrepancies are confirmed also at the GDP level in territorial profile, respectively in absolute and relative terms calculated by the NBS on each territorial administrative unit and finally in the regional aspect (Figure 1)

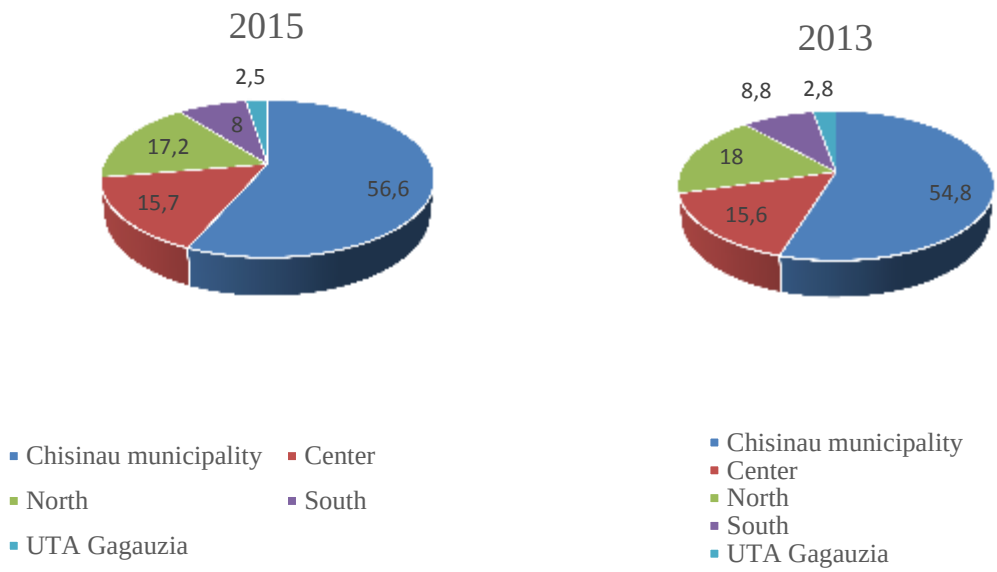

Figure 1. Development regions contribution to Moldova's GDP, Percentage
*Source: based on data from National Bureau of Statistics

About 55% of GDP is concentrated in Chisinau municipality, over 8% in Balti municipality, which is also part of the North-East Region, and UTA Gagauzia - 2.8%. In fact, a geographical concentration of the economy shows that the share of a region in a territorial profile corresponds to its share in the national economy as a whole. The geographic concentration of agriculture on a territorial or regional level is much more homogeneous than in the industrial sector. Thus, to have a similar base in the given sector, statistical data were used on each district on average fruit per hectare for the main agricultural crops in Moldova - wheat, barley, maize and sunflower. The agricultural sector, unlike the industrial one, is important for the economies of all districts. Respectively, the same statistical data show that productivity indicators in agriculture are more or less similar to those in the industrial sector. Even though the agricultural sector has recently shown contradictory tendencies to move in the same direction, regional differences in this sector remain well below those of industry, largely due to weather conditions.

Table 2. Contribution of development regions to agriculture, forestry and fishing as part of Moldova's Regional Gross Domestic Product, thousands MDL

	2013		2014		2015	
	Value, thousands MDL	Share, %	Value, thousands MDL	Share, %	Value, thousands MDL	Share, %
Total	12383120	100	14619434	100	15001568	100
Chisinau municipality	215860	1,75	319511	2,20	304463	2,03
Center	4068450	32,85	4898207	33,50	5220385	34,80
North	4383730	35,4	5137942	35,14	4968889	33,12
South	2982674	24,09	3386151	23,16	3542722	23,62
UTA Găgăuzia	732406	5,91	877623	6,00	965109	6,43

*Source: based on data fron National Bureau of Statistics

The largest value of agriculture, forestry and fishing as contribution to Regional Gross Domestic Product is obtained in the Center and Northern area (about 34%). The Southern area in the analyzed period is 23%, UTA Gagauzia - 6%, Chisinau 2% (Table 2). Among the major causes that led to the increase in disparities can be mentioned: location and scale of foreign investments in development regions, loss of competitive capacity of enterprises, both on domestic and foreign markets, due to the high moral and physical wear out of technologies and equipment (especially in regions located in the eastern part of the country) and limited access to finance for SMEs. At the same time, economic development leads to the emergence of a phenomenon manifested by the emergence and outline of areas of economic decline that is accompanied by a social decline in which there is a profound contradiction between two important factors of growth: labor and capital. The immediate consequence is rising unemployment and falling living standards in the area. Such areas are becoming underdeveloped and become potential sources of social conflict (NBS, 2016, 2017). The social dimension of regional development focuses on the analysis of the social disparities in the quality of education, health, consumption and social relations related to the components of structuring or conditioning regional differences, namely urban - rural, accessibility to services, infrastructure and employment. These taken as a whole are the basic components of the space of the economic and social development inequalities manifested at the regional level (NBS, 2015). Thus, aspects related to the social component of regional development in the Republic of Moldova can be appreciated by the estimation of the number of existing early education institutions. As shown by the most endowed calculations with such institutions are the Center, and North regions followed by the Southern region (Table 3).

Table 3. The distribution of the early education institutions by development regions, 2012-2016, number of units.

	2012	2013	2014	2015	2016
Chisinau municipality	157	159	160	160	161
North	465	473	477	484	487
Center	467	469	486	484	489
South	269	269	270	273	273
UTA Gagauzia	60	60	60	60	61
Total	1418	1440	1453	1461	1469

*Source: based on data fron National Bureau of Statistics

Not less important are the dynamics of pupils and teachers in the primary and secondary general education institutions. There is a trend of permanent decrease in the number of pupils per 10000 inhabitants in the period 2013-2017 on average in the republic, but also in the regions North, Center, South, ATU Gagauzia. Only in Chisinau the number of students per 10000 inhabitants increased from 980 in the 2013/14 study year to 1014 pupils in 2016/17. The number of pupils to a retiring teacher during the analyzed period did not suffer major changes ranging from 10 to 13 students. Differences between regions in terms of water supply and sewerage infrastructure imply specific approaches to regional development. The best equipped are the households in Chisinau, the Center region followed by the households in the North and South Regions. Thus, economic development is achieved not only to meet basic material needs but also to provide resources to improve the quality of life in health, education and a better environment.

Conclusions

Until recently, the regional economic disparities were subjectively identified, currently they are relevant and objectively, including on the basis of official statistical data. The data show differences in enormous economic development that separates the Chisinau region from the rest of the country, whose GDP per capita represents almost 60% of the rest of the country. The country's economy is highly polarized and with pronounced regional gaps. The geographical concentration of population, levers of economic growth, industrial production and services, as well as investments from all sources are located in Chisinau or its municipality. This enormous gap starts from the accumulation in the largest city of services with an intensive added value, while the industrial sector continues to develop. The agricultural sector, unlike the industrial one, is more important for the economies of all districts. Respectively, the same statistical data show that productivity indicators in agriculture are more or less similar to those in the industrial sector. Even though the agricultural sector has recently shown contradictory convergence trends, regional differences in this sector remain well below those of industry, largely due to climatic conditions. Regional development must be linked to structural reforms, and implicitly to the size of the most dynamic sectors - manufacturing and service sector. The actual structural disparities between the capital and the rest of the country indicate that in the absence of an economic structure reform of at district level for these branches, is not possible to reach a territorial development convergence. Is important to elaborate at national level a single system for monitoring all interventions within the development regions in order to properly assess their level of development and subsequently to develop sectoral development programs in the context of cohesion policies, taking into account good practices and the experiences of European countries.

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SOYBEAN WAR IN PARAGUAY: THE SOYBEAN BOOM'S FLOW FROM BRAZIL TO PARAGUAY, ITS SPILLOVER EFFECTS AND STATE RESPONSES

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Abstract

This paper identifies the causes and effects of the soybean boom in Paraguay that took place from 1960 to 1990 when the Brazilian farmers massively migrated. The paper analyze the diverse and complex causes of this migration and agriculture land expansion. The Brazilian farmers and their descendants have easy access to financial aid, technical assistance, and technology. Institutional weakness, lack of transparency and low public participation in building policies and corruption is evident in several ways. Violent clashes between peasants and security forces ingnited as a result of displacement of peasants, massive deforestation, indiscriminate use of agrochemicals and lack of governmental assisstance. The Paraguayan government not only has not provided the necessary assisstance to the peasants for decades but also through its little to no control, facilitated the big actors' predation of natural resources and displacement of peasants. The Zero Deforestation Law was examined briefly, showing its impressive results. Enacting of the Zero Deforestation Law and the rigorous enforcement helped reduce the deforestation by 80-90 %. This reduction is a clear example that shows that when the State acts effectively in cooperation with civil society, regardless of its limitations, improvements can be experienced, benefiting thousands of citizens. The soybean crop expansion, although representing an important proportion of the Paraguayan GDP growth, must be regulated carefully considering its social-environment side effects and the violent conflicts that it may install between the actors within the society. Environmental issues must be subject to strict regulation and control to ensure the valuable resources are cared for an sustainable use.

Keywords: *Atlantic Forest of Paraguay, environmental security, deforestation, migration, soybean production.*

Introduction

The massive Brazilian migration to Paraguay started in the 1960s and continued in the next decades. The majority of the Brazilian migrants and their descendants, mostly farmers, remain until today in the Eastern region of Paraguay, bordering Brazil. Many of the pioneers had previously grown crops in Brazil in the nearby provinces such as Sao Paulo, Río Grande do Sul, Santa Catarina, and Paraná. They worked there in an environment similar to the Paraguayan: an ecosystem of subtropical forest that, once dismantled, offered a fertile soil, and a excelent climate. Therefore, the Brazilian farmers found exceptional conditions to reproduce their production system in Paraguay (Souchaud, 2007). Over the years, soybean became the flagship product, tending to monoculture and transforming the productive conditions and the environment of the region. Even though official data is scarce, thus hindering the possibility of accurately magnifying this process of migration and population, an idea can be formed based on the available data and estimations. The first areas to receive this massive immigration were the departments of Amambay and Canindeyú, but since the 1970s, most of the newcomers have established in Alto Paraná (Souchaud, 2007). 45.6 % of the Brazilian immigrants in Paraguay reside in de departments of Alto Paraná, and around 20 % in Canindeyú (Ayala Godoy, 2014). On the other hand, the demographic and economic

changes that came with the new settlers have impacted the concerning areas in numberless ways. The environmental effects of the soybean agriculture cannot be overlooked, as millions of hectares of forest have been lost. The Upper Parana Atlantic Forest was especially affected.

Material and methods

In order to better understand factors linked to the reasons of soybean expansion in Paraguay, we began with documentary research and through systematizing, tabulating, and statistically treating agronomic and demographic data from the Central Bank and Directorate for Census and Statistics. The secondary data research focused on the prices of commodities, prices of lands in Brazil and Paraguay, institutional framework of the environmental sector, corruption index in Paraguay and environmental crimes and the expansion's impacts focus on land use change in Paraguay. The crop area dataset from Paraguay was obtained from the database available at World Wildlife Fund (WWF) - Paraguay. The crop area data set from Brazil was obtained from the Series Historicas database of the Companhia Nacional de Abastecimento (CONAB) and from tradingeconomic.com. This page provides present historical data, chart and statistics. Soybeans present data and historical chart was last updated on September of 2018.The prices of lands in Paraguay were obtained from research done in paraguayan realtor web pages and interviews to certifyed appraisals. Fieldwork was then carried out between February 2016 and July 2016. In-depth interviews were carried out with indigenous farmers of the indian village of Puerto Barra in Nacunday (Mbya ethnicity), and representatives of governmental and non-governmental institutions in the departments of Caaguazu and Itapua. Non-governmental institutions included NGOs (WWF Paraguay, FeCoProd).

Results and Discussion

Causes of the Migration of Brazilian Soybean farmers to Paraguay. The causes of the migration of Brazilian farmers to Paraguay are diverse and complex. There are many aspects to it including economic factors, environmental factors as the good quality of the arable lands available in Paraguay, and institutional weakness in Paraguay, which meant less regulation and enforcement. In the following sections, these factors will be discussed.

Soil Quality. The Agricultural and Forestry Atlas of Paraguay (Vázquez, 2011) clearly shows the increasing soybean production experimented between 1991 and 2008. The cultivated area escalated from slightly above 200,000 hectares to 700,000 in Alto Paraná during this period. Meanwhile, the production grew from 500,000 tons to almost 2,000,000 tons. The mentioned Atlas shows that the best performance of the soil is precisely allocated in the departments where most of the Brazilian migrants live: Alto Paraná and Canindeyú. In some areas in these departments, the performance reached around 6 tons per hectare in 2017[15]. This increase shows the high quality of the Paraguayan soil in these areas.

Land Price Evolution in Paraguay and Brazil. The differences between Brazil and Paraguay are, in this aspect, significant, although the evolution through the years followed similar patterns. Santos Telles, *et al.* (2016) explain that since the last years of the 1960s until the first years of the 1990s, land prices in Brazil experienced successive periods of increases and stabilization. For most of this period, instability was the reality, but the prices tended to increase and on very few occasions they decreased. Croplands' prices escalated from about two thousand dollars per hectare in 1994 to almost five thousand in 2009 (Santos Telles *et al.*, 2016). Meanwhile, in Paraguay, agricultural land also experienced significant increases in its prices. The average prices climbed from 65 USD/ha. in 1995 to 1,300 USD/ha. in 2012 (Capital Campo, 2017; Salcedo, 2014). See this evolution in Table 1. Although the increase

15 The national average these years reached 3.2 tons/ha. ("Sojapar rompió nuevo record", 2017)

has been phenomenal (more than ten fold in less than 20 years), the prices remain lower than in Brazil. Nowadays, prices can range from 12,000 to 15,000 USD/ha.

Table 1. Paraguay's arable land price evolution from 1995 to 2012.

Year	USD/ha	year	USD/ha
1995	65	2004	328
1996	92	2005	388
1997	121	2006	480
1998	149	2007	658
1999	182	2008	700
2000	215	2009	768
2001	248	2010	879
2002	271	2011	1.042
2003	293	2012	1.300

Reference: USD (Dollar); ha (Hectares)

Access to Agricultural Technology and Biotechnology. As Brazilians moved to Paraguay and found good quality lands at comparatively low costs, access to technology became vital to assure high productivity. This explains the transformation of agricultural production in Paraguay. Mechanization extended, and so did the sizes of the farms. Brazilians had an advantage in this aspect, having preferential access to information, technological solutions, training, consultancy services provided by EMBRAPA[16].

Currency Dynamics and its Impact on farmers' Profits. As much of the soybean production that is destined to the international market and so traded in US dollars, the exchange rate has an important influence in the price of the products and the profits made. Brazil and Paraguay experienced devaluations in their national currencies during the 1990s, facing a stronger dollar. In the first years of the new millennium, the Paraguayan Guaraní's value was three times less than its value in 1995 (BCP, 2018). The rise in local prices also reaches the soybean production. However, in this case, farmers get increased incentives due to their products' better prices in the international market (Richards *et al.*, 2012). "*From 1996 to 2002, as global prices for soybeans were stable in US dollars, in Brazilian reales, Paraguayan guaranies, and Bolivian bolivianos, prices were approaching historical heights*" (Richards *et al.*, 2012: 455).

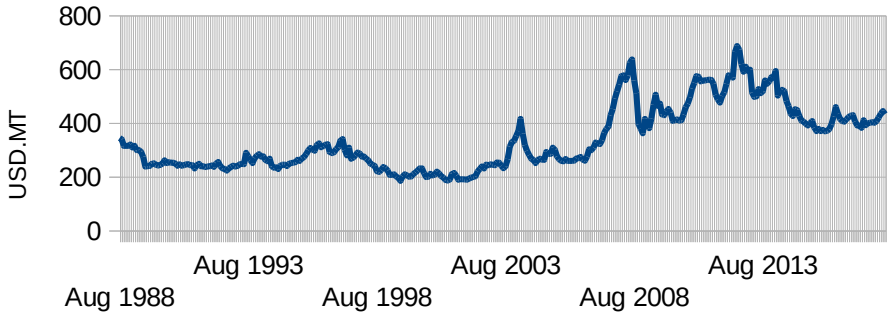
Access to Financial Support. The National Economic and Social Development Bank of Brazil (BNDES), founded in 1952, has played a critical role in financing development in diverse sectors of the Brazilian economy. Companies, rural farmers and entrepreneurs have been subject of the financing mechanisms of the BNDES for decades, and the effects have overflown Brazilian borders. After the 1950s' Government priority in industrialization, many policies oriented to the primary sector followed, leading to an essential increase in rural credit and other mechanisms of support. This resulted in a rapid expansion of crops as soybeans, whose area increased from 0.9 million ha. in 1969 to 6.4 million ha. in 1976. The increasing rates continued in the next decades (CONAB, 2018). The National System of Rural Credit, established in 1965, provide access to credit with low interest rates. The amounts available for such operations increase every year and Brazilian farmers continue to benefit from the strong policies its government is implementing (Lopes *et al*, 2016).

Prices of commodities. From the relatively low prices registered in the 1950s, the increase began to be consistent in the late 1960s. The decade of 1970 witnessed important prices' raise, reaching historic highs in the second half (TradingEconomics.com, 2018). The prices in the next decades showed a relatively stable tendency despite the increases and decreases that the

16 The *Empresa Brasileira de Pesquisa Agropecuária* (Embrapa) is a public enterprise that conducts reaserches in agriculture and cattle.

price experienced. This tendency is shown in Figure 1. The profits, continued to grow, due to the tendency in exchange rates previously described, as well as the lower production costs emerging from the new technology available and the greater productive efficiency.

Figure 1. Price evolution of soybean from 1988 to 2018.



Institutional Weakness and corruption: Although both Brazil and Paraguay have environmental laws, the levels of enforcement are very different. Having lower levels of enforcement and control by the government made it easier and cheaper to produce in Paraguay.

Weak Capacity. The Environmental Secretary (SEAM) was created in 2000 when a significant part of the process of immigration of Brazilian farmers in Paraguay and deforestation took part. The enormous expansion of soybean crops was developed for many years without any environmental regulatory control. Following the creation of the SEAM, there was, a period until its capacity is developed. This is reflected in the institution's budget over the years. In 2003 and 2004, its budget was just above one million US dollars, with low capacity for enforcement. It was only after 2011 that the budget was steadily maintained over five million US dollars, reaching a top USD 9,962,000 in 2018.

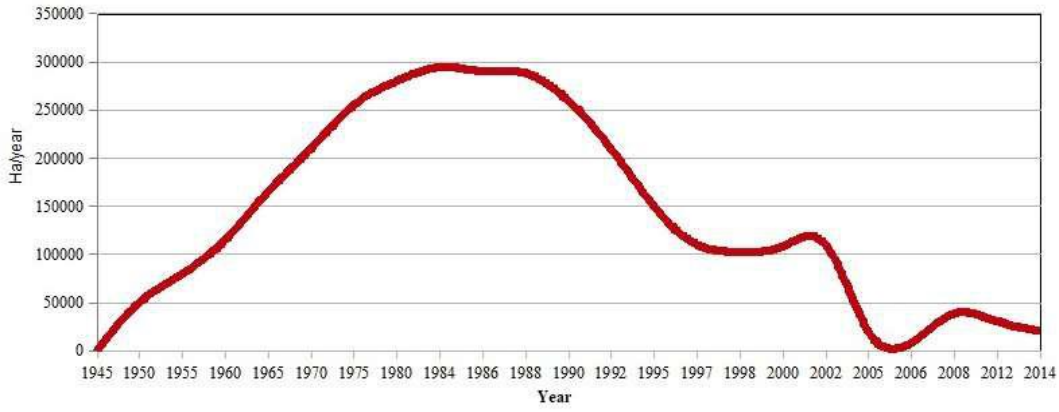
Corruption. Corruption is considered one of the most serious threats to economic processes, social development, the consolidation of democracy, regional integration and to the accessibility of the benefits of globalization. Being the government weak, resource distribution, extraction and management became fertile ground for corruption. The Corruption Perceptions Index (Transparency International, 2016) shows the magnitude of the issue. In the 2016 Index, Paraguay was placed 123 out of 176 countries. Today, the status of environmental governance is characterized by multiple problems: inadequate environmental governance structures; absence of long term planning; lack of links to the National Development Plan and to the Sustainable Development Goals; low public participation and limited availability of information to the public; the preeminence of powerful economic stakeholder groups; and nonexistent independent participation in the decision-making process. In brief, corruption is the major obstacle to environmental conservation in Latin America. (Facetti, 2010).

Environmental Effects of the Migration: Deforestation and Natural Resources Disputes. With the expansion of soybean crops across Paraguay, significant transformations occurred. This expansion of soybean crops became a direct cause of the deforestation the Eastern region of Paraguay suffered since 1960 (WWF, 2016). The deforestation rate reached an enormous 289,000 hectares in 1986. At one point, Paraguay had the second-highest deforestation rate in the world (WWF, 2013). This massively impacted the Atlantic Forest: as much as 7,000,000 hectares were lost due to its conversion into cropland (WWF, 2006). Violent clashes between peasants and security forces ingnited as a result of displacement of peasants, indiscriminate use of agrochemicals. Burning soybean crops, demonstrations, confrontations with security forces resulting in peasants injured and eleven of them killed between 2003 and 2005. As a

response, the government created a Rural Crisis Cabinet, which resulted in a proposal of Land Conversion Moratorium, a Social Pact and a series of programs implemented in the years to come.

State Responses to Environmental Conflicts: Zero Deforestation Law and its Results. There was a precise and impactful response from the State (including the civil society, through WWF-Paraguay and WWF-EPO) to the problem of deforestation through the sanctioning of the Bill Land Conversion Moratorium for the Atlantic Forest of Paraguay. The law aims to *"promote the protection, recovery, and improvement of native forest in the Eastern Region"* (Law 2524, 2004: Art. 1), by prohibiting the conversion of areas covered with the forest for an initial period of two years, which was successively extended[17]. The law was enacted in 2004, and its results were almost immediately seen. From an alarming 110,000 ha. deforested in 2002, the rates dropped to 20,000 ha. in 2005. This represents a drop between 80 and 90 % in the deforestation rates. This effect is shown in figure 2. This law while protecting the native forests has not affected the agriculture production in the region whatsoever, quite the reverse, soy production increased in the follow years (WWF, 2013). Lacerda of WWF's Global Forests Program accurately stated, "*Paraguay provides an example and an inspiration for those countries fighting against deforestation all around the world*" (WWF, 2006).

Figure 2: Evolution of the deforestation rate (Ha./year) in the Atlantic Forest of Paraguay from 1940 to 2014 (Modified from WWF, 2006)



This case study is a clear example of how resources degradation and distribution can generate violent conflicts. Many of the factors identified as primary causes of the Brazilian migration to the Eastern region of Paraguay and the correlative transformation of the landscape, the land use, and the production methods, represent remaining challenges to governance and environmental sustainability. The differentiated access Brazilian farmers have to financial aid, technical assistance, and technology, puts them in an advantageous position against Paraguayan peasants. Brazilians can produce more, due to the better technical management, technology use and the support they receive through their government and its agencies. Unless the Paraguayan government takes serious action to balance this disparity and establish an equivalent aid to local farmers, the unequal opportunities will keep pressing on the peasants, and may result in their lands sold to the bigger and stronger actors. Institutional weakness -and even the absence of it- is evident in several ways. The Paraguayan state not only did not provide the necessary technical and financial support to the peasants but also through its little enforcement facilitated the big actors' predation of natural resources. With the enacting of the Zero Deforestation Law and the rigorous enforcement that followed, the

17 The Law 5045 of 2013 extended its validity period until 2018.

rate of deforestation decreased by 80 % (reaching even more than 90 % in some years). As farfetched as it may sound, it is true, and it was achieved even despite the limited resources that the SEAM had during those years. When the State and then civil society acts effectively, regardless of its limitations, improvements can be experienced.

Conclusion

The soybean production, although representing an essential proportion of the Paraguayan GDP growth, must be regulated carefully considering its negative environmental impacts.

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NATURAL CONSTRAINS VS. FARM'S ECONOMIC EFFICIENCY. EXAMPLE OF FARMS IN POLAND

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Abstract

Natural conditions constitute the foundation for various forms of farming and they determine the production capabilities. They are one of numerous determinants of productivity and profitability. Various natural difficulties may have a significant impact on the economic efficiency of farming. As a consequence, it may lead to abandoning the use of land, reducing the vitality of the rural community, or changing the management system for less environmentally friendly. *The aim of the study is to identify and assess the economic diversification of farming efficiency in areas with natural constraints (ANCs).* The study was based on the Polish FADN data. The analysis included FADN farms with an economic size of 2+ ESU and the data for 2010 and 2015. The analysis covered farms located in lowland zone (I and II), mountain zone and in zone with specific difficulties. Results show significant differences in the efficiency of farming located in individual zones of natural constraints. However, there are no significant differences in the efficiency of farming between farms in the lowland zone and outside the ANCs. The production and financial results of these farms were at a similar level. In turn, the economic efficiency of farms in the mountain zone and in zone with specific difficulties was significantly different from the one of farms outside ANCs. It means that the CAP's ANC subsidies do not compensate for differences in production and financial results of farms in ANCs. To maintain agricultural activity in these areas, we should aim at increasing payments for farms from mountainous areas and from specific areas. This is an important recommendation for the CAP 2020+ that should be taken into account when considering the distribution of CAP funds. This means that the ANC subsidies introduced under the CAP do not compensate for differences in production and financial results of farms operating there, in particular in mountainous areas and the ones with specific handicaps.

Keywords: *farming efficiency, farm incomes, areas with natural constraints, ANC subsidies.*

Introduction

Support for farmers conducting agricultural production in areas with unfavourable environmental conditions has more than forty years of tradition in Europe. The EU member states through the ANC payment system implements various goals of a social, economic and environmental nature. Therefore, the methodology for determining payments, the mechanisms for their implementation and the conditions that must be met in order to obtain them differ in each country (Dax et al., 2003). The purpose of ANC payments in Poland is to provide farmers with compensation for ANCs so as to ensure continuity of agricultural land use, while maintaining the vitality of rural areas and landscape values, promote environmentally friendly agriculture and prevent rural depopulation.

According to Szabo and Grznar (2008), agricultural production in ANCs should primarily meet non-productive objectives related to nature and environmental protection and the generation of new jobs. As Bogdanov (2014) notes, over the last decade the social component of ANC payments has lost its importance, while environmental issues and the maintenance of

specific agricultural practices have become more important. This is undoubtedly connected with global problems of agriculture, including the problem of occurrence of increasingly and more extreme weather events resulting from climate change and the problem of food safety. These problems will require the introduction and improvement of existing measures to ensure continuity of production processes and support for farmers at every production level.
The high share of ANCs in Poland necessitates monitoring whether the instruments aimed at compensating for operating in these areas are effective and accurately distributed. The aim of this study is to identify and assess the economic diversification of farm management efficiency in areas with natural constraints.

Materials and methods

The study was based on FADN data. The analysis included farms with an economic size of 2 and more ESU, which in 2010 and 2015 were conducting agricultural accounting for the needs of the Polish FADN (Table1).

Table 1. Characteristics of the study sample

Specification	Years	
	2010	2015
Number of farms in the sample	11,004	12,105
Number of farms without ANC payments (N_ONW)	5,404	7,453
Share of farms without ANC payments	49.11%	61.57%
Number of farms with ANC payments (W_ONW)	5,600	4,652
Share of farms with ANC payments in the type:	50.89%	38.43%
Lowland I (W_ONW_I)	35.06%	26.43%
Lowland II (W_ONW_II)	13.30%	9.94%
With specific constrains (W_ONW_SU)	1.47%	1.36%
Mountainous (W_ONW_GO)	1.05%	0.70%

Source: own study based on FADN data.

The largest share of farms that received ANC payments were farms located in lowland zone I and II. Farms with specific constraints constituted less than 2% of all farms located in ANCs, while farms located in the mountain zone constituted about 1%. In 2010, farmers received PLN 31.02 million for operating in ANCs, while in 2015 the value of these subsidies amounted to PLN 21.57 million. The analysis included the production and economic results of farms with natural constraints, which were divided into four groups according to the ANC category. The study includes: a) farm production potential; b) production effects and; c) profitability of farms.

Results and discussion

The analysis of farms located in and outside of ANCs showed significant differences in their production potential. ANC farms were characterized by a smaller average area of agricultural land compared to non-ANC farms. The smallest were farms in mountain areas, the largest the ones in lowland. In the analysed period, the average size of mountain farms and the ones with specific constraints increased. The largest increase was recorded in mountain farms (Table 2). A grow in the average land size may lead to increased competitiveness and economic efficiency of these farms. The rise in the average area of arable land was accompanied by the increase in the share of leased land. The only exception are lowland ANC lowland farms with a decrease of 5.51% in the share of leased lands in 2015 compared to 2010. The share of permanent grassland increased. The research shows that permanent grassland is much more important in the structure of land use in ANCs. Outside ANCs, the share of permanent

grassland in the land use structure constituted on average 89% in 2010 and 12.77% in 2015. This increase may result from the need to meet the conditions associated with greening. A slight increase in the share of permanent grassland was also recorded in farms located in lowland areas. The largest share of permanent grassland was recorded in ANC mountain farms and lowland II. These are farms dominated by entities specializing in dairy cattle and herbivorous animals. In 2010, compared to 2015, there was a significant decrease in the share of permanent grassland in the structure of land use in ANC mountain farms and the ones with specific constraints. At the same time, a significant increase in the share of arable land was noted. This may result from the desire to improve profitability. This is also the result of the cattle population declining for many years, including in particular dairy cows (GUS 2016). This situation causes a reduction in the efficiency of using meadows and permanent pastures. This brings specific damages, both economic and environmental, since permanent grasslands have both production function and numerous environment protective functions (Jankowska-Huflejt, Domański, 2008). The research results indicate that farms located in ANCs are characterized by a smaller share of fallow land in the area of arable land. The smallest share of fallow land was found in lowland farms, while the largest in the case of mountain farms and the ones with specific constraints. In 2010, compared to 2015, there was a smaller share of fallow land in lowland II farms and farms with specific constraints. On the other hand, the share of fallow land in lowland and mountain farms increased. The farms lying in ANCs were characterized by a much worse quality of soils compared to non-ANC farms. This has a decisive impact on the possibility of obtaining high income from agricultural production. Farms with the lowest potential for land productivity are farms located in the ANC lowland and mountain areas. The indicator of the quality and usability of soils in these farms was half as high as in non-ANC farms. Hence, it is not surprising that in these farms, the high share arable land is formed by fallow land. Running production on weaker land makes it impossible to generate sufficient income. Low quality of soils leads to cultivation of extensive plant species, which bring lower income. Moreover, they can lead to soil degradation (Czapniewski, Niewęgłowska, Stolbova, 2008). As a consequence, it may lead to abandonment of production in these areas. The conditions in ANCs significantly affect production intensity. The highest average production costs are borne by lowland farms. In 2015, compared to 2010, the total production costs of lowland farms increased.

Table 2. Farm production potential of ANC and non-ANC farms

Years	ANC type	UAA (ha)	Share of leased UAA	Share of arable land in UAA	Share of grassland in UAA	Share of fallow land in UAA	Full-time labour (AWU)	Total production costs (PLN '000)	Production costs per ha (PLN '000)	Fertilizer cost per ha (PLN)	Soil class index
2010	N_ONW	34.75	22.57	85.18	9.89	1.51	1.72	175.61	14.85	981.14	1.05
	W_ONW_I	38.04	22.16	79.02	18.11	0.82	1.70	167.36	6.89	510.74	0.71
	W_ONW_II	31.99	20.73	71.78	26.67	0.95	1.73	139.48	4.84	370.95	0.47
	W_ONW_SU	27.80	24.30	68.60	23.83	1.32	1.72	92.24	8.34	553.81	0.79
	W_ONW_GO	24.40	27.23	38.61	53.98	1.04	1.83	100.18	6.98	201.98	0.48
2015	N_ONW	36.79	23.25	83.29	12.77	1	1.69	212.82	11.93	1014.09	0.93
	W_ONW_I	36.45	20.94	78.54	18.47	0.95	1.72	192.63	8.10	720.38	0.69
	W_ONW_II	29.12	21.71	71.57	27.20	0.58	1.72	152.00	5.62	494.47	0.45
	W_ONW_SU	28.74	26.34	74.76	20.22	0.64	1.66	127.51	8.50	591.61	0.76
	W_ONW_GO	27.06	33.14	74.72	21.60	1.21	1.88	87.19	4.60	147.65	0.41

Source: own study based on FADN data.

Full-time employment in ANC farms did not differ significantly. Relatively higher employment was recorded in mountain farms. It was on the average 1.85, and in the remaining groups 1.70. Employment growth was recorded in lowland I farms. The biggest decrease in employed was seen in units with specific constraints. This could be due to a change in the organization of production. As indicated by the reduction of the average area of permanent grassland, and at the same time an increase in the average area of arable land.

The economic and financial results of the farms varied depending on their location. ANC farms were characterized by much worse results than non-ANC farms (Table 3). In the group of ANC farms, the highest value of production was achieved by lowland farms, while the lowest was obtained by mountain farms and the ones with specific constraints. In these farms the labour productivity was also the lowest. In all ANC groups, labour productivity decreased with the exception of farms with specific constraints. On the other hand, in non-ANC farms, labour productivity increased, which further deepened the difference between farms. Land productivity measured by the ratio of the value of production to the area of arable lands in ANC farms was at a lower level than in non-ANC farms. When assessing land productivity in particular types of ANCs, it should be noted that lowland and mountain farms show significantly lower land productivity than non-ANC farms. Mountain farms and the ones with specific constraints distinguished themselves by the highest change dynamics, but the land productivity decreased. ANC farms achieved a relatively lower efficiency of management compared to non-ANC farms. The lowest level of production profitability was characteristic of farms with specific constraints. In the analysed period, the production profitability in all farm groups was noticeably lower, while the largest decreases were recorded in lowland farms (around 10%). In such conditions, the use of areas of low agricultural utility in food production decreases. This raises the risk of land abandonment and losing its agricultural utility (Bański, 2008). Based on the conducted study, it can be concluded that the return on assets varies considerably depending on the location. The worse the conditions for farming, the lower the income assets generate, and therefore they are less effective. The return on equity ratio for ANC was low. In 2015, compared to 2010, it significantly deteriorated. This may mean that in the future some of these farms will cease to conduct agricultural production. As Gołaś indicates, the ability to accumulate capital by generating profits is a sine qua non condition, i.e. a decisive factor determining the continuation or cessation of economic activity in the long-term (Gołaś, 2009).

Table 3. Farm productivity indicators for ANC and non-ANC farms

Year	ANC type	Production (PLN '000)	Land productivity (PLN '000/ha)	Labour productivity (PLN '000/AWU)	Production profitability (%)	ROE (%)	ROA (%)
2010	N_ONW	229.58	19.30	149.79	131.88	1.12	9.43
	W_ONW_I	211.95	8.97	142.40	126.94	1.85	1.55
	W_ONW_II	173.52	6.08	109.06	122.24	0.88	0.58
	W_ONW_SU	113.79	11.12	74.24	120.31	- 0.93	- 1.12
	W_ONW_GO	125.55	8.82	78.58	126.24	- 1.87	- 1.66
2015	N_ONW	251.39	15.74	154.23	118.67	- 0.80	- 0.95
	W_ONW_I	217.69	9.35	136.03	111.44	- 1.18	- 1.54
	W_ONW_II	171.36	6.33	101.51	109.16	- 1.44	- 2.09
	W_ONW_SU	146.48	10.47	91.46	117.17	- 4.08	- 3.99
	W_ONW_GO	104.40	5.97	60.07	122.34	- 2.70	- 2.76

Source: own study based on FADN data.

The average annual income of ANC farms was characterized by high diversity. The worse the condition for conducting agricultural production, the smaller the farm income. The income of farms from the lowland I area was closest to the non-ANC ones. The average lowland farm income is about 50% higher than the one of farms from other ANCs. The largest differences in incomes are visible for farms with specific constraints and mountain farms. In the analysed period, the income of all farm groups decreased, with the exception of farms with specific constraints (Table 4). The greatest decreases were recorded for ANC farms, including lowland ones. The difference in income between ANC and non-ANC farms decreased. In particular, in the case of mountain farms and farms with specific constraints. Research also shows that mountain farms and the ones with specific constraints were characterized by higher land profitability than lowland farms. In farms with specific constraints, the land profitability was close to the profitability ratio of non-ANC farms. This may be due to the fact that some farms

in mountain areas or the ones with specific constraints could have received ANC payments higher than the once required to compensate for the existing constrains.

Table 4. Income generation and distribution indicators for ANC and non-ANC farms

Years	ANC type	Farm income (PLN '000)	Land profitability (PLN '000/ha)	Subsidies (PLN '000)	Share of ANC payments in farm income (%)	GVA (PLN '000)	Labour productivity (PLN '000/AWU)
2010	N_ONW	95.28	5.73	-	-	134.08	50.17
	W_ONW_I	93.52	3.42	5.23	12.56	132.25	48.42
	W_ONW_II	78.75	2.69	6.37	10.09	111.39	40.98
	W_ONW_SU	60.05	4.31	5.43	10.42	88.70	33.25
	W_ONW_GO	64.17	3.58	5.55	31.35	88.36	31.96
2015	N_ONW	85.08	5.25	-	-	126.81	46.66
	W_ONW_I	76.00	2.76	4.71	11.84	115.06	40.99
	W_ONW_II	63.24	2.32	5.77	17.17	92.63	35.25
	W_ONW_SU	65.61	4.27	4.92	16.42	89.35	33.35
	W_ONW_GO	63.34	3.30	5.83	14.03	88.11	33.43

Source: own study based on FADN data.

The amount of ANC payments received varied depending on farm location. In 2015, compared to 2010, the average amount decreased (on average by 10%), with the exception of subsidies for mountain farms. This was due to changes in the level of payments in relation to the 2007-2013 programming period. In the period 2014-2020, the mountain ANC payment rate increased by 40%. In 2015 the share of ANC compensatory payments in farm income was on average 11.48-17.17%, while in 2010 it was 10.09-31.35%. This is the effect of introducing in 2015 a more restrictive degressivity of payments. In the analysed ANC farm groups, the land profitability was much lower than in non-ANC farms. The lowest land profitability was observed in lowland II and mountain farms. The land profitability index decreased for all ANC types. The biggest decreases were seen for lowland farms (16% on average). A slight decrease in land profitability was recorded in case of farms with specific constraints (0.93%). Labour productivity measured by net value added per person in full-time employment indicates a significant diversity between ANC and non-ANC farms. It ranged from PLN 50,170 to PLN 31,960 in 2010 and from PLN 31,960 to PLN 33,430 in 2015. A much lower level labour productivity was characteristic for farms with specific constraints and mountain ones. In the analysed period, labour productivity decreased in lowland farms, slightly increased in farms with specific constraints (0.30%) and mountain farms (4.60%). Research shows that the difference in labour productivity of non-ANC farms and mountain farms and farms with specific constraints decreases. ANC farms were characterized by diversified efficiency of inputs. Gross value added determining the increase in the value of goods produced in agricultural holdings was the lowest in mountain farms and the ones with specific constraints. In mountain farms the efficiency of inputs slightly deteriorated (a decrease in GVA by 0.28%). On the other hand, in farms with specific constraints, it increased by an average of 0.73%, while in lowland farms, it decreased significantly (by 13% - lowland I; by 16.48% - lowland II). Despite ANC compensatory payments, the ANC farms had worse efficiency than non-ANC ones. This indicates the low effectiveness of subsidies and their impact on the improvement of the economic situation of ANC farms.

Conclusion

The obtained results indicate the existence of significant differences in the efficiency of farm located in different ANCs and the lack of significant differences between farms located in the lowland zone and farms located outside the ANCs. The economic results of these farms were at a similar level, which means lowland ANCs received too high support. The economic effectiveness of farms located in mountain zone and the one with specific constraints

significantly differed from the efficiency of lowland and non-ANC farms, showing that ANC payments do not compensate for differences in production and financial results of farms from ANCs. The data indicate that the main objective of maintaining agricultural activity and stopping depopulation in mountain areas and the ones with special difficulties in the long term may not be achieved. These farms were characterized by much worse economic results than farms from other ANCs. In order to maintain production continuity within these areas a greater focus should be placed on support for mountain areas and the ones with special constraints. On the basis of the conducted research, it can be concluded that in farms receiving ANC payments in Poland in 2010-2015, the efficiency of management has not changed significantly, therefore the economic effect of ANC payments is small. Therefore, it is necessary to start work on introducing changes to the current ANC payment system in Poland.

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COMPARISON OF POLISH DAIRY FARMS AGAINST SELECTED FARMS FROM OTHER EU COUNTRIES USING THE MALMQUIST INDEX

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Abstract

The aim of the paper was to assess the effectiveness of Polish dairy farms (type 45) against a background of similar farms from selected European Union countries and to determine their ability to compete. The studies covered farms from the following countries: Poland, Hungary, Lithuania, Austria, Germany, Denmark, the Netherlands and France. The analysed data covered the 10-year period from 2006 to 2015. The source of research materials was data from farms from the European Farm Accountancy Data Network (FADN). In order to measure effectiveness, the Malmquist productivity index was used. In terms of the Malmquist index, the Polish farms had the lowest value (-2.9%), while the Danish farms had the highest value (2.5%). Such disproportions were mainly due to very slow changes in the technical progress of the Polish farms. In the analysed farms there was a slight decrease in productivity measured by the Malmquist index (by 0.3%). The decrease in the productivity value of the dairy farms was caused by a drop in technical progress by 0.4% with an increase in technical effectiveness by 0.2%. The slight changes in productivity were a consequence of the functioning of milk quotas in the EU. This mechanism has effectively limited the increase in milk production, so the producers could only optimize the inputs.

Keywords: *Dairy farms, effectiveness, Malmquist productivity index.*

Introduction

In Poland, in the years 2005-2015 the agricultural production increased by 73%[18]. The crop production increased by 85.5% while the livestock production by about 65%. The result was the increased share of the crop production in the commercial production by 2.8 p.p. from 38.7 in 2005 to 41.5% in 2015, with the decreased share of the livestock production by 2.8 p.p., from 61.3 to 58.5%. In the livestock production, an important role is played by farms specialising in rearing animals fed on roughage where the production of cow's milk and beef cattle. The share of this group in the commercial livestock production increased from 42 in 2005 to 46.9% in 2015. In the cattle production, the dominant position is occupied by the milk production. Its share in the analysed period was fairly stable, within the range: 32.2 – 33.8%[Gołaś 2017]. In 2016, cattle rearing in Poland was dealt with by 343.2 thousand farms, i.e. about one third of all farms with an area of 1 ha and larger [Characteristics of farms, 2017].

Poland is also a major producer of milk in the European Union. In 2017, it was fifth with the share of 7.5%, after such countries like: Germany (20.4%), France (16.2%), United Kingdom (9.6%) and the Netherlands (9.2%) [Market analyses, 2017].

The objective of the study is to assess the efficiency of the functioning of Polish dairy farms (type 45) against a background of similar farms from the selected European Union countries.

[18] Statistical Yearbook of Agriculture, CSO 2016

Materials and methods

Analysis covered the farms from: Poland, Hungary, Lithuania, Austria, Germany, Denmark, the Netherlands and France. The selection of countries was deliberate. Hungary, Lithuania and Austria have been selected because of the similar size and structure of the farms and the production intensity level. On the other hand, Germany and France have been selected as the largest producers of milk and Denmark and the Netherlands as the countries with the highest milk production intensity level.

For analysing the efficiency of dairy farms in the years 2005-2015, the input-oriented and CCR-based Malmquist productivity index has been used. As the output, the total production value has been assumed, as inputs – the labour costs (the product of working hours and the rate for paid labour on the farm), the activity costs (total costs reduced by costs of salaries and depreciation) and the value of assets. The Malmquist productivity index takes values higher than unity in the event of the increased productivity. The changes in the components of the index, i.e. changes in the efficiency in technical progress, are interpreted exactly in the same way. For the Malmquist index, the productivity is defined as a ratio of the production expressed in the given output value to all inputs applied [Coelli 1998].

The technical efficiency (changes in the technical efficiency) expresses the ratio of obtained outputs from given inputs to the possible maximum outputs achieved by selected entities. Changes in technology (changes in technical progress) determine the change in the manufacturing technique in a given period. The source of the study materials was the data of farms from the European FADN[19].

Results and discussion

Table 1 provides the costs of using own production factors: land, labour and capital in dairy farms (type 45). Despite 11 years of presence in the structures of the European Union's common market, there are still significant disparities in both the potential of farms of the so-called "Old Union" and its new members, including Poland [Kleinhanss 2015]. These differences are evidenced just by their different representation of dairy farms in the six SO classes[20]. The class 2 farms were in the field of observation only for Poland, Lithuania and Austria, while the largest farms dominated in Denmark, the Netherlands, Germany and Hungary. Attention should be paid to the persistent large differences in the level of land use costs, expressed by the amount of rent. The land use costs for the German class 3 farms were 2.6 times higher than in the Polish farms, however, we can observe a slightly higher growth rate in the newly admitted countries. Even greater differences have been observed in the case of the labour costs, by far the highest rates were applicable in the Danish farms, they exceeded the level of EUR 20 per hour, being twice as high as in the German farms and 10 times higher than the level of this rate in the Polish farms. In all the countries analysed, the costs of paid labour were growing as the economic size class increased. It should be stressed that the level of payment for farm labour was in all countries slightly lower than paid in the whole economy, the biggest differences can be observed in the lowest size classes, especially in the Polish, Austrian and French farms.

[19] The network of the farms covered by the studies.

[20] Standard output is the 5-year average production value of the specific production activity (crop or livestock) obtained during 1 year from 1 ha or from 1 animal (exclusive of: edible mushrooms – 100m^2, poultry – 100 head, bees – 1 bee colony i.e. 1 bee family), in the production conditions which are average for the given region.

Table 1. Costs of own production factors: land, labour and capital in the analysed dairy farms depending on the economic size of the farms in the years 2013-2015

Countries	Farm size in SO (thousand EUR) type 45									
	8-25 (2)		25-50 (3)		50-100 (4)		100-500 (5)		≥500 (6)	
	Average[a]	Index of changes[b]	Average[a]	Index of changes[b]	Average[a]	Index of changes[b]	Average[a]	Index of changes[b]	Average[a]	Index of changes[b]
	Costs of land (EUR/ha)									
Poland	58.0	1.34	80.9	1.54	87.1	1.42	94.2	2.04	-	-
Hungary	-		-		78.4	1.79	93.9	1.29	115.8	1.11
Lithuania	11.8	2.10	20.4	2.23	13.6	1.58	16.9	1.66	-	-
Austria	115.0	1.13	143.8	0.97	177.76	1.27	231.0	1.24	-	-
Germany	-	-	204.1	1.17	227.1	1.28	281.3	1.20	251.5	1.86
Denmark	-	-	-	-	-	-	499.9	99.5	616.7	94.1
the Netherlands	-	-	-	-	-	-	704.5	1.15	930.0	1.36
France	-	-	68.4	0.56	109.6	1.08	151.2	1.13	-	-
Countries	Costs of labour in agriculture (EUR/h)									
Poland	2.04	1.18	2.24	1.17	2.25	1.22	2.70	1.11	-	-
Hungary	-	-	-	-	2.79	1.30	2.78	1.22	5.65	1.36
Lithuania	2.67	2.07	2.84	1.42	2.90	1.53	3.39	1.39	-	-
Austria	4.94	1.65	6.98	1.01	6.56	1.14	6.31	0.91	-	-
Germany	-	-	12.43	3.00	11.68	1.46	11.84	1.55	13.35	1.33
Denmark	-	-	-	-	-	-	21.69	1.03	22.82	1.01
the Netherlands	-	-	-	-	-	-	13.62	1.03	16.56	1.14
France	-	-	7.92	1.0	11.46	1.17	12.77	1.11	-	-
Countries	Average salary in the economy [a] (EUR/h)									
Poland	4.29									
Hungary	3.59									
Lithuania	3.11									
Austria	14.02									
Germany	15.67									
Denmark	25.52									
the Netherlands	16.0									
France	14.94									

[a] Average of the years 2013-2015, [b] Index of changes 2015/2010
Źródło/Source: [Ziętara, Adamski 2013, 2017]

As already mentioned, the dairy farms in the selected countries, in addition to the level of labour payment and land use costs, still differ significantly in the production scale. From the data in Table 2 it results that in 2010, on average, the dairy farm in Poland kept 5.9 cows, while the Lithuanian farm kept 4.1 cows, and in the Austrian and Hungarian - 11.3 and 21

cows, respectively. The biggest herds of cows in that year were kept in Denmark and the Netherlands, respectively: 132.2 and 74.7 cows. In Germany and France, the average herd was 45 cows. In 2013, when compared to 2010, the number of cows on the farm increased in all analysed countries (from 11% in the Netherlands to 23.8% in Hungary) apart from France, where it decreased by 10%. The differences between the countries have remained unchanged over the analysed years. In 2010, the average cow herd in the German farms was 7.8 times larger than in the Polish farms, whereas in 2013 – 7.7 times. The corresponding figures in the Danish farms in relation to the Polish farms were 24.0 and 24.4. The average cow herd size does not reflect the complexity of the phenomenon which the milk production concentration is. The more complete picture is provided by the farm structure by cow rearing scale. The share of small farms (keeping up to 9 cows) in Poland, Hungary and Lithuania was within the range from 78 to 92%. In Austria, the share of those farms was around 50%, while in other countries it was between 2.4% (Denmark) and 12.9% (Germany). In Poland, the small farms kept about 30% of the cow population, in Lithuania – about 42%, in Austria – about 16%, and in Hungary – 10%. In other countries from 0.03% (Denmark) to 1.8% (France). Poland belongs to the leading producers of milk in the European Union. With the production of 12.74 billion litres, isfourth, after countries such as: Germany, France, United Kingdom. There are also significant differences in the milk yield of cows. In 2013, the average annual milk yield of cows in Poland was similar to the yield in the Lithuanian farms, amounted to about 5,500 litres and was by about 38% lower than in the Danish farms. In other countries, the milk yield ranged from 6,400 litres (Austria) to 7,700 litres (the Netherlands).

Table 2. Number of dairy farms, cow population and milk production in the analysed countries in the years 2010 and 2013

Countries	Years	Number of dairy farms, thousand	Number of cows, thousand	Cow herd size/farm	Share of small farms[a] (%)	Share of cows in small farms (%)	Milk production, million litres	Average milk yield, litres/cow
Poland	2010	452.8	2,505.6	5.9	82.5	32.2	12.43	4,854.0
	2013	334.5	2,343.51	7.0	77.7	26.8	12.74	5,532.0
Hungary	2010	11.4	239.0	21.0	81.5	10.6	1.68	7,050.0
	2013	9.5	250.0	26.0	78.0	9.4	1.78	7,091.0
Lithuania	2010	85.0	352.6	4.1	85.7	44.9	1.73	4,815.0
	2013	65.0	318.1	4.9	91.8	40.8	1.72	5,447.0
Austria	2010	47.7	540	11.3	54.1	19.6	3.25	6,115.0
	2013	42.2	536.0	12.7	49.3	15.4	3.42	6,407.0
Germany	2010	89.8	4,164.8	46.4	12.9	1.4	29.63	7,085.0
	2013	78.8	4,251.4	54.0	12.4	1.2	31.34	7,343.0
Denmark	2010	4.3	568.2	132.2	4.6	0.1	4.91	8,569.0
	2013	3.0	582.3	157.4	2.7	0.03	5.09	8,963.0
the Netherlands	2010	19.8	1,487.6	74.7	3.8	0.1	11.95	7,866.0
	2013	18.7	1,552.9	83.0	3.7	0.02	12.64	7,769.0
France	2010	82.6	3,720.0	45.0	10.2	0.7	23.93	6,464.0
	2013	92.5	3,737.2	40.4	12.6	1.8	26.65	6,607.0

a – small farms keeping up to 9 dairy cows

Sources: Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten, 2015, Landwirtschafts Verlag, Münster

Changes in the technical efficiency of dairy farms in the selected countries

Table 3 presents the results of assessing the efficiency of the Malmquist index for dairy farms from the selected EU countries. In the years 2006-2015, the index value decreased by -0.3%, and thus not much. The index level was more affected by the decreased changes in technical progress by -0.4%, with the increasing efficiency (0.2%). The year which should be considered worst in the entire decade is 2009 when the index value decreased by 10.6% and the best year is 2010 when the index increased by 15.8%. In the whole analysed period, the index decreased 5 times (2006, 2008, 2009, 2012 and 2015) and also increased 5 times (2007, 2010, 2011, 2013 and 2014), this may indicate the high variability of the production conditions, fluctuations in the buying-in prices of milk as well as of means of production. In the analysed period, the milk production quota system was functioning, which administratively limited the increase in the production within a Member State. Therefore, the farms were able to increase the milk production by using the unused limit of another farm or to exceed the limit by incurring the risk of imposing fines. It should be noted, however, that these limitations applied on a uniform basis in all Member States, which makes it possible to compare them among themselves.

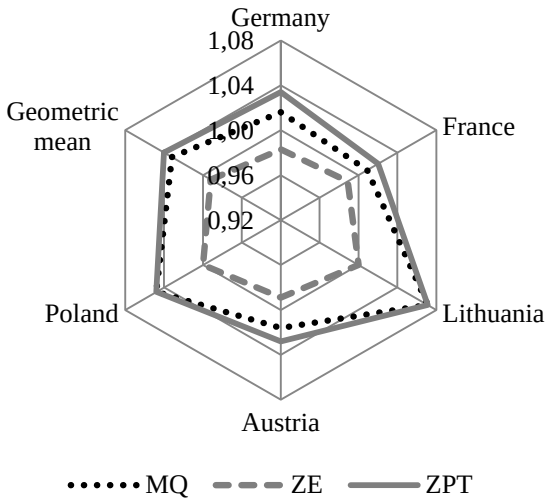
Table 3 Average Malmquist index coefficients in the years 2006-2015 for dairy farms from the selected countries, type 45

Year	Malmquist index	Changes in efficiency	Changes in technical progress
2006	0,977	1,037	0,943
2007	1,08	1,001	1,078
2008	0,954	0,986	0,968
2009	0,849	1,005	0,845
2010	1,158	0,996	1,163
2011	1,056	1,011	1,044
2012	0,982	1,001	0,981
2013	1,024	0,991	1,034
2014	1,012	1,002	1,01
2015	0,913	0,987	0,925
Geometric mean	0,997	1,002	0,996

Source: Own study based on IERIGŻ data

In the further part of the paper, we analysed the Malmquist index level in the farms of the individual EU countries from 2006 to 2015. This comparison has been carried out on the selected sufficiently representative samples from the dairy farms in three different economic size classes. In the group of the farms with the size of EUR 25-50 thousand, the MQ index value shown in Chart 1 rose by 3.2%, with the decreased efficiency of the farms in Germany, France and Austria by, respectively, 1.7%, 1.1% and 1.1%. In the farms of all countries, there has been a positive impact of changes in technical progress on the MQ index. The biggest changes in this area have been recorded in the Lithuanian and Polish farms, by 7.1% and 4.8%, respectively.

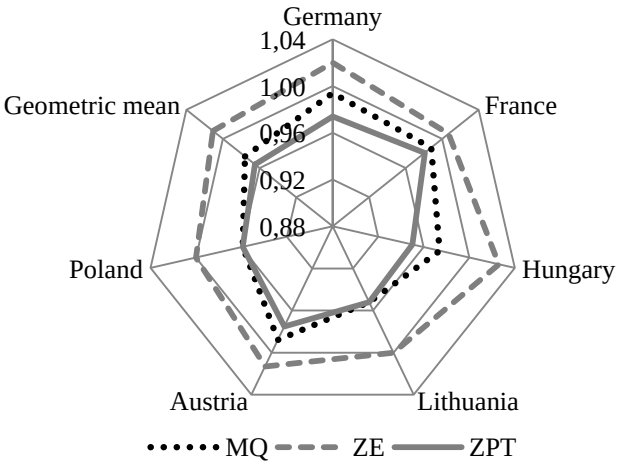
Chart 1. Malmquist index coefficient for the analysed class 3 farms, EUR 25-50 thousand in the years 2006-2015



MQ –*Malmquist Index*, ZE –*changes in efficiency*, ZPT –*changes in technical progres*
Source: Own study based on IERIGŻ data

For dairy farms in the fourth size class, with the standard output between EUR 50 and 100 thousand, the MQ productivity index decreased by 2.4% (Chart 2). This was mainly due to decreased technical progress (decrease by 3.5%) in this group, with the favourable changes in the efficiency (increase by 1.1%). Due to decreased technical progress, the MQ productivity index in the Lithuanian and Polish farms decreased by 4.8% and 4.1%, respectively. Those farms were also the weakest in terms of changes in the efficiency. This does not mean, however, that the efficiency has not improved there, but the level of this increase with respect to the Hungarian or German farms was significantly lower.

Chart 2. Malmquist index coefficients for the analysed class 4 farms, EUR 50-100 thousand in the years 2006-2015

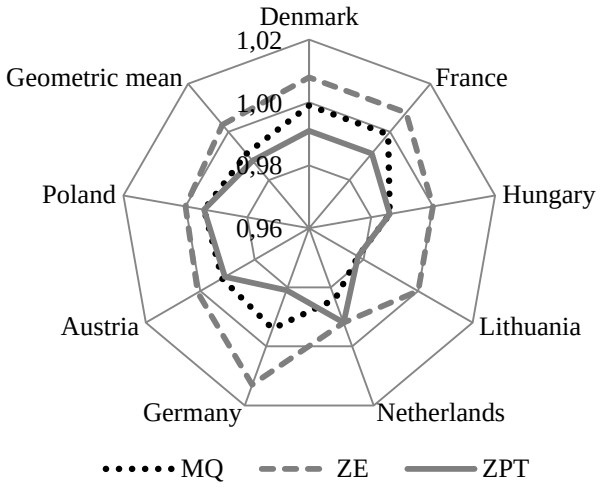


Source: Own study based on IERIGŻ data

In the case of the dairy farms with the very large production scale, classified into the fifth size class with the standard output between EUR 100 and 500 thousand (Chart 3), there has also been no increase in the productivity index. The MQ index has decreased by 0.9% in this

group. The weakest result in these terms was that of the Lithuanian, Dutch and Austrian farms, where the productivity decreased by 2.2%, 1.6% and 1.4%, respectively. Just like in the class 4 group, the decrease in the productivity resulted from decreased technical progress, with the increased efficiency. However, it should be noted that that the increased efficiency of activity in the analysed period did not apply to the Dutch farms (decreased by 0.8%), while it was highest in the German (1.3%), Danish (0.8%) and French farms (0.8%). In terms of changes in technical progress, the lowest values were obtained by the Lithuanian, German and Hungarian farms for which this index decreased by 2.2, 1.9% and 1.4, respectively.

Chart 3 Malmquist index coefficients for the analysed class 5 farms, EUR 100-500 thousand in the years 2006-2015



Source: Own study based on IERIGŻ data

Conclusions

In the period from 2006 to 2015, in the analysed farms there was a slight decrease in the Malmquist index productivity (by 0.3%). The decrease in the productivity value of the dairy farms resulted from decreased technical progress by 0.4% with the increased technical efficiency by 0.2%. The slight changes in the productivity were a consequence of the functioning of dairy quotas. As the production quoting had a limiting impact on the increase in the milk production, the decisive impact on the production output was that of the buying-in price the amount of costs incurred.

The Polish farms in terms of the average value of the Malmquist index were weakest (-2.9%), while the best were the Danish farms (2.5%). The main reasons for such disparities were too slow changes in technical progress of the Polish farms in a large part of small farms. On the other hand, these results mean that the Polish farms have great opportunities to improve their productivity.

The best, in terms of the increased productivity, were the class 3 farms with the size from EUR 25 to 50 thousand. The average index value in this group amounted to 3.2%. The leaders in this group were the Lithuanian (7.1%) and Polish (4.8%) farms. The increased productivity of those farms was due to the changes in technical progress by 4% with the decrease in the efficiency by 0.8%.

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THE TENDENCY CONCERNING THE EVOLUTION OF OILSEED MARKET IN ROMANIA

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Abstract

Agriculture is the main branch of Romania's national economy, an important sector, assuring agro-food product for export, materials for the industry and also, food for population. In the growth a country, an important role plays the international trade because it express the capacity of providing certains services and to produce goods. Romania's agricultural production in 2016 compared to members states of the EU, places Romania, for both cultivated area and output for the sunflower crops, on the first place. Also, rapeseed production has recorded a continuous increasing trend in the analyzed period. The increased price is justified by demand/offer ratio. The main purpose of the paper was to analyze the foreign trade activity of Romania with oilseeds and the trends between 2007-2016. Were used statistical data referring to land surfaces sown with oilseeds, productions, the average yield per hectare, prices, import and export and also, the imports coverage degree by exports.

Keywords: *oilseeds, production, export, import, price.*

Introduction

Agriculture is an important economic sector assuring food for population, raw materials for processing industry and agro-food products for export. Its contribution to GDP is 5.6%. It registered a continuous development in the last decade, and its future depends on a modern technical endowment, investments, employment of high qualified persons, a corresponding farm structure able to assure a higher productivity, economic efficiency and competitiveness (Popescu , 2015).

The Romanian agricultural competitiveness is a debate full topic in the context of the late sectorial reforms during the country accession and convergence to the EU-28 agricultural model (Popescu et al., 2017). The agricultural sector holds a major place in the Romanian economy, with an important contribution to Gross Domestic Product (GDP) creation and also a key role in international trade. The importance of agriculture in Romanian economy results from its share in GDP, labour force and rural community's impact (Ciutacu et al., 2015). The analysis of the foreign trade activity is, like in the case of the other economical branches, of a major importance for establishing efficiency, identifying trades and the justification of specific decisions in this activity (Anghelache, 1999). The external trade has a determinant role in Romania's trade balance, for both exports and imports, especially due the accentuated dependence for the imported food products. In Romania, the land is cultivated with crops which are competitive on world market: maize, wheat, oil crops and barley. These four categories accounts for almost 80% of arable land and have high competitiveness indices of 7.94, 7.52, 3.51 and, respectively, 9.81. Triticale and tobacco are also competitive on world markets, with Balassa indices of 6.65 and, respectively, 4.8, but their shares in arable land are lower 0.87% and, respectively, 0.01. The same products: maize, wheat, oilseeds and barley account for significant shares in agro-food exports: wheat holds the main share of 19%, followed by oilseeds, with 15%, and maize with 14.3%. This structure of exports contains almost the same agro-food products as other studies report (Gheorghe et al., 2017). Vegetables and fruits are foods of plant origin with an important role in the diet, because of

their sensory characteristics and precious nutrients they contain, in the form of carbohydrates, vitamins, organic acids, mineral salts, etc. (Cîrstea et. al, 2013). As (Arghiroiu et. al, 2015) Romania was a net importing country of agricultural products. In 2013 the total trade balance has become a surplus. However, we can say that Romania has become conjectural self-sufficient, because we are surplus to only 5 of the 24 groups of agro-food products. We know a positive balance for cereals, oil seeds and oleaginous fruits, tobacco, live animals, products with raw material nature, and for the remaining agro-food groups we import massive, especially meat, sugars and sugar confectionery, fruits etc. The situation seems to be improving in recent years in terms of the total balance of trade balance, due to the major influence exerted by cereals and oil seeds and oleaginous fruits trade. An important indicator that influences the world, demand and supply is represented by the price over the international market. It has a strong informational consignment, being the basis of economic agent decisions (Angelescu et. al., 2010; Bordean et. al., 2010; Ursu, 2010). Romania's main trading partners in trade with oilseeds are the EU States members, but also we can observe that we import soya beans from Argentina, Brazil and Canada, linseeds from Turkey, India and we export sunflower in South Africa and Pakistan (Armenița Arghiroiu et. al., 2015). Romania is an important pawn over oilseeds market because it produces a significant quantity of sunflower for export. One of the main risk factors in obtaining sunflower crop with stable production is the appearance and evolution of the broomrape. In Romania, more than 60% of the sunflower cultivated area is infested with broomrape. The three more spread broomrape populations in the largest area cultivated with sunflower, are very different regarding the virulence and dissemination of the parasite The race G was definitely found in Tulcea and Constanta counties in Romania and latest surveys showed possible appearance of even more virulent race (Pacureanu et. al., 2009b). As (Pricop et. al., 2011) the race identification must be a continuous process to support farmers, by recommending sunflower hybrids based on the information concerning the parasite spread and virulence throughout the territory. The identification of the parasite physiological races also supports breeders to develop strategy for improvement programs. An evaluation should be made in this context as follows: when the domestic demand of raw materials for processing increases, exports will be reduced (www.agravista.md, 2013). As (Balasu et al., 2014) the production losses caused by soybean bacterial burning (*Pseudomonas savastanoi* pv. *Glycinea*) are major when seed treatment is ignored and the environmental conditions are favorable for the attack.

Material and Methods

In order to make this research, were used statistical data referring to land surfaces sown with oilseeds, productions, the average yield per hectare, prices, import and export and the import coverage degree by export (an indicator of economic competitiveness) (Anghelache C., 2008).

$$GA = \frac{E}{M} x100$$

GA - represents the imports coverage degree by exports;
E - values of exports;
M - values of imports.

This indicator shows the percentage of the value of imported goods covered by the value of exported goods, showing the surplus, the equilibrated or deficit trade balance.

Multiannual average (A): $A = \frac{X1 + X2 + X3 + ...Xn}{n}$;

Growth rate (%):

$$G(\%) = \frac{Xix100}{A} \quad 100$$

G (%) - Growth rate;
Xi - the main indicator used in the analysis as cultivated area, yield, production, etc.;
A - Multiannual average (A).

These data were given by FOASTAT and the National Institute of Statistic and also obtained from the Ministry of Agriculture and Rural Development.

There were also consulted a series of books, magazines and special studies in order to show as concise as possible the evolution of the oilseeds market.

Results and Discussion

As an European Union member since 2007 and a NATO member since 2004, Romania is currently one of the most dynamic large markets in Europe and plays a unique and important part in European agriculture.

Table 1. Surface evolution for the main oilseeds in Romania, during 2007-2016 (thousand ha)

Specification	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Average (thousand ha) 2007-2016 growth rate (%)
Sunflower	835.9	813.9	766.1	790.8	995.0	1067.0	1074.6	1001.0	1000.0	1016	936.0
	growth rate (%)										
	-10.7	-13.0	-18.2	-15.51	+6.3	+14.0	+14.8	+6.95	+6.84	+8.6	100
Rapeseed	364.9	365.0	419.9	537.3	392.7	105.3	276.6	406.7	383.0	471.0	366.8
	growth rate (%)										
	-0.5	-0.5	+14.5	+46.49	+7.1	-71.3	-24.6	+10.9	+4.4	+28.4	100
Soybean	133.2	49.9	48.8	63.9	72.1	79.8	67.7	79.9	128.1	127	85.0
	growth rate (%)										
	+56.7	-41.3	-42.6	-24.8	-15.2	-6.1	-20.4	-6.0	+50.7	+49.4	100

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

Main oilseed crops cultivated in the EU are rape and turnip rape, sunflower and soya. The production was 31.1 million tonnes in the EU in 2016 which is in line with the 5-year average (– 0.8 % if compared to the 5-year average). In 2016, the rape and turnip rape seeds production was 20 million tonnes and it was the most common oilseed crop in the European Union despite its sharp decline since 2014 (-17.1%). The EU-28 sunflower seed production in 2016 was 8.8 million tons and decreased by -14.8% compared to 2014, followed by increase of 10.7% between 2015 and 2016. In 2016, the EU-28 soya production accounted for 2.5 million tons and it is steady increase since 2012 (Statistical Books, Eurostat, 2017 Edition).

In Romania, oilseeds crops register a high weight of the total cultivated area. In Table 1. is presented the evolution of oilseeds surface in Romania, between 2007- 2016. There were analyzed three oilseeds crops, such as: sunflower, rapeseed and soybean.

Between 2007-2016, the sunflower cultivated area varied between 766.0-1074.6 thousand ha. The largest surface cultivated with sunflower was of 1074.6 thousand ha, in 2013. During this year, the sunflower cultivated area increased with 14.8% than multiannual average (936.0 thousand ha). Concerning the area cultivated with rapeseed, had an oscillatory evolution, the largest surface was in 2010 with 537.3 thousand ha. In 2012 decreased over the total with 71.3% than multiannual average (366.8 thousand ha). Rapeseed has become a more and more attractive crop for farmers due to the EU subsidy (Euro 45/ha) provided since 2005 for encouraging bio fuel production (Zahiu et al., 2010). Soybean cultivated area has varied from a period to another but generally it has continuously increased from 48.8 thousand ha in 2009 to 128.1 thousand ha in 2015, with 50.7% than multiannual average (85.0 thousand ha).

Table 2. The evolution of oilseed production (thousand tons) and medium production per hectare (kg/ha) in Romania, between 2007-2016

Specification		2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Average 2007-2016
Sunflower	thousand tons	546.9	1169.7	1098.0	1262.9	1789.3	1398.2	2142.1	2189.3	1758	1954	1530.8
	growth rate (%)	-64.3	-23.6	-28.3	-17.5	+16.9	-8.7	+39.9	+43.0	+14.9	+27.7	100%
	kg/ha	654	1437	1433	1597	1798	1310	1993	2187	1758	1923	1609
	growth rate (%)	-59.4	-10.7	-10.9	-0.7	+11.8	-18.6	+23.9	+35.9	+9.3	+19.5	100%
Rapeseed	thousand tons	361.5	673.0	569.6	943.0	739.0	157.5	666.1	1059.1	959.0	1336	746.4
	growth rate (%)	-51.6	-9.8	-23.7	+26.3	-1.0	-78.9	-10.8	+41.9	+28.5	+79.0	100%
	kg/ha	991	1844	1357	1755	1882	1496	2408	2604	2530	2836	1970.3
	growth rate (%)	-49.7	-6.4	-31.1	-10.9	-4.5	-24.1	+22.2	+32.2	+28.4	+43.9	100%
Soybean	thousand tons	136.1	90.6	84.3	149.9	142.6	104.3	149.9	202.9	262.0	262.0	158.5
	growth rate (%)	-14.1	-42.8	-46.8	-5.4	-10.0	-34.2	-5.4	+28.0	+65.3	+65.3	100%
	kg/ha	1021	1817	1726	2345	1980	1308	2216	2539	2045	2047	1904.4
	growth rate (%)	-46.4	-4.6	-9.4	+23.1	+4.0	-31.3	+16.4	+33.3	+7.4	+7.5	100%

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

Sunflower production has increased from 546.9 thousand tons in 2007 to 2189.3 thousand tons in 2014, with 43.0% more than the multiannual average (1530.8 thousand tons). Rapeseed production has recorded a continuous increasing trend in the analyzed period. In comparison with 361.5 thousand tons carried out in 2007, in 2016, Romania achieved 1336 thousand tons, with 79% than the multiannual average (746.4 thousand tons). Rapeseed production started increasing since 2007 at the moment when the European Union decided to expand energetic crops for bio fuel. Therefore, production performance has been determined both by the increased cultivated surface as well as by the increased yield (Table 2). Concerning the soybean production, had an oscillatory evolution during the analyzed period. In comparison with 84.3 thousand tons carried out in 2009, in 2015 and 2016, Romania achieved 262.0 thousand tons, with 65.3% more than the multiannual average (158.5 thousand tons).

The medium production of sunflower per hectare varied between 654-2187 kg/ha. In 2014, medium production of sunflower increased over the total with 35.9% in comparison with the multiannual average. Rapeseed medium production varied between 991 and 2836 kg/ha. In 2016 it recorded an increase of 43.9% than in 2007. Soybean medium production varied between 1021 and 2539 kg/ha. The large production was reached in 2014.

Table 3. shows the evolution of Romanian oilseeds export, during 2007-2016. The exported quantity of sunflower seeds varied between 382.6 and 1420.1 thousand tons. In terms of value, the year 2013 registered the highest income from sunflower seeds export (550.7 EUR millions). Rapeseed quantitative export varied between 68.2 in 2012 and 1461.9 thousand tons in 2016. The most significant soybean exported quantity was registered in 2016 with 108.9 thousand tons.

Table 3. Evolution of Romania's oilseed exports, during 2007-2016

Specification	2007		2008		2009		2010		2011	
	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)
Sunflower	382.6	105.4	471.3	192.2	564.2	146.1	557.4	214.8	1182.8	508.3
Rapeseed	279.1	77.7	564.0	246.0	782.1	223.7	1052.3	334.0	577.2	273.2
Soybean	22.0	4.7	38.9	13.5	10.4	3.0	36.9	13.2	72.7	28.4

Specification	2012		2013		2014		2015		2016	
	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)
Sunflower	652.4	335.6	1420.1	550.7	1321.9	452.5	1099.3	452.2	1183.7	489.9
Rapeseed	68.2	41.8	471.9	192.5	989.1	338.3	773.4	773.4	1461.9	549.0
Soybean	89.5	41.8	38.8	21.9	40.0	21.4	92.9	40.3	108.9	43.0

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

In Table 4. is presented the evolution of Romania's oilseeds imports, during 2007-2016. The quantitative import of sunflower varied between 66.6 and 1972 thousand tons, and the value oscillated between millions euro 32.5 and 138.4. Rapeseed quantitative import varied between 241.0 and 9.7 thousand tons. The quantitative import of soybean oscillated between 15.6 and 168.3 thousand tons.

Table 4. Evolution of Romania's oilseed imports, during 2007-2016

Specification	2007		2008		2009		2010		2011	
	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)
Sunflower	66.6	32.5	89.5	52.2	141.0	72.9	208.2	109.7	237.3	142.6
Rapeseed	9.7	7.9	76.3	35.8	70.4	28.1	241.0	88.1	70.6	50.2
Soybean	68.5	23.7	94.3	38.0	20.7	7.9	15.6	5.9	34.3	12.9

Specification	2012		2013		2014		2015		2016	
	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)	Quantity (thousands tons)	Value (millions EUR)
Sunflower	131.2	108.9	93.3	101.3	118.9	99.2	189.2	127.7	197.2	138.5
Rapeseed	59.4	42.8	28.8	21.3	38.9	29.7	38.3	26.5	28.1	32.2
Soybean	63.3	29.8	117.2	53.7	102.6	41.8	168.3	65.8	122.3	52.5

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

In Table 5., the import coverage degree by export for sunflower, varied from 324.71% in 2007 to 200.46% in 2009, to 543.81% in 2013 and 456.41% in 2014, for rapeseed, except for the year 2012, when trade is highly low compared to the rest of the analyzed period, the coverage is 97.65%, the exports value covering in the year 2014 is 1138.51% out of imports value. For soybean, the imports coverage degree by exports varied from 19.79% in 2007 to 40.8% in 2013, to 220.87% in 2010 and 219.35% in 2011.

Table 5.The imports coverage degree by exports (G_a(%)) for sunflower, rapeseed and soybean, during 2007-2016

Specification	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
	%									
Sunflower	324.71	368.15	200.46	195.77	356.48	308.12	543.81	456.41	354.14	353.8
Rapeseed	983.47	686.93	794.45	379.12	544.32	97.65	903.81	1138.51	2912.4	1703.2
Soybean	19.79	35.55	38.48	220.87	219.35	140.15	40.80	51.11	61.3	82.04

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

Table 6. Average purchasing prices for oilseed, during 2007-2015 (RON/kg)

Specification	2007	2008	2009	2010	2011	2012	2013	2014	2015	Average (RON/kg) (%)
Sunflower	0.84	1.12	0.86	1.19	1.58	1.84	1.59	1.26	1.5	1.31
	growth rate (%)									
	-35.9	-14.5	-34.4	-9.2	+20.6	+40.5	+21.4	-3.8	+14.5	100
Rapeseed	0.79	1.20	0.97	1.25	1.62	1.83	1.57	1.34	1.64	1.36
	growth rate (%)									
	-41,9	-11.8	-28.7	-8.1	+19.1	+34.6	+15.5	-1.5	+20.6	100
Soybean	0.78	0.97	0.96	1.23	1.3	1.71	1.83	1.43	1.33	1.29
	growth rate (%)									
	-39.5	-24.8	-25.6	-4.7	+0.8	+32.6	+41.9	+10.9	+3.1	100

Source: Romania's National Institute for Statistics Report; 2011, 2016; Own calculation.

Oilseeds price presented in Table 6. reflected a large variation from a year to another, but mainly a continuous increase starting from 2007. The increased price is justified by demand/offer ratio.

Conclusions

During 2007-2016, the sunflower cultivated area varied between 766.0-1074.6 thousand ha, with an increased production from 546.9 thousand tons in 2007 to 2189.3 thousand tons in 2014. The exported quantity of sunflower varied between 382.6 and 1420.1 thousand tons, the highest income from sunflower seeds export being reached in 2013 with 550.7 millions euro.
Concerning the rapeseed cultivated area, had an oscillatory evolution, the largest surface was reached in 2010 with 537.3 thousand ha. Rapeseed production has recorded a continuous increasing trend in the analyzed period, compared with 361.5 thousand tons carried out in 2007, Romania achieved in 2016, 1336 thousand tons. Rapeseed quantitative export varied between 68.2 in 2012 and 1461.9 thousand tons in 2016. Rapeseed exports had values situated between 41,8 EUR millions in 2012 and 549,0 in 2016. Soybean cultivated area has varied from a period to another but generally it has continuously increased from 48,8 thousand ha in 2009 to 128,1 thousand ha in 2015. Concerning the soybean production, in 2015 and 2016, Romania achieved 262.0 thousand tons. The most significant soybean exported quantity was registered in 2016 with 108.9 thousand tons.
Oilseeds prices reflected a large variation from one year to another but mainly a continuous increase starting from the year 2007. The imports coverage degree by exports for sunflower, varied from 324.71% in 2007 to 200.46% in 2009, to 543.81% in 2013 and 4456.41% in 2014, for rapeseed, except for 2012, the trade was low compared to the rest. For soybean, the imports coverage degree by exports varied from 19.79% in 2007 to 40.8% in 2013, as a deficit balance, to 220.87% in 2010 and 219.35% 2011 reaching a surplus.
As a conclusion, in the coming years Romania will continue to become a more and more important oilseeds producer and exporter in the European Community.

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THE EVOLUTION OF THE RECORDING OF MAIZE HYBRIDS IN ROMANIA

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Abstract

The registration of a variety means decades of community work, studying all plant varieties created by Romanian or foreign research, without which no hectare of land can be cultivated in Romania. The system of registering new creations in the Official Catalog is based on a precise description of the variety, on the establishment of uniformity and stability following field examination of the characteristics required by the conditions required for registration. This system makes possible to evaluate all varieties under common ambient conditions. This facilitates control of the interaction between varieties and environmental conditions and it is possible to describe the candidate varieties and reference varieties under the same climatic conditions. The purpose of the test is to establish an optimal expression of the phenotype of the variety as a technical basis of assessment to establish its originality with the aim of determining the status of the variety for the new plant creation and the introduction into cultivation of varieties with valuable biological potential. The state food security is reached, among other things, by a proper system of testing, recording and multiplication of varieties, as well as certification of seed produced in accordance with the domestic and international regulations in force. By rigorous testing of maize hybrids and the recording of the performance of hybrids with resistance to unfavorable climatic conditions to diseases and pests in the Official Catalog of Romanian varieties of plants, farmers are given the advantage of obtaining large data, to cover production costs in crop production and to provide a benefit.

Keywords: *registration, testing, hybrid, corn.*

Introduction

Registering a variety means ten years of community work, of study all plant varieties created by Romanian or foreign research, without which no hectare of land can be cultivated in Romania. Testing varieties for their registration is based on the results of official examinations, covering a large number of features through which the variety is described. Field testing consists of two tests that determine the distinctiveness, uniformity and stability of the variety (test DUS) and, for field crop species, agronomic value and use (test VAU). Tests on distinctness, uniformity and stability are carried out on the basis of the Protocols of the Community Plant Variety Office (CPVO), the guidelines of the International Union for the Protection of New Varieties of Plants (U.P.O.V.). Testing varieties for registration in Romania is governed by Law no. 266/2002 on the production, processing, control and certification of quality, marketing of seeds and and planting material, as well as the registration of plant varieties – republished. The State Institute for Variety Testing and Registration (I.S.T.I.S) is the only specialized body of the Ministry of Agriculture and Rural Development and is responsible for the technical examination of the Romanian and foreign varieties for which registration is requested in the Variety Register and in the Official Catalog of Varieties (www.istis.ro). I.S.T.I.S is the only link between research institutions, where new plant varieties and seed producers are created, respectively the Romanian cultivators. Field examination is carried out in varieties testing centers.

A variety tested for registration is studied for 2 or 3 years, depending on the species, in the 24 Center for Testing Varieties (C.T.S.), which represents the entire diversity of the climatic and soil conditions of Romania and where the optimal agricultural production is achieved, the units being necessary for checking the agronomic value of the varieties. Romania's pedoclimatic conditions make it possible to ensure, in the case of crop species, comparable and competitive productions with those obtained in other European Union countries, to ensure the internal consumption needs as well as export. The performance varieties, with superior qualities, adapted to environmental conditions, are one of the means of obtaining high and stable production. The use of a biological material capable to exploit to the fullest the applied technology represent a dynamic factor for increasing production. In Romania, there is an official system for testing new plant varieties for registration in the National Official Catalog and in the European Union catalogs for marketing and / or granting legal protection.
This system makes it possible to evaluate all varieties under common ambient conditions. This facilitates control of the interaction between varieties and environmental conditions and it is possible to describe the candidate varieties and reference varieties under the same climatic conditions. Correct testing of varieties, according to international regulations, contributes to the development improvement sector by creating hybrids with high production potential and valuable physiological features, which allow the growers to achieve exceptional results. Considering the wide spread of maize both in Romania and other countries, its cultivation in the area where stress factors produce significant losses of production, an important contribution is the creation and cultivation of resistant hybrids in the view of reducing the damage. In this respect, the strategic and methodological improvements of the process of creation of inbred lines and of resistant maize hybrids can be considered of great practical interest. Due to the chemical composition of all component parts, maize is a basic forage for animal feed and a valuable raw material for industry, but it is also of particular importance to humans in some areas of culture. As a result, complex corn amelioration programs aiming at obtaining improved commercial hybrids, which correspond to the increasingly demanding market demands for production capacity and a complex of other characteristics, are carried out in all crop areas from the world (Martura et al., 2016).

Materials and Methods

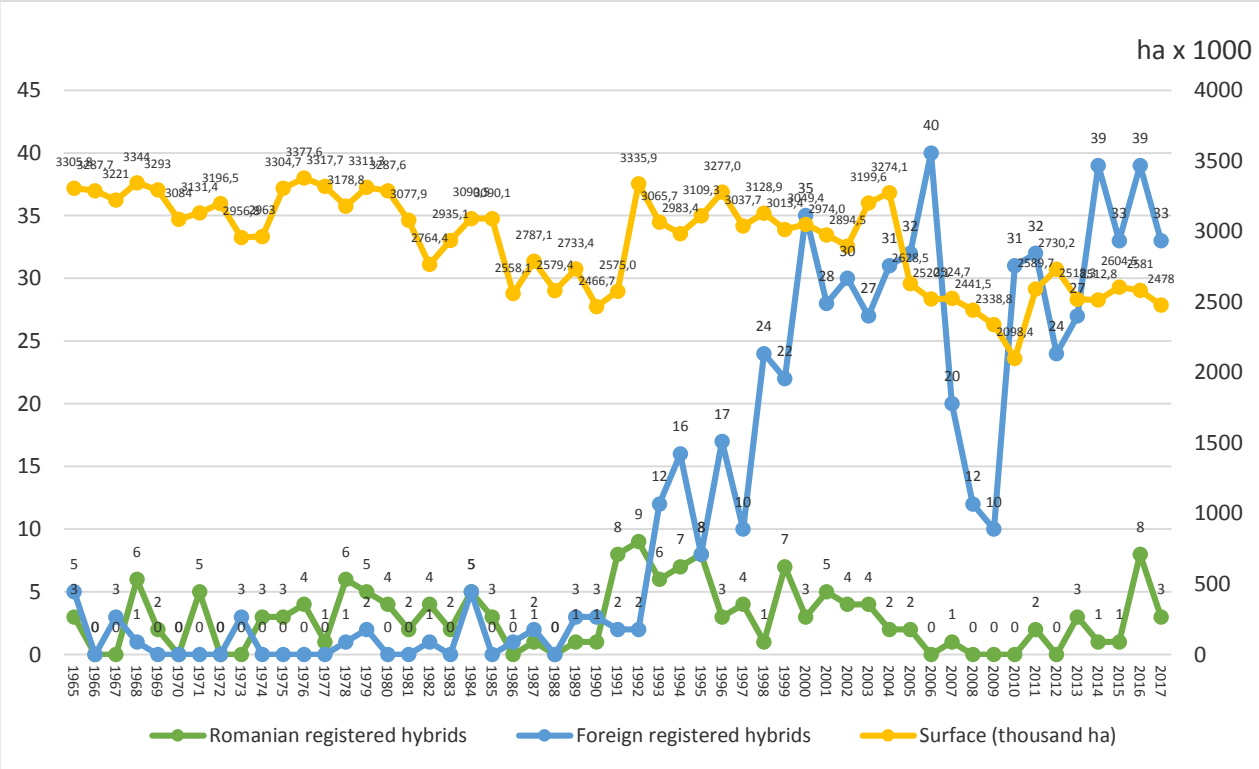
This paper is based on the analysis of the data offered by the Institute for Variety Testing and Registration, respectively the Variety Register (for the period 1965-1969), the Official List (for the period 1970-1999) and the Official Catalog of Cultivated Varieties of Romania (for the period 2000-2017).
This evolution of maize hybrids testing in Romania was carried out for the period 1965-2018, in the context of the main events in Romania, namely the 1989 Revolution and EU accession.

Results and discussions

The first systemic improvement works in Romania were made by the private, farmers Lazaar Laszlo from 1885 and M. Varady in 1902, who created two improved varieties Lapusneag and, respectively, Ardelenesc Varady. After 1910, improvement began in the state institutions, where they were created six varieties improved. Since 1930, the responsibility for the improvement of corn has returned from the Institute of Agronomic Research of Romania (I.C.R.C) established in 1928, which organized the study of the existing varieties and their zoning and created 8 new varieties (Cristea et al., 2004).
The first Romanian maize hybrid was created in 1962 at Improvement Laboratory at Research Institute for Cereals and Technical Plants-Fundulea (I.C.C.P.T) and The agricultural research resort-Turda and was approved in 1965 by the State Commission for Testing and Variety Certification in Romania (currently ISTIS) by registering it with the name HD208 (double

hybrid) in the State Register of Varieties and Hybrids of Agricultural Plants. This result was the effect of the long work done by Vladimir Mosneagă at the Institute of Agronomic Research of Romania (I.C.A.R.) in the field of inbreeding.
On this basis, it was possible to generalize the cultivation of double hybrid maize in a period of only seven years, which places Romania among the countries that have very quickly achieved the transition from the use of free-pollination varieties and local populations to hybrids between inbred lines.
The diversification of the initial material used in the creation of inbred lines, by including alongside the local varieties and populations of genotypes from other parts of the world, and gene infusions from related species, as well as the continuous improvement of breeding methods, have made it possible to make continuous progress in terms of production capacity and adaptability to the hybrids created in the next stages (http://www.incda-fundulea.ro).
The evolution of maize hybrids testing in Romania was quite oscillating, referring to 3 important periods for Romanian agriculture, namely: the communist period, the period after the revolution in December 1989 and the period after Romania's accession to the EU.

Table 1. The situation of Romanian and foreign maize hybrids registered in the Variety Register, in the Official List and in the Official Catalog during 1965-2017



The data in the table was taken from the Variety Register, the Official List and the Official Catalog of Cultivated Varieties of Romania; Own calculation.

The evolution of the recording of maize hybrids in Romania between 1965-2017, has seen a considerable fluctuation in agriculture**.** In the period 1965-1990 the number of maize hybrids tested in Romania was not significant, in 1968 and 1978 most hybrids were, respectively 7, of which one foreign and 6 Romanian. During 1969-1972 and 1974 and 1977 there was a lack of maize hybrids tested in Romania.

The hybrids recorded between 1971-1990, a period marked by the expansion of simple hybrids between inbred lines, surpassed previously created hybrids of 13-14%, and the hybrids created between 1981 and 1985 have secured a further increase of 3-4%. At the same time, other important attributes have been improved, among which the resistance to breakage, fall, drought and scorching heat and diversification of grains quality. After the Revolution of December 1989, the foreign creators of varieties have tested in Romania maize hybrids that they considered to be suitable for the pedoclimatic conditions in order to be placed on the Romanian market. Thus, since 1990, there has been a substantial increase in foreign maize hybrids tested in Romania, reaching the height in 1991, when 40 hybrid foreign maize hybrids were tested in Romania. Romania has entered the EU with an agricultural sector that shows a series of major structural differences to the landscape of European agriculture. Though blurred, the differences continue to manifest at the present time. The reason is founded: policies are not tailored to the size of a single country, but to respond as much as possible to the common needs of all. Romania, however, was largely out of the pattern of the European agricultural system, so it needed a major adjustment to get the most benefice of the European support instruments. 10 years are not enough to reform a country known since ancient times as an agricultural land. Changes have occurred, but not at the expected expected pace. Certainly, the new route - competitiveness and efficiency - that will follow the Romanian agriculture has been imprinted, and the results can not be fully evaluated at a certain moment, but they are visible in time (Albu *et all*., 2018). The highest number of maize hybrids tested in 2006, respectively 40, one year before Romania's accession to the EU.Starting with the year of Romania's accession to the European Union, respectively 2017, all foreign maize hybrids tested in Romania for registration in the Official Catalog of Romanian varieties of cultivated plants allowed the marketing of hybrids registered both on Romanian territory and on other EU Member States. During 2008-2010, there is a large decrease in the number of foreign maize hybrids tested and the lack of Romanian maize hybrids , but starting with 2011, corn hybrids begin to increase reaching the year 2016 to 47 maize hybrids, of which 8 Romanians and 39 foreigners. The potential of Romanian agriculture as well as the entry into the EU of Romania have led to the increase of the number of foreign hybrids tested in Romania, hybrids with a performance genetics that stirred the interest of the big farmers.

Due to continuous genetic progress, Romanian maize hybrids have made an essential contribution to the country's corn production, proving its competitiveness even under the strong competition of foreign hybrids.

Conclusions

Testing allows the introduction into crops only of varieties that have proven resistance to unfavorable environmental conditions in the winter, drought and heat resistance and tolerance to disease and pest attack during the test years.

The introduction of new varieties into culture encourages innovation in the field of plant variety improvement. Establishing the characteristics related to the adaptability of the tested varieties to the pedoclimatic conditions specific to the different parts of the country allows the cultivators to choose the most productive varieties within the precocity groups in the crop areas. Testing the varieties leads to the emergence of competitiveness between native and foreign varieties and influences the Romanian research for the creation of productive and quality varieties. A functional, free and competitive market for seed of the officially registered varieties is developing to ensure not only the needs but also the protection of the beneficiaries at to the European Union standards.

Testing is the basis for the development of the associative system, collaboration and partnership between seed participants of productive and qualitative high-quality varieties.

The current regulations lead to technical progress by introducing into the crop the well-proven, tested and intellectually protected varieties, as well as to the constant information of the cultivators for the widest use of the material of the new varieties.
A country's food security is provided, inter alia, by a proper system of testing, recording and multiplying varieties and certifying the seed produced in accordance with the domestic and international regulations in force.
The introduction of the best varieties into culture and their careful follow-up in the process of multiplication over the years lead to the preservation of morpho-physiological and initial quality features that are very important in achieving high-level production.
By rigorously testing new plant creations and registering only valuable varieties with resistance to adverse climatic conditions to diseases and pests in the Official Catalog, farmers are given an advantage in getting bigger productions, to cover the production costs in the respective crops and to provide a benefit.
In the current context in which modern agriculture must ensure food security for the population, it is necessary to increase the quality and quantity of agricultural production by removing all the causes that lead to the reduction of cereal crops and the improvement of agricultural techniques. A safe way to reduce crop losses is to create hybrids resistant to unfavorable environmental factors and optimize plant culture technologies to adapt them to the peculiarities of agroecosystems. Measures to mitigate the impacts of climate change, respectively to improve agricultural technologies to ensure clean air and food begin with the use of a high biological and cultural value seed resistant to abiotic and biotic stressors. The maximum level of seed quality is reached in a complex of conditions in which a number of factors are identified, with particular reference to those relating to genetic particularities and the preservation environment.
Considering the wide spread of maize both in Romania and other countries, its cultivation in the area where stress factors produce significant losses of production, an important contribution is the creation and cultivation of resistant hybrids in the view of reducing the damage. In this sense, the strategic and methodological improvements of the process of creation of inbred lines and of resistant maize hybrids can be considered of great practical interest.

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IMPACT OF KEY RESOURCES AND ATTRACTIONS ON COMPETITIVENESS OF RURAL DESTINATIONS IN SERBIA AND HUNGARY

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Abstract

All destinations are an amalgam of tourism products aimed at providing consumers with a unique, integrated tourism experience. Destination resources and attractions, as factors of tourist offer, represent real incentive force that creates a tourist demand. The aim of this paper was to explore how key resources and attractions – physical and geographic elements of the environment, cultural heritage, opportunities for sports, leisure and recreation, accommodation facilities and their authenticity, gastronomy, general infrastructure and tourism infrastructure, safety and security - affected competitiveness of rural tourism destinations in Serbia and Hungary. Tourism experts from Serbia (163) and Hungary (175) were asked to evaluate the current condition of 24 attributes affecting the competitiveness of rural tourism destination. Likert scale of five marks was used in order to determine the effect of key resources and attractions on the competitiveness of Serbia/Hungary as a rural tourism destination. The research showed that the rural tourism sector in both countries had plenty of natural resources and special attractions. However, the accompanying services and contents were very limited and this could affect the reduction of attractiveness, and therefore the ability of the sector to highlight its potential. In the absence of ancillary infrastructure and resources, possible outcomes for the destination can range from service delivery below the expectations of visitors to major failures, thereby preventing the long-term development of the destination. The three basic elements have the potential to increase the length of stay and tourist consumption, which are: folk tradition, gastronomy and opportunities for sports and recreational activities in nature.

Keywords: *key resources and attractions, rural destination, competitiveness, Serbia, Hungary.*

Introduction

Rural tourism is one of the priorities in the tourist development of many European countries. The rural tourism market is on the rise, while at the same time the future of many rural areas is uncertain, due to changes in agricultural production or the attractiveness of urban areas due to a higher standard of living. Rural tourism is considered as one of the appropriate instruments for the revitalization of rural areas and ensuring their sustainable future through job retention or job creation, support for agricultural holdings, preserving nature, or preserving rural crafts as a tourist attraction. Destinations of rural tourism are based on a complex tourist product consisting of several partial products (accommodation, transport, food, trade, attractions, etc.) and offered as such to visitors (Roberts, Hall, Mitchell, 2003; Demonja, Ružić, 2010; Sidali, 2011). These individual tourism companies are interdependent and interconnected, and these are primarily small and medium-sized enterprises that carry the activity. Problems in rural tourism that contribute to the reduction of competitiveness are reflected through the existence of strong competition instead of cooperation among providers of tourism products and services in rural areas. Considering the importance of tourism for

rural areas, determining the factors that influence the improvement of the competitive position in the tourism market is of great importance for their further development.

Key resources and attractions – theoretical framework

All destinations are an amalgam of tourism products aimed to provide consumers with a unique, integrated tourism experience. Tourist destination products include a range of facilities and services offered at the local level, along with all socio-cultural, natural resources and public goods (Buhalis, 2000). Dwyer and Kim (2003) suggested that tourism resources and attractions should be the basis of destination competitiveness. An analysis of previous research on the attribute of the tourist destination (Buhalis, 2000; Kim, 1998) clearly indicated a wide range of attributes adopted by researchers to determine the attractiveness of tourist destinations, although some attributes are common for many research (e.g. climate, landscape, activities, accessibility). A review of literature (Seddighi, Theocharous, 2002; Navarro, 2015) found that there is no universal set of tourist attractions and resources to determine the attractiveness of a tourist destination or its competitiveness. For the purposes of this paper, the basic resources and attractions identified in the Crouch-Ritchie competitiveness model have been selected with the exception of links to emitting markets, as these elements are in line with the main research of destination attractiveness. The factor "Safety and security" was added to the group of key resources and attractions of the destination. Consequently, the "Key Resources and Attractions" is comprised of seven main factors:

1. *Physical and geographic elements of the environment* - Natural attractiveness consists of physical and geographical elements of the environment, important for rural tourism: relief, climate, hydrographic elements, plant and animal world, protected natural areas and nature parks. Especially interesting are the areas that have certain forms of relief from horizontal and vertical diversity. The climate impact is reflected in the stimulation of the development of certain forms of tourism, for example, the mountain with many snowfalls for winter, ski tourism. The flora and fauna are also important factors that attract and direct tourists to specific areas. And the animal species that are kept in some areas, increase its tourist appeal. The richness of wildlife in the forests and fish in the waters not only beautifies and makes the area interesting, but also provides opportunities for the development of various touristic contents such as hunting and fishing, observing birds, exploring, taking pictures, taking care of their maintenance and other activities.
2. *Cultural heritage* - Many cultural heritage resources, either material or non-material, include unique features that reflect their history, lifestyle or the environment, and are ideal to become tourist attractions (McKercher, Ho, du Cros, 2004). Rural culture has been, and is still, determined by specificities that help in shaping rural life style and society. Architectural heritage provides a rich source of evidence of rural culture, as well as their aesthetic values - rural houses and supporting structures reflect a way of life and a local history that has contributed to the creation of rural culture through centuries. Music, play and theater performances as part of narration can form the basis of rural social life. Religious festivals and pilgrimages are most often conducted in rural areas. The rural regions of Europe have a strong local identity, unknown to urban areas. The rich heritage of tradition and customs transmitted through centuries has a value that is valued across the continent.
3. *Opportunities for sports, leisure and recreation* - This group includes those types of attractions that are not directly related to natural or cultural characteristics and "artificial" are created, which are recreational activities and manifestations and festivals. In rural tourism, there are frequent combinations of sports and recreational activities and tourism. In this regard, they differ: (1) breaks with elements of sporting content and (2) business trips with elements of sporting content. In both of these sectors, sporting participation can be either active or passive. The most important, recreational and tourist

activities in a large number of rural environments have dramatically transformed from relatively passive to active and very important factors of natural, economic and social change.

4. *Accommodation facilities and their authenticity* - It is considered that a tourist destination with built accommodation capacities is not limited to day-tourists, but can attract visitors who will stay there for a long time (Kelly, Nankervis, 2001). In order for a tourist destination to be successful, the accommodation facilities must be available in sufficient numbers and meet the needs of the coming tourists. Apart from the adequate quality and quantity of accommodation capacities, the concept of authenticity is of great importance in rural tourism, and is intensively applied in the catering sector. The authenticity of the accommodation object is related to the material used in the construction, the architectural style, the attitude towards the environment and the appearance of the interior.

5. *Gastronomy* - Many actors in tourism (e.g. hotels, restaurants, local communities) have understood the importance of gastronomy as a driving force for tourists on one hand, and for, stimulating local, regional and national economic development on the other. Planning and conceiving of additional tourist facilities in rural areas should emphasize some functions of rural settlements, taking into account everyday activities and lifestyles of the local population. One of these activities is food preparation, i.e. local specialties, respecting the authenticity, as well as the protocol of hospitality. Gastronomy with its activities can be of great help in creating the content of the stay. These activities can be daily as: participation in the preparation of food; preparation and production of bread in a rural way; baking brandy in the night; preparing cheese and for the market, and more.

6. General infrastructure and tourism infrastructure - Infrastructure development is one of the important segments of rural policy. Well-developed infrastructure is considered to be one of the most important prerequisites for rural and overall economic development. Developed infrastructure contributes to: the economic aspect of the lives of rural communities by reducing regional inequalities and improving access to the market and, consequently, lower transport and transaction costs, increased trade exchange with other regions, and an increase in rural household income. The volume of investments in rural areas, as well as the volume of revenues from other non-agricultural branches of rural economy, such as tourism, is increasing. Lack or inadequate infrastructure (e.g. lack of tourist signaling) can diminish the atmosphere of tourists at the destination. Infrastructure is basic element in the tourism development of rural areas, so building, equipping, improving and upgrading infra and substructures should be a permanent strategic goal.

7. *Safety and security* - Safety and security are the primary condition for a successful tourist destination. To make tourists feel safe and secure before and during travel is essential for the competitiveness of tourist destinations. Negative impressions of a destination can cause a decline in tourist traffic even if the destination has high-quality tourist attractions (George, 2003). As in any other business activity, as well as rural tourism or agri-tourism, there are certain risks that must be faced by those who want to deal with this business. Risks in agritourism can be found in both the service provider and the tourists who use these services. For example, tourists who come to farm can accidentally be infected by some plant or animal diseases or, on the other hand, tourists can be the cause of death of animals or plants. In order to minimize risks, many farms create galleries from which a production process can be observed without direct contact. Risk is also growing due to conscious (deliberate) and unconscious (unintentional) tourist activities.

Materials, method and the targets of the paper

The aim of this paper is to examine the impact of key resources and attractions on competitiveness of rural destination. The authors analyzed views of stakeholders important for the development of rural tourism on the supply side in Serbia and Hungary (direct

providers of services in rural tourism, employees in tourist organizations and tourist agencies, employees in municipal and provincial services, employed in ministries, teaching staff at faculties). Tourism experts from Serbia and Hungary were asked to assess the current state of seven factors which affect/could affect the competitiveness of rural tourism destinations in Serbia/Hungary. Likert scale of five scores was used, where 1 means "very bad condition of the factor", 2="bad condition", 3="average condition", 4="very well" and 5="excellent condition of the factor". In this paper, survey research covered all those tourism experts who have the knowledge and/or experience relevant to the subject, and whose area of research and action is related to rural tourism and competitiveness of tourist destinations. In Serbia (163 experts) and Hungary (175 experts), interviewed tourism experts are as follows: teaching staff at higher education institutions, employees of the tourist organizations, employees of national and provincial institutions of importance for the development of tourism, tourism managers of travel agencies, owners of tourism enterprises in rural areas (farms, restaurants, ethno-houses, museums, wineries, souvenir shops, event organizers) and others (societies, associations, clusters). The survey was conducted in two ways - a personal interview (technique of "face to face") and by sending a questionnaire via e-mail. Selected tourism experts in Serbia and Hungary were interviewed in the period from March to May 2017. The response rate in both countries is about 60%. Statistical analysis of the data collected through the survey was done in the software statistical program SPSS 20.

Results and discussion

Table 1 presents the differences on the first scale for factors that belong to the determinants "Key resources and attractions" (arithmetic mean, standard deviation, value, and significance). At the significance level $p<0.01$, statistically significant differences were achieved with the factor "Physical and geographic elements of the environment", "Accommodation capacities and their authenticity" and "General infrastructure and tourist infrastructure".

Table 1. T-test for dependent samples - comparison of the results between Serbia and Hungary for the determinant "Key resources and attractions"

Factor	Country	Arithmetic mean	Standard deviation	Value	Significance
Physical and geographic elements of the environment	Serbia	3.5001	.55830	-7.422	.000
	Hungary	4.0058	.56773		
Cultural heritage	Serbia	3.5257	.66477	-2.184	.030
	Hungary	3.7099	.72649		
Opportunities for sports, leisure and recreation	Serbia	3.7426	.73783	-2.535	.012
	Hungary	3.9562	.65157		
Accommodation facilities and their authenticity	Serbia	3.0931	.65256	-7.602	.000
	Hungary	3.6788	.62021		
Gastronomy	Serbia	3.9669	.83267	-.002	.998
	Hungary	3.9672	.90180		
General infrastructure and tourism infrastructure	Serbia	2.9326	.70196	-9.736	.000
	Hungary	3.7117	.61785		
Safety and security	Serbia	4.0478	.75738	1.342	.181
	Hungary	3.9192	.81852		

The obtained results for factors in which there is a statistically significant difference show that the use of rivers, lakes and channels in rural tourism in Hungary is much more intensive and organized than in Serbia, and protected nature areas and nature parks are more represented in the rural tourism offer, that is, a large number of organized programs involving natural areas, as well as a series of activities organized in these areas. Also, there is a significant difference in the factor "Accommodation capacities and their authenticity", especially the difference in

the average ratings expressed between Hungary and Serbia when it comes to the authenticity of accommodation units. The tourist offers, that is, the owners of accommodation facilities in Hungary, take care that the different contents and the appearance of the building increase the attractiveness of the building. The condition, that is, the quality of basic infrastructure in Hungarian villages is better than in Serbian, while the differences between the quality of basic infrastructure in agritourism facilities is not so pronounced. In Figure 1, Serbia is the closest, according to results, in terms of gastronomy, opportunities for sport, entertainment and recreation in rural areas and cultural heritage. This is not surprising, since once part of Serbia (Vojvodina province) and Hungary share a part of history, and that a significant number of Hungarians live in the territory of Vojvodina nurturing their culture and tradition. It is interesting that the only factor of the model that has a higher average rating in Serbia than in Hungary is "Safety and security". In further research, it is necessary to examine for what reasons the safety and security in Hungary is estimated lower than in Serbia, and this advantage can be used for participation in the Hungarian rural market and for attracting their tourists.

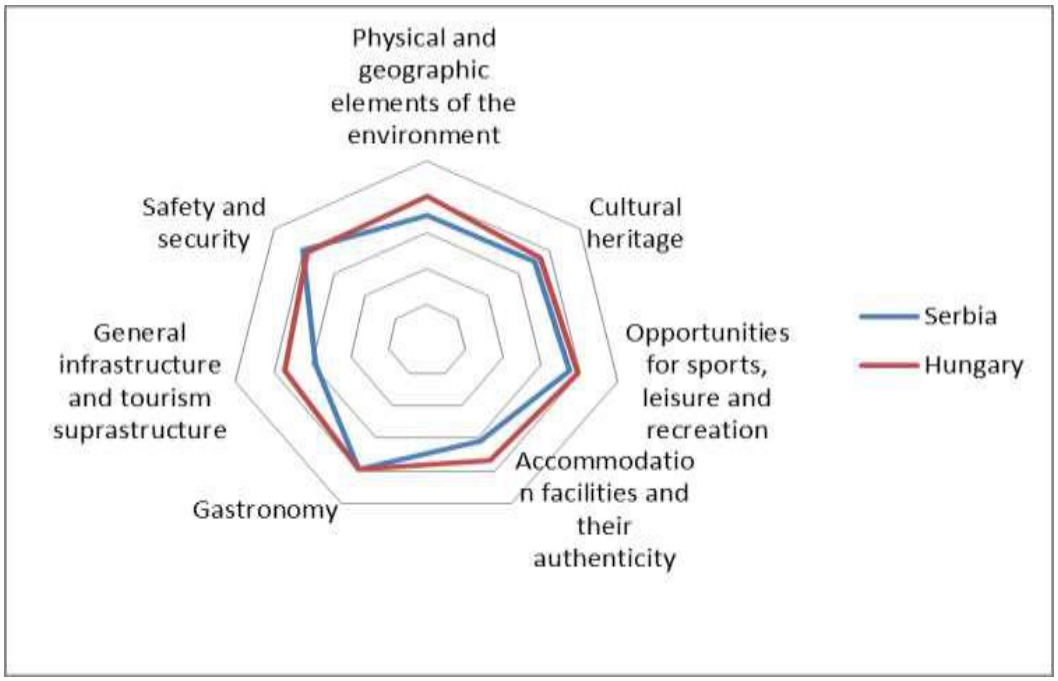

Figure 1. Performance of Serbia and Hungary for factors within the determinant "Key resources and attractions"

Conclusion

The strategy for the development of rural tourism in Serbia should have a special connection with the resources and attractions of the destination, while the priority should be given to maintaining all aspects of security and safety, the continuous improvement of services, the benefits of multicultural environment, the diversity of gastronomic products and the application of the principles of sustainability in environmental management. It is increasingly important to recognize and accept the need for resources to be managed in a sustainable way in order to be preserved for future generations. The rural tourism sector in Serbia has plenty of natural resources and special attractions. However, the accompanying services and content are very limited and this can affect the reduction of attractiveness, and therefore the ability of the sector to highlight its potential. In the absence of ancillary infrastructure and resources, possible outcomes for the destination can range from service delivery below the expectations of visitors to major failures, thereby preventing the long-term development of the destination.

The three basic elements have the potential to increase the length of stay and tourist consumption, which are folk tradition, gastronomy and opportunities for sports and recreational activities in nature. Developing key elements can stimulate visitors of rural areas of Serbia to stay longer, spend more money and participate in a wider range of activities.
The most important competitors to Serbia as a destination for rural tourism are Hungary, Croatia and Slovenia (and increasingly Romania). By comparing the results of the survey between Serbia and Hungary, it can be concluded that the level of rural tourism development in Hungary is significantly higher than in Serbia, since there is a statistically significant difference in the assessment of almost all factors (apart from factor "safety and security"). Tourism experts of Hungary do not see Serbia as their competitor, which leads to the conclusion that tourism policy holders should look at Hungary as a market that requires greater investment and significant efforts to meet the demands of their sophisticated rural tourists, which can not happen in a short period of time. In the future, Hungary should be seen as a partner and Serbian stakeholders should develop joint projects with their stakeholders, which will increase the quality of the supply of rural tourism in Serbia. At this moment, the Hungarian rural tourism market should be seen as an example of good practice. In the meantime, attention and efforts should be directed towards foreign tourist markets with a possible entry into the countries that Serbia has good traditional connections (Montenegro, Bosnia and Herzegovina, Greece, Russia).

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POTATO PRODUCTION CHARACTERISTICS – COMPARATIVE ANALYSIS: SERBIA, MACEDONIA AND ENTITY OF REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA)

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Abstract

The parameters of potato production in Serbia, Macedonia and Entity of Republic of Srpska (Bosnia and Herzegovina) were analysed in the period 2005-16. Sown areas, yields and total production were analysed. Quantitative analysis was performed by using descriptive statistics method, and we used average annual rate of changes to discover the tendencies of changes in the analysed period. In Serbia, there were 63,646 hectares of potato in average, in Macedonia 13,402 hectares and in Republic of Srpska 14,858 hectares. Serbia showed a trend of decreasing areas of potato with average rate of -3.38% per annum, Macedonia had tendency of increasing area by the annual rate of 2.45%, and Republic of Srpska also decreased areas of potato by average yearly rate of -2%. The average potato yield was 13.4 tons per hectare in Serbia, with variation coefficient of 29.8%, in Macedonia, 13.9 t/ha (variation coefficient 6.8%) and in Republic of Srpska 11.2 t/ha (variation coefficient 12.8%). In the observed period, there was a growth tendency in yields: 0.64 in Serbia, 2.30 in Macedonia and 1.31 percent per year in Republic of Srpska. The average annual potato production in Serbia was 803,669 tons (variation coefficient of 18.1%), in Macedonia 186,770 tons (variation coefficient of 6.2%) and 166,221 tons in Republic of Srpska, (variation coefficient of 14.9%). Potato production showed tendency of growth in Macedonia, 4.79%, while it decreased in Serbia, -2.74 and in Republic of Srpska -0.72 percent per year in average.

Key words: *potato, production, Serbia, Macedonia, Republic of Srpska.*

Introduction

Truck farming represents a significant area of crop production for the agriculture in Serbia, Macedonia and Republika Srpska. The main characteristics of olericulture are:

- relatively short production process, which enables two or more crops in a year, and consequently better land use
- intensive production – large investments into irrigation and technology resulting in higher yields
- the need for manure activates cattle breading
- high level of economic effectivity as measured with the value of production and profit per hectare

The object of exploration in this paper is comparative analysis of performances of potato production in Serbia, Macedonia and Republika Srpska. Analysis of potato area harvested, potato yield per hectare and total production is performed for the period of 12 years, i.e. from 2005 to 2016. The aim of investigation is to compare yields, production of potato and its share in total crop production, and tendencies of these indicators in the analyzed countries. From comparative analysis, we can derive conclusions about the importance of potato production and positive/negative tendencies in the analyzed period; our investigation will reveal factors of these movements. The authors of this paper have been recently dealing with this problem.

Novkovic et al. (2008) analyzed vegetable production in Serbia and Vojvodina between 1981 and 2007. They found that the vegetable area is less than 300,000 hectares in Serbia. This includes 80,000 hectares in Vojvodina. In Serbia vegetables are produced on around 8.5% of total arable land, so Vojvodina lags with its 5%. On the other hand, areas under vegetables are stable, with slow tendency of growth in Serbia and decline in Vojvodina. Mutavdzic et al. (2011) analyzed the results of vegetable production in Serbia in the period 2001-2010, and these results were compared with those obtained for the previous decade (1991-2000). During these years, the production of every vegetable type increased: garlic for 2%, red pepper for over 26%, green peas for around 56%. Novkovic et al (2011) analyzed changes in sown areas, yield and total production of several important vegetable types in Vojvodina in the period 2000-2009. Due to increased yields per hectare, the total production increased, despite reductions in the total sown area. Novkovic et al. (2012) obtained similar findings about vegetable production in Republika Srpska. A great number of author has published findings concerning analyses and forecasts of production of different types of vegetables (Novkovic et al., 2012a, 2013, 2014, 2014a, 2015, 2016; Илин et al. 2014; Ivanisevic et al. 2015; Miljanovic et al. 2014; Mutavdzic et al. 2013, 2016).

Material and Methods

Quantitative explorative methods were used in this paper. Quantitative analysis included data on sown areas, yields and total potato production in Serbia, Macedonia and Republika Srpska in the period 2015-2016. The next statistics were calculated: arithmetic mean – average ($\overline{X}$) and rate of change (r).

Given the values of a time series Y with length n, the average index of change is:

$$G = \left(\frac{Y_n}{Y_1} \right)^{\frac{1}{n-1}}$$

and the average rate of change:

$$r = (G - 1)$$

where

r is the average annual rate of change

G is the average annual index of change

Y_1 is the absolute value of the first member of the time series

Y_n is the value of the last number of the time series

n is the length of the series (number of years)

Data published by official statistical bureaus of Serbia, Macedonia and Republika Srpska were used in our analyses. At first, analysis of individual data about potato-production was completed, and that was followed by comparative analysis between the Republics. Comparison was performed using the index-method; when the observed changes had opposite directions between Republics, a qualitative comparative analysis was done instead of calculation of indices.

Results and Discussion

Table 1 displays performances of potato production in Serbia. Data show that there are negative tendencies in areas under potato but on the contrary, yields were growing, which results in slower decreasing of annual production. High value of variation coefficient indicates there were serious influences of factors from nature on average yields and production. Variations in sown area are less, but still high, and this point out the fact that economic and market risks are present in this kind of production.

Table 1. *Basic data on potato production in Serbia, 2005-2016*

Production indicators	Average	Interval of variation		Variation coefficient (%)	Average rate of change (%)
		Minimum	Maximum		
Area (ha)	66,646	40,105	93,897	29.8	-3.38
Production (t)	803,669	577,966	1,015,017	18.1	-2.74
Yield (t/ha)	13.4	6.6	17.8	25.7	0.64

In Table 2, basic characteristics of potato production in Macedonia are shown. Average rates of changes are positive and similar for areas and yields, resulting in progressive tendencies in total production. Stability of potato area in Macedonia is confirmed by the fact the corresponding variation coefficient is relatively low. The variation coefficient for yields is higher but still low, showing that there is some influence of natural factors and production conditions, and in the same time, the production is intensive, meaning that external factors are well controlled.

Table 2. *Basic data on potato production in Macedonia, 2005-2016*

Production indicators	Average	Interval of variation		Variation coefficient (%)	Average rate of change (%)
		Minimum	Minimum		
Area (ha)	13,402	12,926	13,829	1.9	2.45
Production (t)	186,770	165,975	204,778	6.2	4.79
Yield (t/ha)	13.9	12.1	15.4	6.8	2.30

The results of descriptive statistics of performances of potato production in Republika Srpska are presented in table 3. There are negative tendencies in changes of potato area. Nevertheless since the yields are enlarged, decreasing in total production is lessen, the fall is minimal, with the rate of under 1% in average. The variation coefficients of the area, yields and total production are somewhere between data for lower Macedonia and higher Serbia.

Table 3. *Basic data on potato production in Republika Srpska, 2005-2016*

Production indicators	Average	Interval of variation		Variation coefficient (%)	Average rate of change (%)
		Minimum	Maximum		
Area (ha)	14,858	13,284	16,886	8.8	-2.00
Production (t)	166,221	124,144	208,447	14.9	-0.72
Yield (t/ha)	11.2	8.2	14.2	12.8	1,31

The results of comparative analysis of potato area are shown in Table 4. The largest potato area was sown in Serbia, almost five times as much as in Macedonia, and four and a half times as in Republika Srpska. Total area was decreasing in Serbia and Republika Srpska, but increasing in Macedonia.

Table 4. *Average potato area in Serbia, Macedonia and Republika Srpska 2005-2016*

Indicator	Republic			Index Macedonia = 100	
	Serbia	Macedonia	R. Srpska	Serbia	R. Srpska
Average area (ha)	66,646	13,402	14,858	497	111
Rate of change (%)	-3.38	2.45	-2.00	-	-

Average potato yield and the results of the comparative analysis are given in Table 5. In every republic, yields had growing tendencies. The largest average yield was achieved in Macedonia, while in Serbia it was for 4% lower and in Republika Srpska for 19% lower. The average growth rate of potato average yield was highest in Macedonia, and that exceeded growth rate in Serbia 3.6 times and in Republika Srpska for 75%.

Table 5. Average potato yield in Serbia, Macedonia and Republika Srpska, 2005-2016

Indicator	Republic			Index Macedonia = 100	
	Serbia	Macedonia	R. Srpska	Serbia	R. Srpska
Average yield (t/ha)	13.4	13.9	11.2	96	81
Rate of change (%)	0.64	2.30	1,31	28	57

Table 6 contains results of comparative analysis of average annual potato production. Serbia had the largest average annual potato production, which amounted to 160 thousand tons of potato per year. This quantity is 4.3 times as much as in Macedonia and 4.8 times as much as in Republika Srpska. In the same period, Serbia and Republika Srpska had negative average rates of changes of potato production, while the rate was positive in Macedonia.

Table 6. Average annual potato production in Serbia, Macedonia and Republika Srpska 2005-2016

Indicator	Republic			Index Macedonia = 100%	
	Serbia	Macedonia	R. Srpska	Serbia	R. Srpska
Average annual production (t)	803,669	186,770	166,221	430	89
Rate of change (%)	-2.74	4.79	-0.72	-	-

We can get a better insight into the importance of potato production for a country through analysis of production per capita. Results of comparative analysis of potato production per capita are shown in Table 7. The largest potato production per inhabitant had R. Srpska, the next is Serbia with 24% less production p.c. and Macedonia with 36% less production p.c.

Table 7. Average annual potato production per capita, 2005-2016

Indicator	Republic			Index Macedonia = 100%	
	Serbia	Macedonia	R. Srpska	Serbia	R. Srpska
Average annual production/Number of inhabitants (kg/p.c.)	111.8	90.9	141.6	123	158
Number of inhabitants in thousands (2011 census)	7,186	2,054	1,174	349,9	57,2

Conclusions

The results of comparative analysis of potato production in Serbia, Macedonia and Republika Srpska in the period 2005-2016 were as follows:

- The average potato area was 63,646 ha in Serbia (variation coefficient 29.8%), 13,402 ha in Macedonia (variation coefficient 1.9%) and 14,858 ha in Republika Srpska (variation coefficient 8.8%)
- The average annual rate of decline of potato area in Serbia is 3.38%, average annual rate of growth is 2.45% in Macedonia, and average annual rate of decline in Republika Srpska is 2%.
- The largest average potato yield per hectare was achieved in Macedonia with 13.9 t/ha, that was followed by Serbia with 13.4 t/ha and Republika Srpska with 11.2 t/ha.
- The greatest value of rate of growth of average potato yield was in Macedonia with 2.3%, than Republika Srpska with 1.31% and Serbia with 0.64%.
- Serbia has the largest total potato production per year (800,000 tons), the next are Macedonia (190,000 tons) and Republika Srpska (170,000 tons).
- Macedonia has the only positive rate of change of potato production (average annual rate of growth is 4.79%), while there is decline in Republika Srpska (-0.72%) and Serbia (-2.74%).
- Republika Srpska has the highest position among the three republics concerning potato production per inhabitant (142 kg p.c.), then follows Serbia (112 kg p.c.) and Macedonia (91 kg p.c.).

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CARBON DIOXIDE EMISSIONS IN RETAIL FOOD

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Abstract

Increased attention has been paid recently to the analysis of the effects of applying the concept of sustainable development in retail. In that context we have particularly considered greenhouse gases emission in retail food. This is achieved through the use of modern ecological technology in business – through the whole food value chain. The ultimate goal is to achieve the planned reductions of carbon dioxide in retail food, which positively reflects the overall performance of retailers food, environmental in particular. The costs of carbon dioxide emission reduction affect the performance of retailers food. Continuous empirical research shows that almost all global retailers food achieve a significant reduction in carbon dioxide emissions from year to year. Empirical research conducted in this paper on the example of global retailers food in the United States, Europe and the European Union, the United Kingdom, and Serbia shows significant and planned reduction of carbon dioxide emissions in retail food, especially in countries with developed market economies. This empirical research is mainly based on the analysis of the original sustainable (environmental and ecological) reports officially disclosed by selected retailers food, primarily from the countries of a developed market economies, which they started to publish with regular annual financial statements. They are now an integral part of the so-called integrated reporting on performance of global retailers food. Due to the general importance, harmonized regulations on sustainable retail food reporting are being increasingly applied as a data source for more efficient environmental management. In perspective, this will ease the comparative analysis of the carbon dioxide emission of global and other retailers food. (*Jel Classification: I10, L81, M14, M41, Q42, Q56, Q57*)

Key words: *greenhouse gas emissions, carbon dioxide, CO_2 emission sources, renewable energy sources, sustainable reporting*

Introduction

The overall goal of global retailers food is to reduce carbon dioxide emissions through the entire food value chain. The subject of research in this paper is the significance and trend of carbon dioxide emissions in retail food. The costs of carbon dioxide emission reduction are significant and affect the performance of retailers food. The problem of comprehensiveness of the research on carbon dioxide emissions in retail food is that, at the time being, there is no unified system of sustainable (environmental) reporting for all retailers. In addition, many retailers food still do not publish this report, what as a consequence, has an incomplete "comparability" of data on carbon dioxide emissions by individual retailers food. Nevertheless, knowledge of the importance and trend of carbon dioxide emissions from global retailers food is very important in order to manage overall, integrated and, in particular, environmental performance in (concrete) retail food. In view of the global, other retailers food will increasingly publish reports on sustainable development (with data on carbon dioxide emissions). In this way, they will increase its information base for more efficient management of total business, including environmental protection. This will have a positive impact on the achievement of the target profit. This paper attempts to make thorough analysis of specific issues of carbon dioxide emissions in the retail sector, firstly on the example of global

retailers food from different countries, primarily developed market economies, which, due to the general importance of the matter, publish reports on sustainable development with regular annual financial reports. This practice of global retailers food provides them with more reliable information base for efficient management of carbon dioxide emissions through the whole value chain.

Material and Methods

There is voluminous literature devoted to analyzing the way company's performance is affected by general problems and effects of carbon dioxide emission reduction through the whole food chain (Kahn, 2014; Congcong, 2016; Li, 2016; Clune, 2017; Wang et al., 2017; Ji, 2017; Linda, 2014**;** Eriksson, 2017; Lukic, 2013, 2014, 2016, 2017; Lukic et al., 2018). The general research hypothesis in this paper is that the reduction of carbon dioxide emissions positively reflects on overall (integrated, especially environmental) performance of retailers food. The methodology of the study of the given hypothesis is primarily based on the comparative analysis of the carbon dioxide emission of global selected retailers food from various comparable countries of the developed market economy.

Main data sources for the research of the treated problem in this paper are literature, articles, and, in particular, officially disclosed annual financial and sustainable reports of global retailers food. They were processed in such a way that is easy to comprehend the significance and trend of carbon dioxide emission in retail food.

Results and Discussion

The carbon dioxide emission through the entire food value chain is shown in Table 1.

Table 1. Emissions of carbon dioxide through the life cycle of food after farm

Life cycle stage post-farm gate	Number of GWP (global warming potential) values	Median ($kgCO_2$-eq/kg)	Mean ($kgCO_2$-eq/kg)	Stdev	Min ($kgCO_2$-eq/kg)	Max ($kgCO_2$-eq/kg)
Processing meats	5	0,59	0,66	0,14	0,54	0,87
Processing vegetables	15	0,06	0,07	0,04	0,01	0,013
Packaging	8	0,05	0,06	0,06	0,01	0,21
Transport to RDC (Regional Distribution Centre	21	0,09	0,13	0,19	0,02	0,95
Retail	20	0,04	0,10	0,25	0,01	1.14

Note: The table is compiled on the basis of various relevant studies.
Source: Clune et al., (2017)

The data in the given table show that, on average, emission of carbon dioxide is higher in the processing of meat than vegetables. It is also higher in transport than in retail, and is the lowest in the packaging phase. This is in line with the nature of the activities concerned. Figure 1 shows sources of carbon dioxide emission throughout the value chain, with an emphasis on Tesco's participation in carbon footprint.

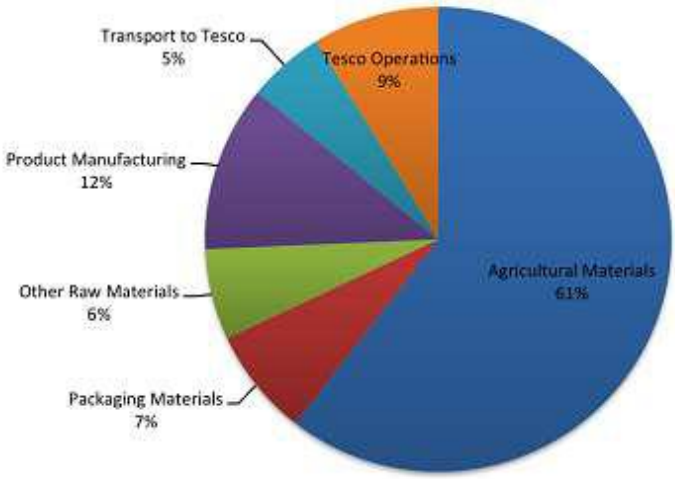

Figure 1. Emissions of carbon dioxide by source in Tesco

Source: Tesco - Our Carbon Footprint, https://www.tescoplc.com/tesco-and-society/sourcing-great-products/reducing-our-impact-on-the-environment/our-carbon-footprint/ (May 24, 2017)

Different is the carbon dioxide emissions of individual retailers food. This is shown by the results of the research in this paper.

At **Wal-Mart** (United States of America, Dominant operational format: Hypermarket / Supercenter / Superstore), a great significance is given to reducing carbon dioxide emissions (Table 2). This is achieved by: investing in renewable energy sources, reducing energy demand, improving energy efficiency, improving refrigeration in stores and maximizing the efficiency of the vehicle fleet.

Table 2. Carbon dioxide emission (Scope 1 and 2) and retail area at Wal-Mart, 2005-2014

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Carbon dioxide emission (million ton CO_{2e})	18,9	19,3	20,1	20,8	20,3	20,6	20,8	21,2	21,0	21,9
Retail area (million square meters)	740	805	867	921	952	985	1,037	1,072	1,102	1,134
Carbon dioxide intensity (million tons CO_{2e}/million m^2)*	0,025	0,024	0,023	0,022	0,021	0,021	0,020	0,020	0,019	0,019

Note: *Calculations performed by the author
Source: Wal-Mart Stores, Inc. 2016 Global Responsibility Report, https://cdn.corporate.walmart.com/9c/73/3f9abcef444397f2c771e081e095/2016-global-responsibility-report.pdf#page=58&zoom=auto,-130,628 (May 5, 2017)

In generating greenhouse gas emissions, Wal-Mart participates with: electricity supply 69%, refrigeration 18%, fuel transport 5.9%, fuel on the site 7% and mobile refrigerators with 0.1 % (Wal-Mart Stores, Inc. 2016 Global Responsibility Report, https://cdn.corporate.walmart.com/9c/73/3f9abcef444397f2c771e081e095/2016-global-responsibility-report.pdf#page=58&zoom=auto,-130,628 (May 5, 2017). Therefore, the main source of greenhouse gas emissions in Wal-Mart is electricity supply. With the increased application of the

ecological operation principles, Wal-Mart reduced carbon dioxide emissions from year to year, which reflects favourably on its overall performance, especially environmental.
At **Tesco** (United Kingdom, Dominant operational format: Hypermarket / Supercenter / Superstore), as with Wal-Mart, considerable attention is paid to the research and control of carbon dioxide emissions. This positively reflects on its overall performance, including the surrounding ones. In Figure 2, an illustration of the specificity of carbon dioxide emissions measurement at Tesco is shown.

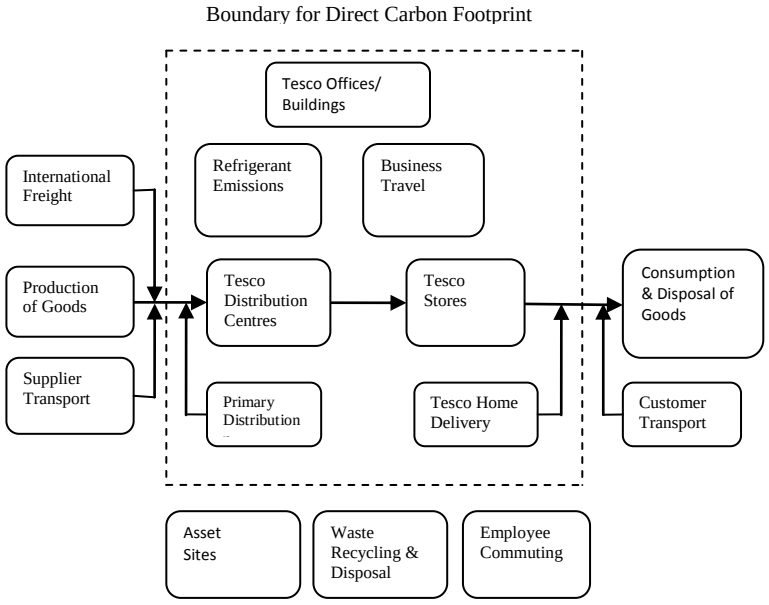

Figure 2. Tesco's emission limit

Source: Carbon Footprint 101: A Guide for Food Retailers, https://www.fmi.org/docs/sustainability/carbon-footprint-101-a-guide-for-food-retailers.pdf?sfvrsn=4#page=11&zoom=auto,-121.85 (May 10, 2017)

Therefore, Tesco participated in total emission of carbon dioxide through entire value chain with 9%. Table 3 shows ecological performances at Tesco.

Table 3. Global ecological performances at Tesco

	2016/17	2015/16	2014/15	2013/14
Carbon dioxide (million ton CO_{2e})	3,9	5,1	5,26	-
Emission of CO_2 reduction (stores and distributional centres) compared to 2006/07	40,5%	39,5%	38,3%	-
Emission of CO_2 reduction (distribution) compared to 2011/12	19,7%	17.4%	14,47%	7,8%
Direct water consumption (million m^3)	23,5	25,5	32,6	32,9

Waste percentage (food and non-food) which is recycled, used again or turn into energy	93%	88%	84%	86%

Source: Reducing our impact on the environment, https://www.tescoplc.com/tesco-and-society/sourcing-great-products/reducing-our-impact-on-the-environment/ (May 3, 2017)

The data in the given table show that the intensity of carbon dioxide emissions in Tesco is decreasing from year to year. Tesco has tendency to improve ecological performances (carbon dioxide emission reduction, direct water consumption reduction and waste treatment improvement). This reflects favourably on its market, economic and financial performances.
At **Ahold** (Germany, Dominant operational format: Supermarket), considerable attention is paid to the reduction of carbon dioxide emissions. This is shown in Table 4.

Table 4. Carbon dioxide emission at Ahold

	2008	2009	2010	2011	2012	2013	2014	2015
Carbon dioxide emissions (thousand tons)				2,176	2,106	2,107	2,090	2,019
Carbon dioxide emissions (kg CO_2/m^2 sales area)	567	574	543	507	480	473	465	420
Sources (%)								
Electricity								49%
Refrigerant appliances								29%
Fuel								12%
Gas								10%

Source: Ahold - Responsible Retailing Report 2015, https://www.aholddelhaize.com/media/1934/ahold-responsible-retailing-report-2015.pdf (May 9, 2017)

Carbon dioxide emissions at Ahold has been decreasing year after year. Since 2016, Ahold has been operating under the name of Ahold Delhaize. In view of this, Table 5 shows data on carbon dioxide emissions for Ahold Delhaize (Belgium, Dominant operational format: Supermarket) in 2016.

Table 5. Carbon dioxide emission at Ahold Delhaize

	2016 Actual	2020 Target
% reduction in CO_2 equivalent emissions per m^2 of sales area (from 2008 baseline)	-22%	-30%
Total CO_2 equivalent emissions per m^2 of sales area – location-based approach	496	n/a
Total CO_2 equivalent emissions (thousand tonnes) – location-based approach	4,505	n/a
Total Scope 1 CO_2 equivalent emissions (thousand tonnes) – location-based approach	1,940	n/a

Total Scope 2 CO_2 equivalent emissions (thousand tonnes) – location-based approach	2,420	n/a
Total Scope 3 CO_2 equivalent emissions (thousand tonnes) – location-based approach	144	n/a
Offset CO_2 equivalent emissions (thousand tonnes)	241	n/a
Avoided grid electricity CO_2 emissions (thousand tonnes)	31	n/a

Source: Ahold Delhaize Supplementary report on Sustainable Retailing performance 2016, https://www.aholddelhaize.com/media/3984/supplementary-report-on-sustainable-retailing-performance-2016.pdf (May 9, 2017)

Significant reduction in carbon dioxide emissions by 2020 (30%) is expected at Ahold Delhaize. This will be achieved by using so-called "green energy" in business operations. Sources of carbon dioxide emissions were: electricity 60%, cooling devices 31% and transport 9% (Ahold Delhaize Supplementary Report on Sustainable Retailing performance 2016, https://www.aholddelhaize.com/media/3984/supplementary-report-on -sustainable-retailing-performance-2016.pdf (May 9, 2017). Delhaize Serbia is also part of Ahold Delhaize which employs the same sustainable development strategy and environment reporting as well as company at its higher organizational level.

Conclusions

A growing number of retailers (food) in the world are increasingly publishing reports on sustainable development. By their reputation, and because of its importance, other retailers will certainly tend to publish this report in the future. It provides the basis for a comparative analysis of environmental performance in retail food from various aspects. In this report, special significance is given to trend of greenhouse gas emissions, in particular, carbon dioxide. Carbon dioxide emissions in trade, in total and by sectors, vary by country. It is significantly higher in China than in Europe or the European Union. Likewise, carbon dioxide emissions are significantly higher in trade of France, Germany and Great Britain than in Greece, Croatia, Turkey and Serbia. Carbon dioxide emissions are higher in Croatia's trade than in Serbian. These differences are due to the application of various ecological measures in business.Carbon dioxide emissions differ in individual stages of the product life cycle, retail companies and product categories. Carbon dioxide emission generators in retail companies are: electricity, transport, ventilation, heating and cooking, refrigeration, and waste. The goal of all retailers is to take appropriate measures, primarily ecological in nature, to reach a planned reduction of carbon dioxide emissions in the future. Among other things, this is achieved with the increasing use of electricity from renewable sources (so-called "green energy"), by using modern ventilation, heating and cooking systems, refrigeration units, green logistics (ecological vehicles) and more efficient waste treatment. The effect of this is to improve the overall performance of retail companies (food), especially environmental.

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PROFITABILITY AND RISKINESS OF CATTLE FATTENING OPERATION IN SERBIA

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Abstract

Cattle production is the most important type of livestock production in Serbia. Dairy production as well as cattle fattening are usually performed at family farms which dominate in Serbian agriculture. Cattle fattening has special status within cattle production because of its long tradition and export potential for baby beef. Therefore combination of dairy production and cattle fattening is common at family farms and could improve economic performance of on farm level. Nevertheless, in recent years cattle fattening is in decline. One of the reasons is that cattle fattening is activity associated with broad range of risks. Therefore, the goal of this research is to determine how some types of risks influence profit in cattle fattening operation at family farms in Serbia. To achieve this goal, profit is calculated on the basis of revenues and costs which are determined for cattle fattening on family farm. Initially, profit is calculated for usual (expected) circumstances. Afterwards, it is analyzed how profit is influenced by changes in some important factors, such as subsidies, price of fattened cattle, price of calves for fattening and corn price. It was determined that profit was influenced the most by potential absence of subsidies, because without state support cattle fattening in Serbia would not be profitable enterprise. On the basis of scenario analysis it is possible to expect that in general cattle fattening should be profitable.

Key words: *Cattle fattening, profit, risk, family farms, subsidies.*

Introduction

Cattle fattening is one of the branches of livestock production with a long tradition in Serbia. However, there is a decreasing trend in the number of cattle so that it has fallen from 920,000 in 2014 to 893,000 in 2016. On the other hand, cattle meat production shows a trend of slight increase from 73,000 tons in 2014 to 77,000 tons in 2016 (Statistical Yearbook of the Republic of Serbia, 2017). Examining longer period (from 1985 to 2011) Dokmanović et al., (2014) determined that (comparing to the period from 1985 to 1990) production of beef decreased by 29.20% in the period from 2006 to 2011. When it comes to possibilities of beef export, there is a tradition of export from Serbia to Italy and Greece, but it has been broken for some time. At the moment, meat export does not have a significant influence within agricultural products export. However, as claimed by Bajramovic et al. (2016), Serbian beef export is larger than beef import. Problem related to cattle meat export is its low competitiveness index in relation to the EU market, while the same index for live cattle is much higher (Živkov et al., 2017). Nevertheless, the same authors claim that "The production of beef is the branch in which Central Serbia has potential to become competitive, primarily due to large pasture potentials, but also due to the access to price competitive animal feed from Vojvodina".

Research, so far, have showed that investments in this production are economically justified in Serbia (Ivanović, 2013), but if there are problems with providing cheap cattle feed these investments turn out not to be economically efficient (Marković et al., 2014). Papers of other authors indicate that cattle fattening operation is influenced by many factors. Syrucek et al. (2017) determined that the most influential parameters in cattle fattening operation were

purchase and selling prices and live weight gains of cattle. López-Paredes et al. (2017) investigated 17 important production traits influencing profitability in a large national beef cattle population, while Kopeček et al. (2009) focused on analysis of the economics of cattle fattening simulating two agricultural policy scenarios. Return and costs in small scale cattle fattening were also analyzed by Sarma and Ahmed (2011), Santana et al. (2016) and Şahin et al. (2009). Having all this in mind, the goal of this analysis is to determine revenue, costs and profit in cattle fattening operation at family farms in Serbia, as well as factors influencing profit the most.

Material and methods

Data used in this paper refer to real costs of cattle fattening at family farms operating in Pancevo municipality. In the example presented in the paper, 40 cattle are fattened in one production cycle. Cattles for fattening are male, Holstein Friesian breed, weighting in average 123 kg per head at the beginning of the fattening period and having 450 kg per head slaughter live weight (projected daily gain is 1,100 g/day). It is assumed that fattened cattle will be sold once a year. Price of calves for fattening, price of fattened cattle and feed price are taken from the STIPS database (System of Agricultural Market Information of Serbia). Amount of subsidies corresponds to current government regulations in this area. Calculation incorporating revenue and costs of cattle fattening is made in the paper. In order to make the calculation, methodology given by Gogic (2014) was used. Sensitive analysis was applied to analyze the influence of certain factors on profit in cattle fattening (various approaches to sensitivity analysis are described by Pannell, 1997), as well as scenario analysis (elaborated by Brigham and Gapenski, 1997) to perform more comprehensive risk assessment.

Results and discussion

In order to analyze economic effects of cattle fattening, a calculation containing the most important types of revenues and costs was made (Table 1).

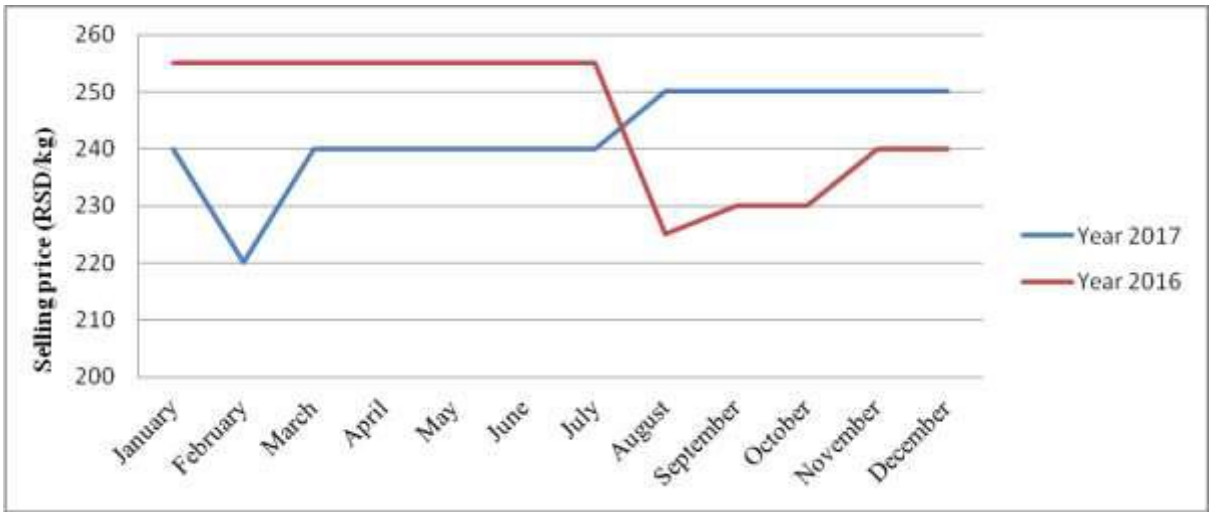
Table 1. Calculation of profit in cattle fattening operation

No.	Element of calculation	Amount (RSD)
I	Revenue	4,540,000.00
1.	Fattened cattle	4,140,000.00
2.	State subsidies	400,000.00
II	Costs	4,212,142.51
1.	Calves for fattening	1,476,000.00
2.	Feed costs	1,764,120.00
3.	Straw	360,000.00
4.	Water	25,000.00
5.	Electricity	20,000.00
6.	Veterinary and medicine	150,000.00
7.	Labor cost	300,000.00
8.	Maintenance of buildings and equipment	10,000.00
9	Depreciation	64,502.00
10	Interest	42,520.51
III	Profit	327,857.49

Source: Authors' calculation

The most important element of revenue is revenue from fattened cattle, while structure of costs is dominated by calves for fattening and total feed cost. It is determined that this is a profitable production, but it is necessary to analyze how some factors influence amount of

profit. Within total revenues of the farm, the largest part is income made by fattened cattle sale. Therefore, it is possible to question how the change of fattened cattle price influences profit of the farm. Fattened cattle price in 2016 and 2017 was between 220 RSD/kg and 255 RSD/kg in regional slaughter houses in which they are sold (Graph 1), while the price used in initial calculations was 230 RSD/kg.



Graph 1. Prices of fattened cattle in Pancevo municipality in 2016 and 2017 (RSD/kg)
Source: STIPS database

Reduction of fattened cattle price from 230 RSD/ha to a minimal achieved price of 220 RSD/kg would lead to a profit decrease from 327,857.49 RSD to 147,857.49 RSD (decrease of 54.90%). However, minimal achieved price of fattened cattle will not lead to losses in cattle fattening. The next question to be answered is if cattle fattening would be profitable without state subsidies. Basic assumption is that a farm is officially registered with appropriate state agency and receives subsidies for fattened cattle. The alternative assumption will be that a farm is not registered and does not receive subsidies (Table 2).

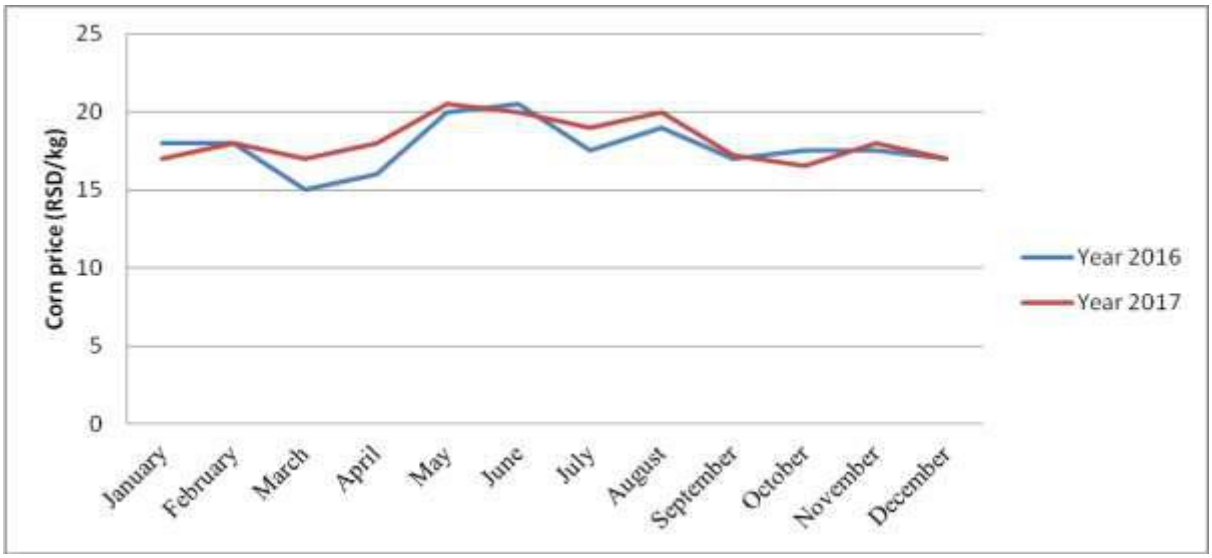
Table 2. Influence of subsidies on profit in cattle fattening

Possibility	Profit (RSD)	Decrease of profit (RSD)	Decrease of profit
With subsidies	327,857.49	-	-
Without subsidies	-72,142.51	400,000.00	122.00%

Source: Authors' calculation

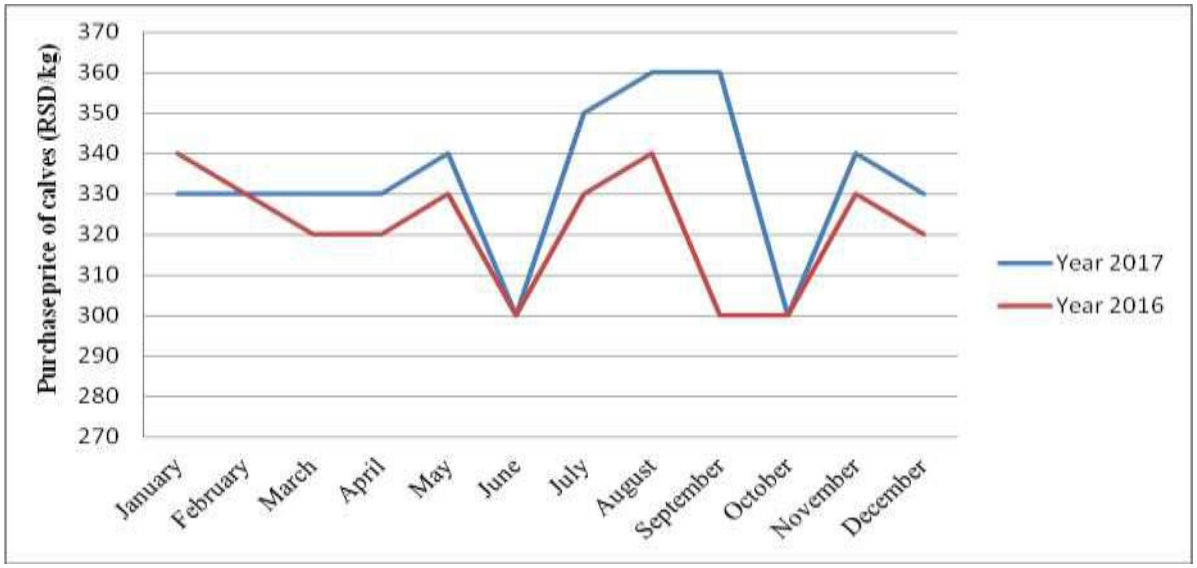
Results show that cattle fattening operation without subsidies results in loss instead of profit. It indicates a significant influence of subsidies on profitability of this production. Keeping in mind a decreasing trend in cattle fattening sector, as well as very low profit per head determined in this research (8,196.00 RSD/head) decrease of subsidies would lead to a further production reduction. That is to say, the amount of subsidies (10,000.00 RSD/head) is higher than profit in this production, which means that farmers use subsidies to cover part of production costs. As it was previously stated, cattle feed costs represent the biggest part of total farm costs. Within cattle feed costs, corn costs are very important element, so fluctuations of corn price were analyzed in the same county during 2016 and 2017 (Graph 2). The highest achieved corn price in the period 2016 – 2017 was 20.50 RSD and the lowest 15.00 RSD. The basic assumption was that corn price is 18.00 RSD/kg. Therefore, it is necessary to determine profit of the farm if the corn price is equal to the highest price in the period observed. Assuming that the corn price is 20.50 RSD/kg it would lead to a profit

decrease from 327,857.49 RSD to 201,977.49 RSD (profit would be reduced for 38.39%), but the operation would remain profitable.



Graph 2. Fluctuations of corn prices in Pancevo municipality in 2016 and 2017
Source: STIPS database

Second type of costs ranked by their amount is cost of calves purchased for fattening. Prices of calves for fattening were fluctuating in 2016 and 2017 and it was necessary to determine their upper and lower limit (Graph 3). As it is showed in the graph, the highest price of calves in the market was 360 RSD/kg and the lowest one was 300 RSD/kg. Therefore, it is necessary to investigate how much the profit would reduce if the highest price of calves was used. It was determined that profit would reduce from 327.857,49 RSD to 75.857,49 RSD (the decrease would be 76.86%), but the production would remain profitable.



Graph 3. Fluctuations of calves for fattening prices in Pancevo municipality (2016 and 2017)
Source: STIPS database

Within previous sensitivity analysis basic assumption was that only one factor was changed, while all other elements of calculations remained unchanged. But, in reality, multiple elements of calculation will change at the same time. Due to that, it is possible to perform scenario analysis which involves projection of three possibilities (scenarios) for future – the best, the worst and the most likely scenario. The best scenario will represent optimistic

approach (all indicators will have the most desirable value) while the worst scenario will assume the least desirable value of all elements of calculation. The most likely scenario equals to basic assumption which is previously presented in Table 1. Within this research two scenario analysis will be performed (because both of them could happen in future), and the only difference is within the worst scenario. The first scenario analysis (Table 3) assumes that state subsidies do not exist in the worst scenario. On the other hand, the other type of scenario analysis assumes that state subsidies do exist in the worst scenario (Table 4).

Table 3. First type of scenario analysis

Scenario	Profit (RSD)	Probability of outcome (%)	Total (RSD)
Best scenario	1,071,936.49	0.25	267,984.12
Most likely scenario	327,857.49	0.50	163,928.75
Worst scenario	-792,378.51	0.25	-198,094.63
Expected profit			233,818.24

Source: Authors' calculation

Table 4. Second type of scenario analysis

Scenario	Profit (RSD)	Probability of outcome (%)	Total (RSD)
Best scenario	1,071,936.49	0.25	267,984.12
Most likely scenario	327,857.49	0.50	163,928.75
Worst scenario	-392,378.51	0.25	-98,094.63
Expected profit			333,818.24

Source: Authors' calculation

It could be seen that in both cases (having in mind three scenarios and their probability of outcome) it could be expected that cattle fattening operation would create profit.

Conclusions

This paper investigated how changes of certain types of revenues and costs influenced profit in cattle fattening at family farms. If each of the elements analyzed is observed separately, their least favorable values will lead to the profit decrease, but they will not create loss, unless there is a total abolition of state subsidies. If the best, most likely and the worst combination of factors observed (prices of fattened cattle, state subsidies, prices of corn and prices of calves) are analyzed through scenario analysis, the worst case scenario will always lead to a loss in cattle fattening activity. However, having in mind probability of occurrence of each scenario it could be expected that in general farm will make profit (even when it is assumed that subsidies did not exist as in the worst case scenario).

Acknowledgement

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FINANCING AS A LIMITING FACTOR OF AGRICULTURAL DEVELOPMENT IN SERBIA

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Abstract

The problem of financing agriculture in Serbia is constantly present and represents the biggest development limitation of this economic activity. The consequences of this problem are visible on the basis of the results of the last 2012 Census of agriculture. According to these data, most farms have four or less animals, irrigation systems cover only 3% of the total arable land, i.e. only 12% farms irrigate their land. The average age of agricultural machinery is about 20 years. A small number of farms has necessary equipment for intensive agricultural production. Out of a total of 631,552 farms, only 542 farms have greenhouses, while the cold store has only on 1,804 farms. Most agricultural entities in Serbia are not profitable, with lack of their own sources of financing, and limited access to the commercial bank loans. The financial resources of the Agrarian budget, as well as other forms of the governmental financial support are insufficient for the operational and development needs of agriculture. For the future development of Serbian agriculture it is necessary to develop new models for financing agriculture in Serbia. To this end, the authors propose the establishment of a specialized (development) agricultural bank and microcredit organizations, as well as the use of securities, financial derivatives, foreign direct investments and EU pre-accession funds.

Key words: *agriculture, financing, agricultural bank, microcredit organizations, financial derivatives.*

Introduction

In the Republic of Serbia, according to the categorization of the Organization for Economic Co-operation and Development (OECD), the rural area is considered 85% of the territory, where 55% of the total population lives. According to the results of the 2012 agricultural census, there are 631,552 agricultural farms in Serbia, with a total of 3,437,423 hectares of agricultural land. In the structure of agricultural holdings, family farms are dominant, with 99.5% of total farm numbers, and the average age of the farm holders of these farms is 59 years. The average size of farm' agricultural land is only 5.4 hectares. In addition to primary agricultural production, in other nonfarm activities are involved 78,301, or only 12% of farmers (Bogdanov & Babović, 2014).

The basic macroeconomic indicators of the importance of agriculture for the economy of a country, are: agricultural participation in the total population, participation of agriculture in total employment, participation of agriculture in gross domestic product and participation of agriculture in foreign trade. According to the data from the Labor Force Survey in Serbia for 2015, the share of employees in agriculture, forestry and fishing in the total number of employees (working age population aged 15-64) was 17%. The share of the rural population in the total population is 40.6%. Based on the data from the agricultural census in 2012, it is evident that only primary agricultural production covers 1,442,628 inhabitants, which makes up over 20% of the total population of the Republic of Serbia.

According to statistical data, the share of agriculture, forestry and fisheries in gross value added at the national economy level, expressed at current prices, was 11% in the period from

1996 to 2015. Average share of agriculture in foreign trade, i.e. in exports of goods in the period 2010-2015 was 22%. It is important to emphasize that unprocessed agricultural products prevail in exports while the products with a higher degree of processing are imported. By analyzing these indicators, it can be concluded that agriculture has great economic and social significance in Serbia.

Due to the specificity of the production cycle, low turnover of capital, low profitability, as well as the lack of own sources of financing, a constant financial and credit support in Serbian agriculture is required. Financing is the biggest and most complex problem of agriculture in the Republic of Serbia, which is constantly actual (Radović, 2009). The consequences of the unresolved financing problem are visible on the basis of the results of the last 2012 Census of agriculture. According to these data, most farms have four or less animal units, while only 522 farms have more than 100 animal units. The irrigation systems cover only 3% of the total arable land, i.e. only 12% farms are irrigating their land. The average age of agricultural machinery is about 20 years. A small number of farms has necessary equipment for the intensification of agricultural production. Only 542 farms have greenhouses and 1,804 farms have cold storage facilities.

Whether viewed in the narrow sense, as primary agricultural production, or in a wider sense, as a multifunctional agriculture, agriculture is "privileged" and has financial support in the market-developed economies. Multifunctional agriculture, in addition to primary agricultural production, includes all the activities that relate to it (food industry, trade, crafts, tourism, etc.). By solving the problems of financing multifunctional agriculture would create development opportunities, increase employment, and stop migration from rural to urban areas in the Republic of Serbia. The aim of the paper is to present financing agriculture in the previous (pre-transitional) period and the current (transitional) period, as well as to propose possible ways of financing agriculture in the Republic of Serbia.

Previous (pre-transitional) period. The economic position of agriculture analyzed through the primary distribution was very unfavorable in the entire pre-transition period due to the policy of depressed prices of agricultural products. The existence of a disparity in the price of agricultural, food and industrial products, so called a "price scissor" is from need to ensure the country's food security. It is estimated that in the 1990s in this way about $ 5 billion was "moved" out of agriculture, which permanently disrupted the economic position of agriculture. The essence of the problem of financing agriculture in the previous (pre-transitional) period was the inadequate relationship between agricultural investment through state budget allocations, and the contribution of agriculture to the creation of gross value added product (Radović, 2009). Financing of agriculture until 1994, that is, until the adoption of the Monetary System Reconstruction Program on 24th January 1994, was entirely dependent on the state, which had a decisive influence on the support directed towards financing agricultural production. The support was mainly related to financing under the beneficial loan conditions, primarily under the beneficial interest rates, and the source of credit was the primary issue many from the National Bank. Credit placements of commercial banks in the agrarian sector, which were financed from own sources of commercial banks' funds, were also under considerable influence of the state. For this period it can be said that both short-term and long-term lending to agriculture was under the state control, the funds in agriculture are administratively directed. The placements of selective loans to domestic agriculture in the 1980s and 1990s, according to the claims of some authors, were based, as this partially improved the unfavorable economic position of agricultural entities (Bjelica, 2001).

Financing of agriculture throughout the primary money emission by the National Bank was a financing method that respected the specifics of agricultural production (the seasonal character of production, the need for funds to be placed in the short term, and staying long-

tied). The price of selective loans was adjusted to the low payment capacity of agricultural entities. The disadvantage of this method of financing was primarily the insufficiency of resources for needs of the agrarian sector.

By adopting the Monetary System Reconstruction Program, on January 24, 1994, the privileged position of agriculture in terms of its financing from the primary money emission by the National Bank was abolished. With the abolition of privileged loans to agrarian sector, no other source of financing was provided, which would be an adequate replacement, and because of this, the agricultural entities were forced to borrow in very unfavorable conditions, because of the restrictive supply of money, the interest rates in the banking market were high.

Current (transitional) period. The primary way of financing agriculture in the Republic of Serbia in the transition period is the agrarian budget. The agrarian budget is a key instrument of agrarian and rural policy. As a safe and permanent source of financing for agriculture, it was established by the Decision on the formation of the agrarian budget, which was adopted in late 1995, and became an integral part of the state budget in 1996. This type of agricultural support exists in all agrarian developed countries, but also within the framework of a unified EU budget.

The share of the agrarian budget in the total state budget was the highest in 1996 (8.3%) and in 2004 (6.1%). Average participation of the agrarian in the total state budget in the period 1996-2015 was only 4.8% (Radović, 2015).

The agrarian budget has a very important qualitative dimension, as it is a key instrument for implementing the measures of agrarian policy. It should be noted that the agrarian policy in the Republic of Serbia in the whole of the past period went through sudden and inconsistent changes, which also reflected on the structural (qualitative) changes in the agricultural budget. In order to develop agriculture, it is necessary to increase the quantitative participation of the agrarian budget in the overall state budget in the coming period. Also, in its qualitative structure, greater incentives should support rural development (Vasiljevic et al., 2016).

The Republic of Slovenia can serve as a model for Serbia in terms of the continuous growth of the share of the agrarian in the total state budget in the transitional period. "Continuous growth of funds allocated from the budget of the Republic of Slovenia for agriculture in the period of transition 1996-2003 as well as the accession of this country to the European Union 2004-2010. It testifies to the need to modernize agricultural production through significant investments, to achieve higher yields, and to ensure that this production is realized with respect to all European Union standards so that Slovenian agricultural and food products will be competitive on the "European market" (Radović, 2011).

Financing of agriculture in the Republic of Serbia in the current transition period is based on financing from the provincial budget as well as from the budgets of local economic communities. Also, one of the funding sources for agricultural enterprises is subsidized loans with the support of the Ministry of Agriculture, as well as loans approved by specialized state financial institutions. These are the Development Fund of the Republic of Serbia, the Agricultural Development Fund of AP Vojvodina, the Development Fund of AP Vojvodina and the Guarantee Fund of AP Vojvodina, as well as the Development Agency of Serbia. However, given that the sources of funding are insufficient, the dominant source of financing of agricultural entities are the commercial bank' loans.

In pre Second World War period Serbia has successful tradition and developed Agricultural savings cooperatives, but according to recent Law on cooperatives (Official Gazette of the Republic of Serbia No. 112/2015) this kind of cooperatives is banned in Serbia.

Material and Methods

The conducted research is based on desktop research and methods of analysis and synthesis. Paper is primarily lean on secondary data obtained from national statistical organizations and public institutions.

Interview with relevant expert in the field of agricultural financing, as a method is used.

All results and derived conclusions are in line with the statements from the available national scientific and professional literature.

Results and Discussion

Possible sources of financing of agriculture in the Republic of Serbia are: (a) a specialized agricultural bank; (b) pre-accession funds of the European Union; (C) microcredit organizations, (d) securities and security derivatives; (E) savings institutions; (F) foreign direct investment.

A specialized agricultural bank could unify the functions of the current specialized state financial institutions, i.e. funds that exist in the Republic, province, but also at the level of almost all local municipalities in the Republic of Serbia. Also, through a specialized agricultural bank, subsidized loans could be realized by the Ministry of Agriculture through commercial banks. Given that this is a new financial institution, it is necessary to adopt a special law, which would precisely define its establishment, functioning, as well as sources of funds. This specialized development financial institution could, with the continuous supervision of the National Bank of the Republic of Serbia and with the management of placements based on the appreciation of regional and local needs and specificities, be able to provide the development possibilities of the domestic agrarian sector (Radović, 2014).

Savings institutions are increasingly active in the agricultural sector. Government subsidies of interest rates for agricultural loans proves to be effective tools for development of this financial market.

Micro-credit organizations are financial institutions with potential to actively engage in financing agriculture, as they play a significant role in supporting the development of entrepreneurship and self-employment of population categories that do not have access to the classic banking market. Therefore, these financial institutions could be an important source of funding for small family farms, which are also predominant in the structure of agricultural entities in the Republic of Serbia. The limiting factor for the application of lending through microcredit organizations in the Republic of Serbia, in the current period, is the lack of a normative framework for their functioning. Therefore, microcredit organizations must place their loans through commercial banks, which significantly increases this type of financing (Radović, 2015).

Securities and financial derivatives (futures and options) can be a significant source of financing and risk management for agriculture, but for that purpose it is necessary to establish the necessary normative framework in the Republic of Serbia, develop financial markets (primary and secondary), as well as ensure continuous information and education of agricultural entities (Kovačević & Zakić, 2016).

Pre-accession funds of the European Union are also a potential source of funding, primarily the IPARD program, but for their use, it is necessary to meet the EU's administrative requirements, as well as to provide sources for pre-financing projects, since the funds are provided on the principle of refund. To that end, it is necessary to support the state that could be realized through a specialized agricultural bank.

Pre-harvest financing as an alternative model of agricultural financing are established in Serbia and Ukraine as a pilot project for this kind of financing system in agriculture for EU[21].

[21] Both pre-harvest financing systems are developed with support of European Bank for Reconstruction and development.

Pre-harvest financing system is based on a future product, which is still in the field in the process of production, as collateral for short-term loan. The instruments of pre-harvest financing are a form of forward contracts. Contracts, by which the agricultural production is financed, in exchange for delivery of agricultural products later. This system is with great success established in Brazil in 1990s. In Serbia it was established in 2015 by the Law on pre-harvest financing, while in Ukraine pre-harvest financing is established as a pilot project in one region (Kovačević & Zakić, 2016).

Establishment of Agricultural savings cooperatives could be significant source of financing for agricultural sector in Serbia. Serbia had successful practice of agricultural savings cooperatives. For development of this kind of financial institutions legal framework in Serbia is required.

One of the possible ways of financing agriculture in the Republic of Serbia are foreign direct investments. Factors of importance for attracting foreign direct investment are: (a) characteristics of tax policy (amount of taxes and contributions to earnings of employees); (B) the quality and the cost of labor; (C) legal protection of investors and the efficiency of issuing the necessary documentation; (D) the quality of the infrastructure; (E) access to overseas markets; (F) the image and political stability of the state; (G) the development of financial markets; (H) subsidies and privileges to foreign investors (Vasiljević & Kovačević, 2014).

Conclusion

Financing of agriculture in the Republic of Serbia should be realized at the current moment with the strong financial and credit support of the state, bearing in mind the underdevelopment of this economic activity on the one hand, as well as the production resources, the importance of agriculture for the national economy and the overall employment of the population on the other. According to the results of this paper the proposal proposes the establishment of a specialized state development financial institution (specialized agricultural banks) that would prioritize the financing of agriculture and rural development in the Republic of Serbia under the beneficial conditions. This type of financing is needed because most agricultural entities in the Republic of Serbia do not have the capacity to self-finance, due to the lack of their own accumulation, and the conditions for lending on the banking market are unfavorable for them.

Agricultural savings cooperatives are important financial institutions in developed countries and it could play important function in for Serbian agricultural sector.

Possible source for the financing of agriculture in the Republic of Serbia are pre-accession funds of the European Union, microcredit organizations, securities and financial derivatives. For the purpose of their implementation, it is necessary to provide administrative conditions, as well as the appropriate legal framework. Of course, foreign direct investment is also a potential way of financing agriculture and rural development, and in order to secure them, it is necessary to have the necessary favorable conditions.

Important mechanism is pre-harvest financing system which needs to be further promoted and developed.

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THE PROPERTIES OF THE FAMILY FARMING CONDUCTING DAIRY FARMING IN THE CITY OF KONYA AND THE FACTORS AFFECTING THEM

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Abstract

More than three billions of people live in rural areas in the world. Approximately 2,5 billion of them provide for the agricultural sector. The agricultural sector is the most important element of the economic growth for most countries and especially the developing countries. The majority of the businesses in agricultural sector is conducted in family farming. Studies have been started by conducting the official introduction of the family farming in United States of America and some South American countries for the purpose of making the production of the family farmers conducting traditional production sustainable and productive. In the study, based on the definition of the family farming, the factors affecting the properties of the family businesses have been detected. Logistic regression model has been used in the determination of the factors affecting the properties of the family farming. According to the model results, agricultural income ratio, equity capital ratio, property land ratio and labor force potential, among the factors affecting the properties of family farming, have been found meaningful. The businesses in the study region are in the state of the businesses with competent income. The businesses to have an income in competent level will facilitate the budgeting for the newly met techniques and technologies and ensure the development of the businesses.

Keywords: *Family Farming, Logistic Regression, Konya.*

Introduction

Agricultural sector is the most important element of the economic growth for most countries and especially the developing countries. The developing countries form three out of four of the world agricultural production and the agricultural sector forms 30% of the GDP in most of these countries (FAO, 2013). 51% of the total population in the world and 23% of the population in Turkey form the rural population (Oğuz, 2010; TÜİK, 2014; FAO, 2014c; 2017). This population provides for the agricultural sector. The agricultural sector has a share of approximately 30 and 60% of the GDP in almost 2/3 of the underdeveloped and developing countries (Doğaner, 2016). The majority of the population working in the agricultural sector is in the position of family business (family farming). Family farming which is conducted by the family members without considering the genders, based on family labor force, sensitive to the environment, protects the environmental resources, supports the sustainable agriculture, provides high yield and quality and is dynamic has an important structure generally in the world. Family farming is a structure providing the sustainability without considering any purpose of sole profit by protecting the cultural elements as well as helping the economic development by forming labor force and income (Blekesaune, 1997; Hoppe, 2010; FAO, 2014a). 500 million out of 570 million agricultural businesses in the world are operated by the family farms and 475 million businesses have a production area less than 2 hectares (Hazell et al., 2010; FAO, 2013). However; these businesses superior in terms of number have a less area in the world in terms of agricultural area (Lowder et al., 2014). They cultivate 68% of the agricultural areas in EU countries, 85% in Asian countries, 83% in North American countries, 18% in South American countries and 62% in African countries. They produce 56% of the total agricultural production (FAO, 2014b). Studies have been started by conducting the

official introduction of the family farming in United States of America and some South American countries for the purpose of making the production of the family farmers conducting traditional production sustainable and productive. The concept "family farming" is widely used in the countries with high income such as European Union countries, some European countries and Latin America and Caribbean countries (Gras, 2009; EUROSTAT, 2010; Économie, 2011; Krasniqi, 2012; Hoppe ve MacDonald, 2013; Garner ve de la O Campos, 2014; Lowder et al., 2014). However; no definition of family farming has been commonly made in the world. According to the definition of FAO, family farming is the vegetative, animal production, fishing and forestry activities conducted by the family members without considering the gender differences with the use of family labor force. The definition of family farming in Turkey is the businesses conducting activities based on the labor force, property and administration of the family without considering the gender differences, having a field between 0-500 decare or 0-49 bovine animal existence, conducting vegetable, animal and mixed production and gaining the majority of their income from these activities (Yener, 2017; Yener and Oğuz, 2018). Besides, the definition of the family farming has been made in the city of Konya. Family farming for the city of Konya is the businesses which do not consider any gender difference, which is conducted by the family members, whose administration belongs to the owner of the business, which have a field of 0-500 decare or 0-49 bovine animal existence, which conduct vegetable, animal and mixed production, whose more than 80% of the total field amount is property land, whose 15-49 aged labor force potential is above 70%, which attains more than 85% of their total income from agriculture and whose equity capital is above 90% within the total capital (Yener, 2017; Yener and Oğuz, 2018). In this study, the factors affecting the properties of the family farming have been analyzed by taking into consideration the definition of family farming made for the city of Konya.

Materials and methods

The main material of the study consists of the primary data collected via questionnaires from the dairy farming businesses in the city of Konya. Moreover; the studies previously conducted regarding the subject and the data collected by the related institutions and organizations have been benefitted. In the study; stratified sampling method out of the simple random sampling method has been used for the purpose of increasing the accuracy of the findings collected from the businesses and providing the sufficient representation of the different parts in the population (Yamane, 1967;Güneş and Arıkan, 1985). Sample size has been calculated as 128 within 95% reliability range with 5% error margin and the businesses taking place in the sample size have been selected according to the principle of random volunteering.

Table 2. Distribution of the businesses conducting Dairy Farming according to their number of animal (Sample Size)

Business Width Groups	Sample Size (Number)
0-25	84
26-75	29
76-+	15
Total	128

Logistic regression model has been formed for the determination of the factors affecting the properties of family farming. The analysis methods that could be used in the event that the dependent variable is binary (0,1) are limited. The widely used models are Logistic Regression (LR), Probit and Linear Probability Model (Cankurt et al., 2010). Logistic regression is a method benefitted in the determination of the cause and effect relation with the explanatory variables in the event that the response variable is observed in the categorical,

binary, triplet and multiple categories. It is a regression method in which the expected values of the response variable as probability when compared to the explanatory variables (Özdamar, 1999). The assumptions foreseen for the linear regression model are distorted in the event that the dependent variable is continuous and it is a discrete variable including two or more levels and the observation variances are not equal due to the fact that the error term shows a binomial distribution. The use of logistic regression analysis is suggested because the data cannot be examined with linear regression analysis in this situation (Bagley et al., 2001).

Logistic regression model is as follows.

$$E(y_i) = \eta = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} \text{ .....} + \beta_k x_{ki}$$

If $E(y_i)$, it takes a value between $-\infty$, ∞.

Assumptions regarding the two-level logistic model are as follows:

i) $y_i \in (0,1)$, i=1,2,3,.....N

ii) $$P(y_i = 1/ \; x_i) = \frac{\exp(\beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} \text{.....} \beta_k x_{ki})}{1 + \exp(\beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} \text{.....} \beta_k x_{ki})}$$

iii) $y_1, y_2, \text{.....}, y_n$ are statistically independent.

iv) Independent variables are independent of one another (Aktaş and Erkuş, 2009).

The odds ratio used in the interpretation of the logistic regression model is a value attained by dividing the observation probability of the phenomenon into the non-observation probability. It is calculated as follows.

$$Odss\ Ratio = \frac{\text{Probability}}{1 - \text{Probability}}$$

Odds ratio expresses
the decrease in the event that Odds ratio<1,
that there is no relation in the event that Odds ratio=1
the increase in the event that Odds ratio>1 (Süt and Şenocak, 2007).

In the study; status of being a family business (Family Business:1, Other Business:0) has been taken as the dependent variable; and agricultural income ratio within the total income, ratio of equity capital within the capital, property land ratio within the total field, labor force potential, animal number and field have been taken as the independent variables.

The relation graphics (Scatter/Dot Charts) in SPSS program have been used in the determination of the relation between the total land amount and property land amount of the businesses conducting family farming. In addition; the relation between the total land amount and the status of producing forage crop has been determined with Chi-Square analysis.

Results and discussion

The definition of the family farming in the city of Konya has been made as the businesses which do not consider any gender difference, which is conducted by the family members, whose administration belongs to the owner of the business, which have a field of 0-500 decare or 0-49 bovine animal existence, which conduct vegetable, animal and mixed production, whose more than 80% of the total field amount is property land, whose 15-49 aged labor force potential is above 70%, which attains more than 85% of their total income from agriculture and whose equity capital is above 90% within the total capital (Yener, 2017; Yener and Oğuz, 2018). According to this definition, 50% of the businesses in the study area conduct family farming (Table 2). 45,24% of the businesses with 0-25 bovine animal existence, 48,28% of the businesses with 26-75 bovine animal existence and 80% of the businesses with 76-+ bovine animal existence conduct family farming.

Table 3. The Status of Being A Family Farming for the Businesses (Piece, %)

Business Groups	Family Businesses		Other Businesses		Total	
	Number	%	Number	%	Number	%
0-25	38	45,24	46	54,76	84	100,00
26-75	14	48,28	15	51,72	29	100,00
76-+	12	80,00	3	20,00	15	100,00
Total	64	50,00	64	50,00	128	100,00

Total income of the businesses conducting family farming is 74.113,40 $ and 91,61% of them is attained from agricultural income. Agricultural income is defined as the total of in-cash and kind values which is supplied by the business manager and his/her family from the agricultural activity attended by them with mental and physical labor force as well as their capital and whose responsibility is also undertaken by the and could be consumed by the business without narrowing down the production capacity (Talim, 1974). The agricultural income attained at the end of a production period in the agricultural businesses are expected to meet the total of the normal interest provision of the equity capital imbursed to a business in return for the labor force of the business manager and his/her family. Also; the agricultural income should be more than the total of the price calculated for the family members working in return for equity capital interest in terms of being able to give opportunities for new investments (Parlakay and Esengün, 2005). According to the notice (Notice No: 2003/20) regarding the determination of the agricultural business extent with competent income, annual processing income with competent income has been calculated as 1.783.222.746,48 $. Annual business income with competent income for 2017 has been calculated as 5.008,07 $ by taking the inflation and emitting of 6 zeros into consideration (The year 2007 has been taken as the basis) (Anonim 2013). In this respect, the businesses taking place in the study are in the status of businesses with competent income. The businesses to have an income at competent level will facilitate the separation of the budget for new techniques and technologies and will ensure the development of the businesses.

Table 4. Definitive Statistics for the Factors Affecting the Properties of Family Businesses

	Family Businesses	
	Amount	%
Agricultural Income ($)	67.891,62	91,61
Total Income ($)	74.113,40	
Equity Capital ($)	698.501,64	98,00
Total Capital ($)	712.791,69	
Property Land (da)	196,30	99,04
Total Land (da)	198,21	
Family Labor Force Potential (FLF)	967,42	71,68

According to Table 3, equity capital ratio is 98% and property land ratio is 99,04%. The average land amount in the businesses is 196,30 da and the majority of the lands are watery lands. According to the Law on Soil Protection and Land Usage no. 6537, it has been specified that the businesses with 65 da watery land and 176 da dry land for the city of Konya have an agricultural land extent with competent income (Anonim, 2014). In this respect, the businesses in the study area have agricultural lands with competent income. However; this situation is observed when the capital distributions of the businesses are considered. The capital amount and the elements forming the capital are very important in the businesses (Aksöz, 1972). The greatest share in the active capital in Turkish agricultural businesses belongs to the land capital. The reason for this is that land capital is higher than that of other capital groups. This situation is an indicator of extensive agriculture. Capital usage per unit

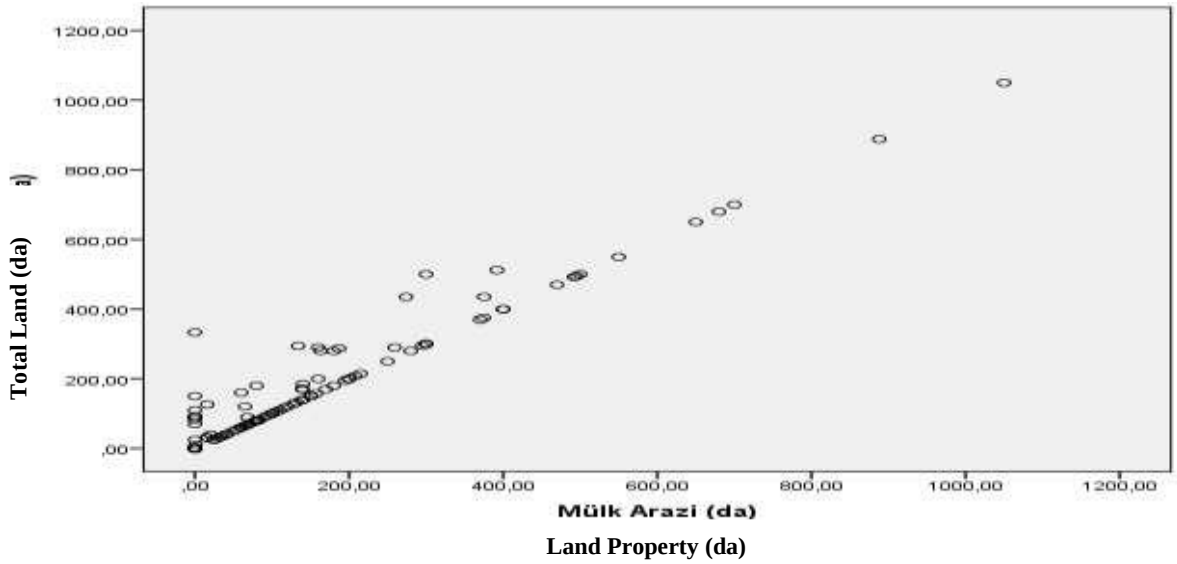
land is not intensive (Cinemre, 1999). The reasons for this situation could be cited as not being able to healthily detect the money capital and material capital, lands to be a tool for social prestige, farming not adopted as a life style and the land value to be far above the production capacity of the land (Cinemre et al., 1995). Family labor force potential is 71,68%. The most important cost criterion following the forage cost is the fee for permanent labor force (shepherd etc.) in the businesses conducting animal production. Family labor force potential to be high has a positive impact on the agricultural income. The increase in the agricultural income is very important in terms of the sustainability of the family farming. The land amount (da), animal number, property land ratio, capital ratio and agricultural income ratio used in the definition of family farming are the important factors affecting the family farming. For this reason; the factors affecting the properties of family farming in the study area according to the definition of the family farming for the city of Konya have been detected with Logistic regression model.

Table 5. Analysis of the Factors Affecting the Properties of Family Businesses

Name of Variable	B	SE	Wald	DF	Sig	Exp(B)
Agricultural Income Ratio	,078	,019	17,483	1	,000	1,081
Equity Capital Ratio	29,668	7,895	14,123	1	,000	7,671
Labor Force Potential	3,784	2,184	3,001		,083	43,987
Land Ratio	-,023	,012	3,669	1	,055	,978
Animal Number	,001	,010	,012	1	,912	1,001
Land Amount	,000	,002	,057	1	,811	1,000
Constant	-34,276	7,900	18,882	1	,000	,000
-2 LogLikelihood64,944Cox&Snell R Square 0,585 Nagelkerke R Square 0,780						

According to the results of Logistic regression model; agricultural income and equity capital ratio have been found positively meaningful at 1% importance level and labor force potential has been found positively meaningful at 10% importance level. Property land ratio has been found negatively meaningful at 10% importance level. The increase in the agricultural income among the equity capital and total income virtually belonging to the business manager and used in the agricultural production fortifies the definition of family farming. However; the importance of agricultural income has been emphasized if the family farming definitions in the world are considered (Calus ve Lauwers, 2009; Chaddad ve Jank, 2006; FAO, 2012; SAGARPA, 2012). But; as the property land ratio increases, the status of conducting family farming decreases. Small businesses conducting animal production increase their income via land renting and the big businesses increase their production area of forage plant. The production of field and horticultural plants is conducted in 55,50% of the rented lands and the production of forage plants is conducted in 44,50% of them. Therefore; a negative relation has been detected between the family farming and property land ratio in the study area. Besides, the relation between the total land and property land is given in Graphics 1.

Graphics 1. The Relation Between Total Land and Property Land



As the total land amount increases in the businesses, the property land ratio increases (Graphics 1). However; this property land is not sufficient for the forage plant production and the businesses apply to the rentin of the lands. A positive relation has been detected according to Chi-square analysis in the study area between the total land amount of the businesses and the status of the production of the forage plants (χ^2= 5233,700, p<0,01). In other words; as the total land amount increases, production area of forage plants increases. This situation is a status desired for the businesses conducting animal production. The status of the businesses for the production of forage plants on total land is 53,51%. But; this production is not sufficient and businesses apply to the renting of the lands and decrease their cost for forages. For this reason; land property ratio has been negatively meaningful. The final factor affecting the properties of the family farming is labor force potential. In contrast to the vegetable production, there is a need for permanent labor force during the production period. As the family labor force potential increases, foreign (permanent) labor force amount will decrease and this situation will increase the agricultural income. As the agricultural income rises, it will increase the desires of the businesses to use new techniques and technologies (Türkyılmaz et al., 2003; Kutlay ve Ceylan, 2008; Sezgin et al., 2010; Oğuz ve Yener, 2017). The sustainability of the family farming could only be ensured by adapting to the changing techniques and technologies for the businesses. Business income becomes very important for the businesses to be able to follow and purchase the new techniques and technologies. Besides; in similar studies, it is emphasized that the income levels of the farmers be sufficient in terms of being able to apply the modern agricultural techniques in rural areas (Kalanlar, 2005; Rogers, 2010; Ohe, 2012; Melesse ve Jemal, 2013; Ryan et al., 2014).

Conclusion

Agricultural income ratio, equity capital ratio, property land ratio and labor force potential from the factors affecting the properties of family farming have been found meaningful. The businesses in the study area are in the status of the businesses with competent income. The businesses to have an income at competent level will facilitate the separation of budget for new techniques and technologies and will ensure the development of the business. As in the average of Turkish businesses; also in the study area, the highest share among the active capital is taken by the land capital. However; the property lands of the businesses are not sufficient and the rent the lands. Besides, a negative relation has been detected between the property land ratio and family farming. The production of forage plants is conducted in

44,50% of the rented lands. This cost item will decrease as long as the production areas of forage plant expand due to the fact that the forage cost is the most important cost element in the dairy production. Furthermore; the most important cost item following the forage costs in animal production is the cost of labor force (shepherd etc.). As the family labor force potential increases, foreign labor force amount will decrease and this situation will decrease the permanent labor force cost item. Also in the model, labor force potential has been found positively meaningful. Family farming is very significant in terms of the provision of sustainability in agricultural production, food safety, prevention of the migration from rural areas to the cities and the assessment of labor force. For this reason; definitions of family farming has been made for the sustainability of the family farming in many countries. When the properties family farming is considered, it is seen that it has the same criteria in almost all over the world. There are changes only in the ratios of the criteria. The definition of family farming should be made on the basis of the countries and regions by taking these criteria into consideration and the businesses conducting family farming should be taken into account while making policies.

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DEVELOPMENTS ON GEOGRAPHICAL INDICATIONS AND TRADITIONAL PRODUCT NAME REGISTRATION IN TURKEY

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Abstract

Interest in the origin of products and geographical indications in Turkey has recently increased in parallel with global developments. Geographical indications have the functions of preserving traditional knowledge and cultural values, combating product imitation, supporting local production and rural development, becoming a marketing tool, and guaranteeing the production methods and product standards. Traditional products, especially in underdeveloped regions, have the potential to create new employment opportunities for women and the young people and bring new initiatives for rural development. In this respect, it is of great importance to build the capacity of cooperatives and raise producer and consumer awareness and this can be achieved through both the public and the private sector. The Decree Law No:555 was implemented in Turkey as the basic regulation on the protection of geographical indications until Industrial Property Law No: 6769 came into force on January 10, 2017. The publication of the National Geographical Indication Strategy Document and the Action Plan on July 4, 2015 in the Official Gazette has been a significant development in terms of determining national policies. Turkey has a good potential for traditional products and products with geographical indications. 329 products have been registered by the Turkish Patent and Trademark Office as of April 2018, and 329 products are currently at the application stage. This study explains the current situation in Turkey on geographical indications and traditional product names and examines the practices in the application and registration process.

Keywords: *Marketing, Food quality, Rural development.*

Introduction

In global markets, it can be achieved through registered products to protect the producers of traditional/regional/local products and enrich these regions/local areas through these products and provide their sustainability. Turkey has a large export potential on existing and potential products with Geographical Indication (GI) depending on growing interest and demand in the world. Therefore, the importance of marketing strategies and registration of traditional/local food and agricultural products has been increasing in Turkey as in the world. (Çalışkan and Koç, 2012, s.211; Official Gazette, 2015). Protection by registration in these products is provided by the registration of "Geographical Indication" (GI) and "Traditional Speciality Guaranteed" (TSG). Minerals, handicrafts and industrial products as well as agricultural products and food can benefit from this protection.

The Decree Law No:555 was implemented in Turkey as the basic regulation on the protection of geographical indications until January 10, 2017 and, after this date, the Industrial Property Law No: 6769 came into force. The National Geographical Indication Strategy Document and Action Plan which are important for the determination of national policies were published in official gazette on July 4, 2015. The action plan predicts to strengthen the coordination between institutions on increasing the effectiveness of marketing strategies in order to particularly improve the institutional capacity of the relevant institutions, promote scientific studies on geographical indications, raise awareness of geographical indications in all segments of society and increase the added value of the products with geographical

indication. This will ensure the increase in registered products and good governance on geographical indications.

Turkey is a party to TRIPS Agreement, Paris Convention and Madrid Agreement for the Repression of False or Deceptive Indications of Source on Goods. Apart from these three agreements, the country accedes to some free trade agreements in which the provisions of the geographical indications are included ("Official Gazette", 2015, Gürsu, 2008). According to the Paris Convention, a separate application can be made for each country where protection is requested. It is also required to apply to the European Commission for the protection within the European Union (EU). The international registration system on GIs is expressed as Lisbon System (İloğlu, 2014).

By May 2018, Turkey has made an application to the EU Commission totally for 14 products notably Aydın Kestanesi, Inegöl Köftesi, Afyon Sucuğu, Afyon Pastırması, Taşköprü Sarımsağı, Kayseri Pastırması, Kayseri Sucuğu, Kayseri Mantısı and Bayramiç Beyazı. Antep Baklavası/Gaziantep Baklavası with the "Protected Geographical Indication" (PGI). In a similar way, Aydın İnciri and Malatya Kayısısı with "Protected Designations of Origin"(PDO). are the first products registered in the EU respectively on the dates of December 21, 2013, February 17, 2016 and July 07, 2017.

Turkey has a potential of GI approximately on 2500 products with its rich eco-system and workforce which is well-equipped with the skills and knowledge of production (Tekelioğlu et al, 2012). The preparation of the strategy document and action plan on GIs is substantial as it will enable this potential to be used together with the increase of awareness activities. Thus, the protection and recognition of these products will be provided and main producers of the products will economically be supported.

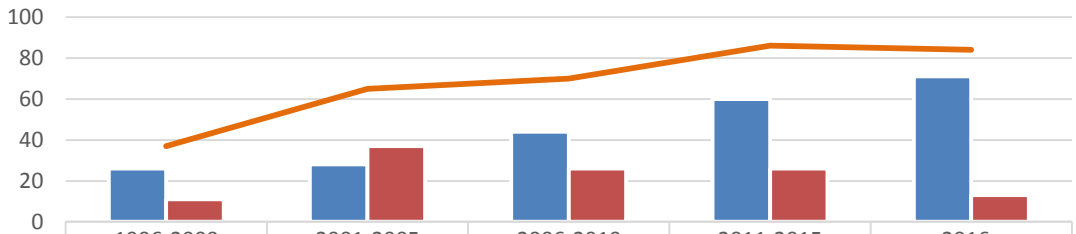
Current Situation in Geographical Indications and Traditional Speciality Guaranteed

As of the date of May 2018, Turkey has a total of 342 registered geographical indications, five of which is of foreign origin. Approximately 80% of the registered geographical indications are food and agricultural products. The meals have received PGI as the definition of TSG has not been done yet by the date of 10 January 2017 on which Industrial Property Law was adopted, (TPE, 2017a).

It has been significant improvements in the registration of geographical indications in Turkey between the years of 1996-2017. A total of 37 geographical indications were registered between 1996 and 2000, 26 of which were PGI. There was a steady increase over the following five-year periods and 84 products were registered in the last three and a half years from 2016 to this date (Graph 1). Due to the fact that the meals are given registration of PGI, 229 products (67%) in total geographical indications have received the registration of PGI.

23% of the 342 products receiving the GI registration in Turkey is made up of meals; 17% is made up of pastries/sweets/candies, and 11% is made up of fruit/vegetables/cereals group (Table 1). Other groups with a high potential of Turkey are handiworks/handicrafts and carpet/rug weaving. The country is far behind the potential in the cheese and olive oils, 4% and 2%, respectively. However, these products have a diversity originating from local and traditional characteristics and a high potential of GI.

Graph 1. Developments on registration of geographical indications



	1996-2000	2001-2005	2006-2010	2011-2015	2016+
PGI	26	28	44	60	71
PDO	11	37	26	26	13
Total	37	65	70	86	84

Kaynak: TPE (2018)

Table1. Products/product groups with geographical indication

	PGI	%	PDO	%	Total	%
Meals	74	*32,3*	5	*4,4*	79	*23,1*
Pastry/Sweets/Candies	53	*23,1*	5	*4,4*	58	*17,0*
Fruit/Vegetables/Cereals	4	*1,7*	34	*30,1*	38	*11,1*
Handcraft/Handicraft products	26	*11,4*	4	*3,5*	30	*8,8*
Carpets/Rugs	27	*11,8*	0	-	27	*7,9*
Sauces/Spices/Pickles	9	*3,9*	5	*4,4*	14	*4,1*
Cheeses	6	*2,6*	8	*7,1*	14	*4,1*
Fabrics/Cloth fabrics	11	*4,8*	1	*0,9*	12	*3,5*
Cookies	2	*0,9*	9	*8,0*	11	*3,2*
Olive/Olive oil	1	*0,4*	7	*6,2*	8	*2,3*
Beverages	0	-	8	*7,1*	8	*2,3*
Drinks	4	*1,7*	0	-	4	*1,2*
Others	12	*5,2*	27	*23,9*	39	*11,4*
Total	229	*100,0*	113	*100,0*	342	*100,0*

Sources: TPE (2018)

The increase in the number of applications made in recent years to the Turkish Patent and Trademark Office indicates the potential of the GI and there are 415 products in the application stage as of May 2018 (TPE, 2018). The increase in the applications filed with the EU has also gained importance and registration applications have been filed for a total of 14 products after registration of Malatya Kayısısı, Antep Baklavası and Aydın İnciri. These products are given in Table 2.

Table 2. Products in the application stage in the EU

PDO	PGI
Giresun Tombul Fındığı	Antep Lahmacunu
Edremit Körfezi Yeşil Çizik Zeytini	Kayseri Mantısı
Bayramiç Beyazı	Kayseri Pastırması
Milas Zeytinyağı	Kayseri Sucuğu
Antepfıstığı/Antep fıstığı	İnegöl Köfte
Taşköprü Sarımsağı	Afyon Sucuğu
Aydın Kestanesi	Afyon Pastırması

Sources: http://ec.europa.eu

Applications for Geographical Indications and RegistrationProcedures

Applications for geographical indication are made to the Turkish Patent and Trademark Office according to the latest regulation. The Agency evaluates geographical indications or traditional product name applications according to the Articles 33 to 37 and 39 of the Law and may request comments from related institutions and organizations for evaluating technical information. Based on this, technical comments can be sought from the Ministry of Agriculture and Forestry on food and agriculture issues. Those who have the right to apply for GI according to the Law on Industrial Property No 6769 are listed below:

1. Producer groups,
2. Public institutions and organizations related to the geographical area in which the product originates, and professional organizations in the form of public institutions,
3. Associations, foundations and cooperatives that are in the public interest or who are authorized to protect the economic interests of their members in relation to the product,
4. Producers concerned, in case that the product has only one producers and provided that this is proved.

Applications for geographical indications in Turkey are carried out mostly by commerce and industry chambers, municipalities, public institutions, local authorities and commodity exchanges (Table 3). While producer organizations in EU has an active role on geographical indications, their role in Turkey has remained limited. Up until today, producer associations and unions have made applications for five products including GiresunTombul Fındığı, Edremit Körfez Bölgesi Zeytinyağı, Güney Ege Zeytinyağları, Antep İşi and Yamula Patlıcanı and these products received GI registration. Bodrum Mandarini, Elazığ Öküz Gözü Üzümü and Tomarza Kabak Çekirdeği were registered with the producer association's channel. The difference of GI protection from brands is that it is an anonymous right that includes all the producers that produce the specific product that is included in the registry, not just the right for registrant. For this reason, it is especially crucial that cooperatives should take part in this system.

Table 3. Distribution of private/legal entities making application for geographical indication

Applicants	PGI	%	PDO	%	Total	%
Chambers of commerce and industry	81	*35,4*	29	*25,7*	110	*32,2*
Municipalities	40	*17,5*	20	*17,7*	60	*17,5*
Public institutions and local governments	36	*15,7*	10	*8,8*	46	*13,5*
Commodity exchanges	16	*7,0*	9	*8,0*	25	*7,3*
Sumer	24	*10,5*	0	-	24	*7,0*
Associations and foundations	12	*5,2*	3	*2,7*	15	*4,4*
Cooperatives/Producer associations	1	*0,4*	15	*13,3*	16	*4,7*
Union of chamber of merchants and craftsmen	9	*3,9*	4	*3,5*	13	*3,8*
Trading companies	5	*2,2*	5	*4,4*	10	*2,9*
Research and training centers	2	*0,9*	6	*5,3*	8	*2,3*
Foreign applications	0	-	5	*4,4*	5	*1,5*
Others	3	*1,3*	7	*6,2*	10	*2,9*
Total	229	*100,0*	113	*100,0*	342	*100,0*

Souces: TPE (2018)

The most common problem expressed in implementation in GI is the inconsistency of registration properties with the Turkish Food Codex. Products with geographical indications are special products and do not comply with the legislation specified the general scope and for this reason it is necessary to make the inspections according to appropriateness to the

characteristics of the registry. The Implementing Regulation of the Industrial Property Law stipulates that the inspection shall cover all activities related to the appropriateness according to the characteristics indicated in the registry and it will be carried out by the inspection authority recorded with the registry and approved by the Turkish Patent and Trademark Office (Article 45). 49th Article of the Law No: 6769 explains the rules of inspections of the use of GI.According to the Law, the inspections specified in GI registration certificate are made by the inspection authority established by the applicant. Provisions relating to the inspection of the use of GIs and the TSG in the Law on Veterinary Services, Plant Health, Food and Feed Law No: 5996 and in other laws are also applied. GI registration procedures start with application and continue with registration and inspection. These steps;

1. Application stage

The information and documents that should be included in the application file when applying to Turkish Patent and Trademark Office are;

- Information and documents proving the conformity of the entity with the name of the desired geographical indication and the information on the product group and information and documents proving appropriateness to the definition of GI.
- Technical information and documents describing the product and its properties.
- Information and documents that clearly define the boundaries of the geographical area.
- Information and documents relating to production methods, local production techniques and procedures and customs.
- Information and documents proving the connection of the quality, the reputation or other characteristics of the product with the geographical area in question.
-.Information and documents related to the historical background of the product in geographical area in question.
- Information and documents detailing the form of the inspection.
- Information describing the use of the geographical indication and, if applicable, labeling and packaging procedures.

2. Evaluation of the application and objections

The application file is examined by the Agency and if the information and documents are incomplete, the applicant is requested to correct these deficiencies. Upon request of the applicant, additional time is given for not exceeding two times to complete the missing information and documents, If the missing information and documents cannot be remedied within the given time, or if the submitted information and documents cannot be met, the application is rejected. Application which has been examined and approved within the scope of Article 38 of the Industrial Property Law No: 6769 is published in Bulletin. If the application is rejected, it can be appealed by the applicant to the Agency in written and justified within two months from the notification of the rejection decision.

On the other hand, from the date of publication to the Bulletin for the accepted application, third parties may object to the application within three months in a reasoned and written manner. The applicant is informed by the Agency of the objection and the opinion is requested. Both of the objections mentioned above are examined by the Board and opinions can be requested from the relevant institutions or organizations for the evaluation of the alleged objections. Taking into the opinions of the institutions consideration, the applications which are deemed appropriate as a result of the examination of the objection; in the case of a change in the form or scope of the application, with the part of the application which has undergone the change, are published in the bulletin. This posting cannot be appealed. If the appeal is rejected, the Decision is published in the Bulletin.

3. Registration and Requests for Changes

Within three months from the date of publication in the Bulletin, the applications for which no objection has been made, the rejection of the objections have been finalized, or amendments

have been made upon evaluating the objections, are recorded in the registry and published in the Bulletin. In addition, if there is a change in the properties of the registered geographical indication, the request for change can be made by those who have interest.

4. Scope, use and inspection of the right

Persons entitled to use the registered geographical indication or the TSG use it on the product or its packaging together with the geographical indication or the TSG product emblem. The emblem shall be used according to the legislation. The inspection shall be carried out by the inspection authority specified in the application and approved by the Agency. The change in the inspection authority on the registry can be made with the approval of the Agency. Inspection reports are submitted to the Agency annually from the date of the publication of the registration to the Bulletin. However, in case of a complaint, the Agency may require that inspection reports be submitted before the end of the period. If the inspection reports are found to be deficient, the registrant will be notified of the registration and be asked to correct the deficiencies within six months. If it is determined that the deficiencies have not been remedied or that the inspection activity has not been carried out in accordance with the procedure, the provision of Article 43 of Law mentioned above shall apply. The registrant may claim costs related to the supervision from the audits.

Conclusions

Traditional/local/regional products in Turkey have been taken under GI protection (with PDOs and PGIs) by specific regional/local name by 2017. The regulation on "Traditional Speciality Guaranteed" was made after this date with the Industrial Property Law No: 6769. There was no regulation regarding traditional products in Turkey while products which is protected under GI with PDOs and PGSs in the EU is reflected with the GI protection as PDOs and PGSs in the Law numbered 555.For this reason, it has been an important step to have an arrangement in the Industrial Property Law No: 6769 in this regard.

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CONSEQUENCE OF BREAD WHEAT COST AND MARKETING PRICES AND RELATIONSHIP WITH YIELD AND SOME QUALITY PARAMETERS

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Abstract

Trakya region is located in the Northwestern part of Turkey and bread wheat is mainly field crops widely grown in the region. Bread wheat production area is almost 550.000 ha and yield is 5 tons per hectare depend on genotypes, environment and agronomic practices. In Trakya region in some year, adverse weather condition, disease or insects may lead to a crop loss and revenue returns that do not cover production expenses. Due to biotic and abiotic stress factors rise in farm input costs and wheat prices has had economic effects on wheat sector in Trakya region. The mean yields from 2008 to 2017 seasons used in this analysis of 4 cultivars (Pehlivan, Gelibolu, Selimiye and Aldane) growing in the region. In the study grain yield and quality parameters of four cultivars from regional bread wheat experiment used from 2008 to 2017 growing seasons. Grain yield, 1000-kernel weight, test weight, protein ratio, gluten, gluten index, hardness, sedimentation value were compared with marketing price and yield. This comparison allows for a sectorial overview of the proportion of Trakya region wheat producers covering their production costs. Across 10 years environments, average yield varied from 450.4 kg da^{-1} in 2016 and 863.6 kg da^{-1} in 2008 seasons. The year of 2008 was more profitable due to higher yield and price and followed by 2014 thanks to higher grain yield. The highest wheat yields, mean prices and mean profit were in 2008 year. Wheat marketing prices and profit continuously have fallen from 2008 to 2017, but in 2009 and 2015 years dramatically dropped. Mean marketing prices of wheat rose to record highs in 2008 thanks to highest wheat yields. In 2016 both wheat yields and mean prices including mean profit were down significantly. In Trakya region in order to get high profit the yield and some quality parameters were in the mainly factors.

Keywords: *Bread wheat, cultivars, price, inputs, costs of production*

Introduction

Bread wheat is the mainly field crops is grown in Trakya region. Due to various environment condition can cause some problem related with yield and quality in wheat production (Öztürk and Korkut, 2017). It is very important issue that for farmers to get high profit from per hectare is the mainly critical factor in wheat production. The cost of production in wheat yield increases with increase in farm size. In Trakya region farm size is not too large. So farmers are not able to sow the crop timely, spend more on fertilizer, and pesticides. Wheat yield is more responsive to this commercial input.

Almost all breeding programs in the world aim to improve varieties with stable yields (Pfeiffer and Braun, 1989). In wheat, grain yield and baking quality are dependent on the environment, genetic factors and the interaction between them (Yan and Holland, 2010). Increasing yield potential without affecting negatively the quality of the grain is difficult, mainly because increases in grain yield are generally accompanied by a decrease in the grain's protein content, which is strongly associated with bread-making quality (Pena 2002; Pena 2008). Grain protein content in the mature grain is largely determined by environmental and farm management factors, with genetics playing a minor role in being either low or high in protein content. By contrast, protein quality is determined by the genetic composition of the wheat variety and also how the environment influences genetic expression (Blakeney et al.,

2009). Although there are many other quality requirements for durum wheat in the international marketing, some physical characteristics such as 1000 kernel weights and hectolitre weights determine the marketing price (Ozberk et al., 2006).
The cost of production analysis indicates that wheat yield, cost of production and gross income per acre increases with increase in farm size. The primary reason for this is that large farmers having their own tractor plough their fields more are able to sow the crop timely, spend more on fertilizer, irrigation and weedicides. Wheat yield is more responsive to this commercial input that is why the gross income increases with farm size as above mentioned input usage increases. The increase in yield is more than the increase in cost that is why the net income per acre, cost per 40 kg and income per 40 kg reduces with farm size. So to increase the usage of these inputs, time availability of these inputs both in term of quality and quantity is ensured at the doorstep of the farmers (Hassan et al., 2005).
The rise in farm input costs and wheat prices have economic effects on wheat sector. A cumulative distribution of forecasted production costs for wheat farms shows that current high wheat marketing prices will allow a greater share of producers to cover their production costs. Because of the various environment condition yields in wheat production is not stable. Due to this fluctuation in some year farmers get lower profit. So, this study was performed with the aim of to determine profit of wheat and relation of yield and quality parameters with wheat price in Trakya region, Turkey.

Material and Methods

This research was established with 25 genotypes in randomised complete block design (RCBD) with 4 replications in Edirne location, from 2008 to 2017 growing seasons. Each plot had 6 meter long, with 6 rows, spaced 0.17 meters apart. A seed rate of 500 seeds m^2 was used. In the study four cultivars (Pehlivan, Gelibolu, Selimiye and Aldane) were selected from the regional bread wheat experiment and their yield and quality parameters from 2008 to 2017 growing seasons were used. Grain yield, 1000-kernel weight, test weight, protein ratio, wet gluten, gluten index, hardness, sedimentation value were compared with marketing price and grain yield. This comparison allows for a sectorial overview of the proportion of Trakya region wheat producers covering their production costs.
Grain yield, 1000-kernel weights and test weight, (Blakeney et al., 2009), protein ratio, grain hardness, gluten value, gluten index, and sedimentation (Köksel ve ark., 2000; Perten H. 1990; Anonymous, 2002; Anonymous, 1990) were investigated. The quality analysis of Zeleny sedimentation test and wet gluten content were determined according to ICC standard methods No. 116/1 and 106/2, respectively (Anonymous, 1972; Anonymous, 1984).
The significance of differences among means was compared by using Least Significant Difference (L.S.D. at a %5) test (Gomez and Gomez, 1984; Kalaycı, 2005).

Results and Discussion

This study's cost and returns analysis of the Trakya wheat sector provides a snapshot of the range of production expenses across the Trakya region and compares this range with the national season average price that farmers received for wheat. This comparison allows for a sectorial overview of the proportion of Trakya region wheat producers covering their production costs. This analysis uses normal yields of four cultivars and also used later in this report in comparison of actual costs and forecasted costs.
Combined analysis of variance across the 10 (from 2008 to 2017) years revealed highly significant variation among years based on grain yield, 1000-kernels weight, test weight, protein ratio, hardness, sedimentation, gluten value and gluten index, shown in Table 1.

Table 1. Mean yield and quality parameters based on years from 2008 to 2017

Years	TKW	TW	PRT	HARD	SED	GLT	IND
2008	43.9	83.2	13.2	51.3	51.3	37.5	76.2
2009	38.6	80.5	13.1	51.0	45.8	37.9	78.2
2010	39.5	80.8	11.0	46.0	50.5	28.9	91.7
2011	34.5	82.7	14.5	55.0	64.3	42.3	87.8
2012	47.0	85.3	12.5	51.5	44.5	39.1	74.9
2013	44.9	79.9	10.7	46.8	43.3	29.9	78.6
2014	48.3	80.7	9.8	46.5	41.3	25.9	93.0
2015	43.0	81.9	12.3	49.8	61.0	35.5	85.0
2016	36.1	83.7	11.2	48.0	41.0	31.7	72.0
2017	47.0	80.9	12.4	48.0	50.0	36.4	73.0
Mean	42.3	82.0	12.1	49.4	49.3	34.5	81.0

Note: Significance at **: P<0.01; *: P<0.05; TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), HARD: Hardness (PSI), SED: Sedimentation (ml) GLT: Gluten (%), IND: Gluten index (%),

According to results there were significant differences among years based on investigated parameters, so test weight ranged from 79.9 kg in 2013 to 85.3 kg in 2012. Mean test weight was 82.0 kg. Grain 1000-kernel weight is the mass of a given number of kernels and is a useful measure of grain size (Blakeney et al., 2009). The 1000-kernel weight varied from 34.5 g in 2011 to 48.3 g in 2014 among years and mean value was 43.8 g. (Table 1). There were also significant differences among years according to protein ratio and varied from 9.8% in 2014 up to 14.5% in 2011 year and mean protein content was 12.1%. Wheat hardness is influenced especially by genetic factors, but can be influenced also by environment and its factors like moisture and protein content (Turnbull and Rahman, 2002). In the study there were significant differences among years according to grain hardness and ranged from 46.0 in 2010 up to 55.0 in 2011 year. Mean of the grain hardness was 49.4 (Table 1). Statistically significant differences for wet gluten content were found among years. The highest wet gluten content (42.3%) was measured in 2011 year, whereas the lowest with 25.9% in 2014 growing year. Mean gluten content was 33.9%. Gluten index ranged from 74.9% in 2012 up to 93.0% in 2014 year. Analysis of variance showed there was a highly significant difference among years for sedimentation value. Average sedimentation value varied from 41.0 ml (in 2016) to 64.3 ml (in 2011). Throughout all the 10 years environments, the mean grain yield was 696.4 kg da^{-1}. Due to favorable environment condition the highest average yield of four cultivars was 863.6 kg in 2008, and in 2014 with 824.4 kg/da. The lowest yield was in 2016 with 450.4 kg da^{-1} due to unfavourable environmental condition (Table 2). Wheat marketing prices and profit continuously have fallen from 2008 up to 2017, but in 2009 and 2015 dramatically dropped. Mean marketing prices of wheat rose to record highs during 2008 so the highest wheat yields, and mean profit were in 2008 year. In 2016 both wheat yields and mean marketing prices including mean profit were down significantly from their 10 year highs.

Table 2. Mean grain yield and marketing price / profit of wheat based on years

Year	Lower Prices ($/kg)	Higher Prices ($/kg)	Mean Prices ($/kg)	Mean Yield (kg ha^{-1})	Mean Profit ($/ha)	Lower Profit ($/ha)	Higher Profit ($/ha)
2008	0.32	0.55	0.42	8636	3627	2764	4750
2009	0.24	0.41	0.31	6116	1896	1468	2508
2010	0.28	0.46	0.38	6719	2553	1881	3091
2011	0.28	0.40	0.34	6660	2264	1865	2664
2012	0.28	0.42	0.34	6947	2362	1945	2918
2013	0.29	0.42	0.35	7198	2519	2087	3023
2014	0.29	0.41	0.35	8244	2885	2391	3380
2015	0.25	0.35	0.29	6885	1997	1721	2410
2016	0.27	0.32	0.30	4504	1351	1216	1441
2017	0.16	0.37	0.27	7729	2087	1237	2860
Average	0.27	0.41	0.34	6964	2354	1857	2904

Regarding the years, mean lower marketing prices ranged 0.16 $ kg, (in 2017) up to 0.32 $ kg (in 2008), and mean was 0.27 $ kg. The highest marketing prices of wheat ranged 0.32 $ kg in 2016 up to 0.55 $ kg in 2008 and mean was 0.41 $ kg (table 2). Across all the 10 years environments mean marketing prices ranged from 0.27 $ kg in 2017 to 0.42 $ kg in 2008 and mean was 0.34 $ kg.

Based on the years and profit of wheat production per hectare, lower profit ranged 1216 $ ha (in 2016) up to 2764 $ ha (in 2008), and mean was 1857 $ ha. The highest profit of wheat varied 1441 $ ha in 2016 up to 4750 $ ha in 2008 and mean was 2904 $ ha (Table 2). Across 10 years environments mean profit ranged from 1351 $ ha in 2016 to 3627 $ ha in 2008 and mean was 2354 $ ha.

Correlation coefficients among yield, quality parameters with marketing price were given in Table 3. To determining the effect of the yield and quality parameters on marketing price the correlations were determined by Pearson's correlation analysis (Table 3). It was found various relations among yield and quality parameters with marketing price and profit. Decreasing in some quality parameters was negatively affected marketing price of wheat. So, there was slightly negatively relation between low price with 1000-kernel weight (r=-0.122), protein ratio (r=-0.125), and wet gluten value (r=-0.189). Increasing in some quality parameters had a positive effect of wheat marketing price. Due to this relation there was positively slightly association between higher price with 1000-kernel weight (r=0.206), protein content (r=0.160), hardness (r=0.117), and gluten index (r=0.136).

Based on investigated quality parameters 1000-kernel weight and gluten index were the mainly factors increasing in profit. Protein ratio in wheat production had a significant effect on marketing price. Also, decreasing in yield led to various levels of reductions in profit. Increasing in gluten index was positively affected mean and high profit in wheat production so there was positive relation between gluten index with mean profit (r=0.287), and higher profit (r=0.162). The higher 1000-kerenl weight had a significant increase in mean profit (r=0458) and higher profit (r=0.482). Grain yield in wheat also positively affected mean (r=0.461) and higher marketing price (r=0.646).

Table 3. Correlation coefficients among yield, quality parameters and marketing prices

Parameters	Lower Price	Higher Price	Mean Price	Mean Yield	Mean Profit	Lower Profit	Higher Profit
Higher Price	0.544						
Mean Price	0.819	0.901					
Mean Yield	0.114	0.646	0.461				
Mean Profit	0.540	0.900	0.842	0.863			
Lower Profit	0.762	0.804	0.864	0.728	0.935		
Higher Profit	0.401	0.918	0.774	0.886	0.979	0.859	
TKW	-0.122	0.206	0.050	0.714	0.458	0.374	0.482
TW	0.287	0.018	0.111	-0.211	-0.042	0.058	-0.054
PRT	-0.125	0.160	-0.029	-0.016	-0.018	-0.083	0.082
HARD	0.111	0.117	0.038	-0.039	0.009	0.061	0.050
SED	-0.089	0.020	-0.042	0.119	0.037	0.014	0.056
GLT	-0.189	0.057	-0.142	-0.050	-0.103	-0.153	0.004
IND	0.315	0.136	0.304	0.250	0.287	0.368	0.162

Note: Significance at **: P<0.01 and *: P<0.05; GY: Grain yield, TKW: 1000-kernel weight, TW: Test weight, PRT: Protein, GLT: Protein, GLT: Gluten, IND: Gluten index, HARD: Hardness, SED: Sedimentation,

Conclusion

Bread wheat is the mainly growing field crops and due to various environment condition and misapplication agronomic practices its yield and quality could be lowered. It is very important issue that for farmers to get high profit from per hectare is the mainly critical factor in wheat production. Across 10 years grain yield varied year by year because of the various environments conditions. In Trakya region the year of 2008 was more profitable due to higher yield and marketing price and followed by 2014 thanks to higher grain yield. The highest wheat yields, mean marketing prices and mean profit were in 2008 year. Wheat marketing prices and profit continuously have fallen from 2008 up to 2017, but in 2009 and 2015 dramatically dropped. The mean marketing prices of wheat rose to record highs during 2008, due to the highest wheat yields. In 2016 both wheat yields and mean marketing prices including mean profit were down significantly from their 10 year highs. In Trakya region in order to get high profit the yield and some quality parameters were in the mainly factors. Based on investigated quality parameters 1000-kernel weight and gluten index were the mainly factors increasing in profit. Also, protein ratio in wheat production had positively effect of wheat marketing price. Also, decreasing in yield led to various levels of reductions in profit. Increasing in gluten index was positively affected mean and higher profit in wheat production. Grain yield in wheat also positively affected mean and higher marketing price.

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AUTOMATIC DETERMINATION OF ALTERNATIVE PARAGLIDING TOURISM FIELDS BY GEOGRAPHICAL INFORMATION SYSTEM (GIS)

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Abstract

In this study, alternative flying fields suitable for paragliding which is one of nature sports within the boundaries of Sivas province, Turkey were automatically determined by Geographical Information Systems (GIS) analyses and the developed user interface program by taking into account the international technical conditions required for flying. The suitability of these determined fields was checked with the flight tests performed in company with the experienced paragliding pilot, and they were proposed as nature tourism areas. With this study carried out, it was ensured that the paragliding fields, which were mainly determined by observational and experimental methods, were scientifically determined in accordance with the international technical specification criteria. Furthermore, a new method has been developed in order to automatically determine alternative paragliding fields in any city with the help of the introduced GIS-based system and user interface program.

Keywords: *GIS, Ecotourism, Paragliding, Spatial analysis, Nature-based tourism.*

Introduction

There are many studies on the use of GIS in the management of recreational resources. Issues such as tourism transport capacity, sustainable tourism infrastructure planning (Boers and Cottrell, 2007), sustainable land-use planning (Bunruamkaew and Murayama, 2012), determination of areas with potential for ecotourism (Bishop and Hulse, 1994; Boyd et al., 1995; Miler et al., 1998; Tseng et al., 2013; Parladır, 2013), nature-based tourism modeling, recreational use potential modeling (Kliskey, 2000) come to the forefront in these studies. This problem is also related to geographical site selection. In the literature, different studies were performed related to site selection by GIS (Şentürk and Erener, 2017; Rutherford et al., 2015).

Paragliding which is one of the alternative nature sports appealing to adrenaline junkies carries a risk by the way it is done. It is necessary for athletes to receive a license to fly on their own. Apart from the pilot experience, the flying field should meet the technical requirements to ensure safe flying. First of all, appropriate hills are required for paragliding. The land structure of the front of the hill and the climatic conditions should be investigated very well, and their analyses in terms of suitability and risks should be performed by experienced pilots. Appropriate weather conditions (fog, wind, snow, rain, etc.) are important in areas considered for paragliding. The predominant wind direction, slope, and altitude of the take-off field are the most important features that should be primarily evaluated for the safety of the take-off field. Another feature that is essential for the safety of the take-off field is that there should be no barriers in areas within a distance that can endanger the take-off, flight and landing safety on the hills to paraglide. Landing areas should be a flat area away from anything that can cause turbulence (Topay, 2003). It is certain that these scientific determinations will provide convenience in far better elevations during flying and going away for kilometers. Although the determination of flying hills and landing areas with appropriate conditions in large geographies is difficult, the examination of all fields by pilots is also impossible. At this stage, GIS-based studies are crucial for determining potential flying fields.

In the determination of the fields for paragliding which is a risky sports branch, attention should be paid to criteria such as the take-off direction, slope and altitude of the hill to paraglide, determination of the required field widths to be able to continue flying for a long time and the fact that landing areas allow for secure landing. With this study, a new method has been developed for a short-term, low-cost and scientific determination of potential flying fields in accordance with the criteria specified in a wide area such as the provincial border. In accordance with the results obtained, an attempt to contribute to tourism in the region was made by recommending new flying fields to paragliding lovers. Furthermore, a new method has been developed to be able to automatically determine alternative paragliding fields in any city with the help of the introduced GIS-based system and user interface program.

Material and Methods

Sivas provincial border (Turkey) was selected as the study area (Fig. 1). When the tourism structure of Sivas province is examined, it is observed that thermal tourism along with cultural tourism mainly comes to the forefront. Mountaineering and trekking attract attention when the nature sports done in the region, which is not known sufficiently in terms of nature sports, are examined (Governorship of Sivas, 2011). However, the city has enough wind potential for paragliding, and it is necessary to determine suitable flying fields in the city to evaluate this feature.

Figure 1. Study Area: The border of Sivas province

The data presented below were used within the scope of this study.

- Energy transmission lines,
- Stand,
- Highways,
- Streams,
- Lakes/ponds,
- Dams,

- Corine land use,
- Protected areas,
- Military areas,
- Airports,
- Digital Elevation Model (DEM),
- Aspect and
- Wind direction.

Firstly, the grid network to be used as the basis of the study was formed in a way to cover all Sivas provincial border. The grid size was determined to be 250 m x 250 m to ensure the sufficient sensitivity in the analyses to be performed. The analysis criteria to be used in the determination of the fields were determined based on the "Flying Altitudes, Conditions and Hill Features" specified in the Turkish Aeronautical Association Flight Training Directive, supported by the International Aviation Federation (Fig. 2). Furthermore, the criteria applied with experiential knowledge for safe flight were extended with the help of experienced pilots (flight instructors with EP license). The analysis criteria created in this context are presented in Fig. 2. The analyses were performed separately for take-off and landing fields.

Different geographical analyses (Statistical analysis, spatial analysis, zonal statistics analysis, surface analysis, proximity analysis, neighborhood analysis, sieve analysis, overlay analysis, visibility analysis) were used to determine the suitable paragliding areas. These analyses, which is included in decision making and planning techniques, is based on the elimination of unfavorable locations within certain criteria starting from the most unfavorable criteria in determining location suitable for the purpose of the study. Furthermore, the criteria applied with experiential knowledge for safe flight were extended with the help of experienced pilots (flight instructors with EP license). The analyses were performed separately for take-off and landing fields.

In this study, although the criteria given in Fig. 2 were used, the relative criteria that may vary according to the flight pilot's professionalism were presented as the parameters that the user could enter in the developed user interface program depending on his/her preference. Furthermore, the user interface program was developed with the Model Builder in ArcGIS 10.1 GIS software environment (Fig. 4) so that it could be used anywhere in the world, on condition that the data listed above were provided. The variable parameters considering in this user interface program were given in Fig. 3.

Factor	*Analysis Type*	*Analysis Site*	*Suitability criteria*
Slope	*Slope*	*Departure*	*18° - 30°*
		Landing	*0° - 5°*
ΔH= departure-landing	*Surface*		*60 m < - ≤ 350 m (amateur)*
			60 m < - ≤ 600 m (moderate)
			200 m < - ≤ 1400 m (professional)
Viewshed	*Viewshed*	*Departure*	*Horizontal view 225°*
			Horizon Upper / Lower +90° / -90°
			Visibility distance 2000 m
View x Wind			*Headwind or wind from sideways (45°)*
Flight Distance			*2 km – 8 km*
	Distance		
Stand			*≥ 200 m*
Lake			*≥ 300 m*
Dam – Pond			*≥ 500 m*
Stream			*≥ 300 m*
Power line			*≥ 500 m*
Road			*≤ 200 m*
Airport			*≥ 6 km*
Protected Sites			*> 0 m*
Marsh			*> 0 m*
Naked rocky			*> 0 m*
Thana			*> 0 m*

Figure 2.The criteria applied with experiential knowledge for safe flight

Variable Criteria		
Grid sizes	*Distance from stand*	*Distance from Marsh*
Province	*Distance from lake*	*Distance from road*
Slope	*Distance from dam / pond*	*Wind direction*
ΔH	*Distance from power transmission line*	
Flight distance	*Distance from stream*	

Figure 3. Variable criteria

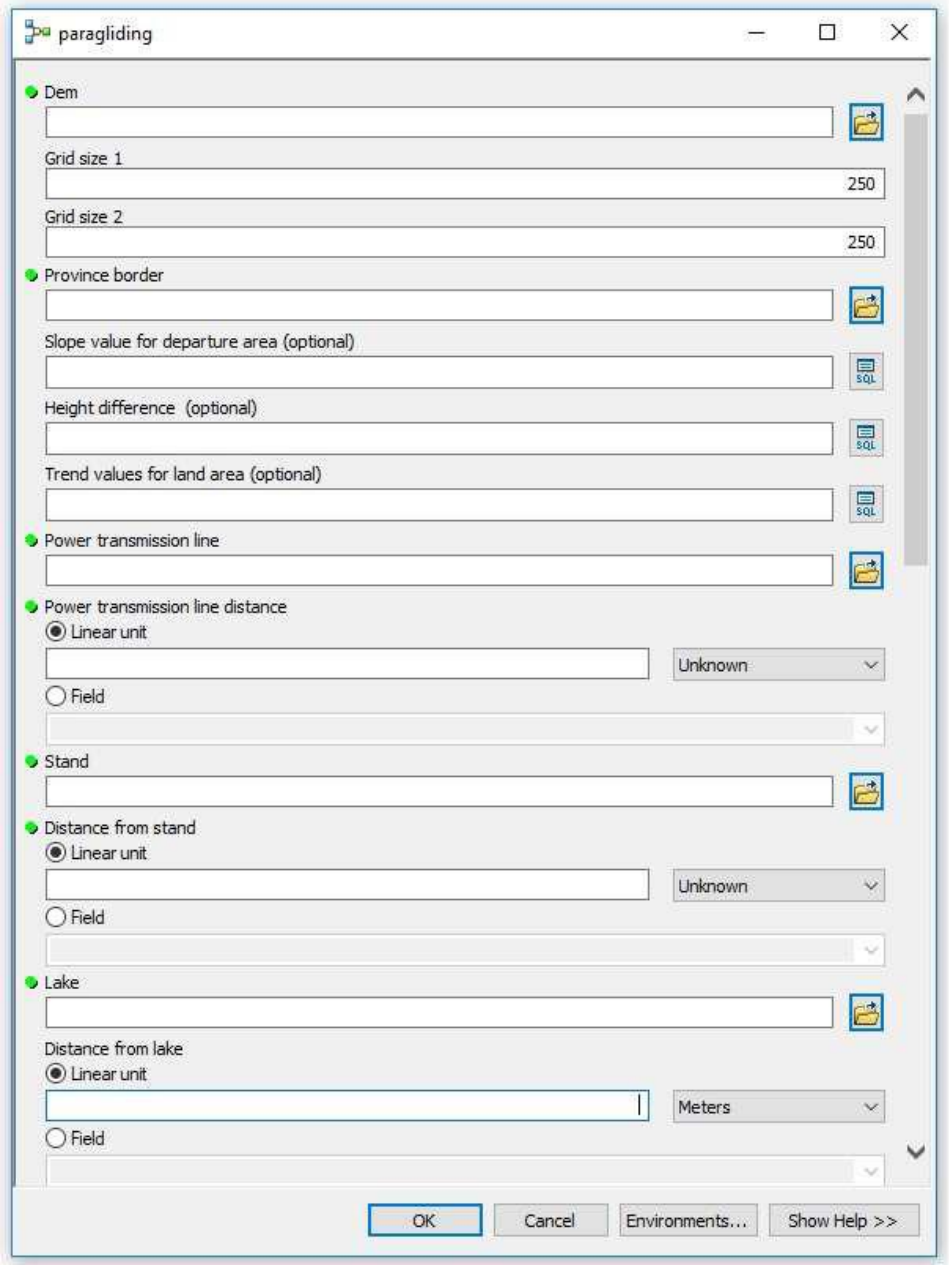

Figure 4. The user interface program developed on ArcGIS software

Results and Discussion

It was proceeded to the final stage of the study after determining automatically the fields suitable for flying in the GIS environment by the user interface program developed. 32 fields were determined as the fields suitable for paragliding (Fig. 5). 8 out of the designated target points were randomly visited (Fig. 5), and the suitability of the region for paragliding was validated. The test flight was performed in the field by the professional paragliding pilot with EP license (Fig. 7), and the acceptability of the field as a paragliding field was decided. According to the results obtained; all fields were determined as flight areas from the point of topography but one of them was not accepted due to the missing data of power transmission lines in the area.

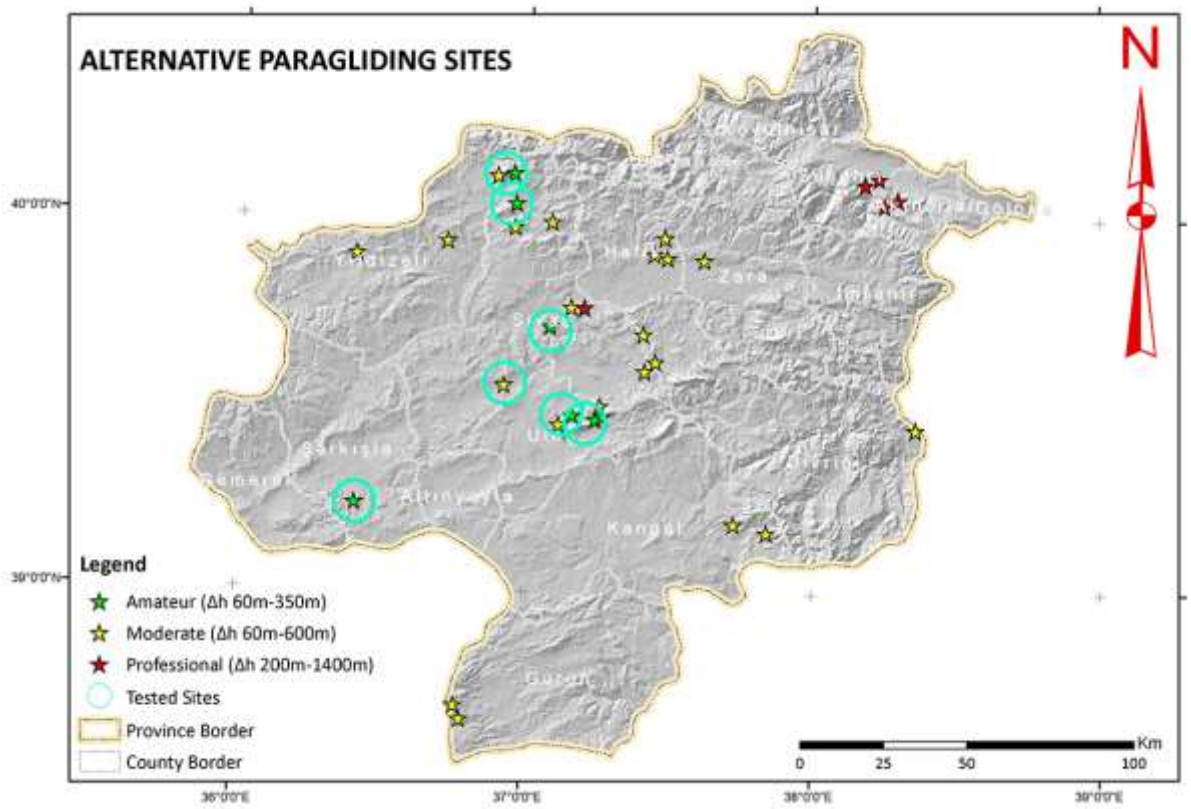

Figure 5. Suitable paragliding fields map

Conclusions

GIS is an extremely important tool in geographical analysis and provides a great deal of convenience to its users. It is seen that the large areas which cannot be analysed via observations, can be analysed objectively and scientifically by the help of GIS. However, the accurate and up-to-date data is of great importance in such GIS studies.

The outcomes of the study have indicated potential new tourism areas by bringing many fields that have not yet been discovered by paragliders to the agenda. It is thought that these fields that can be transformed into attraction centres with various promotional and demonstration flights over time will make positive contributions to the socio-economic development of the region. On the other hand, the outcomes of the study suggest new alternative flying fields that can be reached more easily and quickly by paragliding lovers. This will provide economic benefits by reducing the time and cost that athletes spend to do paragliding and also allow the sports branch to reach larger masses.

Acknowledgement

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ABATEMENT OF AGRICULTURAL GREENHOUSE GAS EMISSIONS IN THE EUROPEAN UNION: A REVISED ANALYSIS OF MARGINAL ABATEMENT COST

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Abstract

Avoiding the adverse effects of climate change has become a key priority for the European Union. Marginal abatement cost curves of greenhouse gas emissions are essential to assess the potential for efficient reduction from a cost-effectiveness perspective. We process simulations based on an agricultural supply-side model calibrated against six economically contrasted sets of annual data (2007-2012). The paper characterises how the year-based uncertainty surrounding the MACCs influences both the initial level of emissions, as well as the carbon price elasticity of emissions. The assessment is made at the European Union level, both at regional (FADN regions) and infra-regional (farm types) levels. The paper analyses the distribution of regional and farm-types abatement rates and estimates the price elasticities of abatement rates and emissions for the different years and different carbon prices. The economic context draft by annual data accounts significantly in the variations, yearly sets of prices and tax effects playing an important role when estimating the cost of GHG emission abatement. In this regard, the elements of economic contexts condition the marginal cost curve at two levels: i) they determine the level of emissions in the absence of regulation (at zero emission prices) and ii) they determine the opportunity cost of reducing with a unit the emissions and the response that can be expected from the introduction of emission pricing.

Keywords: *Marginal abatement costs, Greenhouse gas emissions, Carbon price*

Introduction

The European Union (EU) has an ambitious agenda for addressing the effects caused by the climate change. In particular, the European Commission has introduced fixed key targets to reduce greenhouse gas (GHG) emissions. Compared to 1990, the EU should reduce GHG emissions to 40% by 2030 and to 80% by 2050. Agriculture represents a major and complex source of GHG emissions. In 2016, the EU agricultural GHG emissions accounted for approximately 10% of total EU-28 GHG emissions (European Environment Agency, 2018).

The heterogeneity that characterises agriculture, combined with many potential mitigation techniques, allow cost-effective non-CO_2 GHG abatement. In this regard, marginal abatement cost curves (MACCs) are an important and useful tool for assessing the potential for efficient reduction in the sector. A significant effort has been made to evaluate such curves in different contexts (Ragnauth et al., 2015; De Cara et al., 2005; De Cara & Jayet, 2011; Moran et al., 2010; OCDE, 2015; EPA, 2013; Eory et al., 2014, Vermont & De Cara, 2010).

To appreciate the uncertainty surrounding MACCs, several authors have proposed sensitivity analyses of the curve to several key parameters. In order to study the strength of marginal abatement cost curves, Klepper & Peterson (2003) used the computable general equilibrium model DART (Dynamic Applied Regional Trade). Vogt-Schilb et al. (2015) applied the software MACTool developed by the World Bank, in order to assess the uncertainty of MACCs. On the other hand, according to Kesicki & Strachan (2011) and Kesicki & Ekins (2012), MACCs can play an important role in the decision-making process when introducing a cap-and-trade system or a carbon price.

In order to analyse the GHG emission reduction potentials both at sectoral and regional levels, Van Vuuren et al. (2009) used six various models (three recursive-dynamic computable

general equilibrium models, two energy system models and a macro-econometric non-equilibrium hybrid simulation model). De Cara et al. (2005) showed that an emission tax of 55.8 €/tCO_2 leads to an 8%-reduction (207 MtCO_2eq) in total agricultural emissions as referred to emissions from reference-year 2001. When fixing a 10% EU abatement target, De Cara & Jayet (2011) highlighted that cap-and-trade systems could play a major role, this target being achieved at a price of 32–42 €/tCO_2.

In this paper, we are taking into consideration the direct GHG emissions sourced by agriculture, relying on the two main agricultural gases, nitrous oxide and methane respectively. By using a supply-side model, an emission tax (expressed in €/tCO_2eq) is introduced. The six years 2007-2012 on which this study is based, are characterised by very contrasted economic contexts (e.g. agricultural and fertiliser prices, changes in agricultural policy). The paper analyses the distribution of regional and farm-types abatement rates and estimates the price elasticities of abatement rates and emissions for three emission taxes, 30, 50 and 100 €, respectively.

Materials and methods

The model used in this paper is the European agro-economic model AROPAj (De Cara et al. 2005, Galko & Jayet, 2011) which simulates the agricultural supply in the European Union. AROPAj is a mathematical programming model, being built on a set of linear programming models. The Farm Accountancy Data Network (FADN) represents the main source of data for AROPAj. The model is based on *farm types* or *farm groups*, which represent a group of farms with similar features grouped by a clustering method, depending on four key variables (main types of farming, altitude, irrigation and economic size).

The paper is based on the actual V5 version of the model, calibrated against six sets of annual FADN data, more precisely 2007-2012. In order to maximise the total gross margin (π_k), in the model, the supply level and the input demand are chosen by each farm group k. The generic form of the model is:

$$max_{x_k} \quad \pi_k(x_k, \theta_k, \varphi) \qquad \text{s.t. } x_k \in A_k(\theta_k, \varphi)$$

x_k represents the vector of producing activities for farm group k, including variables such as the area of each crop or the number of animals in each category. The greenhouse gas emissions are a component of x_k, being calculated endogenously. The production constraints are indicated by A_k, the matrix of input-output coefficients. The vector θ_k relates to k-specific parameters, and the vector φ refers to common parameters. In the model, the main constraints are represented by the crop rotation constraints, the availability of utilized agricultural area, the animal feed requirements and the implementation of the Common Agricultural Policy instruments.

The paper is based on the methodology developed by De Cara & Jayet (2011). Therefore, the abatement rate is defined as:

$$abat_i(p) = 1 - \frac{E_i(p)}{E_i^0} \tag{1}$$

where E_i^0 refers to the base-year agricultural i-GHG emission in FADN region i and E_ip represents the GHG emissions for region i reported to the carbon price p introduced. The following specification for the abatement rates is used:

$$i = 1, n: \qquad abat_i(\mathrm{p}) = \alpha_i \left(1 - e^{-\left(\frac{p_i}{\tau_i}\right)^{\beta_i}}\right) \tag{2}$$

Several conditions are placed, e.g.: $0 < \alpha_i \leq 1$, $\beta_i \geq 0$ and $\tau_i > 0$. The term α_i refers to the i-asymptotic level of abatement rate ($\lim_{p\to\infty} abat_i$ (p) = α_i). In order to analyse the price response of the abatement rate, we are using two indicators, the price elasticity of the abatement rate and the price elasticity of emissions.

Results and discussion

By using the AROPAj model, our results are based on the temporal variability of the six contrasting years, bringing a quantitative clarification on MACCs. As shown in Figure 1, the lowest GHG emission abatement rate was recorded in 2012 and the highest in 2010. We can observe that a significant gap ranging from 0.25 to 0.35 being observed for a carbon price of 200 €.

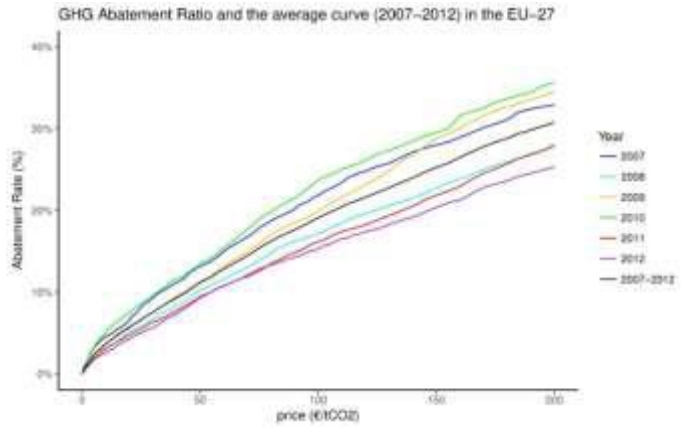

Figure 1. EU abatement ratio (2007-2012) and the average abatement rate for a carbon price up to 200 €.

Our analysis takes into consideration the fact that the initial level of GHG emissions is different for each of the six years, the highest level being in 2010 (see Figure 2).

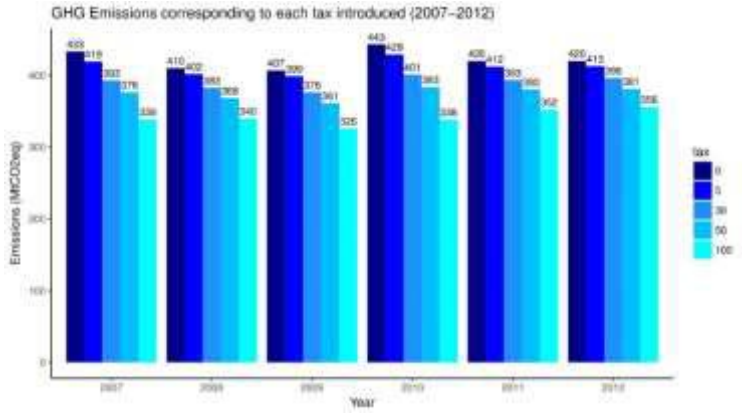

Figure 2. The EU initial emission levels (2007-2012).

If we focus on the EU aggregated results, the estimated parameters show that a 10% decrease of EU agricultural emissions implies a price elasticity of abatement rate ranging from [0.75, 0.92] and an absolute value of the price elasticity of emissions ranging from [0.08, 0.10].

In order to assess the regional distribution of abatement costs, both spatial and temporal dimensions were analyzed, allowing for a clear assessment of the significance of the year effect, as well as the tax and region effect. In doing so, Figure 3 displays the distribution of EU GHG emission abatement rates for three given carbon prices and each of the six years, as well as the average European rate of each year and the average of the six years.

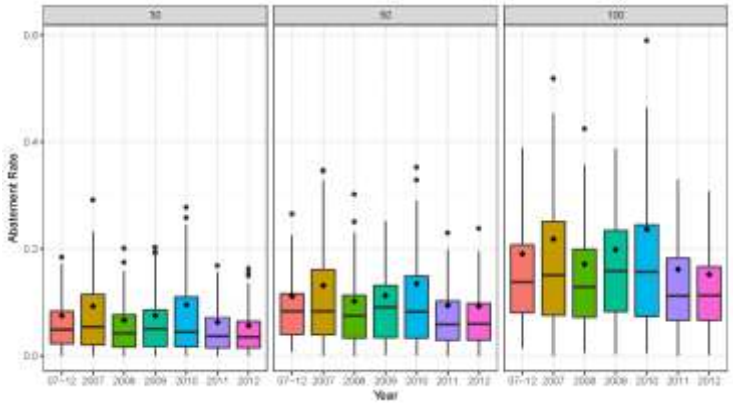

Figure 3. Distribution of GHG emission abatement rates by region, year and three tax levels.

The variability of abatement rates between regions is higher than the variability of rates between years. Depending on years, for a carbon price of 30 €, 50 € and 100 €, respectively, the median varies from 4 to 6%, from 6 to 9% and from 11 to 16%. For a tax of 100 €, by taking into account the average of the six years, 50% of regions are reducing less than 14%. Compared to the average calculated over 2007-2012, we can observe that in 2007, 2009 and 2010, the median is superior to 14%, while in 2008, 2011 and 2012, is below.

Although it can not be precisely located within which FADN region belongs, in the AROPAj model, each farm type has its place in a particular FADN region. The distribution of farm-type abatement rates plotted against the cumulative baseline emissions, for each emission tax introduced (30, 50 and 100 €/tCO2eq respectively) shows a significant variability from one tax to another for the 6 years (2007-2012) (see Figure 4). As the tax increases, the range of variability of the 6-year curves is higher both on the ordinate and on the abscissa. There is a shift of curves to the right, which suggests a shift in the concentration of abatement efforts toward farmers with the highest abatement rates.

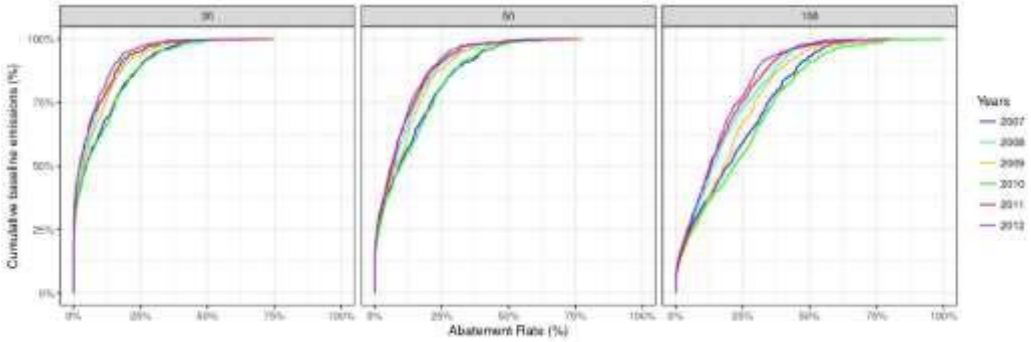

Figure 4. Distribution of farm-type abatement rates, over the period 2007-2012, in the EU-27, when emission tax is equal to: 30, 50 and 100 €/tCO_2eq respectively.

In 2010, year characterised by a low crop price, a minimum level of the fertilisers price, and, implicitly, a high fertilisers consumption, farmers who reduce their emissions by less than 15% represent approximately 76% of baseline emissions, in the case of an emission tax of 30 €/tCO2. The percentage of emissions drops to 60% for a tax of 50 €/tCO2 and to 37% in the case of a tax of 100 €/tCO2. At the extreme opposite, in 2012, year described by high crop and fertilisers prices, farmers who reduce their emissions by less than 15% represent approximately 87% of baseline emissions, in the case of an emission tax of 30 €/tCO2. For a tax of 50 €/tCO2, it drops to 76% and in the case of a tax of 100 €/tCO2, to 58%.

Due to the fact that the distribution of baseline emissions among farm-types and regions can play an important role, regional and farm-types abatement rates are plotted against the cumulative baseline emissions. Thus, the distribution can be observed for each of the FADN

regions and farm-types considered in the analysis, and this, for each of the six years and for each of the three emission taxes introduced (see Figure 5). Regions and farm-types characterised by higher abatement rates are located to the right of the graphs. Our results are robust by reference to the results obtained by De Cara et al. (2005), whose analysis was based on the year 2001.

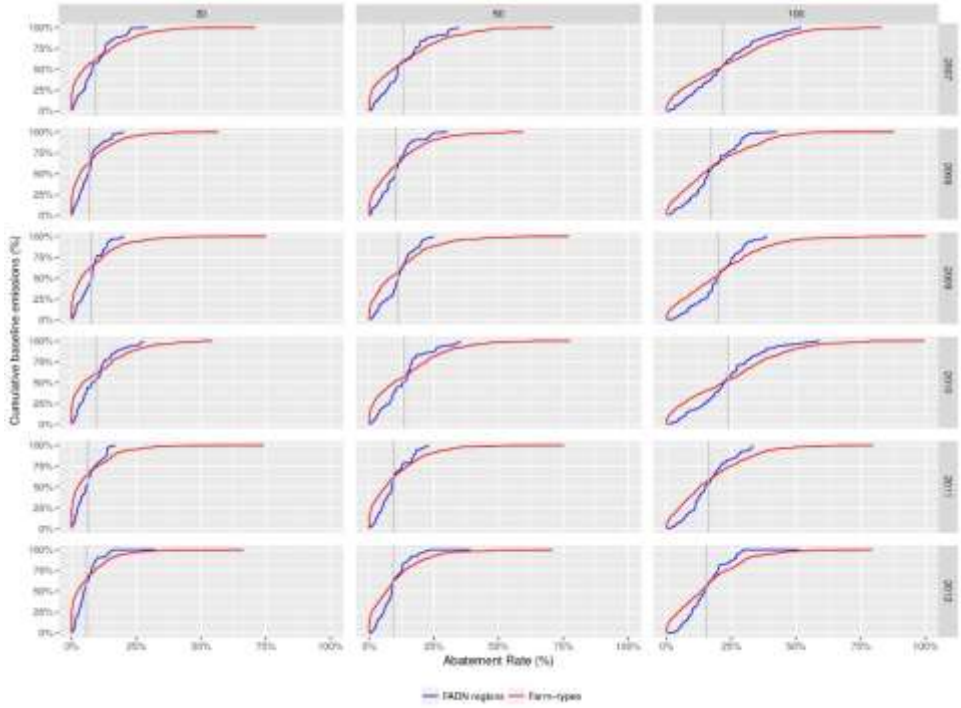

Figure 5. Distribution of regional and farm-type abatement rates, in the EU-27, for three tax levels.

The distribution of regional and farm-type abatement rates shows that the farm-type level variability is larger than regional variability. A part of the abatement cost variability remains unseen due to the regional aggregation. The farm-type curve is less concentrated around the abatement rate corresponding to the intersection between the two curves, a rate that varies depending on the level of the emission tax introduced.

Conclusion

One of the strengths of our paper consists in giving sensitivity to economic context, having the means to produce a wide estimation on important elements, such as the abatement costs, the price elasticity for the various functions of reducing emissions. This study provides an assessment of abatement costs at the European level, both at regional and infra-regional levels, showing that, when estimating the cost of emission abatement, years and tax effects have an important role, the EU being characterised by a large spectrum of individual abatement ratios against a given price. A 10% reduction of EU agricultural emissions implies a price elasticity of abatement rate of approximately [0.75, 0.92] and an absolute value of the price elasticity of emissions ranging from [0.08, 0.10]. The implied price elasticity of abatement rate for a decrease of EU base emissions by 30% ranges from 0.59 to 0.71. The distribution of regional and farm-type abatement rates shows that the farm-type level variability is larger than regional variability.

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INDIGENOUS AGRICULTURAL PRODUCTS AND BIODIVERSITY FOR THE DEVELOPMENT OF REGIONAL AREAS. ITALY CALLS BOSNIA AND HERZEGOVINA

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Abstract

In 1992, the Earth Summit in Rio de Janeiro considered biological diversity by introducing the term 'agricultural biodiversity' or 'agro-biodiversity', conceived as the set of varieties that each farmer recognizes and considers as part of the collective heritage of his land of origin. Italy holds a record of biodiversity for almost all the most important crops used for food, only partly due to physical and geographical factors. Apulia, in Southern Italy, is witnessed by numerous small details such as the countless units of land surface and quantity of agricultural products, the thousand dialects, habits and customs of the small towns. Apulia seems to be a happy island for biodiversity and needs to be protected with about 500 agricultural varieties between horticulture products and vegetables (Polignano carrots, onions from Acquaviva, and so on). The Southern part of Bosnia and Hercegovina, region of Herzegovina, is administratively divided to Western and Eastern Hercegovina having Neretva river as natural border between two. Due to difficult post-conflict transitional period, disputable approaches undertaken in revitalisation of the agriculture and ongoing political disagreements, the rich agro-biodiversity of the area has been partially lost. But regardless all difficulties, Herzegovina farmers, holding a strong cultural identity with food, helped saving from disappearance some of the oldest and most traditional products such as cheese in sack, kajmak (creamy cheese) in sack, roga green bean or poljak bean, all of them recognized by Slow Food movement to be 'arc of taste' products. The contribution, in addition to verifying the state of the art, intends to evaluate the different approaches regarding the subject outlined with regard to management and practices in Italy and in Bosnia and Herzegovina.

Key words: *Agriculture, Biodiversity, Development, Italy, Bosnia and Herzegovina.*

Introduction

The impact of human action on the environment has not always been negative. Since the first forms of agriculture, man has operated a 'controlled disturbance' in natural ecosystems, choosing and cultivating some plant species, rather than others. And then, selecting those that gave him more chances of survival. In this way, in harmony with the natural environment, it has created numerous varieties of fruit, olives, vines, vegetables, legumes, cereals, which have been the basis of its diet and of the food traditions of its communities, together with cause and effect of the environmental and climatic characteristics of the places inhabited by it.

The enormous wealth of local varieties, however, has been progressively reduced with the advent of intensive agriculture, according to the almost exclusive criterion of higher productivity. As a consequence, the local varieties, well adapted to their thousand cultivation environments, have been progressively and inexorably replaced by new varieties and hybrids, which allow high productions, long shelf life and greater suitability to be transported over long distances. The local food has therefore become global. The Earth Summit in Rio de Janeiro in 1992 considered biological diversity by introducing the term 'agricultural

biodiversity' or 'agro-biodiversity' understood as the set of varieties that the farmer and the inhabitant of that place recognizes and it places itself among the most cherished objects of the collective heritage of its land of origin, and the inheritance that nature leaves to itself and which contributes to making a place different from the other. The main objectives of the Conference are: conservation of biological diversity; durable use of its components; the fair and equitable distribution of benefits deriving from the use of resources genetic through, inter alia, adequate access to genetic resources and the appropriate transfer of relevant technologies, taking all rights into account on these resources and technologies, and through appropriate funding.

In the Mediterranean context, Italy holds a record of biodiversity for many of the most important crops for food use and this is only partly due to physical and geographical factors. The diversity of the climate, the orography (i.e. the reliefs), the differences between the land and the water availability of the various areas of the country can justify the cultivation of many species, but not so much the birth and spread of such a large number of cultivar locals. The richness of the varieties is, however, linked to historical and anthropic factors, invasions and dominations, productive and rural economy, religion, language, roads and transport, local customs and traditions. This in particular is analyzed for Italy in Puglia. Bosnia and Herzegovina also has a large production and local variety. The paper highlights the importance of Herzegovina with reflections on the structural difficulties of the countryside and isolation, linked to the reference context. So that talking about agriculture and biodiversity is strongly linked to the historical, political and territorial problems of a country that is being directed towards a planning and valorization of agricultural products. So that talking about agriculture and biodiversity is strongly linked to the historical, political and territorial problems of a country that is slowly taking its own path towards planning and valorization of agricultural products. The Slow Food initiative is from Italy interested in Bosnia and Herzegovina to support diversity and to make economy with agriculture by giving farmers the opportunity not to abandon the campaign. The contribution, therefore, in addition to verifying the state of the art, intends to evaluate the different approaches compared on this subject with regard to attention, management and practices. Italy calls Bosnia and Herzegovina because for some years we are working on methods, land planning and enhancement by the writers and this is a further step towards collaboration and cooperation. Finally the Introduction and the Conclusion are to be attributed to R. Grumo, Materials and methods to S. Giordano, S. Samardzic, G. Radovanovic, and Results and Discussion are to be attributed to all the authors.

Materials and methods

Biodiversity and best practices: the project BiodiverSo in Puglia Region

Starting from the definition of Biodiversity as a complex of communities of ever-changing plants, animals and micro-organisms interacting with the surrounding environment and influenced by human action, it is possible to note how those ecosystems with a wider number of species are better preserved; according to this evidence, the EU acts in order to reduce high species extinction rates by 2020 and restore natural ecosystems as much as possible.

In accordance with the International Treaty on Plant Genetic Resources for Food and Agriculture (RGV), it is fundamental to refer to two RGVs: cultivated varieties and spontaneous species. To date, a capillary and systematic survey of the present active RGV has not been carried out in Italy as well as in Puglia Region. Germplasm conservation actions are often entrusted to the initiatives of individual farmers, who hand down seeds from generation to generation, and to the ex situ conservation that is normally carried out by public entities, such as NRC (National Research Council) and Universities. The revaluation of local varieties genotypes and/or their rehabilitation is vital in order to widen the genetic base of the

horticultural sector, allowing better tolerance to stresses, to safeguard the health of consumers and the environment, as well as to enhance some typical local products. Puglia Region is particularly rich in cultivated varieties of vegetables: carrot of Polignano and Sant'Ippazio; onion of Acquaviva and Margherita; cauliflower, broccoli and black cabbage, curly kale, 'mugnoli' and 'cime di rapa'; immature melon and cucumber named 'carosello' or 'barattiere'; chicory of Molfetta and Galatina; the tomato 'Regina' and the one from Manduria. Some of these cultivated varieties were included in Annex 8 of the Puglia RDP 2007-2013, which includes 14 local varieties of vegetables at risk of genetic extinction (only six of them received applications from custodian farmers for funding measures aimed at preservation), others are included among Traditional Agri-Foodstuffs (PAT).

In Puglia Region a remarkable initiative is represented by the project 'Biodiversity of horticultural species of Puglia' (BiodiverSO), which is part of the Rural Development Program for Puglia - FEASR 2007- 2013 (EC Reg. 1698/2005) - Measure 214, Action 4 Sub-Action a) 'Integrated Biodiversity Projects'. The main purpose of the integrated BiodiverSO project is to contribute to the achievement of a significant reduction in the current rate of erosion of the biodiversity of the Apulian horticultural species, especially by intervening on all the local varieties listed in Annex 8 of the Puglia RDP 2007-2013 and other species.

A first phase consisted in an i depth research aimed at finding the horticultural genetic resources at risk of erosion, and the consequent draft of a catalogue, aimed at conserving and characterizing them. Each variety is identified by means of an identification code, combined with other information (place of collection, farmer coordinates, etc.), together with the so called 'passport data'. All these data univocally identify an accession (i.e. an entry in a database), in order to avoid cases of homonyms, overlaps and other elements of confusion. An integrated strategy was applied throughout the Project including, with mutual support, ex situ conservation, in situ and on farm ones.

Ex situ conservation is carried out in different ways: collections of plants in the field (with specific reference to catalogued fields of onion, chicory, 'carosello' and 'barattiere', 'Regina' tomato, etc.); collections of seeds kept in double, from both the IBBR-CNR and the DISSPA-UNIBA, in their seed banks; collections of propagation material kept in vitro, at DISAAT-UNIBA. The following phases are related to the organization and monitoring of in situ/on farm conservation: collection of information on existing local varieties (inventory) and collection of propagation material intended for preservation of ex situ safety and characterization activity; identification of different areas to be allocated primarily to in situ/on farm conservation (choice of those areas where priority, promotion, organization and monitoring activities should be implemented); characterization and evaluation of the differences held by local varieties; assessment of population size and genetic structure of local varieties maintained in situ/on farm; construction and management of an information system related to the in situ/on farm conservation, construction and management of communication devices, in particular the project website. The main purpose of in situ/on farm conservation is to maintain the current and potential usefulness of genetic resources, so as to meet the needs of present and future generations, intervening also in the farming activity of the so called custodian farmers, and to protect the rights of farmers themselves. The knowledge system and database is strengthened through the formation of an efficient regional biodiversity network that links above mentioned custodian farmers (holders of local varieties), farms, stakeholders (farms, agro-food industry, catering) and local authorities to the enhancement of environmental, cultural and historical and architectural resources. All this aims will at an effective development of the territory both from an environmental and economic point of view, with new micro-supply chain supporting those already existing. The integration of the different actions and methodologies developed within the mentioned Project will allow to obtain a series of tools that can be used at a macro-level in order to promote knowledge

regarding the precious heritage of horticultural agrobiodiversity in Puglia Region and to increase the awareness that the protection of genetic resources is essential.

Preservation of agrobiodiversity in BIH after the conflict, specifically in Eastern Hercegovina as case study area

Bosnia and Herzegovina, even being one of smallest countries in Europe, is one of the five European countries richest in species, what makes it an important center for biodiversity. Unfortunately, about 19% of all plant species are thought to be under significant threat, what Bosnia and Herzegovina put among countries having one of the highest proportions of threatened species of any country in Europe. Many of these species are part of traditional foods, cultivated in local way by implementing local know-how and methods of cultivation. One of major reasons for placing these species in danger to disappearance is depopulation of rural areas, to which remarkably contributed the last war in BIH. The impact which war had on agrobiodiversity state of BIH was twofold. Obviously, negative ones because large part of fertile land and cultivations were in the conflict area, thus it was not possible to be cultivated and got mined while, incredible, but there was one positive effect of the war for the preservation of the agrobiodiversity. Due to war circumstances and under sanctions, not receiving foreign seeds, inhabitants were forced to use local resources and local knowledge about plants and food in order to survive. Reproduction of local seed varieties proved to be the best practice which helped the population survival and insured the preservation of local agrobiodiversity. While in 80ies and 90ies Europe and the rest of the world started to pay attention to loss of biodiversity, take the first decisions and put in practice instruments aiming to undermine the damages, in the years after the conflict, 1996-2003, BIH found itself in a very different context in which the emergency strategy for the state agriculture recovery was production of food in whichever way it was possible. After a period of many different methods undertaken and projects launched which did not take into consideration the value of local products and biodiversity, only in 2004, thanks to specific projects of Italian cooperation agency, new approaches were made and more attention was afterwards paid to typical local products and preservation of agrobiodiversity while the first ex-situ conservation of local plant varieties was insured in 2005 by SEEDNET project and with support of the Government of the Republic of Srpska. In 2009 Institute for genetic resources of Republic of Srpska is founded which continue the work of ex and in situ conservation of seeds in RS.

Southern part of Bosnia and Herzegovina, called Herzegovina, occupies about 20% of the whole country. Herzegovina is administratively divided in two entities, Federation of BIH and Republic of Srpska. Its Western and Northern parts are characterized by upper mountains and continental climate while Southern part has all characteristics of Mediterranean region. Thanks to such a diverse relief and presence of various climates, Herzegovina is richest part of Bosnia and Herzegovina in plant species and animal breeds. But, unfortunately, Herzegovina is at the same time the region where population decline faster than in rest of B&H what put its flora and fauna in a really big danger of extension, what entails loosing local dishes and receipts and local know-how. Complicated governing administration in B&H, not yet established valid system of protection of Geographical Indications, further aggravates the situation. Due to luck of Rural development strategy at the state level, EU IPA pre-accession funds for rural development were blocked until this year when state strategy is finally adopted. Practically, in the previous ten years, the biggest contribution in making awareness for the need of safeguarding of the agrobiodiversity and local food was made by local, non-formal association 'Slow Food Convivium Trebinje, Hercegovina' which is part of the largest international and today well known network Slow Food International that operates in more than 160 countries all around the globe. Slow Food International is doing it through two main projects: Arc of Taste and Presidium project. Arc of Taste is a world catalogue of

endangered traditional foods while Presidium product and project establishes a group of producers and helps them to preserve and valorize arc of taste product. Within ESSEDRA I project 2012-2013 which Slow Food implemented in Balkan countries, are identified and catalogued local tipical products and breeds, mostly localized in Hercegovina region: Cheese in sack, 'Kajmak' from the sheepskin sack, 'Herzegovina honey', 'Livno cheese', 'Pramenka' sheep, Dry figues, Poljak bean and Popovo polje corn variety 'Stodanac'. Lately, closer cooperation is established between technical offices of Regional office of RS Ministry of Agriculture and Slow Food Trebinje, Hercegovina Convivium what brought to new applications and new breed and vegetable varieties for Slow Food Arc of taste catalogue. 'Gatacko cattle' breed, 'Roga' green bean and Trebinje tomato 'Jabucar' are the last recognized local breeds and vegetables. From Bosnia and Hercegovina 17 products are presently in Slow Food Arc of Taste catalogue among which 10 are administrated by Convivium Slow Food Trebinje, Hercegovina. The list is not finished yet. Old varieties of legumes are identified and new applications are in preparation.

Results and Discussion

In Puglia Region a remarkable initiative is represented by the project 'Biodiversity of horticultural species of Puglia' (BiodiverSO), which is part of the Rural Development Program for Puglia - FEASR 2007- 2013 (EC Reg. 1698/2005) - Measure 214, Action 4 Sub-Action a) 'Integrated Biodiversity Projects'. The main purpose of the integrated BiodiverSO project is to contribute to the achievement of a significant reduction in the current rate of erosion of the biodiversity of the Apulian horticultural species, especially by intervening on all the local varieties listed in Annex 8 of the Puglia RDP 2007-2013 (and therefore, as mentioned above, on cauliflower, broccoli, artichoke, tomato, batata, carrot, chicory, melon), as well as on 'carosello', 'barattiere', chicory, onion, carrot of S. Ippazio and other species. An important result is given by the programming that is favored in this area both nationally and regionally. In fact, the experience of the Puglia Region is one of the best practices in Italy. Therefore one can speak of a harmonization of the different levels (European, national and regional) of competence, as expressed in the contribution. Even being one of the Balkan countries with richest biodiversity, therefore being rich in food diversity, Bosnia and Herzegovina is among last European countries in sense of valorization of its food heritage. In B&H there are only five food products protected with the sign of geographical indication, of which one is Herzegovinian honey. It is due to bad political situation in B&H. Unfortunately, one of victims is agro food sector. There are two institutions who have conflict of jurisdiction who of them is in charge to do the job of protection of geographical indications. That situation discourages producers to organize themselves and do something to protect their products. From the other side process of emigration that is pronounced In Herzegovina in its worst form additionally aggravates the situation with loosing rich culinary heritage of Herzegovina. One of chances for saving our food diversity is indubitably work with Slow Food, an Italian initiative that is expanding internationally and Institute of genetic resources of RS. This model for Herzegovina proved to be the best till the moment. In addition, there are two new structures created contributing to preservation of local products and providing overall better conditions for development of agriculture in Eastern Hercegovina – Trebinje Agricultural Fund and Center for agricultural development of Mediterranean part of Eastern Hercegovina. Thanks to adoption of Rural strategy at state level, EU IPA funds for rural development are deblocked giving possibility for proper projects to be launched aiming agrobiodiversity conservation. In particular regarding the Slow Food experience permanent awareness campaigns and continuous Slow Food Trebinje, Hercegovina work on preservation of the local varieties made the best impact to those who kept the tightest cultural link with local

agricultural inheritance - local farmers, the real custodians of local varieties of Hercegovina agrobiodiversity.

Conclusion

The analysis of the theme in the two territorial realities in Italy and in Bosnia Herzegovina poses different interpretations in relation to the different agricultural systems both on the structural and the management level.

On the structural level the starting conditions that are physical-geographical and historical must be evaluated. It is well known that biodiversity and the large number of local cultivars are not only linked to the climate and the orography, the difference between land and water availability, but also to various historical and anthropogenic factors, invasions and dominations, productive economy and rural, religion, language, roads and transport, local customs and traditions. Not entering into detail it can be said that the agricultural sector has had over time both in Italy and in Bosnia and Herzegovina a different evolution even if both have experienced phases of depopulation of the countryside. Today however the attention the support and the revitalization of the sector, the return to the origins and the conservation of agrobiodiversity are decidedly common themes. On the management side, in Italy, coordinated actions have been undertaken for decades. Starting from the National Strategy on Biodiversity in Agriculture must have the following priority of intervention: to disseminate eco-compatible and sustainable agricultural practices aimed at diversifying them productions, protecting the rural landscape (reducing simplification and fragmentation of habitats), ensure the diversity of agroecosystems, the management of the territory, the environmental complexity of agricultural areas, management

soil contamination; integrate the visions, the actions and the instruments of the 'Environment' and of 'Agriculture' share criteria and methods among the local authorities called to apply the National Strategy. Furthermore, Italy is mainly assisted in the regions whose development is lagging behind with the European Union and CAP. Currently the CAP is increasingly oriented towards preventing the risks of degradation environmental. Agricultural activity is increasingly seen as an activity that it can produce 'Environment' and 'Landscape' and in this sense the farmer is increasingly framed in the role of guardian of the environmental and landscapes elements of a territory. The process of integrating environmental objectives in the CAP, started in the eighties, still continues today inside of Cap reform process that defines more broad the role of the farmer recognizing him also a producer of public goods. Bosnia and Herzegovina still lives unfortunately elements of political-administrative division and fragmentation, as witnesses refer, and yet with the presence of a huge variety of crops there is the need to support even more and raise awareness among the population and farmers in particular in this sense. In fact complicated governing administration in B&H, even is not yet established valid system of protection of Geographical Indications, further aggravates the situation. Fortunately there are experiences that are shared by the two countries and that combine to change old approaches in the name of tradition, innovation and sustainability. One of chances for saving our food diversity is indubitably work with NGO such as Slow Food. This model for Herzegovina proved to be the best till the moment. Certainly Herzegovina should keep the hope that European integrations will force institutional levels of BiH administration to trace the path to protect local products in a more adequate way. Finally it can be said that the two experiences, as case studies, have shown on the one hand that there is a greater attention on the part of the Government in Bosnia Herzegovina towards the initiatives that support agriculture according to a more sustainable model that enhances local products, This position it was unthinkable a few decades ago, considering the political events that have affected this country. On the other hand, Bosnia also looks at Italy as a partner for the possibility of transfer of know-how and importing models of

agricultural development such as the 'BiodiverSO' program to apply good practice and strengthen the links between Italy and Bosnia Herzegovina, as the Slow Food experience is showing.

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WILLINGNESS TO PAY FOR GEOGRAPHICAL INDICATION PRODUCT ON THE INTERNAL MARKET: CASE OF ATTIÉKÉ IN IVORY COAST

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Abstract

This paper analyses the issues of Geographical Indication (GI) on the internal market in the case of Côte D'Ivoire (Ivory Coast). Conjoint analysis is used to know the local population's willingness to pay (WTP). In Côte D'Ivoire, attiéké is a local food elected to GI process. Five regions (Abidjan, Dabou, Jacqueville, Grand-Lahou and Yamoussoukro) were concerned about the production and 238 consumers from these regions were investigated. As results, consumers were focused on hygienic conditions and the size of grain. The price was not so important because 41% of consumers did not look at the level of the price.

Keys words: Geographical Indication, internal market, attiéké, willingness to pay, conjoint analysis.

Introduction

Many studies have been conducted on the role played by the Geographical Indications (GI) in regional development: The link between quality and place (Brian Ilbery and al 2008), the synergy with others activities (Barjolle, Thévenod-Mottet, 2004), debate about GI issue for USA (Tina Monten, 2005). In these studies, at first glance, GI's products are important because its prices rise in international trade compared to conventional products. Several products elected in the process of certification GI saw their price increase in a short time. The famous examples of GI success stories are in France: " Puy, green lentil" price increased to 273 % between 1996 and 2002 (Agritrade, 2005). Another example about wine in the same country, between 1991 and 2013, the price of wine minus "champagne" one increased by 75% in current value, and 29% after adjusting for inflation. Between 1991 and 2013, the price of wine minus "champagne" one increased by 75% in current value, and 29% after adjusting for inflation (MAAF, 2014). The statistics also show that France is among the best consumers of their own GI's products. FranceAgriMer (2015) showed that between 2003 and 2013, the proportion of consumption of wine called "rosé" has increased to 50% and at the end of 2013, France was the first consumer of this wine in the world.

The arguments about the necessity of selling GI's products at a bargain are spread in developing countries. Africa star's one is Ziama coffee that would be estimated at more than 10,000 CFA francs instead of 6,500 CFA francs following its acceptance (OAPI, 2014). Unfortunately, African's GI products are not focusing on the internal market. Few studies were led to the acceptance of GI products in the internal market. The drawback of non-taking account of the internal market can be the fall of local population well-being and the non-sustainability of trade in the case which the international prices oscillate. In this paper, we aimed to address consumers' willingness to pay for GI's products in the internal market and the higher price that can be charged by producers without losing their internal customers. Some crucial questions are underlined: What characteristics of existing products are

appreciated or not? Is GI's label claim by local consumers? What is consumers' willingness to pay in order to make distinction between different qualities?

Barjolle and jeanneaux (2012) tried to treat localized firms and understood how they worked, how they set a price on the market. So, we aimed to go far and study the acceptance of a product in GI's certification by the local populations and the price they would be able to pay for that. The concept of Willingness To Pay (WTP) is used to this aim and defined as the maximum price that consumers gave consent to pay for a given product or service (Marine Le Gall-Ely 2009). In ordinary, we suppose that when the prices rise, sales fall. But in most cases, consumers win of the exchange in the market due to the difference between the reservation price and the market price. This win was called Consumer's surplus by Alfred Marshall (1890). Study WTP allows setting a price that maximizes the producer profit without threatening the number of its clients. In other words, the WTP allows setting a market price higher for which we have the approval of the consumer in exchange for a reduction in the uncertainty about the production's conditions, product's information and the inclusion of some consumers' aspirations.

However, the extent of the WTP requires several methods for its assessment. WTP can be calculated in different ways depending on the data source and / or expected results. When the sources are real data coming from the market, elasticity method and hedonic price can be used. If there is incitement, Vickrey auctions and BDM methods or Becker Degroot and Marschak lotteries can be used. And when prices are non-existent, we mostly resort to an investigation for fix the price. In this case, we have conjoint analysis, contingency analysis, psychological price and simulated purchases.

In the case of this study, data source are non-existent, so we face to range of choices. We chose conjoint analysis to estimate directly the maximum price that the consumer wants to pay for the acquisition of property and know at the same time the characteristics he researches. Conjoint analysis is evaluated by two main methods, namely the Conjoint analysis of the complete profile inspired from Paul green and adaptive conjoint analysis (ACA) invented by Richard Johnson through his company Sawtooth Software in 1985. Complete profile analysis is an instantaneous analysis and requires a greater intellectual sagacity to design the importance of the proposed profiles and consistently ordered while the adaptive analysis follows a dynamic evolution and a playfulness that allows interviewees to take their time in expressing their answers with automatic recording of the above. We will choose the method of conjoint analysis in the complete profile that despite its modern adaptation through nested logit method or hierarchical Bayesian analysis stays the most appropriate for our developing countries.

Materials and methods

Consumers have to make a choice between similar products which differ simply with the place, nature or date of production. Most of the time these attributes are mixed and consumers must reveal their preference by choosing appropriate products. This individual choice revealed is "path worth utilities". In conjoint analysis, a defined set of combinations of path worth utilities of each survey are ranked, organized, selected and then put on model to find the path worth utilities that can help to know the willingness to pay. As a bonus, we can know the importance of each attribute in consumers' willingness to pay. In practice, it is set in three steps:

Step 1: the preparation

We investigate about the main attributes of the products. After that, we combined a set of attributes. For instance, if we have three attributes, the number of combinations will give us eight. For K attributes we find 2K combinations. All these combinations of the attributes are present, but with their variants. Suppose that attribute one has two variants, we mark the

presence of variant one of parameter +1, and we mark the missing of variant one or the presence of variant two by parameter -1. The list of combination is called "design matrix" and the use of all combinations is called 'full factorial design". Now, we produce a matrix with the new value of each combination. We get a matrix with n line and m column. Back to our example n= 3 and m= 8. Enter this matrix there will be the number link to all variants of attributes like compose with +1 or -1.

Step two: the realisation

In this step, we go investigate the sample of surveys targeted by our study. For that, we put numbers on the set of attributes combined on the card and asked the investigated to rank order or set a logic action with these cards. And we noted the numbers on the surveys' choices.

Step 3: the utilities function and relative weight of attributes

a- utilities function

We used the model of Green P.E. and Srinavasan V. (1978)

$y_k = \mu + \sum\sum \beta_{jm}.x_{jm}$ (1)

In this model, y_k is the logic action of ranking for example, β_{jm} is the path worth utilities of attribute j with its variant or factor m, x_{jm} is a parameter of the design matrix link attribute j with its variant or factor m and μ is a constant. We remark that $\sum\sum \beta_{jm}.x_{jm}$ is the expression of the "design matrix" we talked previously and this expression (1) can be rewritten like:

$y_k = \mu + X.\beta_{jm}$ (2)

In this second model, y_k is dependant variable, X is independents variable, β_{jm} is a parameter or coefficient of the model. We are familiar with the equation (2) because it's linear and it can be solved with Ordinar Least Square (OLS) method to find the expression of β_{jm}.

b- Relative weight of attributes

To calculate the relative weight of each attribute: (i) Firstly, we used the coefficient β_{jm} of each attributes found in the equation (2); we multiplied by 2 and put in absolute value. (ii) Secondly, we summed all the values found and (iii) finally we divided each multiplied value by the total sum. Another manner to find the same result is to identify the minimum between all the values β_{jm}, we can note it ${\beta_{jm}}^{jM}$. After that, we look for relative value by making the difference between each value and the minimum of all the values computed. Let us call it ${\beta_{jm}}^{*}$with expression ${\beta_{jm}}^{*} = \beta_{jm} - {\beta_{jm}}^{jM}$. And then we calculate the weight ($\hat{\beta}_{jm}$) of each expression whit the following equation :

$$\hat{\beta}_{jm} = \frac{{\beta_{jm}}^{*}}{\sum_{j=1}^{j} max({\beta_{jm}}^{*})}$$

Data

GI is not yet realised in Côte d'Ivoire but we are trying to set the process on sixteen local's products. "Attiéké" is among these products. It's a steamed manioc very appreciate in Côte d'Ivoire and sub regions. Previously, it was done by people call lagoon's AKAN but now it is produced in and out borders by Benin, Burkina Faso, Taiwan and so far. Unfortunately, this imitation goes with a loss of quality of attiéké. Original producers- lagoon's people from Côte d'Ivoire are still doing attiéké in the traditional way. The original process of production is difficult, more costly for producers and the difference in quality proposed on the market are not distinguishable. Consumers complain many times about the non-constancy of taste. To solve this problem and avoid the relinquishment of activity by original producers, AFD and Ivorian's authorities is setting a process of GI.

In this process, lagoon's AKAN are targeted. They have been localised in four regions: Ebrié in Abidjan, Adjoukrous in Dabou, Avikam and Agni-Baoulé in Grand-Lahou and Alladjans in Jacqueville. In additional we chose Yamoussoukro which is not next to these regions and not original producers, but produce attiéké in huge quantity.

We chose two villages in each region basing on criteria:

- villages should not be distant from more than 45 km of chief of the region
- And have a legal producer group in the village.

The formula of François D. Giezendanner 2012 allows us to calculate the number of surveys. It was 238 for p= 0.9, 1-p= 0.1 with a confidence level of s= 99%. So, the tolerable error margin was e = 0.05 and t = 1.96. Surveys (25 per village) were investigated and led to take 250 persons in the sample.

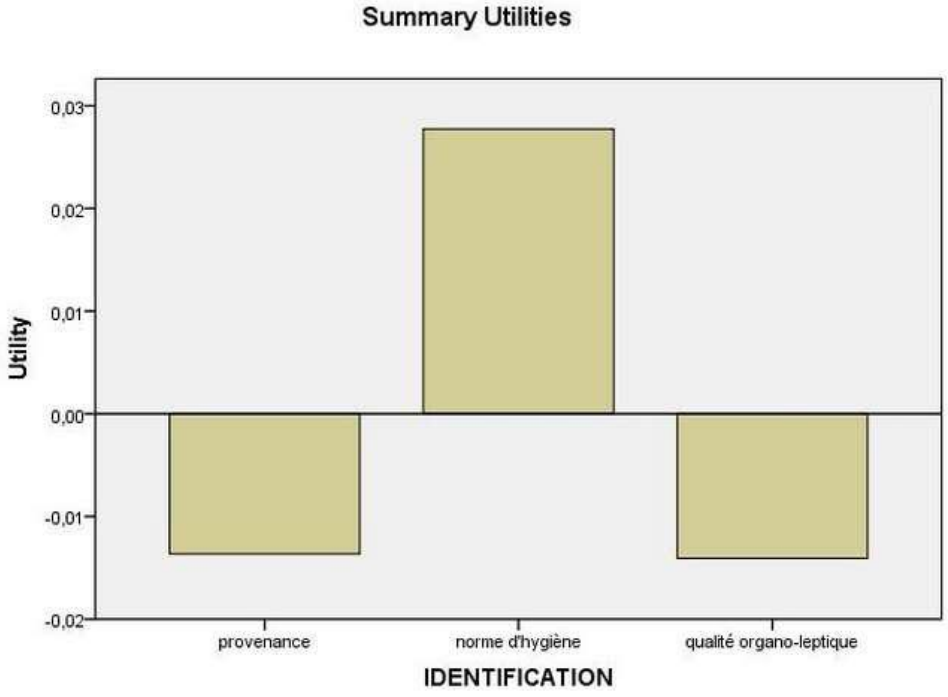
During our first investigation, people seemed to focus on some characteristics called identification (origin, organoleptic quality, level of hygiene), price (200, 250, 300), packaging (simple or sophisticated packaging), area of the distribution (open market or supermarket) and the type of attiéké (long grain, medium grain, small grain, Agbodjama, Garba or any type). We combined these attributes and proposed surveys to choose five and order them according to their preferences. Results were obtained with SPSS 17.

Results and discussion

Among 250 peoples were investigated, only 232 agreed to fill our card of conjoint analysis. It means that (250-232)/232 = 0.077 or 7%persons have not participated in our analysis. Such refusal may be interpreted as personal considerations, and psychological. However, we noticed that Non responses are not absolute non-consumers.

Some people dislike attiéké, to avoid giving false information, they didn't take part in our analysis. Some others, rich now, don't like too much attiéké because it was their main feed when they were poor and don't want to remind this period and others consider attiéké as a food for the population less fortunate. The last category of non-responses is due to the fact that some people don't want to see the price of attiéké raise because attiéké is social food, which helps many Ivorian to access food cheaply. For those who accept to take part in the survey, we have the following results.

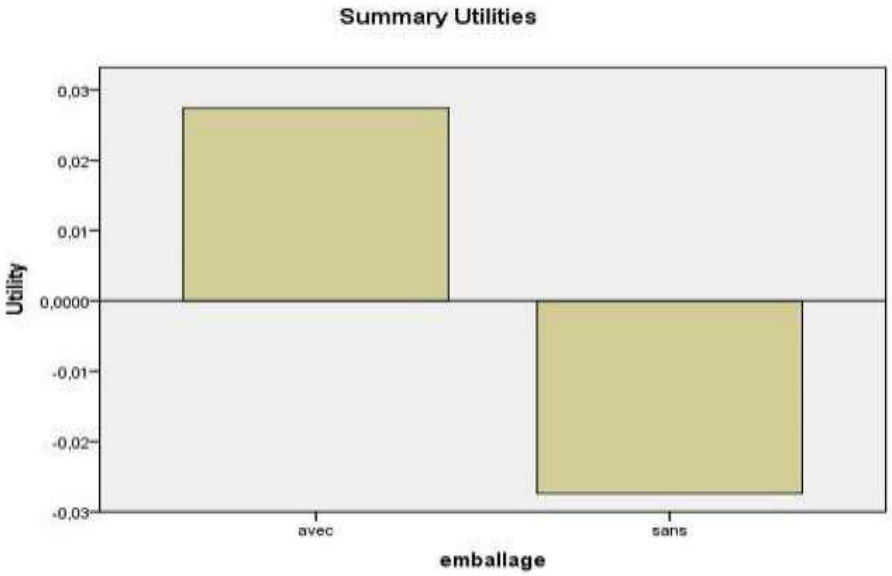
Graph 1. Identification



Source: author with SPSS 17

Identification has positive and negative scores. Hygiene has positive score (0.28) origin and organoleptic quality has same negative score (-0.014). It means that hygiene is more important for consumers than the origin and organoleptic quality. They must be sure that eating attiéké can't make them ill.

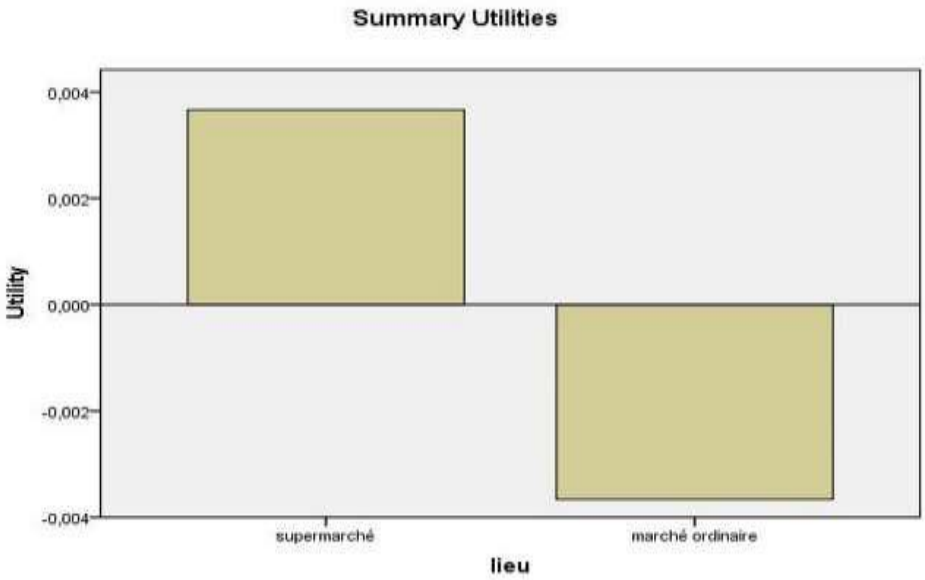
Graph 2: packaging



Source: author with SPSS 17

Packaging has also positive and negative score: positive for sophisticated packaging and negative for simple package. We also see that the absolute score is the same for both 0.027. Sophisticated packaging is the most desired because it's beautiful and seems safety.

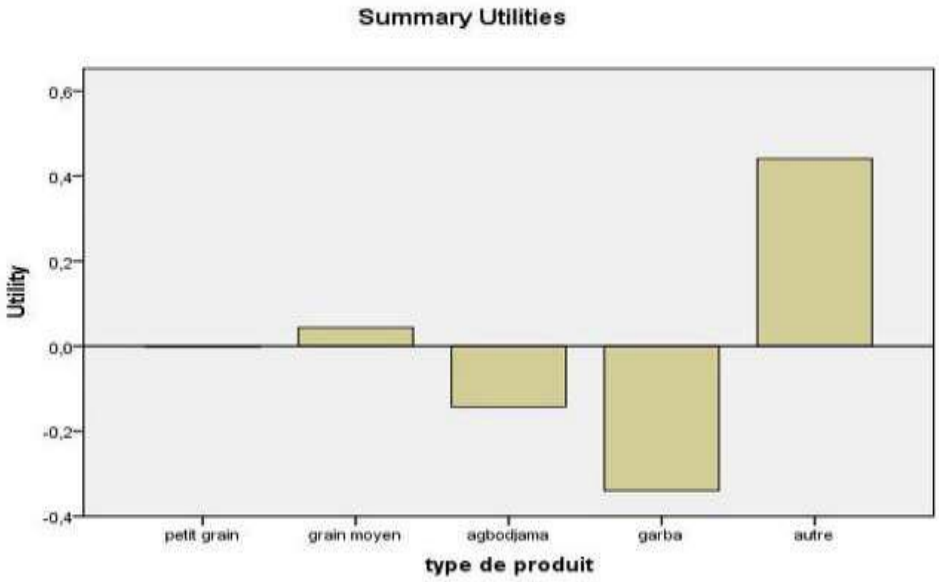
Graphic 3: Place of distribution



Source: author with SPSS 17

Concerning the **area of distribution,** the value of the score is positive for supermarket (+ 0.004) and the value of the score is negative for open market (-0.004). Like the packaging, the absolute score is the same. Consumers prefer a specific market of attiéké and think that supermarket is more hygienic than the open market.

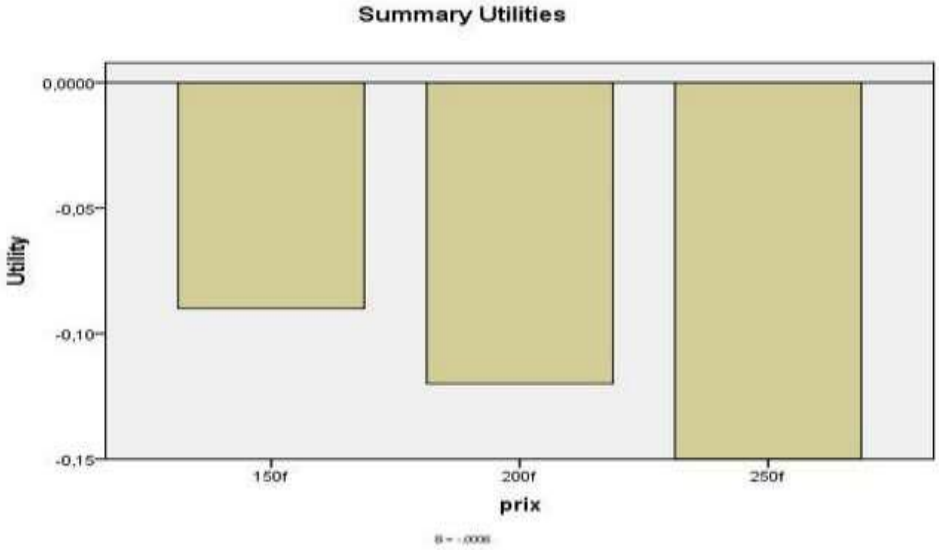
Graph 4: Type of attiéké



Source: author with SPSS 17

We have positive and negative scores. Among the positive scores, the high score is for any type of attiéké (+0.441), it is followed by medium grain (+0.043). For negative scores, we note at lower place small grain (-0.002), follow by Agbodjama (-0.143) and finally Garba (-0.339). In this case, the analysis showed that consumers preferred medium grain than any type of attiéké. If they don't find any type and medium grain, they resort firstly to small grain, secondly to Agbodjama and thirdly to Garba.

Graph 5: Price



Source: author with SPSS 17

Table 1: Reversal

Reversal Summary

N of Reversals	N of Subjects
1	96

Source : author with SPSS 17

Table2: Coefficient

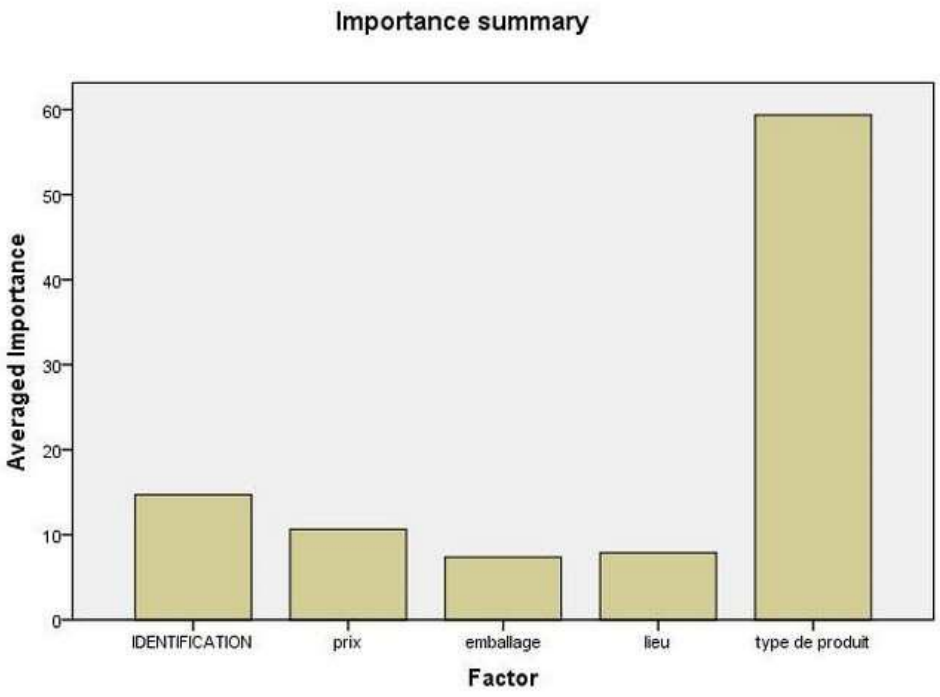
Coefficients

	Coefficient B Estimés
VENTE	-.0006

Source : author with SPSS 17

Price has only negative scores. The lowest is 150 (-0.90), follow by 250 (-0.150) and finally 200 (0.120). Price is quantitative and has a coefficient of regression is estimated to -0.0006. Consumers are not ready to pay more for attiéké in general, but if they have to pay they will pay 200f, 250 and 150 F CFA. This order means nothing, but it show just that attiéké is a normal product which price is not flexible. In another way, 41.37 % of the survey are ready to pay more than the median price.

Graph 17: Relative weight of attributes



Source: author with SPSS 17

We find that attributes have not the same weight. The most important is type of attiéké (60%), followed by the identification (15%), price (11%), instead of the distribution (8%), packaging (7%).

Model specification

Table 1: test of correlation

Correlations[a]

	Value	Sig.
Pearson's R	.978	.000
Kendall's tau	.840	.000
Kendall's tau for Holdouts	.857	.001

Source : l'author with SPSS

All the standard errors are inferior to one. This can be good for our model because it means that values find are not far from the mean/real. The coefficients R of Pearson, Kendall's tau, Kendall's tau for Holdouts are all significant at 1%, so the variables are not correlated and the model is well specified. We find that attiéké is a common food, for that, people are not ready to pay more. Unfortunately, the original producers are leaving the arduousness of job to practice other activities more easily and lucrative; we notice a decrease of the quality level of attiéké. The results show that some people dislike attiéké and some others would like to improve some attributes. The type of grain is the first criteria of choice of attiéké. The customer prefers medium grain than others. After the type, he looks at the origin of attiéké, hygiene is the most important in this case. We can note that the sum of these both attributes is 75%. In Côte d'Ivoire, each region has his type of grain and reference to grain led to one or several specific regions, but the confusion need to be overcome in order to give more precision on the region they talk about. Type of grain and origin can represent the need to have a Geographical indication of the level of 75%. After that, he looks for the price and is ready to pay until 250 but generally he dislikes pay more for attiéké cause at each time price rise only to 1% his preference fall to 0.0006. However, there is a considerable percentage (41.37 %) which wants to pay more than market price to have quality. We must identify the characteristics of this group to well-know them. With the knowledge of each score, we can compute and make a choice between a kind of products we want to sell on the market. It's a marketing tool to choose ideal product and it can help also to know what type of product is needed in a specific market or a specific group if we add more complementary studies. In this case, let take two different products which are able to be sold in our market. Let call Product 1: attiéké Agbodjama from known area, sold in supermarket at 250f with a sophisticated packaging and Product 2: attiéké with medium grain from known area, sold in supermarket at 250f with a sophisticated packaging. We can compute the part worth utilities of the both attiéké with the scores found. Let us do it

U (product 1)= u (attiéké Agbodjama) + u (origin) + (supermarket) + u(price 250f) + u (sophisticated packaging)+ constant

U(product 1)= (-0.143)+ (-0.014) + (0.004) + (-0.15) + (0.027)+ (-5.392) =(-5.668)

U (product 2) = u (attiéké medium grain) + u (origin) + u (supermarket) + u (price 250f) + u (sophisticated packaging) + constant

U (product 2) = (0.043) + (-0.014) + (0.004) + (-0.15) + (0.027) + (-5.392)= (-5.482)

We note that U (product 1)< U (product 2) because (-5,668)< (-5,482). This show clearly that product 1 is more appreciated than product 2 in the region of the survey.

Conclusion

We tried to get Scientific's basis of GI's setting. If internal market is not taken into account, opportunities given by GI can turn into disadvantages. We have to manage with the promise of international trade and deal with the local customers. Therefore the preference of local

customers can help the producers improve the product attributes and be more competitive in international trade.

Acknowledgements

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GOVERNANCE REGARDING THE SERVICE OF POTABLE WATER

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Abstract

Social representations are visions of the everyday world that is historically constructed alongside the dissemination of media intensifies their audiences. In this sense, the printed media availability and water policy has not only been systematically reduced to opinions by the press, but also two logics have been grown on the credibility of the information and the verifiability of it. In this sense, this study exposes the lines of discussion for the analysis of tandem policies and agenda setting in the availability and supply of water. The results show frames from newspaper audiences are considered promoters of a relative deprivation that is the conformism of the service quality of public water supplies.

Keywords: *compliance, performance, availability, supply rate.*

Introduction

In the context of participatory democracy, the media play an important role in establishing an agenda and eventually propaganda against or in favor of a political regime centered on the security and sustainability of natural resources and public services corresponding. It is a phenomenon in which the axes and themes of discussion, although established by the traditional media such as television, radio or the press, it is citizenship that, when processing such information, generates two rationalities, one informative and other reasoned with respect to the problems inherent to natural resources and public services. In this way, civil society as a user of public services, evaluates the performance of its authorities according to the information available in the traditional media. In this sense, the study of a problem such as scarcity, shortage or unhealthiness of water resources and services can be approached as a phenomenon of establishment of agenda and propaganda of the rectory of the state, institutional protection and civil despair. The phenomenon in question lies not only in the establishment of an agenda and the diffusion of propaganda in the matter of scarcity, lack of supply and conflict over water use and consumption, but also in the attribution of responsibility to the State as the guiding principle of security and sustainability, as well as institutions for non-compliance with their ethics of care and the consequent attribution of defenselessness of civil society. However, in the face of the hypothesis of State rector and civil despair, a proposal for active, deliberative and participatory citizenship emerges which rationalizes information in order to be able to express an opinion based on the degree of credibility of the information and verifiability. There are three theoretical frameworks from which the phenomenon in question has been explored: 1) theory of agenda setting, 2) theory of media culture, and 3) theory of social representations. The agenda-setting theory points to the framing of the media as biases or systematic tendencies to disfavor or enhance the state's leadership in matters of security and sustainability. It is a process in which the television, radio or the press focus their interest in evidencing the capacity of a government based on the popularity or scientificity of its strategies and programs. A direct consequence of the establishment of the agenda is the axes and topics of discussion in public opinion, essentially in the discourse of leaders such as communicators, analysts or columnists. From this fact, the

media culture theory emphasizes the information that has been emitted by an opinion leader and its resonance in the opinion of citizens who are not experts in the axes and subjects of discussion, but who cite their leaders to construct a opinion. This phase of formation of civil opinion is known as a social representation and this consists of producing meanings around referring symbols or archetypes of information, linked to local customs and customs, values and regional norms. It is the case of security as the axis of discussion and central topic of debate in the agenda of traditional media, citizenship and rulers. It is possible to appreciate words like "sicario", "ajuste de cuentas" or "lugarteniente" in the speeches of these actors, which reflect: a) the establishment of an agenda of public insecurity, b) the cultivation of information related to delinquency organized and c) representations of the State in the exhibitions of captured criminals or representations of the defenseless citizenship before reports of political corruption linked to drug trafficking. The relationship between authorities and users of public services has been a central issue on the agenda of the media. In the case of water as a strategic resource for local development, quality of life and subjective well-being, in recent years it has been widely reported by the media (Anaya, 2014). Particularly the print media with national circulation has included reports about the imbalance posed by water scarcity and water shortages associated with the corruption of local authorities and increased rates deregulated (Gomera et al., 2013). The impact of media coverage regarding the shortage and poor health has been a central issue in the public health agenda (McCombs, 1996). It is estimated that about six million children die from diseases hidrotransmitadas in countries with high rate of water shortages, but this means not only disseminate information but also associate the performance of the authorities in terms of management. Thus, in the suburbs of large cities the problem of water supply has been addressed by a system of tandem in which increasingly restricted water consumption and substantially increase rates (Gudynas, 2010). Approximately 20% of incomes in vulnerable or water exclusion situation, is assigned to the purchase of water products, but print media rather highlight the lack of infrastructure and attention to contingencies such as drought or frost (Perez and Soler, 2013). Thus, the media have a particular view of the problem of water scarcity and shortages of attributing responsibility to the authorities and exonerating users who have regular access to drinking water or justifying the soft drink consumption or pharmaceutical industry. It is a process in which the systematic dissemination of biased information regarding the relationship between availability and management of water resources is known as agenda setting (Carreon et al., 2014). An agenda is a result of information exchange between the agents involved, but the differences between them generates information biases that are spread in the media and the information is used to justify rate increases by the authorities, waste by users, conflicts among actors or, tandem or policies restricting water (Flores, 2013). However, the agenda is also historic, as it involves the reaction of organized civil society groups regarding the performance of its authorities on quality public services (García, 2011). It is power relations that have grown and are activities to the less informative provocation, but in the case of the print media is a process more complex elaboration that this letter sets as an overview of water availability in reference to consumption human. If the media presented the conflict as central themes of water problems because the authorities responsible for the inefficiency of rates and expose closures avenues, boycotts pipe facilities or kidnappings as evidence of ungovernability that inhibit local sustainable development. Consider the following premises for addressing administrative corruption on supply and collection of public drinking water:

Premise 1. The availability of resources to be scarce generates an administration of austerity and shortages financed by a steady increase in the unit price of water in terms of political relations between local governments and civil society organizations;

Premise 2. The rates reflect political negotiations between members and militants with regard to election time and the proximity of local elections, but also in terms of management capabilities of civil society organizations;
Premise 3. Conflicts between civil society and local government in the field of municipal water services are a reflection of systematic dissemination of shortage and quality of drinking water in the media with particular emphasis on national newspapers.
Formulation: What is the proposed media for the sustainability of supply and collection system considering his framing of the facts?
Hypothesis: The journalistic framing printed media around scarcity, and water management is established from the systematic diffusion of responsibility of local officials.

Materials and methods

Design. Non-experimental, retrospective, documentary and exploratory study.
Sample. Notes the daily newspaper of national circulation during the period from 2010-2015. The sample of informative notes was made using the data mining technique of the search engine google.com, considering the keywords: "shortage", "tandem", "conflict", "leaks". The selected journals were: "El Universal", "La Jornada" and "El Reforma" (see Table 1).
Instrument. Matrix analysis of content including references consulted and processed information as shown, instruments and results.

Table 1. Index Mediatisation Leak

Newspaper	*Indicator*	*Coding*	*Weighing*	*Interpretation*
El Universal	Press releases	0 = null framing; 1 = very little frame, 2 = little frame, 3 = frame somewhat evident, 4 = very clear frame	30 to 40 points	Diffusing agenda of the direct and significant relationship between scarcity, tandem and conflict between rulers and ruled
La Jornada	Press releases	0 = null framing; 1 = very little frame, 2 = little frame, 3 = frame somewhat evident, 4 = very clear frame	30 to 40 points	Diffusing agenda of the direct and significant relationship between scarcity, tandem and conflict between rulers and ruled
El Reforma	Press releases	0 = null framing; 1 = very little frame, 2 = little frame, 3 = frame somewhat evident, 4 = very clear frame	30 to 40 points	Diffusing agenda of the direct and significant relationship between scarcity, tandem and conflict between rulers and ruled
			IML = 90 a 120	Propaganda diffusing the direct and meaningful relationship between the rectory of the state, institutional protection and civil defenselessness

N = 30 press releases. IMF = Leverage Mediation Index established by the average of the evaluations of 10 judges with respect to press releases that framed the information about the scarcity, the tandem and the conflict between authorities and users of the potable water service, focusing the problematic in municipal and residential leaks. *Source: self-made

Procedure. From the google.com data mining technology, press releases were identified regarding the scarcity, tandem and conflicts arising from municipal and residential water leaks. The Delphi technique was used to compare and integrate information concerning the framing of press releases. Ten expert judges were used in the problem: 3 officials, 3 journalists, 3 citizens and the evaluation of the researcher. Estimated impact of press releases considering its content and frequency of keywords or indicators of scarcity, shortage and cost of drinking water.

Validity. The degree of coincidence between the informed deliberation and the reasoned deliberation of the judges who were assured in writing the confidentiality and anonymity of their answers, as well as the warning that the results of the study would not affect negative or positively their economic, political and social status.

Coding. Each one of the headings of the press releases was evaluated by the judges, considering a scale that goes from 0 = null framing to 4 = very evident framing. For example, consider: "In Iztapalapa there is water for the next 50 years" was considered by the 10 judges as a biased or biased header since, if it is related to the discovery of a well, its degree of contamination and water inaccessibility limit the supply even of the colony in which the well is located. Therefore, it was evaluated with 4 points.

Weighing. The Leverage Mediation Index was established based on the average of 10 judges 'evaluations of the headings of the press releases, following the criterion of validity regarding differences and similarities between the judges' responses. Such a weighting strategy allows us to move towards multivariate estimates that require averages, standard deviations and variances to establish differences between groups, but whose purpose has not been that of the present work, which only presents a first approximation to the explanation of the phenomenon. **Interpretation.** Since the options "frame something evident" and "very clear frame" reflect a position in favor of considering the establishment of an agenda in each newspaper and the emergence of a propaganda by the three newspapers, the sum of these options formed a range ranging from 30 to 40 (90 to 120 for the case of summation of options 3 and 4 with respect to the total of 30 notes). It was considered that the summations inferior to the rank would reflect a null establishment of the agenda for each newspaper and consequently, a null propaganda campaign for the case of the three newspapers.

Results and discussion

Table 2 shows a synthesis of the findings related to the establishment of an agenda and propaganda regarding scarcity, tandem and conflicts over municipal and residential leaks.

Table 2. Index Mediatisation Leaks

Newspaper	*Indicators*	*Coding*	*Weighing*	*Interpretation*
El Universal	10 news headlines identified in the google.com data mining system	0 = null framing; 1 = very little frame, 2 = little frame, 3 = frame somewhat evident, 4 = very clear frame	27 points from 30 minimum and up to 40 possible to assume the frame, bias or information trend	Null agenda of diffusion of the significant relation between scarcity, tandem and conflict between the authorities and the users of the public service
La Jornada	10 news headlines identified in the google.com data mining system	0 = null framing; 1 = very little frame, 2 = little frame, 3 = frame somewhat evident, 4 = very clear frame	31 points from 30 minimum and up to 40 possible to assume the frame, bias or information trend	Diffusing agenda of the direct and significant relationship between scarcity, tandem and conflict between rulers and ruled
El Reforma	10 news headlines identified in the google.com data mining system	0 = null framing; 1 = very little frame, 2 = little frame, 3 = frame somewhat evident, 4 = very clear frame	23 points from 30 minimum and up to 40 possible to assume the frame, bias or information trend	Null agenda of diffusion of the significant relation between scarcity, tandem and conflict between the authorities and the users of the public service
			IML = 81 points from 90 minimums and up to 120 possible	Null advertisement of diffusion of the Governing State and the institutional protection in the matter of supply

Source: self-made

It is possible to appreciate the null establishment of an agenda by El Universal and El Reforma, but in the case of La Jornada there is a bias that implies the social representation of the source of information and the problem, would consist in associating the prestige of the newspaper with the context of scarcity, tandem and conflict between authorities and users.
However, if the index is analyzed in its entirety the bias of the press does not reach a minimum level of frame that allows observing a propaganda of the State, institutional protection and the civil despair.
The weights of notes and images as well as their mutual correspondence concerning the coverage of water leaks Iztapalapa. The indices show that the items appear to be biased as they reached a total value of 4 points of 40 possible. Only in notes III (Alistan constitution water Iztapalapa) and V (is lost in leaks 35% water) reporters tried to externalize their opinion on the issue. The note V journalist quotes two officers: David Robles and Hector Reyes to support the header of your report regarding the volume is wasted by visible and invisible leaks without establishing a percentage distinction between the two issues and link it with 35% announcing at the top of the note. Regarding framed pictures, media coverage seems to be high since it reached a total of 36 points of 44 possible. A quarter of the selected notes reached the maximum weighting relative to framing. IX and X notes included images out of context without any connection with the note. Such notes did not try to influence or confuse readers. However, the notes I, II, III, IV, V, VI, VII, VIII and XI included images that seems evident extreme shortages; public service users storing water in drums, jars and buckets. Or, images where principal photography leaks seem to be uncontrollable.
In the case of the relationship between discourses and images, the index was 22 Framed Hybrid which was considered moderate as only the notes III, VI, IX and X included images that did not correspond to the briefing notes.
However, if the note III who headed: "ready incorporation of water in Iztapalapa" included images of water users away. That is, "El Universal" describes a leak to argue the need for a legal agreement and illustrates his message with images relating to a possible consequence: the improvised water storage. The results showed a moderate mediation except Framed Images Index (IEI), which was close to a very high degree of media coverage. Such findings are consistent with those found by Garcia (2011) who found moderate levels of media coverage regarding the participation of users in situations of scarcity and shortage. Finally, the index Mediatisation Leak scored a total of 81 of 120 possible to settle at a moderate level. That is, the press coverage about the shortage prompted by leaks seem to have an unusual bias in relation to the description and illustration of the water situation is diffused.
The contribution of the present study to the theoretical frameworks of the establishment of the agenda and propaganda of the rectory of the State and civil defenselessness lies in the proposal of measurement of the level of framing of the print media regarding the problem of scarcity, shortages and activated conflict for municipal leaks.
However, the type of sample selection through google.com data mining and the Dephi technique limit the results to a specific study context since it is necessary to extend the information search to the subsidy, remission or tariffs of the public service, as well as to carry out a representative study with citizens of at least two demarcations, one with high and another with low availability of water in order to be able to compare the findings in different contexts and samples. With respect to the theoretical and conceptual frameworks related to the establishment of the agenda, media culture and social representation, the present work has shown that this process is partial since the IML reached a moderate level, but above all, the phenomenon that is going of diffusion of scarcity, tandem and conflict seems to show only part of a more global and extensive problem, namely: the anthropocentrism that consists in the management, distribution and consumption of water in a region or locality considering it as

the exclusive patrimony of its inhabitants and rulers without considering the needs and capacities of future generations of animal and plant species.

It is necessary to include categories of anthropocentrism, biosferism or ecofemminism in order to be able to establish the agenda and the propaganda of the State, but not only with respect to security but with respect to sustainability. This will allow us to anticipate scenarios of authoritarianism or informational totalitarianism, where the central agency version prevails, unilateral communication, impersonal defenselessness and unidirectional demotivation, the main indicators of a context of null civil participation.

Conclusion

The phenomenon of diffusion relative to the rectory of the State in the matter of the management of water resources and services before the scarcity, tandem and conflict exposed in the press and established as axes of discussion in a local agenda as in a campaign of conjunctural propaganda supposes: 1) the analysis of the impression or bias of the print media in a given electoral period, 2) the observation of the impact of the framing on the civil opinion, its preferences and voting intentions 3) the discussion concerning the level of framing of the press and its effects on the speeches of public and private actors and sectors before, during and after the local or federal elections. This exercise will allow us to infer the degree of deliberation, participation and responsibility of the civil actors regarding the information on the performance of their authorities and the reflection of their anthropocentric or biospheric logic and ethics in the supply and collection of drinking water service.

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MECHANISMS AND ECONOMIC CONSEQUENCES OF PUBLIC INTERVENTION IN AGRICULTURAL HOLDINGS IN POLAND DURING THE PERIOD OF EU MEMBERSHIP

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Abstract

The concept of public intervention in agriculture can be understood very broadly, as any act or omission of the operation of public state institutions. The article discusses the economic reasons for the public intervention, then presents the types and effects of intervention implemented by the CAP as well as the impact of intervention policy on economic decisions of agricultural producers. In the further part of the article, based on the statistic data from 2004-2016, were presented the changes in agriculture sector. A characteristic feature of this process is the deagrarisation the national economy and the development of rural areas. Throughout this process, it is extremely important for the ongoing structural changes to result in the improvement of the competitive position of farms and long-term and sustainable rural development. Poland's accession to the EU has generated new economic and organisational conditions to support structural changes in the broadly defined food economy and rural areas. Policy instruments implemented within the CAP create chances for the stabilisation of structural policy conditions over the period of several production cycles, thus stimulating the desired changes in the area structure of farms, the improvements in the competitiveness of production, environmental protection and multi-functional development of rural areas. Thus they are a fundamental instrument supporting the process of modernisation of Polish rural areas and agriculture. The article conclusions refer to structural changes and to competitiveness of agri-food sector.

Keywords: *public support policy, competitiveness, intervention in agriculture.*

Introduction

The active role of government and justification for state intervention in economic process results from the conviction about market failure (Bator, 1958). This concept suggests that in the realities of the market economy the processes of allocation of goods and services show a number of frictions. As a result, the state of actual equilibrium achieved by the market is not compliant with Pareto optimum. In broader terms, the concept of market failure identifies the scope and circumstances of observed defects of market mechanisms that lead to the perpetuation of market imbalances (Baumol, 1952). In this context, it emphasizes the positive aspects of market intervention by public authorities (Stiglitz, 1989). Economic theory distinguishes a number of reasons for market failure. The mechanism and the logic of intervention in modern agriculture is shown in figure 1.

Arrow (1983) was one of the first to point out that, in fact, one can distinguish two different states of efficiency in the allocation of goods depending on the degree of fulfilment of the Pareto demands. The first approach suggests that each allocation of goods in equilibrium meets only the demand of the so-called "poor efficiency" in the sense of Pareto.

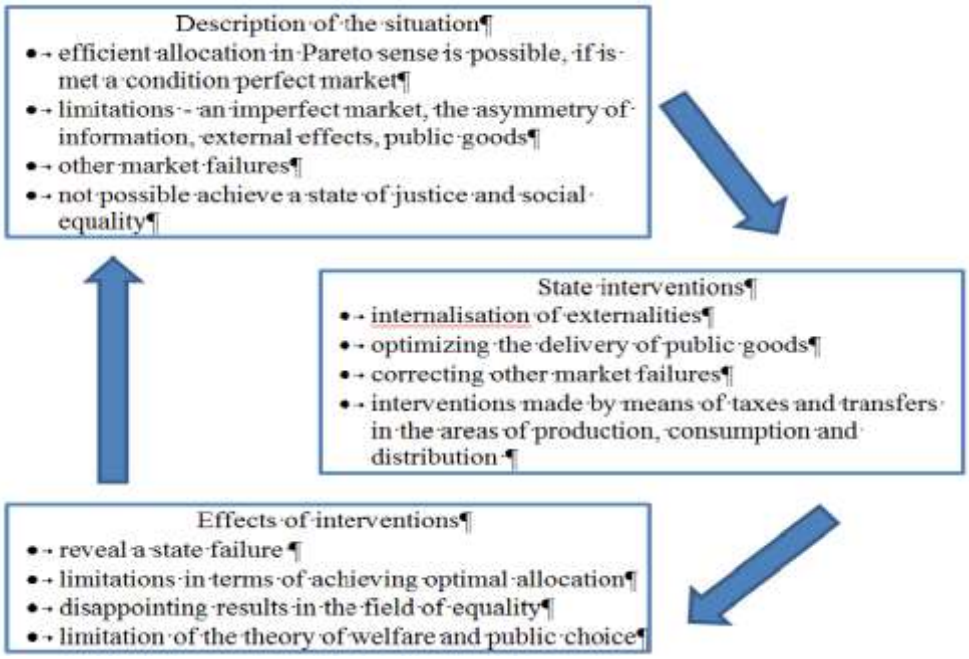

Figure 1 The mechanism of the modern intervention in agriculture

Source: Own study based on a literature review of studies of welfare economics and public choice economics

The global experiences prove that the market and the state have to co-exist and the state intervention should be always limited to support market mechanism and not replacement thereof. The state should interfere only when it has a clear advantage over the market mechanism; hence only when the market fails to protect the general interests of the society (Woś, 2004). The contemporary global economy often rejects the thesis on the perfect market (Czyżewski, 2007) thereby justifying the role of state intervention. When explaining the main reasons for intervention in the modern global agriculture point to the high level of risk linked to agricultural activity and lack of effectiveness in prevention of this risk. The risk results from e.g. changing climate conditions, lack of sufficient information and underdevelopment of agribusiness structures, including also consultancy. The need for interventions in the agribusiness sector is justified also by: the phenomena of external costs and effects, low price elasticity of supply, lower level of labour productivity than in other sectors of the national economy, low mobility of the workforce employed in agriculture, the need to provide public goods, implementation of the sustainable development concept. Implementing the objectives of CAP support has important impacts on food economy. The current objective set of the CAP, according to the "EU 2020" strategy, is that agriculture should contribute to smart, sustainable and inclusive growth. Government policy measures have static effects, risk-related effects and dynamic effects on production, and different transfer efficiency on farm income depending on policy tools applied. The impact of agricultural subsidies on income distributional effects depends on their type, the structure of the markets and the existence of market imperfections. Most of the studies investigate the direct impacts of subsidies on prices, output, income, the environment, etc. by assuming that subsidies do not alter the structure of agricultural markets and do not interact with market institutions. In reality, government policies may have various unintended effects (they can change the structure of market organization or crowed out some market institutions) (Forgasi et al., 2014). The objective of this study is presentation of the mechanisms and economic consequences of public intervention in agriculture in Poland during the period of EU membership which seems to be very important for rural economy as well as whole economy of Poland.

Material and Methods

The basis for the research was studied literature, especially in the field of main stream economics, welfare economy and public choice theory, referring to the question and to the consequence of public support in agriculture. Documentation studies were carried out also in the field of literature consolidating issues related to public support and process of structural changes, innovation transfer, the development strategy of determining the directions of the policy and the main acts forming the regulatory environment. Assessment of the importance of structural changes in agriculture was carried out using methods of descriptive and comparative analysis. The empirical material was the statistical data of the Central Statistical Office (CSO) for the years 2004-2016 and Agency for Restructuring and Modernisation of Agriculture (ARMA).

Results and Discussion

The specific nature of the agricultural sector and its entities boils down primarily to the limited mobility of production factors involved in this sector. This particularly regards land, family labour resources, and, to a significant extent, the capital adjusted to agricultural activity. Agriculture and its entities are deprived of the benefits from transferring resources to more effective sectors, which determines the nature of competitiveness. Following the accession to the EU, there have been major changes in the agrarian structure, which continued long-term trends. In the period preceding accession to the EU (1996-2002), large agricultural holdings (20-50 ha of utilised agricultural area - UAA), which took over arable land from small and medium agricultural holdings, but also from holdings of over 50 ha, developed dynamically. The growth in numbers was accompanied with the decrease of surface. The arable land of holdings below 1 ha grew, but their number dwindled. Following the accession to the EU, the number of holdings decreased by 28%, and their surface – by 3%. The development of large holdings lost dynamics, but still arable land was taken over by holdings of the surface of 20 to 50 ha, for both smaller holdings, of which the number and area decreased, and bigger, the number of which, despite the decrease of the area, grew. The number of holdings taking over land slightly increased. The number of holdings smaller than 1 ha decreased by 27%. To a slightly lesser extent the number of small and medium holdings decreased (from 25% to 16%). Their area also shrunk, including, to a largest extent, in the group of 10 to 20 ha (by 8%). These changes indicate that the Polish agriculture, despite major changes, is still to a large extent dispersed. Figure 2 shows the use of land in different groups of farms in 2016.

The basic source of reduction in production cost is the change to the relation between production factors – particularly the relation between capital on one hand, and land and labour on the other. In Poland, the equipment of labour with land and capital is much lower than in most EU countries, which determines relatively low labour and land productivity (Figure 3). Low cost of labour in Poland is the primary factor that contributes to the advantage of Polish agriculture over agricultural products of the majority of EU countries with regard to cost and prices. Low remuneration for labour, however, cannot be the basis for maintaining competitiveness of agri-food sector product on the European and global market. Improvement in efficiency of use of land and labour means also the improvement in the quality of production conditioned by technical and technological progress, and the level of producers' knowledge. At the same time, the land cultivation system, which is less intensive in Poland than in most EU countries, is a kind of competitive advantage because Polish agriculture can become "sustainable" agriculture faster that agriculture of other EU countries, which is the EU objective in the 2050 perspective.

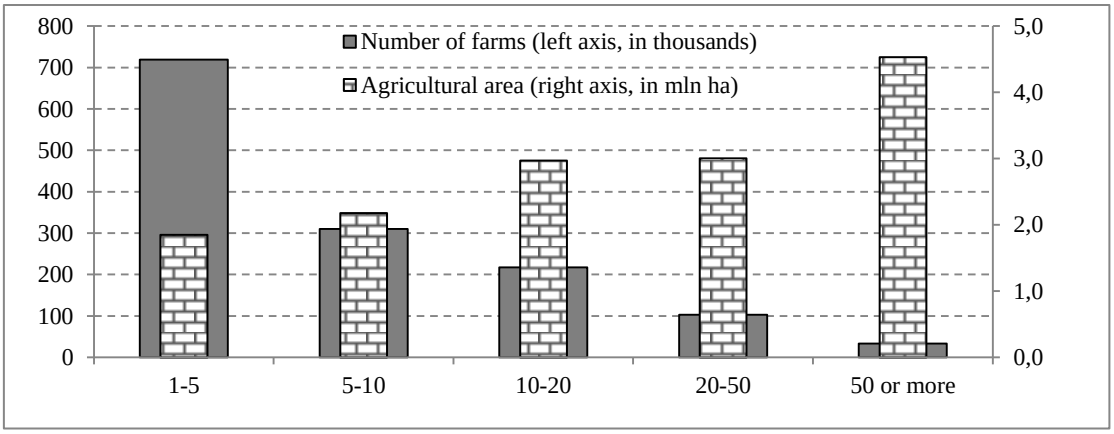

Figure 2. Land use by groups of farms in 2016.

Source: Agriculture in 2016. CSO data, Warsaw 2017.

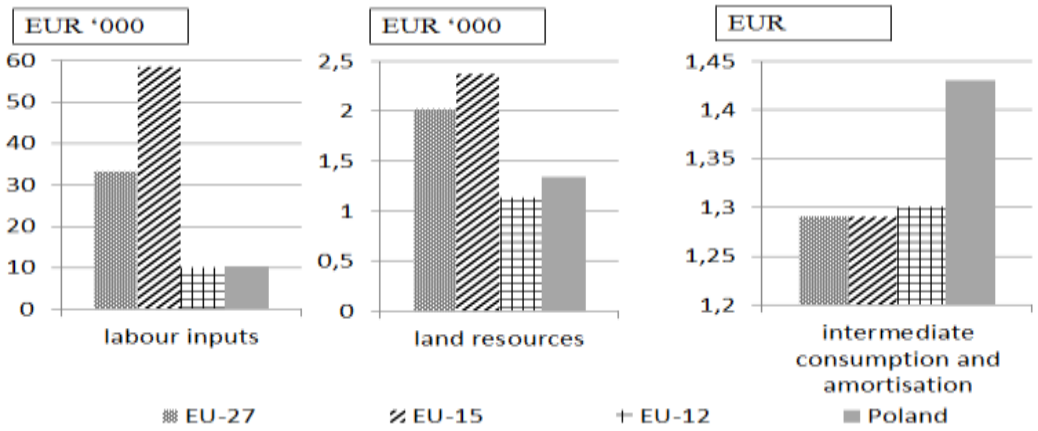

Figure 3. Productivity in Poland against average values for groups of EU Member States in 2016.

Source: Eurostat 2017.

The EU funds had a significant share in the financing of transformations in agriculture until Poland's accession to the EU. The direct payments are the most common type of support, each year about 1.4 million of farmers use this form of support. The value of payments in the 2004-2016 period increased and fluctued betwen ca. Euro 1.5 billion to Euro 3.5 billion per year. When calculated per one farm it reaches an average of ca. Euro 2.3 thousand, and this form of support is used by 87% of farms having an area of more than 1 ha. An equally important source of income (regardless of production, and only based on the farm's location) are payments for less-favoured areas (LFA). Each year these payments are granted to ca. 700 thousand farmers, i.e. half of those receiving direct payments. The land surface covered with LFA payments amounts to ca. 6.9 million ha. The manner of spending of the resources is not subject to settlement. Smaller farms usually allocate the granted payments to current needs and means of production (fuel, fertilisers), while the bigger ones also make investments.
The resources earmarked for investments are also an important source of aid for farms. So far, the financial resources for investments in farms, available under SAPARD, SOP "Agriculture", RDP 2004-2006, RDP 2007-2013 and RDP 2014-2020 were used in their entirety. The SAPARD programme was aimed at preparing the Polish agri-food sector to the accession, in particular in the adjustments to the sanitary, hygienic and environmental protection requirements of the EU. After 2004, the strategic objectives of agricultural policy implemented via SOP Programme "Agriculture" and RDP 2004-2006 covered: improving the

competitiveness of the agri-food sector, sustainable development of rural areas, improvement of the condition of the natural environment, improvement of the quality of life and diversification of economy in rural areas. In the next programming period, the RDP 2007-2013 has become the programme to support the implementation of the concept of multifunctionality of agriculture and rural development. It assumed economic strengthening of farms and an increase in the competitiveness of the agri-food sector, while assuring instruments for diversification of economic activities towards the acquisition and the creation of alternative sources of income for the rural population. RDP 2014-2020 for Poland focuses on three main objectives, i.e. supporting competitiveness and productivity in the agri-food sector, ensuring sustainable management of natural resources and climate action, as well as achieving sustainable territorial development of rural economies through the development of local infrastructure, investments in the field of education, culture and public services, creation of new and maintenance of existing jobs.

In the 2004-2016 period, the cumulative value of support for the agri-food sectors from three main sources of support: the payments from the EU budget, a grant from the national budget to KRUS and grant from the national budget, exceeded in total PLN 581 billion (EUR 145 billion) (Figure 4).

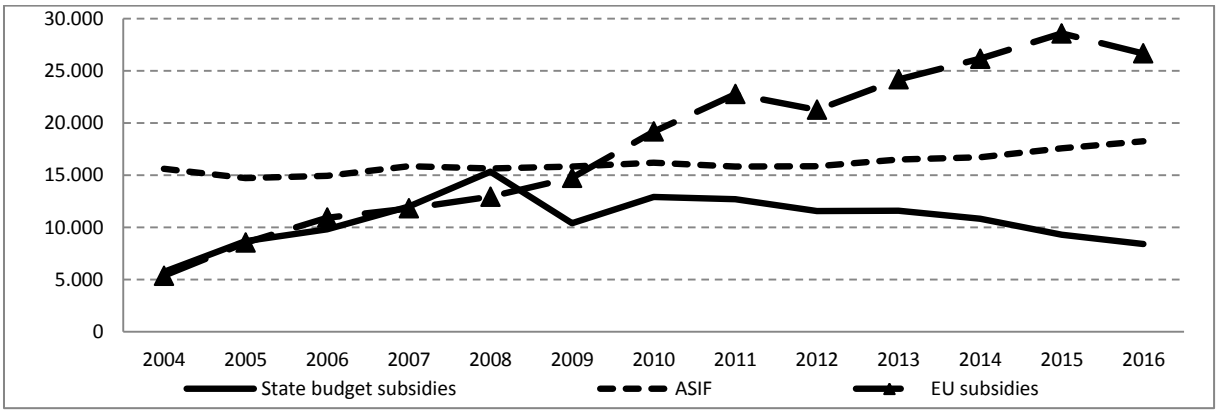

Figure 4 Budget spending on the agricultural sector in 2004-2016 (PLN '000 000).
Source: Authors' own calculation according to Budget Act (different years).

Rural development programmes, and indirectly also direct payments, are the primary source of support for investments in the Polish agriculture[22]. The role of the latter is particularly significant in farms with a large area. The area-related nature of direct and supplementary payments and LFAs[23] means that each year, farms receive cash they can spend on any purpose they want. The importance of these payments in the support for income, indirectly also for investment, is evidenced by the fact that they are received commonly. In order to obtain the payments, a farmer is only required to properly fill in an application and keep land in a good agricultural condition.

Conclusions

In the last decade the structural changes taking place in the Polish agriculture became more dynamic. The most important among them cover: a drop in the number of farms with

[22] Direct payments in agriculture fulfil a number of functions, i.e. the income function (they compensate the farmers' increased production costs), the stimulating function (they can define the direction of agricultural production), the modernisation function (they can be used for co-financing of investment), the information function (they indicate the areas of production).

[23] About half of the land used for agricultural purposes in Poland is located within LFAs. These include e.g. the areas where there are difficult climatic conditions, rainfall is too big or too small, there is a disadvantageous topography (e.g. mountains), or the quality of soil is low.

simultaneous growth in the share of the largest farms, which directly influences the increase in the average area of farms, drop in employment in agriculture and progressing production concentration and specialisation. The structural changes are, however, slow and cannot be efficiently accelerated due to non-agricultural circumstances. The Polish agriculture is still characterised by a strong polarization of the agrarian structure.

Policy instruments implemented within the CAP created chances for the stabilisation of structural policy conditions over the period of several production cycles, thus stimulating the desired changes in the area structure of farms, the improvements in the competitiveness of production and multi-functional development of rural areas. Thus they are a fundamental instrument supporting the process of modernisation of Polish rural areas and agriculture (Wigier, 2014a). Today, we already know that CAP has actually caused an increase in support for agriculture, while structural funds have triggered considerable cash flows intended for modernisation of food economy and rural areas development. However, not all investments proved to be effective, which undoubtedly prompts us to reflect on the scale and the value of investments in agriculture.

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REACHING TERRITORIAL COHESION? PRINCIPLES AND PRACTICES OF THE NEW REGIONAL POLICY: THE CASE OF INTEGRATED TERRITORIAL INVESTMENTS AND COMMUNITY LED LOCAL DEVELOPMENT IN POLAND

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Abstract

The emerging concepts and regional policy instruments are focused on balancing socio-economic development in the spatial aspect. They differ from each other not only in the approach to scale and scope of support of individual territorial units, but also in the way and conditions of assistance. New regional policy tools can be considered through the prism of links with the rural development process and policy, and in particular the potential effects that they may have on these areas. The paper analyses new instruments of the EU Cohesion Policy and Common Agricultural Policy (CAP) in a form of Integrated Territorial Investment (ITI) and Community-led Local Development (CLLD). The aim of the research was to determine potential impact of these initiatives on the socio-economic situation of the countryside in Poland. The study showed that both the ITI and CLLD could play a positive role in creating conditions conducive to improving the situation of rural population in Poland. However, their implementation involves a number of barriers and risks. The progress in implementing projects under analysed instruments is limited and does not allow for their comprehensive assessment. The conducted research has been based on literature review, available quantitative data and qualitative information published by the public bodies implementing the ITI and CLLD in Poland.

Keywords: *Regional Policy, Rural Areas, EU, Integrated Territorial Investment, Community-led Local Development*.

Introduction

Phasing out the approach to territories through the prism of borders for the benefit of their functions, specialisations, links, potentials and development barriers is increasingly reflected in the economic and regional policy (Shucksmith and Brown, 2016). This trend also applies to the EU policies, in particular the Cohesion Policy and the CAP (McCann, 2015). Given the challenges and problems in the situation of many rural territories, as an appropriate approach of government intervention, the neoendogenous paradigm is often recommended (Tödtling, 2011). According to this concept, the development programmes and projects should be created at the local level and based on support, knowledge, advice and strategies generated at the regional and central level (Copus *et al.*, 2011). What is widely discussed currently, is the postulate of the rural development policy, which takes into account the properly targeted top-down programmes with bottom-up projects. In the context of designing the Cohesion Policy and CAP and their implementation systems, it is suggested to reduce the support treated as compensation for poor territories and allocation resulting from the "objective" demand, in favour of strengthening the capacity of all local and regional units. In such approach, local stakeholders could shape their own situation independently basing on their own material, intellectual, environmental and social resources as well as on links with external actors (Copus *et al*., 2011). This argument is linked to a belief in the effectiveness of public intervention which is financially consolidated and based on simple principles and objectives. It is argued that new possibilities for intensifying connections between rural and urban areas, defined more by their functional connections rather than administrative classifications, are

provided within the current EU development (the financial framework 2014-2020) (Nurzyńska, 2014). The chances of strengthening contacts motivated by obtaining mutual benefits are to be expressed, *inter alia*, by a relatively deeper decentralisation of financial support from the structural funds, promoting cooperation between self-governments and thematic concentration of interventions. The EU policy mechanism under which these postulates are implemented is the integrated territorial approach, in particular the instruments called ITI and CLLD (Kozak, 2016). The purpose of the paper was to analyse and assess this instruments as the new tools of regional policy aiming at reaching territorial cohesion for the different types of rural terrains, namely: suburbs, intermediate and peripheral areas. This mechanisms were characterised in the context of selected regional and social development theories. Using the data on the implementation of ITI and CLLD in Poland the potential effects for the development of rural areas were considered.

Material and methods

The paper is based on literature review. The presented conclusions were also drawn from the analysis of the strategies and legal documents relating to the implementation of the EU Cohesion Policy and the CAP in 2014-2020 in Poland. In the analyses the information and data published by public bodies involved in implementing the ITI and CLLD in the EU and Poland were taken into consideration as well.

Results and Discussion

A new subject in the EU regional policy is the integrated territorial approach concept. This idea, understood in a narrow sense, applies to the specific tools of the EU Cohesion Policy and CAP. The EU law identifies three integrated territorial approach instruments: ITI, other integrated operations and CLLD. These tools have been regulated at the EU and country level. These instruments differ in terms of the main area of implementation, method of management, functioning and supervision, as well as the size and type of the source of financing.

Within the framework of ITI, there are instruments supporting the sustainable urban development and instruments supporting various areas. The former is described by Article 7 of the Regulation on the European Regional Development Fund (ERDF). The overall objective of the mechanism is the sustainable urban development, which is implemented by investments, separate priority axes or operational programme (*top-down approach*). The allocation under Article 7 of ERDF mainly covers the thematic objective of the Cohesion Policy related to promoting the transition to the low-carbon economy of all sectors, conservation and protection of the environment and resource efficiency, as well as the objective referring to social inclusion and the fight against poverty. ITI supporting various areas, including urban areas is under Article 36 of the general regulation (van der Zwet *et al*., 2014; Łukaszewska-Krawiec, 2015).

In turn the CLLD is implemented using development strategies which are multi-sectoral, integrated and customised to the specific sub-regional area. Its use is a rule for the CAP (at least 5% of the European Agricultural Fund for Rural Development allocation, EAFRD) and is voluntary in the case of the Cohesion Policy (European Social Fund, ESF and ERDF) and maritime and fisheries policy (European Maritime and Fisheries Fund). CLLD is to encourage local communities to take action for the benefit of their area, civic activity and involvement in implementing the development policy objectives (*bottom-up* instrument). The beneficiaries of this instrument are Local Action Groups (LAG), which function based on Local Development Strategies (LDS). The LDS selection criteria are defined by the Member States.

It is estimated that in 2014-2020 the EU Cohesion Policy allocated EUR 15 billion for the integrated territorial approach mechanism, i.e. about 5% of the total budget of this policy (Committee on Regional Development, 2016). The majority of funds for this initiative are

spent from the ERDF (82%) and the remainder is from the ESF (14%) and the Cohesion Fund (CF) (4%) (Łukaszewska-Krawiec, 2015). ITI is implemented by 20 EU Member States, particularly for the projects designed for urban areas. On the other hand, CLLD is based mainly on the LEADER programme implemented within the second pillar of the CAP and financed by the EAFRD (use of this instrument is obligatory for this fund). CLLD as a Cohesion Policy tool is implemented in case of 17 Member States[24] (Committee on Regional Development, 2016).

The regional policy of the EU and Poland is focused on deepening and streamlining urban-rural relations. It is expected that *the effects of ITI and CLLD interventions will not be the same in different rural regions* (Table 1). The ITI will relatively apply most to the *suburban rural areas* as these locations are situated within the strong impact of sprawling cities and most often constitute a part of their functional area. In suburban villages the economic problems resulting from the insufficient level of diversification of local economy are often not so urgent. In turn, they are characterised by single family development, various forms of land use, intensive commuting flows, high landscape values, gentrification and advanced multifunctionality (Bański, 2017). In the case of villages located in the neighbourhood of big agglomerations, the ITI could broaden and increase the intensity of existing urban-rural relations, notably in the aspect of integration of labour markets, education and other public and private services (e.g. by development and modernisation of transport infrastructure). This should result in strengthening the urbanisation process, as well as improving the position of the commuters, in the inward direction i.e. from the places of residence to the location where work and services are concentrated (Śleszynski, 2013; Hadyński, 2015). For some localities, the ITI should also contribute to the intensification of pressure towards the growth of the administrative boundaries of cities, which sometimes may result in local conflicts (Table 1).

Table 1. Integrated Territorial Investments and Community Led Local Development: selected opportunities, barriers and risks for rural areas in Poland

OPPORTUNITIES	BARRIERS AND RISKS
Strengthening urban-rural socio-economic relations and creating the synergy effect	Reduction of importance of rural areas in favour of cities, development centres and their functional areas
Increasing the living standard of the commuters	Local conflicts
Increasing the impact of rural population and local authorities on implementing the cohesion policy	Uneven participation in the decision-making process and distribution of financial resources
Development of cooperation between self-governments and local communities	Orientation towards spending resources and implementation of simple short-term projects
Solution of rural areas' urgent problems	Low scale of activity of the local community
Creation of new markets and development of existing supply and consumption markets	Problems in cooperation between self-governments and local communities
	Development of administrative structures and low inputs for supporting projects

Source: Own elaboration.

The ITI increase the scope and capacity of collaboration between self-government bodies. A formal or informal field of cooperation between communes, which is created and developed within this instrument, will include units of different size and economic and personal potential. This may be accompanied by an uneven impact on the decision-making process, rewarding the urban units and not bringing any benefits to rural communes (Table 1). The observed process of capturing EU support by institutions with the high potential often results in reducing opportunities for obtaining mutual benefits, which, in the case of ITI, means

[24] In most cases it is financed from the ERDF and ESF budgets (11 countries), less often only from the ESF (4 countries). In the case of Lithuania, CLLD is financed only from the ESF and, in the case of Sweden, a separate operational programme has been created (Łukaszewska-Krawiec, 2015).

limited possibility for achieving integrated effects. At the same time, the budgetary constraints and other resources provided for the implementation of collective projects makes the urban influence on their selection and implementation higher. On the other hand, an insufficient level of financial support may result in the selection of operations which are simple, not challenging, problem-solving and oriented exclusively towards spending resources (e.g. investment in unnecessary infrastructure) (Table 1). However, the final shape and effects of undertaken urban-rural cooperations will depend, to a large extent, on the developed ways of collaboration, acquired experience, level of involvement of participants and amount of available financial resources.

For *intermediate rural areas* the ITI instrument will be of relatively lower importance. These areas, though located often far from big cities, are characterised by various economic and social connections with other towns and villages. In this case the impact of urban centres does not eliminate a number of elements typical for rural areas, as well as economic problems stemming from the insufficient effect of agglomeration. Intermediate rural locations are often characterised by the lack of identification of major socio-economic function. For some villages located in these areas, especially those linked with the urban functional areas and oriented towards spatial integration, the ITI should be one of useful development policy tools. In these locations, integrated projects may foster the greater networking of rural economic entities (including farms) by creating or strengthening links with urban markets[25] (Table 1). However, for the majority of villages from intermediate area relatively more adequate seems to be the *CLLD* mechanism. The opportunities and effects created by both analysed instruments will depend on the conditions of financial support adopted at national, regional and local levels, as well as on the level of participation and engagement of given communities and authorities.

A significant distance from urban centres along with a predominance of traditional industries, which are based on the use or management of natural resources (i.e. agriculture, forestry, hunting, tourism and recreation, health services) is characteristic of *peripheral rural areas*. Villages located in these terrains often face poor level of technical and social infrastructure, shallow markets and social problems. At the same time, peripheral locations are highly valued due to their amenities, especially environmental resources, which are appreciated by both locals and tourists. It should be assumed that in case of these villages the impact of the ITI will be limited. Among the analysed instruments the CLLD could play a significant role in supporting development processes in peripheries. However, it is stressed that the problems of rural areas should not be tackled using regional policy tools, which are not adjusted to the challenges faced by poor villages. In this context, it is postulated to maintain mechanisms providing services to the countryside and supporting its development by agricultural and rural development policies (Röhl and Schröder, 2017). Nevertheless, nowadays the idea of integration is nowadays often discussed (Ministry of Regional Development, 2010).

The projects within the ITI formula are implemented in Poland in 24 functional areas. In 17 cases, their implementation is carried out within regional capitals and their functional areas (most important agglomerations), and in 7 cases in sub-regional cities and their functional areas. Delimitation of functional areas was prepared by the Polish Ministry of Development. There are two legal forms of ITI implementation in Poland: ITI associations (10 cases) and ITI Partnerships (14 cases). The partnerships of cities and related functional areas have a form of agreements (14 cases) or associations (10 cases) known as unions. In the formulas of cooperation agreements of communes, the leading role is played by a leader i.e. mayor of the city. A prerequisite for implementing ITI is the development of a cross-sectoral strategy that

[25] For example, benefits of joint initiatives may be profitable for farms oriented towards direct sales in cities and towns, as well as for urban dwellers preferring organic food or agri-tourism as a form of spending free time (Peters and Gregory, 2015).

addresses the needs of the area concerned. In Poland more than 550 local government units are involved in implementing ITI by the end of the 2016 (Żygadło, 2017). The most important source of financing the projects within the ITI instrument is the ERDF and ESF provided for the implementation of regional operational programmes (ROP) – approx. 3.8 bln EUR. In total an allocation for implementation of ITI in Poland amounted to more than EUR 6 bln[26] (Żygadło, 2017).

The CLLD as a new instrument of integrated territorial approach policy principle is aimed at encouraging local communities and various entities in the development process of the given area by creating and implementing the LDS. In Poland this tool is implemented under the CAP, Common Fisheries Policy and Cohesion Policy. This instrument has been indicated as appropriate for implementing the measures in areas of strategic intervention regarding cities and districts requiring revitalisation and rural areas with the lowest level of access to goods and services determining development opportunities. As for the Cohesion Policy, the use of CLLD instrument under the ROP in particular region were determined by the decision of self-government authorities. For the time being, CLLD is implemented only in two Polish regions: Kujawsko-Pomorskie and Podlaskie Voivodeship. The vast majority of LAG in Poland have used so far financial support available under the EAFRD. It is estimated that for the CLLD instrument in Poland in 2014-2020 about 900 million EUR was allocated, of which the vast majority came from the EAFRD (82%) (Gierulska, 2016).

Conclusion

The main instruments of implementing the integrated territorial approach concept in the EU Cohesion Policy are ITI and CLLD. The former is dedicated primarily to cities and its functional areas. Its objective is to support joint projects undertaken by self-governments and local communities. The latter mechanism is addressed for the rural residents in order to assist in taking development projects based on endogenous resources. Both policy tools are different not only in terms of the area concerned, way of management, implementation and supervision but also have various size and main source of financing.

Analyses showed that ITI and CLLD instruments are an important component of the current EU Cohesion Policy. They could bring new opportunities for the rural development, particularly in creating and broadening the multidimensional rural-urban relationships. These build-up connections could be strengthened by projects integrating labour markets, investments in education system and healthcare services, as well as by increase of local social capital. It is expected that the implementation of ITI will be especially favourable for the villages located in suburban areas. Though, the ITI projects will include significant improvements in the condition of technical infrastructure and investments conducing environment protection and public health. In the case of intermediate and peripheral rural areas, the impact of the ITI will be rather limited. However, significant opportunities to start or intensify the bottom-up development processes in these territories should be offered by the CLLD mechanism.

In Polish regional policy in 2014-2020, the integrated territorial approach principle concerned the eastern part of the country, cities with functional areas, rural areas and border areas. New instruments related to this policy concept, namely ITI and CLLD are used in Poland as well. The process of implementing of these tools has been launched. It runs relatively slow. So far, the formal, legal and organisational foundations for using granted financial resources from the EU funds have been created and the implementation of various projects has been started. For

[26] Additional support for the urban transport development and energy efficiency use is provided within the Infrastructure and Environment Operational Programme (IE OP) and Eastern Poland Operational Programme (EP OP) – approx. 2.4 bln EUR. The ITI unions are financed from the Technical Assistance Operational Programme (OP TA) (20 mln EUR).

the time being institutions involved in ITI mechanism face many problems and constraints (weak status, focus on building an institutional capacity rather than implementation of integrated projects, lack of territorial dimension of the projects, allocation is treated as granted). Available data showed that ITI strategies were implemented by different partnerships in the country, covering more than 550 self-government units in 24 urban functional areas. In turn, the LDS were implemented by more than 320 LAG. Institutional delays and the initial phase of introduction of many projects do not allow to formulate assessments concerning the effects of analysed interventions, particularly in solving local problems.

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RISK MANAGEMENT IN OCCUPATIONAL EXPOSURE TO PESTICIDES: THE ROMANIAN PERSPECTIVE

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Abstract

This study aimed to establish the importance of controlling exposure and health effects of toxic chemicals used in agriculture, and to raise awareness in rural communities. Data regarding main categories of pesticides, number of exposed workers, number of occupational diseases caused by these chemicals, and the value of biomarkers in certain exposures were collected from plant protection offices and from official public health reports. Specific health effects in agricultural workers were analyzed following a 14-year retrospective study between 1993 and 2006. On the basis of national data and scientific literature review, educational materials were written, respectively a dedicated chapter in the work health promotion guide and a dedicated module for the training course for practitioners of occupational medicine in the agriculture sector. Currently, there are about 9300 Romanian workers applying pesticides in agriculture, while 3523 are involved in mixing, loading, and storage. Occupational intoxications have decreased significantly until 2006 and occur only accidentally nowadays. Activity of serum cholinesterase - significantly decreased at $p < 0.01$ - is a reliable biomarker to be used in occupational exposure to organophosphates and carbamates. In 2017 the training course was accredited as Continuous Medical Education course at European level and is expected to engage more than 10000 participants. Research and official statistics subjected an informational system created to support agricultural communities to better manage the risk posed by plant protection products.

Keywords: *Pesticide, Agriculture, Intoxication, Risk management.*

Introduction

Agriculture is one of the most hazardous occupations based on occupational fatality rates, nonfatal occupational injury rates, and occupational illness rates (Donham and Thelin, 2006). The main chemical risk is posed by a wide range of substances used to combat and control pests, generally called pesticides or plant protection products. Classification of toxic materials under the Globally Harmonized System includes acute toxicity - i.e., lethal dose for 50 percent LD50, skin irritation / skin corrosion, serious eye damage / eye irritation, respiratory or skin sensitization, mutagens, carcinogens, reproductive toxicity, specific target organ toxicity, aspiration hazard, and chemical mixtures. Usually, the Hazard Communication Standard translated into the Safety Data Sheets must be consistent with the Globally Harmonized System of Classification. For many chemicals there will be a dose called threshold dose bellow which no effect or response is observed in individuals. The regulations on plant protection products focus on the active substance and do not apply to the potential combined effect resulting from the concomitant use of several formulations (i.e., mixture) as applied in practice, therefore it must take into account the cumulative exposure to more than one active substance (Kienzler *et al.*, 2016; US EPA, 2015). However, the best approach is to always keep exposures or the risk of a hazard as low as possible.

The threat for farmers and operators is posed especially by organophosphates, carbamates and pyrethroids. The main target-organs are skin, lungs and central nervous system, and chronic effects are mainly respiratory (bronchial asthma) and neurological diseases (deficits in neurobehavioral performance, abnormalities in nerve function, and Parkinson's disease),

diseases of the reproductive system, as well as different types of cancer (California Department of Public Health, 2015).
Scientific evidence suggests aluminium (heavy metal) is involved in Alzheimer's disease (Dyck, 1994; Yokel, 2000), while pyrethroids are known to cause respiratory and skin problems – asthma and allergies. Organophosphates are by far the most toxic pesticides responsible for neurotoxicity (acute and chronic) *via* inactivating acetylcholine esterase (Frumkin, 2010). In humans, important families of enzymes are involved in xenobiotic metabolism. For example, unusual variants of paraoxonase (PON) have recently been associated with genetic risk to organophosphate exposure, while the variant PON1 may detoxify the pesticide poisoning more efficiently (Kaur *et al.*, 2017).
Carbamates and aluminium phosphide are also associated with acute toxicity. Organochlorine pesticides may have developmental, endocrine and reproductive effects (Frumkin, 2010). Naphthalene, the first registered pesticide in the USA in 1948, is now classified by the International Agency for Research on Cancer as a possible human carcinogen. Used as a household insecticide against moths, it was replaced with 1-naphthyl-*N*-methylcarbamate.
The objective of the present paper is to provide Romanian evidence on occupational pesticide exposure, and to encourage the use and/or development of new instruments to raise awareness in relevant communities involved in risk management and rural economy.

Material and Methods

A retrospective study was conducted on agricultural workers chronically exposed to pesticides between 1993 and 2006 following the eligible criteria of occupational poisoning.
In order to evaluate the current situation in terms of exposure and health effects, quantitative and comparative analysis was done on the basis of the following data sources:
1. Occupational Morbidity in Romania, managed by the Operative registry book of occupational diseases, monthly updated (National Institute of Public Health, 2017);
2. Health and Environment Report (National Institute of Public Health, 2014): number of workers handling pesticides, occupations, geographical distribution, reported symptoms, and reliable biomarkers - activity of serum cholinesterase (CHE). Normal blood values of CHE vary widely between individuals therefore it is best to have individual baseline test results: 20% depression from baseline suggests a possible poisoning; 40% depression of serum CHE suggests a diagnosis of acute poisoning and removal from work exposure (Donham and Thelin, 2016). The Romanian study considered normal limits for serum CHE concentration (U/L) within 4620 – 11500 in males; and 3930 – 10800 in females.
3. *Phytosanitary Authority of Sibiu*: evidence of commercial pesticides approved in 2017, according to European Regulations.
The threshold limit value – time-weighted average (TLV-TWA) is the concentration for a normal 8-hour workday, repeatedly day after day without adverse effect. For example, TLV-TWA = 0.5 mg/mc air for pirimiphos (organophosphate) and zineb (carbamate); and TLV-TWA = 1 mg/mc air for pyrethrum (Romanian Government Decision, 2006).
The preparatory stage for training course included an up-to-date literature review, reports, statistics and publications on good practices on prevention on occupational diseases in agriculture. The original search was conducted in 2015 (Stoia, 2015), and updated in 2017 as part of the AGROSH+ Project (Stoia, 2017).

Results and discussion

The number of occupational poisonings due to pesticides has decreased within the period 1993-2006 (Figure 1); the number of exposed workers was, on average, 7628 ± 1237; and 7% of those workers were exposed to concentrations above TLV-TWA.

Since 2007 no incidence of pesticide poisonings was reported in workers. The reasonable explanation was law enforcement due to harmonization with European Union Regulations, putting in place regulations regarding the distribution, selling and use of pesticides, a proper recording, certifying and labeling of the products, regulations regarding the tolerable amounts in foodstuffs, and proper occupational risk management (Romanian Parliament Act, 2006).

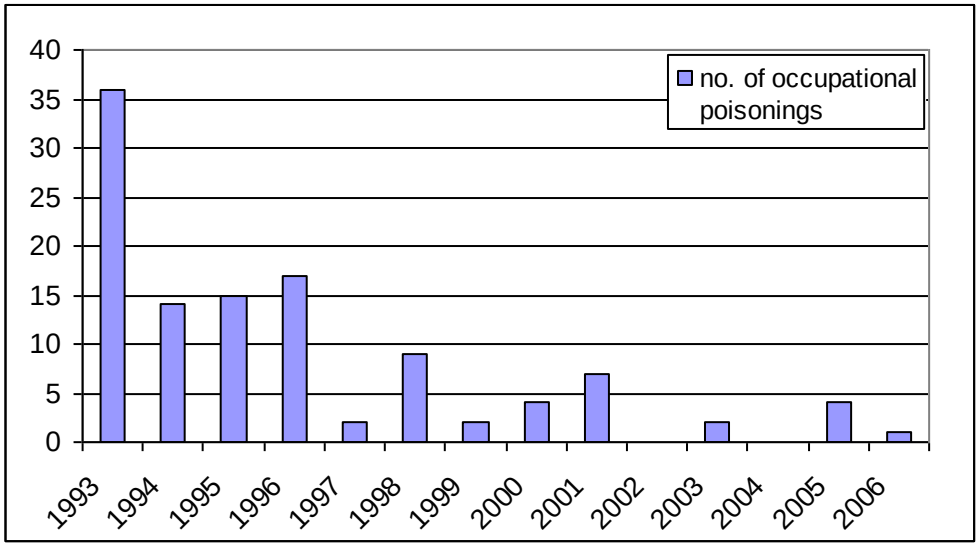

Figure 1. Incidence of occupational poisonings due to pesticides between 1993 and 2006

Since 2005, the most common occupational diseases in Romanian agriculture were respiratory diseases (chronic bronchitis, bronchial asthma, and hypersensitivity pneumonitis caused by organic dust allergens) and musculoskeletal disorders (National Institute of Public Health, 2017). This issue may point out the fact that pesticide poisonings commuted into occupational diseases by target organ systems (respiratory diseases) and the comparative exercise is nevertheless still useful to derive the important trends of the future.

Data reported by the National Institute of Public Health in 2014 show a number of 12823 Romanian exposed workers including 9300 pesticide-users, 1572 warehouse-operators, and 1951 others. Typically, pesticide use is associated with mixing and loading the product, application of the spray solution, and clean-up of the spraying equipment (Damalas and Koutroubas, 2016). The greatest part of users is distributed in the South, i.e. 2182 self-employed cultivators in the Olt County. Skin and eye irritation are the most frequent symptoms workers complain about when handling pesticides.Organophosphates affect the nervous system by altering the efficacy of the enzyme CHE, and enzyme levels may be used as a molecular biomarker in risk assessment (Robson and Toscano, 2007). CHE was shown to be specific for the exposure to organophosphates and carbamates in the Romanian study, exhibiting low concentrations compared with controls but within the acceptable limits, as summarized in Table 1. Sometimes, elevated transaminases occur in liver injury produced by certain pesticides, as well as elevated glycaemia and risk for diabetes (Ki-Hyun *et al.*, 2017).

Table 1. Comparison between the average concentrations of CHE in pesticide-exposed workers and controls.

Group (N = 100)	**CHE (U/L)**	**Standard deviation**	**Student test t**	**p**
Exposed	6773.3	±1596.2	3.09	**< 0.01**
Controls	7714.7	±1355.2		

Source: National Institute of Public Health.

In Romania, control and approval of pesticide products is performed by the Ministry of Agriculture and Rural Development *via* local phytosanitary offices. Commercial plant protection products in use on January 2018 are listed in Table 2.

Table 2. List of plant protection products commonly used in Romanian agriculture.

No.	Chemical class	Common name	Hazard symbol
1	Aluminium derivatives	Delicia Gastoxin, Quickphos-Up, Agroxin	T+
		Aliette 80 WG	Xi
2	Carbamates	Vydate 10 G	T+
		Royalflo 42 S	Xn
		Novozir MN 80, Dithane M-45, Topsin 70 WDG	Xi
3	Organophosphates	Pyrinex 48 EC, Novadim Progress - Sinoratox 400 EC, Nemathorin 10 G	Xn
4	Organochlorines	Bravo 500 SC	Xn
5	Synthetic pyrethroids	Force 20 CS, Poncho Beta FS 453, Faster 10 CE, Fury 10 EC	Xn
		Confidor Oil SC 004, Confidor Energy, Decis 25 WG	Xi
6	Neonicotinoids	Nuprid 600 FS, Picus 600 FS, Seedoprid 600 FS, Calypso 480 SC, Biscaya 240 OD	Xn
		Cruiser 350 FS, Actara 25 WG	Xi
7	Imides	Rovral 500 SC, Merpan 80 WDG - Captadin 80 WDG	Xn
		Captan 80 WDG - Caiman	Xi
8	Triazoles	Impact 125 SC, Caramba 60 SL, Misha 20 EW	Xn
		Score 250 EC, Topas 100 EC	Xi
9	Amides	Armetil Cobre	Xn
		Equation Pro (mix *amide* + *dione*)	Xn
		Infinito 687, 5 SC	Xi
10	Abamectin from *Streptomyces avermitilis*	Vertimec 1.8% EC	Xn
11	Dinitrophenol	Karathane Gold 350 EC	Xn
12	Morpholine	Acrobat MZ 69 WG	Xn
13	Metaldehyde	Agrosan B	Xn
		Optimol, Allowin	Xi
14	Aromatic anilide	Signum	Xn
15	Copper and compounds	Funguran OH 50 WP	Xn
		Copac, Alcupral 50 PU	Xi
16	Sulphur	Thiovit Jet 80 WG	Xi
17	Urea derivatives	Dimilin 48 SC	Xi
18	Methoxyacrylate	Ortiva 250 SC	Xi

Legend: **T+** "very toxic"; **Xn** "harmful"; **Xi** "irritant".
Source: Phytosanitary Authority of Sibiu County.

The term "pesticide" may communicate an unintended negative perception based on fear, therefore terms such as insecticide or herbicide, or even the class of chemical, are more appropriate. Agriculturalists often utilize the term "plant protection products" to help avoid the general negativity associated with "pesticides" (Donham and Thelin, 2016). Reducing pesticide use was demonstrated to rarely decrease productivity and profitability in arable farms (Lechenet *et al.*, 2017). Complementary to risk management in Romanian agriculture, the educational activity was provided by Romtens Foundation of Bucharest in collaboration with experts and consisted in two main results, namely: (1) editing and distribution of a guidance book to 200 occupational health and safety specialists, in 2015; (2) training and providing additional qualifications for about 30 practitioners of occupational medicine in the agriculture sector, in November of 2017, as part of the project "OSH+ for the European Agriculture sector - Stimulating growth in rural areas through capacity building for providers (and beneficiaries) of occupational medicine and OSH services" (AGROSH+)[27]. The training material was peer reviewed and accredited as Continuous Medical Education course at European level. This project aims to have more than 10000 participants who will be getting in contact with the project activities or results. Increasing the role of occupational health services and physicians, including important issues such as training, and legal protection to allow them to do their jobs without fear of sanctions by employers is of great importance in preventing occupational diseases (European Commission, 2013). In the future, it would be very useful to raise the awareness of the national health care system with respect to a comprehensive prevention plan.

Conclusions

The commercial plant protection products used in Romania are harmful, mostly of them, and the health professionals should find out what pesticides are used in their community. In the past, the incidence of occupational poisonings due to pesticides was about 8 new cases per year. Since 2007, specific morbidity in agriculture belongs to respiratory diseases. In particular, pyrethroids are recognized as causal factors for bronchial asthma. At this time, there are a few instruments to measure a specific effect in the human body, and the most reliable biomarker is CHE activity depressed by organophosphates and carbamates. In 100 Romanian agriculturists the enzyme was found within normal range, significantly different in the exposed group of subjects compared to controls. Because pesticides can cause syndromes that are difficult to differentiate from other types of common diseases, the occupational history is essential, as well as identifying the toxic product that the worker has been exposed to. The relative acute human toxicity of insecticides classes from most to least toxic is organophosphates > carbamates > organochlorines > pyrethroids > neonicotinoids. Generally, uncertainty is present in all risk assessments and it occurs because of a lack of knowledge, therefore it is important to detect new adverse occupational health consequences. In the future, it would be very useful to develop new instruments of information and hazard communication in rural communities with respect to their health needs and local economy.

Acknowledgement

The author is grateful to Dr. Theodor Haratau (Romtens Foundation, Bucharest), the project manager of AGROSH+, for partnership and support.

[27] Details are given at: http://www.agrosh.ro (e-learning section including the training manual requires log-in access).

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THE IMPORTANCE OF TV SHOWS FOR FARMERS AND THEIR PRESENCE IN THE MEDIA SPACE OF SERBIA AND REGION

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Abstract

Starting from the importance of television, due to its close relationship with viewers, as a convenient means of informing and education, its offer of special contents that can have an impact on the acquisition of specific knowledge and motivation of agricultural producers in Serbia has been analyzed. The research for this paper was to determine the importance of special programs intended for countryside and agriculture to inform producers as well as to establish the confidence of agricultural producers regarding information provided by such content. A sample that covered 314 inhabitants of rural areas in Serbia was created. A questionnaire was used as a test instrument, which consisted of a group of questions relating to the respondents' views of special programs intended for agricultural producers, as well as assessing the usefulness of the information received and trust in the subjects in the programs. The programs that were analyzed were broadcast on four television channels that have a national frequency, two of which are public and two are commercial. Compared with other ways of informing about news in agricultural production, among agricultural producers, TV shows about agriculture are in second place immediately behind Agricultural Advisory Services. Almost half the respondents (47.8%) singled out interviews with government officials and attachments that recommend different products as the least useful information. When it comes to trust in the subjects that speak in programs about agriculture, the surveyed viewers give priority to experts (almost every second viewer), while in second place are agricultural producers.

Keywords: *television, information, knowledge, agricultural producers*.

Introduction

Television is the most influential medium and the most numerous auditorium (McQueen, 2000), which provides it with deep penetration into all spheres of society. Media theorists speak about the diverse functions that the media perform (fun, escapism, distracting, socializing and ideological), and as the primary ones, the informative and educational functions of television have been identified (Lorimer, 1998; McKuley 1976; McKinn, 2000). The transfer of information using television technology, which includes sound and image, contributes to the formation of a knowledge system in people (Marković, 2010). Information and knowledge are two important factors for rural development. The knowledge of the locality further assists the farmers. Dissemination of information along with new concepts and farming techniques can bring new opportunities for farmers (Mohammad et al., 2010). FAO (2001) reported that television is the most important medium for communicating information among rural people in developing countries. In rural areas, people are facing many problems and obstacles to get information about agriculture. Farmers have little accessibility and access to information in rural areas. In remote rural areas, where direct contact between advisers and farmers is difficult due to underdeveloped infrastructure and inaccessibility, mass media (radio and television) are useful tools for improving the knowledge of farmers (Nazari et al., 2011). The notion of television content intended for the rural population besides being informational and educational must also have a motivational character to encourage the viewers to personal action and the acceptance of contemporary methods of production

(Šarković, 2016). The largest number of studies on the impact of media acquisition and dissemination of specific knowledge in agriculture has been achieved in developing countries where television is still, regardless of the increasing presence of the Internet and its influence, the most important mass media in informing and educating inhabitants of rural areas. Among the areas studied is India, where agriculture accounts for about half the population, that most frequently lives in poor rural areas where radio and television are the most important sources of information and education (Kappor, 2011). Kappor (2011) stated that research evaluating the efficiency of programs for the rural population in the Hisar district showed the following:
1 - emissions for the rural population were not as attractive as most programs because they were based on monologues with low visual content;
2 - many more recommendations by TV specialists were intended for large producers than small farmers who make up the majority of the rural population.
Research which analyzed the environmental awareness of residents in two rural provinces in Turkey (Akca, 2007) showed that television and print are the main sources of information about the environment among the rural population. Television as a medium has the potential to improve the information and knowledge of farmers. There is a need to produce more agriculture-related programs on television and to adapt the broadcasting time to agricultural producers so that they can make better use of television content (Chhachhar et al., 2012). Producers that are more intensive than others follow specialized broadcasts for farmers and show a higher degree of concern for the environment. The level of information also shows a high correlation with the adoption of ecological practices, such as soil analysis and harvesting of harvest residues (Šarković, 2016). Most of the television stations that were part of Yugoslav Radio Television (JRT) used to broadcast on their national channels programs dedicated to agriculture and the village. This can be considered somewhat of a tradition as these are, most often, the longest-running broadcasts in the program scheme.
On the former Television Belgrade and Zagreb, specialized shows for rural communities and agriculture have been broadcast since the year of their establishment (1958). Today, RTS1 broadcasts *Knowing-Having*, and the Croatian National Television HRT1broadcasts "Fruits of the Land". At RT Republic of Srpska (RTRS), one of the oldest programs on agriculture called *Bundle* has been broadcast since 1993. Federal Television (FTV) Bosnia and Herzegovina broadcasts a program called *Green Panorama* on Sundays. Television Slovenia broadcasts the show *People and the Earth*. All programs dedicated to rural communities and agriculture are broadcast on Sundays on Channel 1 of these television stations.
Television has a strong role in informing and educating agricultural producers, having special programs on rural communities and agriculture in public media service programs in Europe. The British public service BBC, on its first channel, has broadcast a show once a week since 1988 called *Countrylife* for 60 minutes. The Austrian Public Service (ORF) broadcasts the *Land and Leute* Program for 26 minutes every day at 16.30 on the ORF2 channel. The Italian National Television RAI (RAI) on its first channel on Sundays at 12:20 pm broadcasts a special program dedicated to agriculture and the development of villages with special themes for each show. The series is called the *Green Line* (Linea Verde), and each show lasts about 56 min. The Hungarian MTVA's public broadcasting service on its first M1 channel once a week broadcasts a program entitled *Magary Boss* for 20 min. All these broadcasts confirm that television in many parts of the world accepts that in addition to its informative function, it has the function to educate viewers.
With this background of widespread agricultural programs targeting rural communities and farmers, the objectives of this research were to determine the importance for rural inhabitants of Serbia of special programs intended for countryside and agriculture to inform producers as well as to establish the confidence of agricultural producers regarding information provided by such content.

Material and Methods

In the theoretical part of the research, scientific papers and professional journals were analyzed. The presence and content of specialized programs for farmers in the media channels in Serbia and the region were also monitored. In the empirical part of the research, a sample of 314 inhabitants of rural areas in Serbia was made for collecting reliable and relevant indicators using a questionnaire. Serbia has four national television channels, two of which are public and two are commercial. The sample included a monthly one-time broadcast throughout 2012. These were "Knowing-Having" - RTS, Broadcasts - RTV, "Good Land" TV show-TV B92 and "Host" show - TV Prva.

Results and Discussion

In the period January-December 2012, the broadcasts/programs for agriculture and the village, which were broadcast on four television channels with national frequencies had ratings of between 2.4 and 1.2 (Table 1). The most-watched broadcast was "Knowing-Having" (ZnanjeImanje) whose rating (2.4) was twice that of Host (Domaćin) (1.2) on TV Prva (Josifović and Senić, 2012). The explanation for these results should be sought in the long-standing tradition of monitoring the Public Service programs of Serbia that has existed since 1958, as this media institution, in its various program contents, is engaged in the field of countryside and agriculture. Of these four programs, the program "Knowing-Having" (ZnanjeImanje) has the longest tradition, having been broadcast since 1972, and has also broadcast "Furrows" (Brazde) since 1975. The other two programs have a significantly shorter tradition of broadcasting (TV B92 - 2002; TV Prva - 2007).

Table 1. Annual rating of analyzed emissions

Broadcast title	Rating	
	Average number of viewers per minute	%
Host	81000	1.2
Good Land	112000	1.6
Furrows	123000	1.8
Knowing Having	166000	2.4

Source: Šarković (2016).

In this survey, informing the population in rural areas was observed through information on new and modern methods of agricultural production that would ensure yields and product quality. The results of the survey, which included 314 inhabitants of rural areas in Serbia, showed that the highest percentage of respondents (22%) were informed about the novelties in agricultural production through the Agricultural Advisory Services (AAS), while a slightly lower percentage of respondents (19.1%) selected TV shows on agriculture.

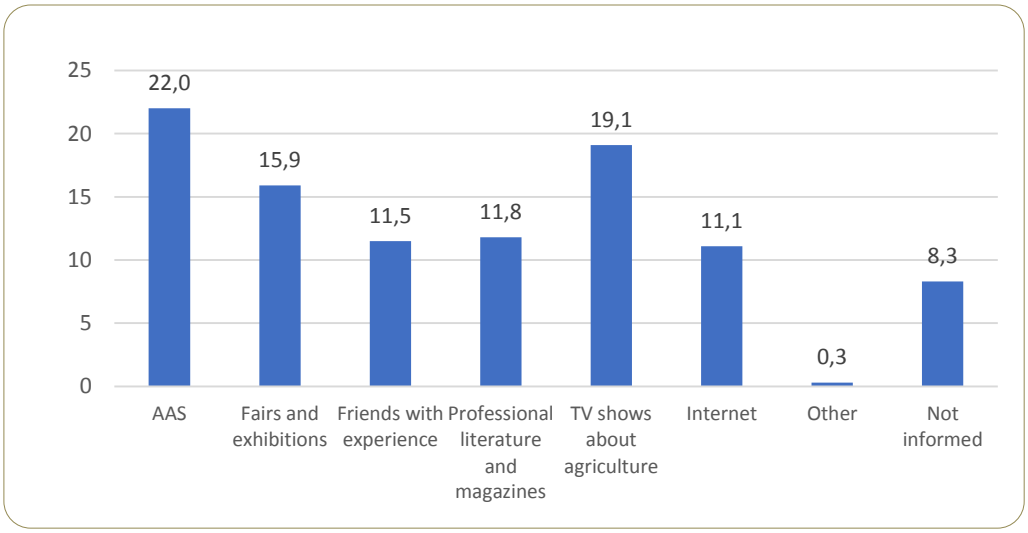
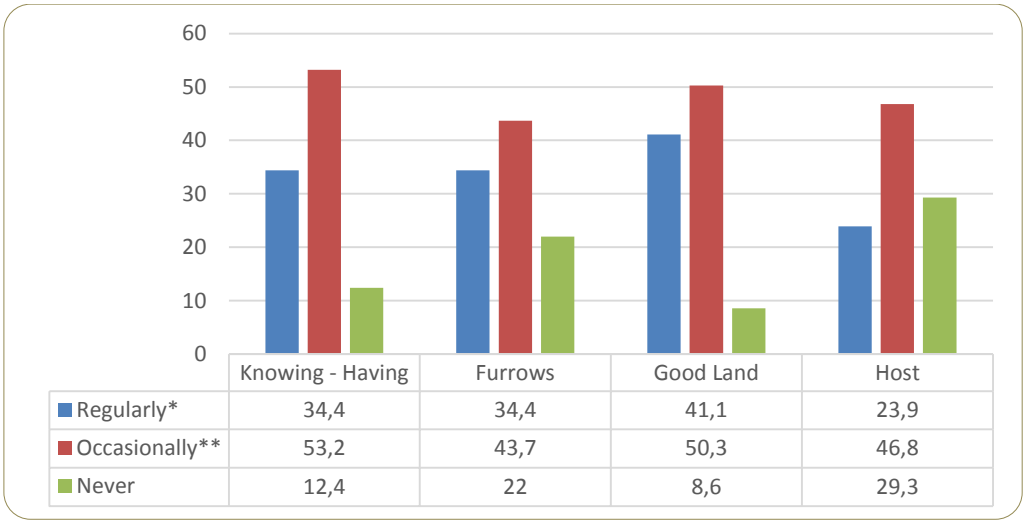


Figure 1. The most important sources of information on news in agricultural production.
Legend: AAS - Agricultural Advisory Service.

The highest percentage of regular viewers (41.1%) was for the program "Good Land" (Dobra Zemlja), and the smallest (23.9%) for the program "Host" (Domaćin), while "Znanje-Imanje" (Knowing–Having) and "Furrows" (Brazde) changed regularly, with the same percentage of respondents (34.4%) being regularly monitored. Further analyzes showed that the total viewers (regular and occasional viewers) for each of the above mentioned shows was more than 70% of the analyzed sample (Figure 2).



	Knowing - Having	Furrows	Good Land	Host
Regularly*	34,4	34,4	41,1	23,9
Occasionally**	53,2	43,7	50,3	46,8
Never	12,4	22	8,6	29,3

Figure 2. Frequency of watching programs on agriculture.
*Regularly - once to four times a month
**Occasionally - once in three months and rarely
Source: Šarković (2016).

Regarding the usefulness of different sources of information for respondents watching the programs (Table 2), almost half of the respondents considered that the least useful interviews were with state officials and contributions suggesting different products (47.8%), and slightly more than a third of viewers (35.6%) thought that all contributions were useful. A very small percentage of viewers (6.1%) identified reports on farmers and their experiences as the least

useful information. When it comes to trust in subjects that talk about topics from different fields of agriculture in agricultural programs, viewers generally gave their preference to experts in these areas (almost every second viewer). The experiences of other farmers were most believed by every fourth respondent, and experts from the Agricultural Advisory Services (AAS) were trusted by around 15% of viewers. Significantly smaller percentages were found for other sources of information. Thus, science, experience and profession give the highest confidence to agricultural producers who viewed these four programs.

Table 2. Trust in the sources of information given in the four agriculture programs

Source of information	%
Experts	46.8
Authorities / government officials	1.3
AAS - Agricultural Advisory Services	15.0
Representatives of the company recommending the product	0.0
Agricultural producers	22.6
Journalists	0.3
To all	1.3
Nobody	3.5
Don't watch agricultural shows	9.2
Total	100

Conclusion

Television as a medium has exceptional potential for transmitting information, advice and opinions from the domain of modern agriculture. At the same time, through image and sound, television directly influences the acceptance of different norms and rules to change behavior and to accept good agricultural practice. Compared with other ways of informing about news in agricultural production, among agricultural producers, TV programs about agriculture come in second place immediately behind the Agricultural Advisory Services (AAS). Almost half the respondents (47.8%) singled out interviews with government officials and attachments that recommend different products as the least useful information. When talking about trust in subjects in agricultural programs, the surveyed viewers gave preference to experts (almost one in two) while agricultural producers were in second place. Television as a medium did not fully use its potentials for transferring knowledge and information to farmers in Serbia. Experts who appear in shows with clear and simple messages will contribute to overcoming the gap between scientists, researchers and agricultural producers as the end users of information. Journalists and editors of specialized programs intended for farmers, creating visually attractive content at the same time, would educate and motivate farmers to adopt good agricultural and environmental practices.

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LAND CONSOLIDATION AND PROFITABILITY RATIOS IN AGRICULTURAL PRODUCTION

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Abstract

Agriculture production nowadays is considered as a commercial activity. Consequently, it must be treated through the market tools of analysis. One among many strategic activities in development of agricultural production is land consolidation. Relations between land consolidation and profitability of agricultural production are complex and not clearly visible. Method for research on those relations could be based on researching relations structure between land consolidation and profitability of agricultural production. The connection between those two phenomena in economics is analysed through the profitability ratios. Profitability ratios in final form are simple equations between two measurable values and they are obtained *a posteriori* after realization of agricultural products on the market. When planning land consolidation numerous unknown parameters exists and it is almost impossible to predict all of them and consequently the prediction of profitability is very uncertain. Bearing in mind the importance of agricultural production and accepting the assumption that in next decades the demands for food will increase or, at least will keep the actual level, it is possible to develop models for prediction of profitability in agricultural industry depending on land consolidation. The analysis is based on micro economic level which encompasses the structure of costs and gains in agricultural production as well as certain intervals in which the parameters of profitability could belong in the future period of time. According to those intervals, the different scenarios were investigated. The profitability ratios were analysed depending on fixed and variable costs where fixed and variable costs were decomposed on the part which depended on land consolidation and part which was independent of land consolidations. The research and results are predominantly in the theoretical context including efforts to be done on the base of available data from practice in Republic of Serbia.

Key words: *Land consolidation, Profitability ratios, Parcel size, Economics.*

Introduction

Land consolidation is a process which is expected to improve agricultural production on the area where it is provided. Measuring effects of land consolidation is very complex and process tied with lots of uncertainties depending on parameters which are intended for measurements. This is a consequence of market nature of contemporary agriculture. On the one hand, the land as a base of agricultural production should be managed very carefully in order to keep and improve its arable characteristics and, on the other side, the market results are also expected in order to provide economically sustainable agriculture. It is obvious that those two principles are opposite and conflicted.

Land consolidation evaluated from the beginning of its appearance in the fourteenth century (Van Dijk, 2003; Uimonen, 2004) with the emergence of legislation in the middle of the eighteenth century. Beginning with the aim to group fragmented land ownership it evolved to one of the most powerful tools for agricultural land management which should provide improvement of agricultural productivity. The following definition include elements which explains land consolidation as a complex process with final aim of increasing agricultural

productivity: "Land consolidation is a land management activity that involves all the procedures for exchanging, rearranging and expanding farmland parcels with the goal of increasing farm productivity" (Asiama et al., 2017). The main aim of this paper is in the concordance with this definition of land consolidation and the research will be provided according to it. The second group of aims is connected with profitability in agriculture. In the contemporary circumstances the agricultural products are realized on the market. If the agricultural production is profitable it could be capable for investments and, consequently, for development. Bearing in mind that profitability depends not only on productivity but as well on the market conditions (which are mostly unpredictable and predominantly tied by prices of agricultural products) it is necessary to analyse the structure of different influences on agricultural productivity and its potential contribution to profitability in agricultural production. The profitability ratios in economy are well known and explicated in simple formulae, but analysing their structure they could provide a lots of information about certain economic activity in agricultural production. Bearing in mind that lot of information are not available in analysing profitability ratios some assumptions are introduced in proposed methodology.

This paper aims to analyse the effects of land consolidation, as a tool for land management which shall meet the both requirements: improvement of economic results in agriculture and savings and improvement of arable land characteristics.

Material and Methods

The paper is based on a model which has been developed to research different scenarios for profitability ratios in agricultural production in case of different levels of land consolidation influences.

The common and well known profitability ratios are:

- Profit Margin on Sales;
- Basic Earning Power;
- Return on Total Assets;
- Return on Equity;
- Return on Investment and
- Earnings per Share.

Bearing in mind that land consolidation predominantly affects the productivity and real assets (land) of agricultural production in this paper the following ratios were investigated: Profit Margin on sales, Return on Total Assets and Return on Investments. Basic Earning Power, Return on Equity and Earnings per Share are dependent on the factors which are not directly influenced by land consolidation and consequently were not included in this research. The basic assumptions in this research are that assets and prices are constant after land consolidation. It is possible to introduce changes in asset and prices of agricultural products and but it require broader approach and it is not main focus of this research.

Profitability ratios are given by following formulae:

$$\boldsymbol{PMS = \frac{NI}{S}} \qquad (\mathbf{1})$$

where:

- $\boldsymbol{PMS}$ - Profit Margin on Sales ratio;
- $\boldsymbol{NI}$ – Net Income and
- $\boldsymbol{S}$ - Sales

$$\boldsymbol{ROA = \frac{NI}{A}} \qquad (\mathbf{2})$$

where:

- $\boldsymbol{ROA}$ – Return on Total Assets ratio;
- $\boldsymbol{NI}$ – Net Income and
- $\boldsymbol{A}$ – Total Assets

$$\boldsymbol{ROI = \frac{IR - IC}{IC}} \quad (\mathbf{3})$$

where:

- $\boldsymbol{ROI}$– Return on Investment ratio;
- $\boldsymbol{IR}$ – Investment Revenue and
- $\boldsymbol{IC}$– Investment Cost.

Analysing the structure of given formulae (1), (2) and (3) immediately follows that land consolidation should increase the net income, Sales and investment costs. Also it is assumed that investment revenues will increase after land consolidation and that assets will remain the same before land consolidation.

Net income, sales, investment revenue and investment costs could be calculated by following formulae:

$$\boldsymbol{NI = n * (p_u - c_u)} \quad (\mathbf{4})$$

$$\boldsymbol{S = n * p_u} \quad (\mathbf{5})$$

$$\boldsymbol{IR = (n + \Delta n) * p_u} \quad (\mathbf{6})$$

$$\boldsymbol{IC = \alpha_{LC} * c_\alpha} \quad (\mathbf{7})$$

where:

- $\boldsymbol{n}$ – number of agricultural production units;
- $\boldsymbol{p_u}$ – price per agricultural product unit sold;
- $\boldsymbol{c_u}$ – cost per agricultural product unit sold;
- $\Delta\boldsymbol{n}$ – increased product units as a result of land consolidation;
- $\boldsymbol{\alpha_{LC}}$ – the land consolidated area and
- $\boldsymbol{c_\alpha}$ – the cost of land consolidation per unit of land consolidated area.

Rearranging formulae (1), (2) and (3) we obtain as follows:

$$\boldsymbol{PMS = \frac{n * (p_u - c_u)}{n * p_u} = 1 - \frac{c_u}{p_u}} \quad (\mathbf{8})$$

$$\boldsymbol{ROA = \frac{n * (p_u - c_u)}{A}} \quad (\mathbf{9})$$

$$\boldsymbol{ROI = \frac{(n + \Delta n) * p_u - \alpha_{LC} * c_\alpha}{\alpha_{LC} * c_\alpha} = \frac{(n + \Delta n) * p_u}{\alpha_{LC} * c_\alpha} - 1} \quad (\mathbf{10})$$

Analysis of profitability ratios shall be also provided before and after land consolidation. This is illustrated by figure 1 and explication of model is given by formulae as follows:

$$\Delta PMS = PMS_a - PMS_b = 1 - \frac{c_u^a}{p_u} - 1 + \frac{c_u^b}{p_u} = \frac{c_u^b - c_u^a}{p_u} = -\frac{c_u^b - \Delta c - c_u^b}{p_u} = \frac{\Delta c}{p_u} \quad (11)$$

$$\Delta ROA = ROA_a - ROA_b = \frac{n_a * (p_u - c_u^a)}{A} - \frac{n_b * (p_u - c_u^b)}{A} \quad (12)$$

$$\Delta ROA = ROA_a - ROA_b = \frac{(n_b + \Delta n) * (p_u - c_u^b + \Delta c)}{A} - \frac{n_b * (p_u - c_u^b)}{A} \quad (12a)$$

$$\Delta ROA = \frac{n_b * (p_u - c_u^b)}{A} + \frac{\Delta n * (p_u - c_u^b + \Delta c)}{A} - \frac{n_b * (p_u - c_u^b)}{A} \quad (12b)$$

$$\Delta ROA = \frac{\Delta n * (p_u - c_u^b + \Delta c)}{A} \quad (12c)$$

$$\Delta ROI = \frac{(n_b + \Delta n) * p_u}{\alpha_{LC} * c_\alpha} - 1 \quad (13)$$

where indexed or exponential letters a and b means the value after and before land consolidation and:

- ΔPMS – difference of Profit Margin of Sales after and before land consolidation;
- ΔROA - difference of Return on Total Assets ratio after and before land consolidation;
- ΔROI - difference of Return on Investment ratio after and before land consolidation;
- Δn – increment in agricultural production units after land consolidation and
- Δc – increment of costs per agricultural unit produced after land consolidation (should be negative value related to costs before land consolidation i.e. the costs should be reduced related to the value c_u^b).

The values of prices (p_u) and assets (A) are adopted as a constant because the prices of agricultural products are not affected by land consolidation and the value of land after land consolidation shall be the same as before it.

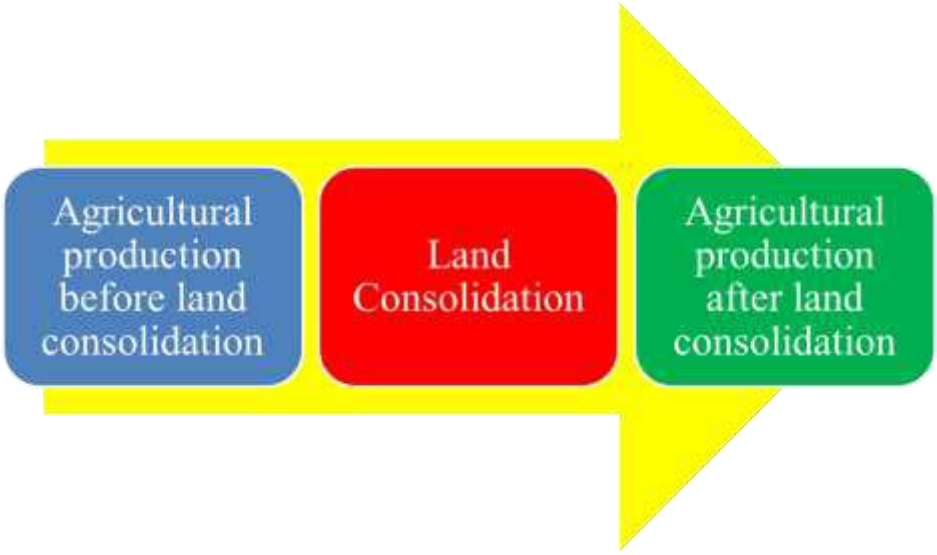

Figure 1. Timeline of land consolidation influence on profitability ratios analysis

Finally, proposed models for research on the influence of land consolidation on profitability ratios are as follows:

$$\Delta PMS = \frac{\Delta c}{p_u} \tag{14}$$

$$\boldsymbol{\Delta ROA = \frac{\Delta n * (p_u - c_u^b + \Delta c)}{A}} \tag{15}$$

$$\boldsymbol{\Delta ROI = \frac{(n_b + \Delta n) * p_u}{\alpha_{LC} * c_\alpha} - 1} \tag{16}$$

Results and Discussion

According to formula (14) it is obvious that the contribution of land consolidation to the Profit Margin of Sales is proportional to and linear dependent on costs reduction per agricultural unit produced. If the cost reduction was explicated as a percentage of the constant price per agricultural production unit, the formula (14) reads:

$$\Delta PMS = \frac{\Delta c}{p_u} = k\frac{p_u}{p_u} = k \tag{17}$$

The graphical interpretation of land consolidation contribution to the Profit Margin of Sales is shown on figure 1.

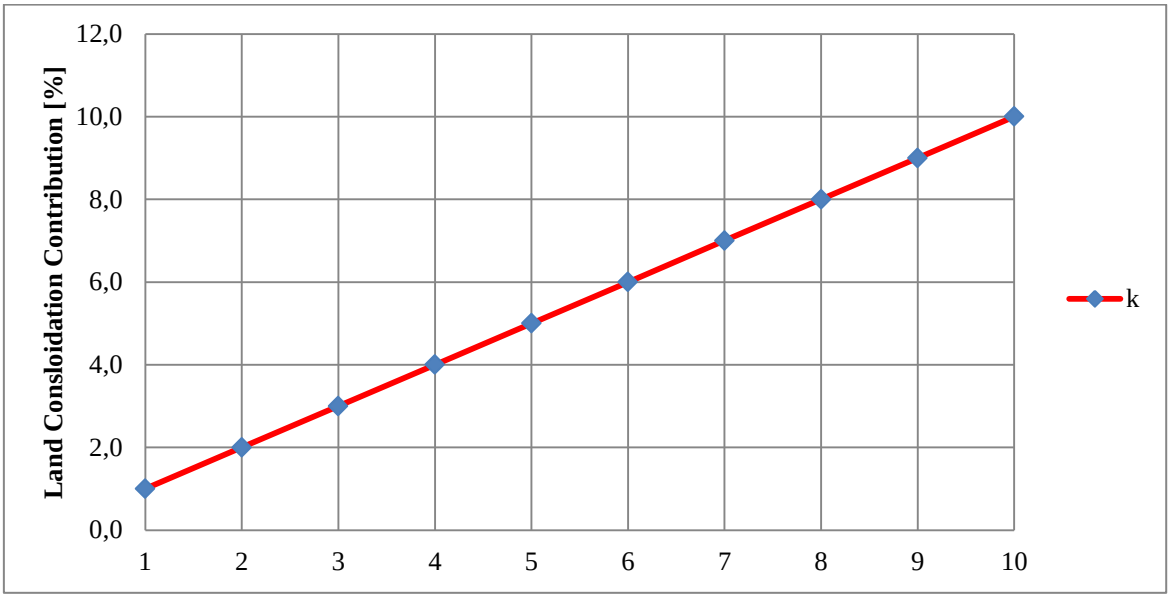

Figure 1. Land consolidation contribution to the Profit Margin of Sales ratio.

Analysis of contribution of land consolidation to return on total assets ratio (15) leads to conclusion that it is dependent on increment of agricultural production and cost reduction. The total assets A shall be constant before and after land consolidation and consequently it should not affect the return on asset ratio from the aspect of land consolidation.

For the accepted values p_u=0.15$ per agricultural unit produced, costs before land consolidation per agricultural unit produced c_u^b=0.10$ and if increment of production belongs to the interval [100,1000] with cost reduction which belongs to the interval [-0.05, -.01] $ per agricultural unit after land consolidation the obtained results for land consolidation contribution belongs to the interval $[\frac{6}{A}, \frac{100}{A}]$. The graphical interpretation of result is shown on figure 2.

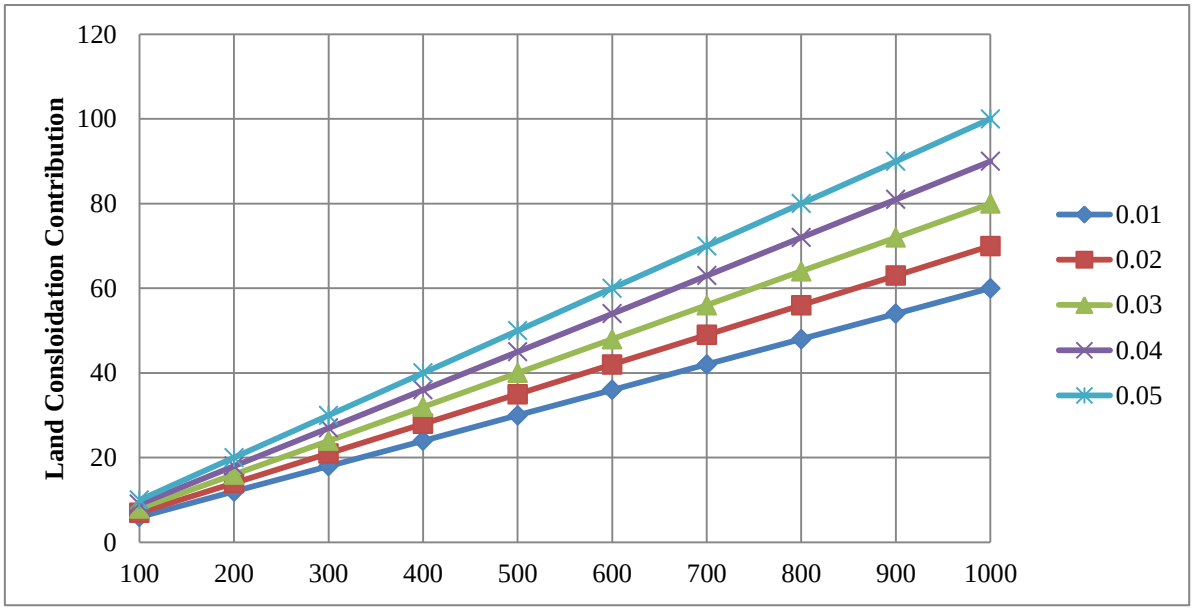

Figure 2. Land consolidation contribution to the Return on Assets Ratio.

Return on investment depends on the land consolidation contribution to the agricultural production explicated by increment Δn and of the land consolidation cost per area unit. The return on investments could be determined per area unit and consequently formula (16) reads:

$$\Delta \boldsymbol{ROI} = \frac{(\boldsymbol{n_b} + \Delta \boldsymbol{n}) * \boldsymbol{p_u}}{\boldsymbol{c_\alpha}} - \mathbf{1} \qquad (\mathbf{18})$$

In that case the Δn and c_α are related per unit of area i.e. per hectare. In the case of accepted values p_u=0.15\$ per agricultural unit produced, $n_b = 5000$ agricultural units produced per hectare before land consolidation, if increment Δn belongs to the interval [100, 1000] and the cost of land consolidation belongs to the interval [200, 600] \$ per hectare the Land consolidation contribution to the Return on Investment ratio will belong to the interval [0.3,3.5]. The results are shown on the figure 3.

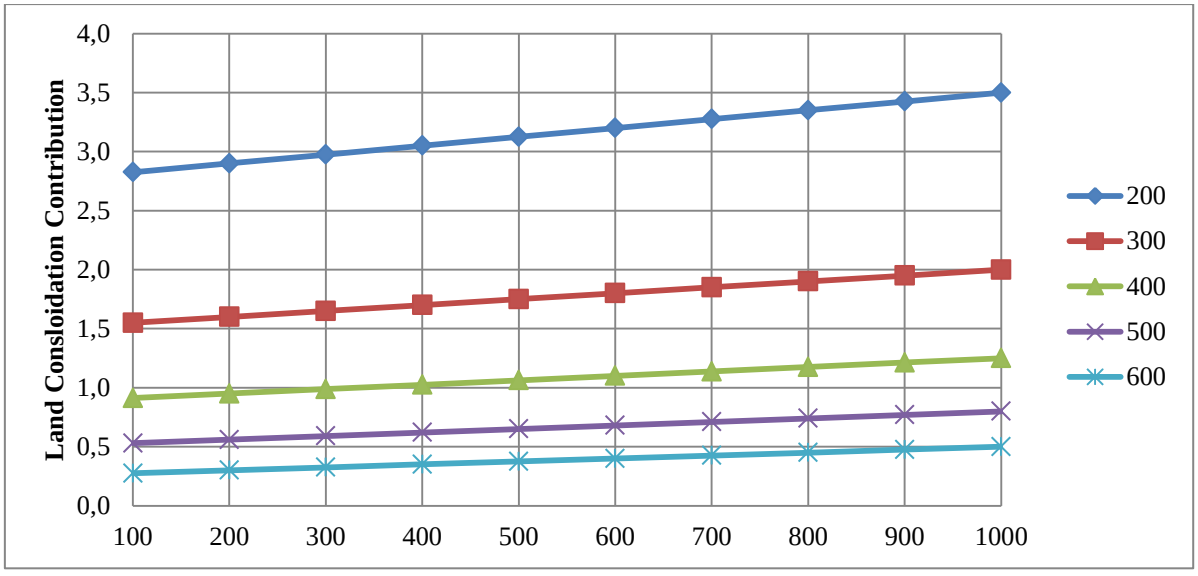

Figure 3. Land consolidation contribution to the Return on Investment Ratio.

Conclusions

The analysis of land consolidation contribution to the profitability ratios resulted by following conclusions:

- Contribution to the Profit Margin of Sales ratio is linear and proportional to the cost reduction per agricultural produced unit caused by land consolidation;
- Contribution to the Return on Assets Ratio is dependent on the increment of agricultural production and the cost reduction caused by land consolidation and
- Contribution to Return on Investment Ratio depends on the increment of agricultural production caused by land consolidation and the cost of land consolidation.

Those conclusions were obtained under the fixed prices because prices of agricultural products are independent of land consolidation and are formed on the market. Further investigation could include research about mutual influences of agricultural prices changes and land consolidation contribution to the profitability ratios.

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CONSIDERATIONS CONCERNING THE PRODUCTION AND MARKETING OF SORGHUM IN ROMANIA

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Abstract

The paper captures trends regarding the production and marketing of sorghum in Romania during 2007-2016. For this purpose, a series of specific indicators were analyzed, among which: the area cultivated with sorghum for consumption and sorghum for brooms, in Romania, on Macroregions and Development Regions; the production of sorghum for consumption and sorghum for brooms, in Romania, on Macroregions and Development Regions and the commercial balance of sorghum for consumption. Worldwide, in 2016, the area cultivated with sorghum was 43,186.79 thousand ha, and the production of 70,212.22 thousand tons. For the year 2025 it is foreseen an increase of the cultivated areas to 44,113.98 thousand ha and the production to 79,123.45 thousand tons. The surface cultivated with sorghum for consumption in the year 2016 in the EU was 123.18 thousand ha and the production of 676.4 thousand tons. The main sorghum cultivars for consumption in the EU in 2016 were: France (47.92 thousand hectares), Italy (43.84 thousand hectares) and Romania (9.16 thousand ha), and in the top producing countries for the year 2016 were: Italy (313.79 thousand tons), France (244.89 thousand tons), Spain (36.36 thousand tons) and Romania (24.41 thousand tons). The data used in this paper was taken up primarily from the National Institute of Statistics of Romania (INS), but also from other international specialized websites. It is necessary to specify that the results of the research are presented in tables and are graphically illustrated.

Keywords: *area cultivated, production, Romania, sorghum.*

Introduction

Sorghum is the world's fifth largest grain crop. The first places in this ranking are occupied by the following grains: wheat; rice; corn and barley. Sorghum along with Amaranth was the basis of the Romanians' diet in the 1970s. This cereal is beneficial for the population because it contains: 43% of the daily protein requirement; 47% of the iron requirement and 55% of the recommended phosphorus value. The sorghum is on the one hand an important source of Mg, Cu, Ca, Zn, K, niacin, thiamine and antioxidants and, on the other hand, is a source of natural fibers (48% of the daily dose). According to the research, the components of sorghum contribute directly to: slowing the aging process; reduce the risk of cardiovascular disease, diabetes, cancer; reduce cholesterol and maintain the digestive tract healthy. (The main benefits of sorghum consumption, https://viataverdeviu.ro/beneficii-consum-sorg-cereala)

Sorghum is a plant with very good tolerance to drought and moderate climate and soil requirements. This is why it can be cultivated in lowland areas affected by drought, with soil poor in nutrients where corn cannot grow. Sorghum is sown 2-3 weeks later than the maize, when the soil temperature at 10 cm is 10-12℃. The sorghum culture needs water until the appearance of the panicle, after that it supports drought. (GRIGORIEV A., Sorghum - culture guide). Currently, it has been found that the use of sorghum culture before the sunflower culture presents a number of positive aspects among which the most representative are: a high degree of nutrient conservation in the soil in order to assimilate them during the spring period and a better structure for the soil. (www.agrointel.ro)

Sorghum bicolor is the most common and most widely grown species of this genus. This species also has several natural varieties and several modern varieties obtained by hybridization: Sorghum for grains (*Sorghum bicolor var. sorghum*); Sorghum for brooms (*Sorghum vulgare var. technicum*); Sweet sorghum (*Sorghum bicolor var. saccharatum* or simplified *Sorghum saccharatum*); Forage sorghum, also known as the Sudan grass (*Sorghum sudanense*). In Romania there are cultivated several types of autochthonous hybrids, but also imported varieties. At the National Institute of Agricultural Research and Development from Fundulea, several hybrid varieties were registered: Hybridized sorghum with Sudan grass – *Tutova, Tereza, Tinca* (changed in *Catinca* by an order of the Ministry of Agriculture in 2007); Sugar sorghum hybrids – *Roza, Doina, Prut, varieties: Fundulea and Carmen;* Broom sorghum hybrids – *Siret, Denisa* and *Donari* (https://ierburiuitate.wordpress.com/tag/matura/)
Worldwide, lately, sorghum is used: for feeding cattle and poultry (because it has nutritional value close to maize); in order to obtain alcohol, starch, sugar, vinegar, beer and biofuel; for traditional braids. The sorghum can be a good substitute for wheat flour, so it can be used by those who do not tolerate gluten. Due to the nutritional properties of sorghum, top restaurants in California and New York have introduced sorghum preparations in their meals. At present, Sorghum is considered versatile and has replaced some rice and quinoa preparations. Another advantage is that it is cheap. It's called the 'wonder grain', as says Chef Michelle Bernstein of Cena by Michy and Seagrape Restaurants in Miami (www.theguardian.com).
An important aspect is that many smaller, higher-yielding sorghum hybrids have been created, which have higher productivity, 20-30% higher than old varieties.
At the EU level, a new orientation has emerged, namely the use of biofuels that are important substitutes for diesel and gasoline. In this case, there is an orientation of the enterprises from agriculture towards the cultivation of energy plants. Currently, sweet sorghum culture is the best bioethanol production culture. (Popescu and Condei, 2014)
Researchers from EUBIA (European Biomass Industry Association) have proposed two optimal options of sorghum cultivation:
- Plantation of at least 1,000 hectares with a production of 6,000 cubic meters of bioethanol per year;
- Plantation ranging from 10,000-50,000 ha, with a production of bioethanol of 60,000-300,000 cubic meters/year.
The proposed options could, at present, provide a lower production cost for bioethanol than in 2005, when the production cost was 600 Euro/tons in the European Union. (Grassi, 2007)
Due to the vast uses of sorghum in the European Union it is noted that it imports annually an average of 160,000 tons. In perspective, in Europe, the development of sorghum culture is expected to reduce the annual import level and even to export significant quantities to the international market. (www.sorghum-id.com/ro/piete-sorgul-o-solutie-sigura/)
Currently, due to climate change, specialists in the field are looking for new solutions for profitable farming activities. The sorghum could be one of these. In Romania it is cultivated for about 50 years, but on small surfaces. It is cultivated especially for brooms, but also as feed. In Romania, there is the tradition to sweep the house with sorghum brooms. Even if the population has modern vacuum cleaners, there is still a sorghum straw broom in people's homes. From the published data, it can easily be noticed that these sorghum strawers brooms have raised the interest of exporters. For example, a company in Bihor has exported this product to Austria and Germany. Salonta is the town with the oldest tradition in Romania, in terms of cultivation and processing of sorghum used for broom breeding. The tradition of producing brooms in Salonta began in 1903, when the Soront Farmers' Salt Producers and Manufacturing Processors of Sorghum was established, which also had a broom brewing workshop with 70 workers. Nowadays, Salonta sorghum broom cleans houses and courtyards from Germany, Austria and Italy. (Tudor, 2017)

Results and Discussions

According to the statistical data published in relation to the situation of the sorghum surfaces grown at the level of the European Union, it can be noticed that these surfaces are smaller compared to the surfaces cultivated with maize. It has been forced to say this because sorghum culture is currently considered by the specialists in the field as an alternative to corn crop, especially for animal feed. In Table 1 is showed the evolution of the areas cultivated with sorghum in the main cultivating countries at the level of the European Union in the period 2010-2017. During the analyzed period, in the European Union it is observed that the surfaces with sorghum have recorded oscillations from one year to another. The largest area cultivated with sorghum in the European Union was registered in 2014 (158.09 thousand ha), and the smallest in 2010 (114.19 thousand ha). According to the data presented in Table 1, the main European Union sorghum cultivator is France. In this country, the areas occupied with sorghum have changed from time to time. The lowest area with sorghum was 42.19 thousand ha (2012), and the largest area was 62.97 thousand ha (2014). In 2017, in France it can be seen that the surfaces with sorghum increased by 18.02% compared to 2010.

Table 1. Evolution of the areas cultivated with sorghum in the main cultivating countries in the European Union in the period 2010-2017

-Thousand ha-

Specification	2010	2011	2012	2013	2014	2015	2016	2017	2017/2010 %
EU 28	114.19	117.03	119.38	145.84	158.09	139.15	123.18	140.91	123.39
Bulgaria	3.54	2.84	5.30	4.34	6.71	6.82	3.29	3.00	84.74
Greece	0.04	0.22	0.14	0.47	1.84	2.86	2.74	2.74	6,850.0
Spain	7.14	8.48	7.73	8.97	7.29	8.38	8.12	6.46	90.47
France	47.65	43.44	42.19	51.25	62.97	53.70	47.92	56.24	118.02
Italy	40.73	41.73	37.10	51.07	51.91	45.37	43.84	46.21	113.45
Hungary	3.16	5.33	4.43	5.33	4.58	4.58	4.45	6.39	202.21
Austria	1.17	1.11	1.19	1.94	2.80	2.92	2.26	2.99	255.55
Romania	10.28	13.08	19.99	21.67	18.85	13.49	9.16	15.75	153.21
Slovakia	0.23	0.50	1.00	0.49	0.85	0.62	0.93	0.65	282.60

*Source: Eurostat, 13.02.2018; own calculations

The next position in the top of the sorghum cultivation countries is occupied by Italy. In Italy, in 2017, 46.21 thousand ha of sorghum have been grown. In this country in 2017, the surfaces cultivated with sorghum registered an increase of 13.45% compared to 2010. On the third place, Romania was with 15.75 thousand ha cultivated with sorghum in 2017. Romania in 2017 cultivated 28.00 % of the surface cultivated with sorghum from France and only 11.17% of the total area cultivated with sorghum in the European Union. In 2017, the areas cultivated with sorghum increased compared to 2010, in most of the presented countries except for Bulgaria and Spain. In table 2 is showed the evolution of sorghum production in the main cultivating countries within the European Union during 2010-2017. After analyzing data on the production of sorghum produced in the European Union, it is found that this varied every year. The largest production made with sorghum in the European Union was 940.51 thousand tons (2014), and the lowest in 2012 (493.90 thousand tons). The significant production of sorghum produced in 2014 at the European Union level is closely related to the area cultivated with sorghum during the same period. In 2017, sorghum production in the European Union increased by 17.81% compared to 2010.

Table 2. Evolution of sorghum production in the main cultivating countries within the European Union, 2010-2017

-Thousand tons-

Specification	2010	2011	2012	2013	2014	2015	2016	2017	2017/2010 %
EU 28	608.86	673.90	493.90	739.84	940.51	728.68	676.74	717.34	117.81
Bulgaria	8.61	5.55	10.80	11.63	18.35	17.04	7.71	6.00	69.68
Greece	0.12	0.84	0.76	1.76	6.63	10.27	6.39	6.39	5,325.00
Spain	36.56	38.64	27.38	44.85	47.66	50.34	36.36	31.13	85.14
France	262.61	280.74	239.91	278.54	395.62	277.80	244.89	326.68	124.39
Italy	271.44	287.82	157.81	316.92	368.78	294.22	313.79	243.60	89.74
Hungary	7.85	16.62	12.44	14.45	19.47	15.53	16.11	10.83	137.96
Austria	7.90	7.48	8.12	10.18	18.53	20.68	16.62	20.33	257.34
Romania	18.68	39.70	37.48	49.83	51.54	31.73	24.41	63.97	342.45
Slovakia	0.53	1.20	1.90	0.96	3.28	1.22	2.11	4.21	794.33

*Source: Eurostat, 13.02.2018; own calculations

Concerning the production of sorghum in the main producing countries, the situation remained unchanged, as in the case of the main sorghum cultivation countries. The top three positions in the top of the sorghum producing countries in the European Union are occupied by: France; Italy and Romania. In 2017, France recorded a production of 326.68 thousand tons, 24.39% higher than in 2010. This year, Italy also made 243.60 thousand tons of sorghum and Romania had a production of 63.97 thousand tons. In 2017, Romania achieved only 8.91% of the EU production and 19.58% of the production made by the leader of the sorghum-producing countries. Bulgaria and Greece obtained the smallest production of sorghum in 2017, below 10.00 thousand tons.

Table 3. Evolution of sale prices for Sorghum in some producing countries in the European Union, 2010-2016

- Euro/100 kg Sorghum-

Specification	2010	2011	2012	2013	2014	2015	2016	2016/2010 %
Bulgaria	17.60	18.43	19.69	15.3	15.32	15.46	13.91	79.03
Spain	18.80	20.00	22.95	19.2	16.30	16.84	15.99	85.05
France	14.52	20.37	29.06	30.39	22.9	21.50	21.00	144.62
Hungary	13.39	17.97	19.51	21.29	14.25	12.63	12.63	94.32

*Source: Eurostat, 04.02.2018; own calculations

Table 3 presents the evolution of sales prices for sorghum in some producing countries in the European Union in the period 2010-2016. From the analysis of the data presented, it can be seen that the sales prices for sorghum in the studied countries recorded changes from year to year. In 2016, in France the price for sorghum was 21.00 Euro /100 kg, registering an increase of 44.62% compared to 2010. The highest price recorded in France was 30.39 Euro/100 kg, while the smallest was 14.52 Euro /100 kg (2010). In the rest of the studied countries, sales prices for sorghum have decreased in 2016 compared to 2010, as follows: Bulgaria (-20.97%); Spain (-14.95%) and Hungary (-5.68%).

In order to present the main tendencies related to the production and marketing of sorghum in Romania, statistical indicators have been presented in the paper at both national and macroregional level. It is necessary to specify that in Romania it is made up of four macroregions and eight development regions.

(https://www.upet.ro/annals/economics/pdf/2010/20100224.pdf)

Table 4 presents the dynamics of the areas cultivated with sorghum in Romania and at the level of the macroregions of development, during 2010-2016. From the data presented and analyzed it may be obvious that the areas cultivated in Romania in 2010 almost doubled compared to 1990. At national level, during the analyzed period we witness a significant change of the surfaces cultivated with sorghum. The largest area cultivated with sorghum in Romania was in 2013 (21,668 ha). On the opposite side, the smallest area cultivated with sorghum was recorded in 2016 (9,160 ha). In 2016, at national level there is a decrease, with 10.92% of the surface cultivated with sorghum compared to 2010. At the macroregional level, the oscillation of the surfaces cultivated with sorghum in the analyzed period can easily be noticed. Macroregion 4 is highlighted by the largest areas cultivated with sorghum. In 2016, over 50.00% of the area cultivated with sorghum at national level was cultivated in this macro-region. At the opposite end, in Macroregion 1, has the smallest areas cultivated with sorghum.For example, only 902 ha were cultivated in 2016, which represents only 9.84% of the area cultivated at national level and 19.61% of the area cultivated in Macroregion 4. In 2016, the surface cultivated with sorghum in Macroregion 2 was of 2,422 ha and 1,216 ha in Macroregion 3. According to data provided by the National Institute of Statistics, in 1990, Romania cultivated 5,195 ha of sorghum. In 2000, the area cultivated with sorghum fell significantly to 1,602 ha. In 2007, which represents the year of Romania's accession to the European Union, only 20.36% of the area which was cultivated in 1990 with sorghum can be found, respectively 1.058 ha. Gradually, after 2007, the farmers in Romania began to turn to the sorghum culture, because this has several advantages.

Table 4. Dynamics of the surfaces cultivated with sorghum in Romania and in the macroregions of development, during 2010-2016

-ha-

Specification	2010	2011	2012	2013	2014	2015	2016	2016/2010 %
Romania	10,283	13,081	19,992	21,668	18,845	13,486	9,160	89.07
Macro-region 1	346	817	1,088	1,716	1,872	1,036	902	260.69
Macro-region 2	3,457	3,312	6,013	7,125	4,829	2,937	2,422	70.06
Macro-region 3	1,111	3,391	5,076	5,162	3,178	1,621	1,216	109.45
Macro-region 4	5,369	5,561	7,815	7,665	8,966	7,892	4,620	86.04

Source: INS, 10.11.2017; own calculations

Between 1990 and 2007, according to data provided by the National Institute of Statistics, in Romania the production of sorghum had the following evolution: 3,522 tons (1990); 1,479 tones (2000), and in 2007, the year of joining the European Union, 1,193 tones.

In Table 5 it is presented the evolution of the sorghum production in Romania and in the macroregions of development, during 2010-2016. From the analysis of the data on the production of sorghum in Romania, it can be seen that it recorded oscillations from one year to the next. The most significant national sorghum production was 51,543 tones (2014) and the smallest of 18,677 tons in 2010. At the national level, in 2016, sorghum production increased by 69.87% compared to 2010.

Regarding the production of sorghum made within the macroregions, it can easily be noticed that there have been changes in production from year to year. Macroregion 4, during the analyzed period, recorded the largest outputs. In 2014, a production peak of 24,948 tones was registered, and in 2010 there was a minimum production (9,944 tones). In Macroregion 1, the smallest production of sorghum is recorded at national level. In this macroregion the production of sorghum varied between 809 tons and 5,043 tons during the analyzed period.

Table 5. The evolution of sorghum production in Romania and by macroregions of development, 2010-2016

-Tons-

Specification	2010	2011	2012	2013	2014	2015	2016	2016/2010 (%)
Romania	18,677	39,696	37,481	49,829	51,543	31,728	24,413	169.87
Macroregion 1	809	2,695	1,774	3,907	5,043	2,767	2,539	342.02
Macroregion 2	5,157	8,841	9,480	15,795	13,943	4,847	5,893	93.98
Macroregion3	2,767	11,379	8,773	11,440	7,609	3,850	3,727	139.13
Macroregion4	9,944	16,781	17,454	18,687	24,948	20,264	12,254	203.78

*Source: INS, 10.11.2017; own calculations

In 2016, this macro-region recorded the highest percentage increase of sorghum production, by 242.02% compared to 2010. Macroregion 1, in 2016, made only 10.40% of the national sorghum production. Regarding the production of sorghum made in Macroregion 2, it can be observed that this has recorded changes every year. A maximum production point was recorded in 2013 (15,795 tones), and a minimum point was in 2015 (4,847 tones). In Macroregion 3, in 2016, sorghum production registered a 39.13% increase compared to 2010.
According to the statistical data on average yield per hectare of sorghum produced in Romania in the period 1990-2007, it was found that it evolved as follows: 674 kg / ha of sorghum (1990); 923 kg / ha (2000) and 1,128 kg / ha in 2007, the year of accession to the European Union.

Table 6. Evolution of the average yield of sorghum per hectare in Romania and in the macroregions of development, during 2010-2016

-Kg/ha-

Specification	2010	2011	2012	2013	2014	2015	2016	2016/2010 (%)
Romania	1,816	3,035	1,875	2,300	2,735	2,353	2,665	146.75
Macroregion 1	2,338	3,299	1,631	2,277	2,694	2,671	2,815	120.40
Macroregion 2	1,492	2,669	1,577	2,217	2,887	1,650	2,433	163.06
Macroregion3	2,491	3,356	1,728	2,216	2,394	2,375	3,065	123.04
Macroregion4	1,852	3,018	2,233	2,438	2,783	2,568	2,652	143.19

*Source: INS, 10.11.2017; own calculations

In Table 6 it is presented the evolution of the average production of sorghum per hectare in Romania and in the macroregions of development, during 2010-2016. In Romania, in 2016, an average yield of sorghum per hectare was 2,665 kg / ha. At the macroregional level in 2016, the following situation was recorded: Macroregion 3 (3,065 kg / ha); Macroregion 1 (2,815 kg / ha), Macroregion 4 (2,652 kg / ha) and Macroregion 2 (2,433 kg / ha).
In 2016, there was a percentage increase in the average yield of sorghum per hectare compared to 2010, both at national level and at macroregional level. At the national level, in the year 2016, there was an increase of 46.75% compared to 2010. At the macroregional level in 2016, compared to 2010, the following increases were registered: Macroregion 1 (+20.40%); Macroregion 2 (+63.06%); Macroregion 3 (+23.04%) and Macroregion 4 (+43.19%). The production of brooms was a traditional seasonal activity (autumn-winter). In each village there were some craftsmen known as Sweepers who, after clearing the dry inflorescences of sorghum (panicles with branches of 100cm long) and sorting them by thickness, tightened them in small bundles which were bounded with wire and after that sew them (http://www.patrimoniuimaterial.md/ro).

During 1990-2007, in Romania, the areas cultivated with broom sorghum developed as follows: 7,162 ha (1990); 5,213 ha (2000) and 4,508 ha (2007) in the year of accession to the European Union. (INS)
Table 7 shows the evolution of the surfaces cultivated with sorghum in Romania and in the macroregions of development, between 2010 and 2016. During the analyzed period, the areas cultivated with sorghum for brooms at the national level and in the development regions recorded changes from year to year. At national level, the largest area cultivated with broom sorghum was registered in 2012 (3,597 ha), and the smallest area was 2,611 ha (2016). Since 2013, the areas cultivated with broom sorghum have been on a declining trend. In 2016, the areas cultivated with sorghum in Romania dropped by 17.19% compared to 2010.

Table 7. Evolution of the areas cultivated with broom sorghum in Romania and by macroregions of development, between 2010-2016

-ha-

Specification	2010	2011	2012	2013	2014	2015	2016	2016/2010 %
Romania	3,153	3,344	3,597	3,185	2,824	2,748	2,611	82.81
Macroregion 1	852	619	887	948	863	761	953	111.85
Macroregion 2	374	395	502	439	373	294	356	95.18
Macroregion3	436	611	625	427	442	447	435	99.77
Macroregion4	1,491	1,719	1,583	1,371	1,146	1,246	867	58.14

*Source: INS, 10.11.2017; own calculations

In Macroregion 4, it is observed that the largest surfaces with sorghum for brooms were cultivated, except for the year 2016, when a surface of only 867 ha was recorded. In this Macroregion, in 2016, the area cultivated with broom sorghum decreased by 41.86% compared to 2010. In 2016, Macroregion 1 cultivated 36.49% of the surface cultivated with sorghum at national level, in Macroregion 2, a percent of 13.63% of the total cultivated area was registered, in Macroregion 3, 16.67% of the total cultivated area was found and in Macroregion 4, 33.20% of the cultivated area was grown.
According to the data presented in Figure 1, Romania's sorghum exports have changed over the period 2012-2017. For sorghum, the highest value exports were recorded in 2014 (4,200,000 Euros) and the lowest exports were 1,358,000 Euros (2013). In 2017, the value of sorghum exports fell by 34.63% compared to 2012.

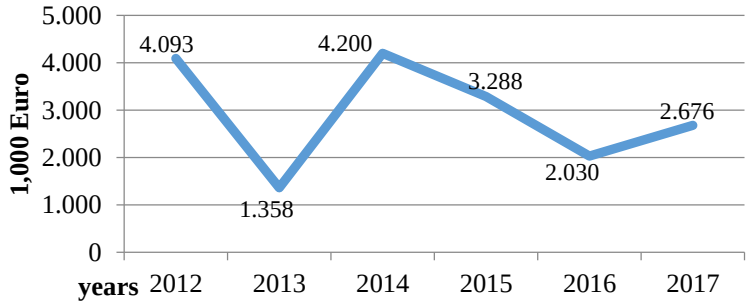

Figure 1. Value of grain sorghum exported from Romania between 2012 and 2017
Source: www.statista.com/statistics/762900/grain-sorghum-export-value-romania, 2018

Between 2013 and 2017, sorghum imports were outpaced by sorghum exports. The largest imports of sorghum were 1,610 thousand Euro (2013) and the smallest were 798 thousand Euro (2017) (see fig. 2).

Currently, in Romania, according to the official data on the production and marketing of sorghum, there is a growing interest in this culture.

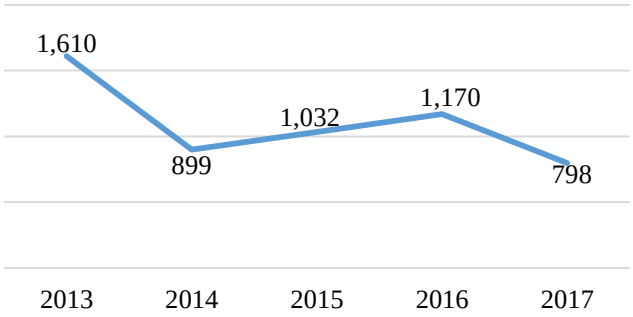

Figure 2- Value of grain sorghum imported from Romania between 2013 and 2017 (Thousand Euro)

*Source: Trade Map, 2018

After analysing data on imports and exports for the Sorghum category, it was noticed that in 2013 there was a trade balance deficit of 252 thousand Euros, but since 2014, a positive balance has been recorded.

Conclusions

Following the analysis of the main indicators referring to the sorghum production and marketing sector in Romania, the following aspects were identified:

- At national level, there is a 10.92% decrease in the surface cultivated with sorghum in 2016 compared to 2010;
- In Macroregion 4 the most significant surfaces with sorghum are cultivated. In this macroregion, in 2016, more than half of the nationwide surface cultivated with sorghum was grown;
- Year 2014, was noted by the highest production of sorghum at national level of 51,543 tons;
- in 2010, the smallest production of sorghum from the analyzed period was achieved, of 18,677 tons;
- In 2014, in Macroregion 4, a production of 24,948 tons of sorghum was obtained, being the highest production from the analyzed period;
- In 2016, the average yield per hectare for sorghum culture increased by 46.75% compared to 2010;
- Macroregion 3 was highlighted in 2016 by the highest average yield per hectare for sorghum crop of 3,065 kg / ha;
- In Romania, both sorghum used for animal feed and for the production of brooms used in the household sector is cultivated;
- In 2012, the most significant surface with broom sorghum was cultivated, of 3,597 ha. On the opposite side, the smallest surface cultivated with broom sorghum was cultivated in 2016 (2,611 ha);
- In Macroregion 4, the most significant areas with broom sorghum have been cultivated within the macroregions of development, with only one exception, in 2016, when only 867 ha were cultivated;
- During 2012-2017, the value of sorghum exports recorded fluctuations from year to year;

- In 2014, the highest export value for sorghum was recorded, 4,200,000 Euro; the smallest value of imports for sorghum was 798,000 Euro (2017);
- In 2013, there was a commercial deficit for the "Sorghum" category, of 252,000 Euros. Also in this year, an import coverage by exports of 84.34% was registered;
- In the period 2014-2017, there was a positive trade balance for the "Sorghum" category;

In perspective, an increase in the areas cultivated with sorghum in Romania is expected because, on the one hand, this culture is suitable for all soil types and resists the drought, and on the other hand the demand for sorghum on the international market will increase.

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FORESTRY AND AGRO-FORESTRY

STUDY OF THE VISUAL QUALITY PARAMETERS OF THE CORK OF SOME CORKFORESTS IN THE EXTREME NORTH-WEST ALGERIA

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Abstract

The cork forests in northwestern Algeria are confronted with a series of environmental constraints that accentuate their degradation, especially fires, drought, conifer afforestation and the health of trees. Initial cork quality analysis (with Coveless device CQ05 n°0017) revealed that the cork is well distributed between the southern and northern zones of the geographical area of production.Cork for producing natural and colmated stoppers (Q-A and Q-B) growmainly in Hafir (69.33%) and M'Sila (49.33%). On the other hand, the cork bark with low thickness (Q-C) and corkwood refuse (Q-D) are individualized respectively on the samples of Nesmoth (40%) and Beni Ouarssous (30.67%). The superimposition of its cork quality values to environmental factors by means of MCA has shown that the effect of biotic and abiotic factors is decisive. In the mountains of Hafir, there are healthy trees (trunk and crown), in full production (circumference and height), which grow on stony ground that give the best quality of cork (Q-A) between the four provenances. On the other hand, in the Beni Ouarssous coastline, trees are declining (trunk and top), invaded by the conifer afforestation and the aftereffects of fires that produce poor quality of corkwood refuse (Q-D). Between its two boundaries, the M'Sila (littoral) and Nesmoth (mountain) stands are well adapted to their natural environment by simultaneously providing Q-B and Q-C cork.

Keywords: *Oak cork, cork, quality, environmental factors, MCA.*

Introduction

The cork is a non-wood forest product, natural and renewable. It is very adapted to its growing area in the seven subericulture countries forming the western part of the Mediterranean and the Atlantic coast (Spain, Italy, France, Algeria, Morocco, Tunisia and Portugal). This area of 2.7 million hectares produces an annual tonnage of 300 million kg / year (Santos Pereira et al., 2008, Aronson et al., 2009). The cork is a highly prized product, once classified as a strategic product of the world economy (Marc, 1930). Indeed, the assets and the outlets of the cork are countless, they concern great panoply of use, from the simple object of leather goods to the complex product used in the cosmetic industry. Its price is very variable between 0.08 and 3.54 € / kg for raw cork. In the case of prepared and treated cork; it ranges from 0.21 to 6.61 € / kg, and in the case of cork products it ranges between 1.80 and 2.40 € / kg (Santiago Beltrán and Elena Rosselló, 2015). But the first use of cork remains indisputably its transformation into a natural cork stopper. A medium-sized cork oak produces about 3.9 kg of cork per year; 74 natural corks of average quality. In terms of the economy, one kilogram of natural corks oscillates between an average price of almost 46 €, and some batches can reach up to 240 €. As a subericulture management, this is consistent with the sustained production of 2500 natural corks of 33 cork oaks forming one hectare of well-managed and valued cork oak. It also implies that the abandonment of this form of management inevitably leads to dieback of the oak groves and the loss of rare biodiversity as a result of scrubbing and fires (Santiago Beltrán and Elena Rosselló, 2009; Varela, 2008). These good practices also nourish an important industrial fabric. The demand from these industries is increasingly focused on corks that can provide high quality corks. The majority

of these transactions take place annually in the forest, several months before harvesting the cork, between industrialists and private or public landowners. They frequently result in very complicated payment methods because of the complexity and heterogeneity of the species itself (Natividade, 1956, Courtois and Masson, 1999). A harvest of cork stored in piles and presented for sale is very rarely free of waste or bad quality boards. The purchaser and the manager can both consolidate losses as profits; it is a product that is valued according to its quantity (the caliber) and its appearance (the visual quality). Experience has shown that these two attributes are very difficult to define and very often lead to very controversial assumptions and conclusions. For a large number of cork professionals, the caliber and visual quality of cork are related to tree characteristics and applied sylvo-pastoral management, especially: trunk diameter, tree height, canopy diameter, height debarking, tree density and stripping number. For others, the geographical situation, the climate, the topography, the altitude, the nature of the source rock are of a very important influence (Bossuet, 1988; Gonzalez-Adrados, 1989; Pereira 2007). Other external factors are already known to influence the growth of cork: age of cork at the time of cork stripping, quality of exploitation, phytosanitary status of trees and fire passage (Montoya 1988, Dehane et al., 2015). According to the same authors, in trees that are too old, the accumulation of wounds and scars and the reduction in the growth of wood and cork with age, lead to a marked decrease in the increases in the cork obtained.

The purpose of this study is to confirm or refute these environmental factors through a tedious investigation carried out on the majority of oak groves in the extreme north-west of Algeria. The work presented in this article is part of other previously undertaken studies, including the evaluation of cork growth and health status of trees, the analysis of cork quality and the physical-chemical parameters of the cork, study of the growth of Algerian cork according to the origin and the mineralogical analysis of the cork (Dehane et al., 2013; Dehane et al., 2014, Dehane and Gheffar, 2017; Chourana and Dehane, 2018).

Material and Methods

Site

In the northwestern part of Algeria, four representative sites of cork oak production areas are selected: the Hafirforets (HA) (Tlemcen, Sabra locality), the Nesmoth forest (NE) (Mascara, locality of Tighenif), the forest of M'Sila (MS) (Oran, locality of Boutelis), the cork forest of Beni Ouarssous (BO) (Tlemcen, locality of Borj Arima). These cork forests come from two types of facies and are characterized by a Mediterranean climate: In mountain, we distinguish a subhumid and fresh bioclimate for HA (650mm / year), semi-arid and hot for NE (400mm / year). In the littoral, the two forests MS, BO are temperate semi-arid (450mm / year, 430mm / year). The analysis of the pH level of the cork oak soils indicates acid soils and it is between 5 and 6.5. The electrical conductivity of soils is low (<120 µs / cm), characteristic of unsalted soils. The nature of the bedrock is siliceous (sandstone in the mountain and schistous in the littoral). With the exception of the MS site which is of the "dehesa" type, all the others are of the "maquis" type with a low density of trees per hectare and with dense and high undergrowth.

Sampling methods

The recent use of the techniques of the estimation of the quality of the cork on tree before the stripping offers a big advantage to sub-growers and to administrators. Indeed, the coveless proposes a multitude of information on the maturity of the cork (thickness and aspect), its quality and its price without passing by the extraction of the samples which are very often harmful for the health of the tree and very prohibited by the forest departments.

Due to the low density of producing trees (reproduction cork) (<20 trees / ha) and dense undergrowth, sampling by itinerary was used. In this type of sampling, the area is scanned

and, generally, at predefined intervals, the sample trees are probed as much as necessary, up to a total of 75 trees. (Gonzalez Montero, 2004; Santiago Beltran et al., 2015). In the case of the present study and to cover a wide variation of trees, topography and reliefs, we approached the itinerary according to three altitude levels in each oak groves (Crest, slope and shallows). In each level, and on zigzagged passages at intervals of 200m in length, the first spotted tree from the front is probed at 1.30m then materialized at the GPS as a starting point until reaching the last subject thus indicating the 75 samples.
Depending on the offered exposure, the sounding by the instrument is performed on an applicable surface of 20cm x 20 cm, similar to traditional cala. With each measurement memorized and validated by the device, various quantitative and qualitative characteristics of the trees are mentioned jointly: diameter and height of the tree, height of stripping, debarking coefficient, trace of fire, conifer afforestation and undergrowth. Other environmental factors are also noted such as altitude, soil texture, surface deposition, pH and electrical conductivity of the soil, silviculture work.
Other visual measurements are noted to assess the overall health status of the trees. This is the foliar deficit in the crowns (slight defoliation: loss of foliage less than 20%, significant defoliation: 20 to 75% of leaves lost, very serious defoliation: more than 75% of leaves lost). The examination of the trunk concerns the wounds caused by the stripping and the ensuing damages such as the holes of insects, the cavities, the dryness of the mother of the cork, the carbonaceous canker. Three health conditions are distinguished: a healthy state (no defoliation, no damage on the trunk), a weakened state (weak defoliation, no cavities or injuries on the trunk), a state of decline (severe defoliation and injury with damage marked).

Quality analysis methods

The device relies on the electrical conductivity of the mother to determine the thickness of the cork and its appearance. The data provided by the instrument is divided into nine quality classes by merging the thickness and aspect orders (IPROCOR, 2006) (Tab.1).

Table1. Commercial Quality Classes (Iprocor, 2006)

	1	2	3	4	5	6	7	8	9
Qualityclass	19ª more. 6ª more.	15-19 5ª more.	15-19 6ª	13-15 5ª more.	13-15 6ª	11-13 5ª more.	11-13 6ª	11less. 4ª more.	Refuse

Each quality class has a value based on the selling price of cork (Tab.). A quality index is calculated for each tree from the percentages of all cork choices and their value (weighted average) (Iprocor, 1994):

$$\mathbf{IQ= (5/100) \times \Sigma_{i=1\text{-}9} (A_i \times Q_i)}$$

Where:

- IQ = corkquality index
- A_i = average value savings / kg of cork categories 1-9 boiled and baled.
- Qi = proportion of the material volume of the given category in relation to the total production volume (%).

Statistical analysis methods

Variance analysis and the chi-square test are used to explain the possible relationships between the different quantified variables. To better understand and explain the impact of the Environmental factors of the four sites on the quality of the cork produced, a Multiple

Correspondence Analysis (MCA) was carried out by the XlStat software. The MCA was based on an observation / variable table of various modalities measured on trees, cork and route. We used 15 variables and 300- observations.

Results and Discussion

Dendrometric characteristics of trees

The table 2 below presents per site the average dendrometric characteristics of the sampled trees.

Table 2. Measurements of sample trees

	(HA) Forest1	(NE) Forest2	(MS) Forest3	(BO) Forest4	Statistical test *
Circumference of the tree (cm)	181,53	102,29	131,82	91,36	F=63,32 ; p<0,000
Height of the tree	15	12	16	18	F=74,66 ; p<0,000
Debarking height (m)	1,70	2,37	1,92	1,70	F=44,57 ; p<0,000
Thickness of cork (mm)	29,60	24,55	32,55	24,37	F=27,99 ; p<0,000

*: Anova test, significant difference (p <0.000)

The table 2 gives the averages of the 300 trees in each forest. In the BO forest, the tree heights are relatively high (18m), conversely the circumferences are reduced (91.36 cm). In this forest, trees are highly competitive with high and dense undergrowth, thus producing a very thin cork (24.37mm). The HA and MS forests consist of trees of average circumference respectively (181.53 cm and 131.82 cm) and heights of reasoned bark (1.70 m and 1.92 m), the thickness of the cork is better on these topics. The NE forest has the lowest tree heights between the four forests (12 m), high bark heights giving a low cork (24.55 mm).

Appearance of cork

According to the IPROCOR standards, the 300 sample trees sampled with the Coveless CQ05 contain the following classes of enlarged and summarized quality (Fig.1 and Fig.2). Both figures show a strong presence of the cork bark with low thickness and cork stoppers respectively proportions of the order of 28.33% and 26.33%. The first proportion is very frequent in the Nesmoth and Beni Ouarssous samples, namely 40% and 35% respectively. The second proportion is more marked on Hafir trees with a rate of around 51% and M'Sila (23%). Thick cork constitutes only 4% of the total and very particularly present in the forest of M'Sila and Hafir (7% and 7%). Raw corkwaste (refuse) absorbs 24% of the samples, very marked at Beni Ouarssous cork (31%) and relatively homogeneous on the other provenances, HA (20%), MS (23%), NE (23%).

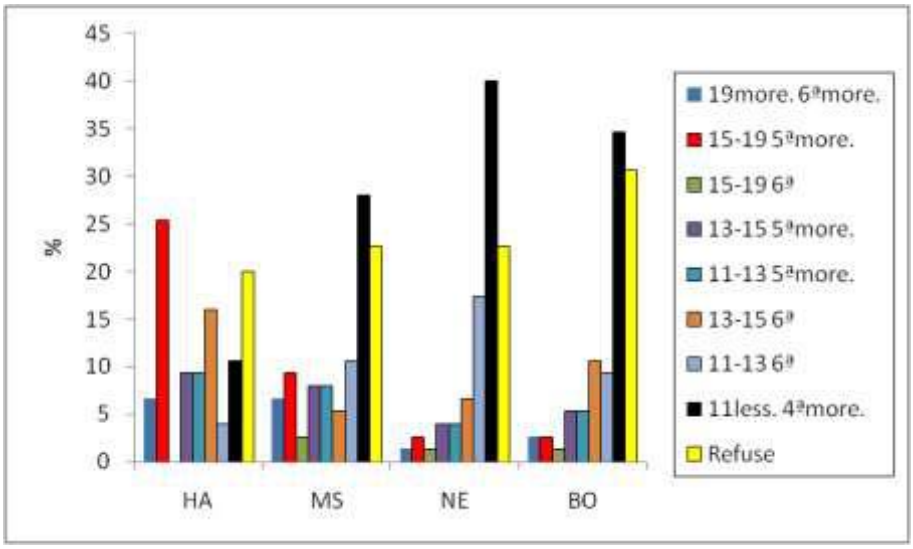

Figure1: Extended quality class of cork

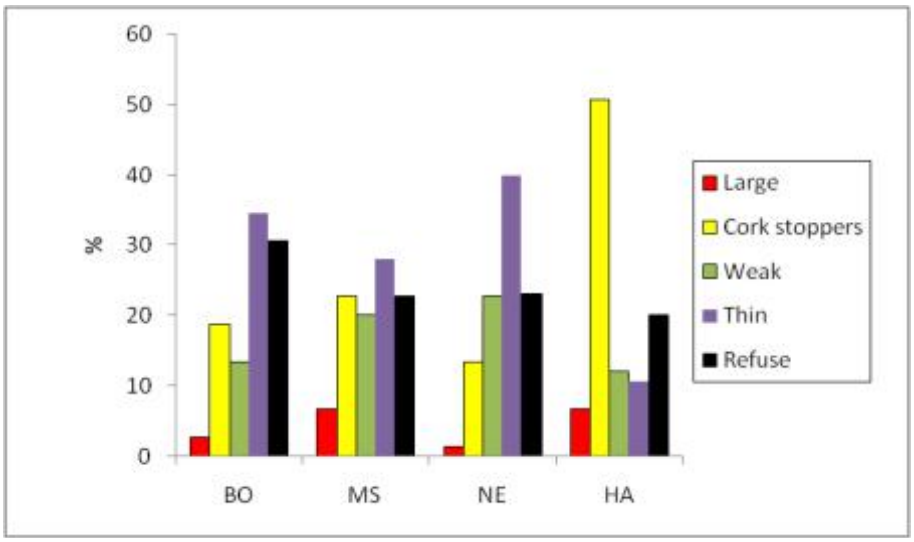

Figure 2: Summary class quality of cork

Industrial grade class

Industrially speaking, the nine extended quality classes and the five summarized quality classes can be merged into two levels of industrial grade classes 1 and 2:

-Industrial grade class 1: These are the primary outlets for cork samples before they are punched in the factory (cork stoppers, refuse, and cork bark with low thickness).

Indeed, the cork stoppers of this level of quality is attributed to classes of cork able to be perforated for diameters of 24 and 21 mm with all classes of minor and ordinary defects they contain. On the other hand, the raw cork waste by excess of major anomalies cannot be perforated. Finally, thin cork despite these minor defects, its small thickness does not allow it to be punched in stoppers.

-Industrial grade class 2: This is the final outlet for cork samples after casing, manual or automatic sorting in the plant (natural corkwood stoppers, colmated natural stoppers, refuse, grinding). Indeed, the cork stoppers of this level of quality are divided into two subclasses. The first subclass, natural stoppers are assigned to classes of cork able to be perforated for diameters of 24 and 21 mm and only for the classes of minor defects not requiring a colmation.

-The second class, colmated natural stoppers is assigned to classes of cork able to be perforated for diameters of 24 and 21 mm and only for the classes of ordinary defects requiring obligatorily work of colmation. On the other hand, refuse cork under defects of major anomalies and intended for grinding. Finally, thin cork despite these minor defects, its small thickness allows it to be cased into discs or washers.
The results obtained from the merger of the extended and summarized quality classes are shown in the table 3.

Table 3. Industrial grade class grades 1 and 2 of corks of sample trees

Provenance	N	V1		N	V2	
BO	26(34,67%)	Cork stopper	A	16(21,33%)	Natural cork stopper	A
	26(34,67%)	Thin	B	10(13,33%)	Colmated stopper	B
	23(30,67%)	Refuse	C	16(34,67%)	Washer	C
				23(30,67%)	Grinding	D
HA	52(69,33%)	Cork stopper	A	43(57,33%)	Natural cork stopper	A
	8(10,67%)	Thin	B	9(12%)	Colmated stopper	B
	15(20%)	Refuse	C	8(10,67%)	Washer	C
				15(20%)	Grinding	D
MS	37(49,33%)	Cork stopper	A	22(29,33%)	Natural cork stopper	A
	21(34,67%)	Thin	B	15(20%)	Colmated stopper	B
	17(22,67%)	Refuse	C	21(28%)	Washer	C
				17(22,67%)	Grinding	D
NE	28(37,33%)	Cork stopper	B	11(14,67%)	Natural cork stopper	A
	30(40%)	Thin	C	17(22,67%)	Colmated stopper	B
	17(22,66%)	Refuse	A	30(40%)	Washer	C
				17(22,67%)	Grinding	D
	118(52,44%)	Cork stopper	B	92(30,66%)	Natural cork stopper	A
	50(22,22%)	Thin	C	51(17,00%)	Colmated stopper	B
	57(25,33%)	Refuse	A	85(28,33%)	Washer	C
				72(24,00%)	Grinding	D
Total	300(100%)			300(100%)		

Legend: N: Number of sample; V1: Industrial Grade Class 1; V2: Industrial Grade Class 2; %: Percentage

Industrial Class 1 encompassing three classes simultaneously: the cork stoppers; the thin cork and the refuse cork are individualized respectively on the samples of Hafir (69.33%), Nesmoth (40%) and Beni Ouarssous (30.67%). The Chi-square test indicates a highly significant difference at the 5% threshold between the four sources (X 2 = 26,69; p=0,0001).

The table also shows that industrial grade classes 2 are heterogeneously distributed within and between the four provenances. Indeed, the cork natural cork (with limited minor defects) and well present in Hafir (57.33%) than the other oak groves. In contrast, cork stopper is very common in M'Sila (20%) and Beni Ouarssous (22.67%). For its part, thin cork for washers is dependent on Hafir (40%) and Beni Ouarssous (53.67%). Lastly, cork with no value and destined for grinding is the majority in Beni Ouarssous (30.67%) than the other origins. The chi-square test at the 5% threshold records a very highly significant difference between the four provenances (χ^2 = 44,26; ddl= p< 0,0001).

Study of the quality parameters of the plain and mountain cork (Beni-Ouarssous-M'Sila VS Hafir-Nesmoth)

The cork processing industry would like to better control their supply and therefore know the factors that favor the production of quality cork, tree and stand parameters, environmental factors, management parameters and better yet, to find objective criteria for measurement of quality easily quantifiable. In the real state of the constraints of the environment in which these trees grow, these findings are very difficult to detect because of the high variability between subjects and provenances. In fact, the cork quality analysis of any cork forest cannot be limited to the identification of quality classes and quality indices.

In fact, these two variables are the consequences of tree growth conditions, that is to say the environmental factors that surround them and also certain parameters used in cork-oak growing. To do this, a multivariate analysis (MCA) was used by taking into account several quantitative and qualitative variables specific to the four oak groves (Tab.4).

Table 4. The modalities adopted for the MCA

Variables	Codes	Description and modalities
Situation of the production area	G	1: North, 2: South
Cork oak area	A	1:Littoral1(MS) ;2:Littoral2(BO) ;3:Montain1(NE) ; 4: Montain2 (HA)
Climate	Cli	1: Semi arid; 2: Sub-humid; 3: Humid
Topography	Tp	1: Ridge, 2: Slope, 3: Bottom
The nature of the bedrock	R	1: Sandstone; 2: Shale
Circumferences (cm)	Cr	1:70-107 ; 2 :107-1140 ; 3:140-170; 4: 170-200 ; 5:>200
Height of trees (m)	H	1:7-12m ; 2:12-14m; 3:14-16m; 4:>16m
Debarking height (m)	H.e	1:<1,5; 2: 1,5-2,5 ; 3:2,5-3,5 ; 4:>3,5
Cork thickness (mm)	Cal	1: Large (>42,75mm), 2: Standard (24,75-42,75mm); 3: Thin (<24,75mm)
Soil	Sol	11: deep, 2: stony
Texture	Tx	11: (sandy), 2: (sandy-silt), 3 :(sandy-clay),4: clay-sandly
Health status	St	1: Healthy; 2: weakened; 3: decaying
Fire Pass	Inc	Inc 1: None; 2: 1 passage; 3 (recurrence)
Conifer afforestation	Er	1: No, 2: weak, 3: medium; 4: Strong
Quality type	Q	1:Q-A; 2:Q-B; 3:Q-C; 4:Q-D

The multivariate analysis represented in the table 4 and the figure 3, tries to explain the impact of certain environmental parameters on the qualitative and quantitative variables of the quality namely the commercial and industrial quality (Tab.5).

Table 5. Eigen values and percentages of inertia (environmental parameters / quality)

	F1	F2
Own value	0,465	0,332
Variability(%)	20,834	14,416
% cumulated	20,834	35,250

Through the table 5, we observe that the first axis represents 20.83% of the inertia, while the second axis shows 14.41%. These two axes together account for 35.25% of the majority of the variation of the point cloud of the sample trees and the modalities taken into consideration (300 subjects and 14 variables) (Fig.3).

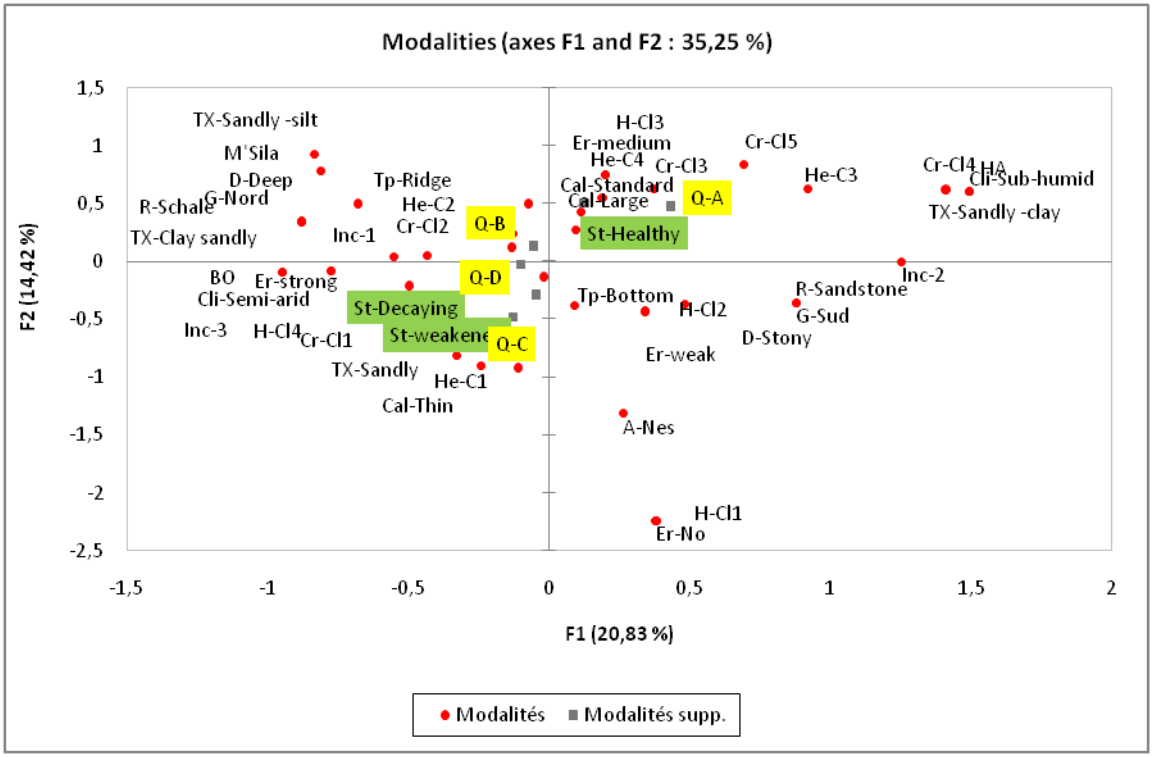

Figure 3. Representation in the factorial plane F1 x F2 of the environmental parameters / quality.

Interpretation of axes

In the first axis (F1), quality cork Q-A is opposed to Q-B, Q-C and Q-D cork. In fact, the type QA favored by the cork processing industry, with the quantitative and qualitative characteristics mentioned (Standard caliber, appearance 5amore, cork stopper with minor waste, natural cork stoppers) seems to depend on the natural area of Montain whether it is in Hafir or Nesmoth. This type of product is significantly related to the health status of healthy sample trees (crown and trunk). On the other hand, the other three types (Q-B, Q-C, Q-D) form a single block and they are more related to the natural coastal area (M'Sila and Beni Ouarssous). These three types are not related to the overall health status of the sample trees. In the second axis 2 (F2), the quality cork Q-A and Q-B are distinguished from Q-C and Q-D. These two products are the most popular on the market both in mountain (HA) and coastal (MS). The significant values at the alpha threshold = 0.05 are mentioned in the table 6.

Table 6. Variables tests of environmental parameters / quality

Variables	F1	F2
G-North	**-15,167**	**6,067**
G-South	**15,167**	**-6,067**
A-Littoral1	**-9,443**	-0,896
A-Littoral2	**-8,071**	**7,901**
A-Montain1	**2,618**	**-13,105**
A-Montain2	**14,895**	**6,1**
Cl-Semi-arid	**-14,895**	**-6,1**
Cl-Sub-humid	**14,895**	**6,1**
T-Ridge	**-0,875**	**6,17**
T-Bottoms	**1,107**	**-4,627**
R-Sand-stone	**15,167**	**-6,067**
R-Schale	**-15,167**	**6,067**
X-Clay-sandly	**-4,647**	0,396
X-Sandly	**-4,548**	**-11,24**
X-Sandly-clay	**14,895**	**6,1**
X-Sandly-silt	**-6,43**	**7,204**
Inc-1	**-6,319**	0,798
Inc-2	**15,282**	-0,011
Inc-3	**-9,443**	-0,896
D-Stony	**9,903**	**-7,404**
D-Deep	**-9,903**	**7,404**
E-Weak	**3,436**	**-4,306**
E-Strong	**-7,854**	-0,819
E-Moedium	**2,867**	**10,837**
E-No	1,939	**-11,425**
Cir-Cl1	**-6,683**	**-8,11**
Cir-Cl3	**2,347**	**3,955**
Cir-Cl4	**7,503**	**3,339**
Cir-Cl5	**3,688**	**4,494**
H-Cl1	1,939	**-11,425**
H-Cl2	**3,436**	**-4,306**
H-Cl3	**2,867**	**10,837**
H-Cl4	**-7,854**	-0,819
Ca-Thin	**-2,846**	**-10,631**
Ca-Standard	**2,513**	**9,578**
He-C1	-1,088	**-9,21**
He-C2	**-2,847**	**5,372**
He-C3	**5,688**	**3,889**
ES-Weakened	-1,763	**-6,553**
ES-Decaying	-0,346	**-2,095**
ES-Healthy	**1,977**	**7,932**
Q-A	**4,95**	**5,469**
Q-B	-0,434	1,041
Q-C	**-3,793**	**-6,243**
Q-D	-0,96	-0,233

According to Courtois and Masson (1999), the results are very succinct in terms of the relationships between the quality of the cork and the factors of the physical environment such as the geographical situation, the climate, the topography, the altitude, the nature of the cork, the bedrock because of the complexity of the growth factors of the species in itself. In the factorial plane (F1), the type Q-A cork (natural stoppers and with minor waste) is Mountain (Hafir and Nesmoth), it is the domain of the southern geographical region of the production zone, particularly in the ridges. It is common in stony, low-fertility sites, on a Sandstone hardens of sub-humid and semi-arid where it usually freezes in autumn and winters.

With reference to Lombardero and Montero (1980), these areas refer to poor and degraded soils but producing a good quality cork with very slow increases with exploitation rotations exceeding the usual 10 years or even 12 to 15 years and which are at the best prices. According to Dehane (2012), mountain cork is dominated by reduced cells. The Q-A cork is very present on tall trees in search of light and photosynthesis (12-14m) and adults of class 3 (140-170cm),4 (170-20cm) and also on large trees of the class 5 (>200cm). The caliber produced belongs to the ordinary and thick commercial class (24.75-42.75mm; 42.75mm). This class of quality is also related to the debarking heights of class 3 and 4. These subjects are moderately invaded by *Pinus halepensis* and *Juniperus oxycedrus*.

As reported by to the literature, the commercial and industrial classification of cork quality according to tree parameters (circumference, height, debarking height) is not fully scientifically identified. in agreement with Montero and Cañellas (2003), the best quality of cork accompanies the subjects in full production that is to say the subjects in the sizes are between the classes of circumference (107-140cm) and (170-200cm) especially the class (140-170cm) at 1.30m from the ground than young (<107cm) and old and very large (>200cm) subjects. The same authors argue that cork extracted from high parts of the tree, despite its good quality is classified as refuse cork (Q-D) or thin cork (Q-C) with all the price losses it generates. The better quality cork (QA) seems to be influenced by the overall health

of the trees (healthy), ie with a leafy crown with more than 75% foliage and a trunk free from wounds and fungi. (parched mother, chancre, cavity, insect holes). For cork type QB, QC, QD (cork stopper, thin (washer) and refuse (grinding) are at least within the range of the northern geographic area of the production area, growing on schistose ground semi arid with high maritime humidity (>60%). In this area, the trees of the Beni Ouarssous oak forest are distinguished by their high sensitivity to the recurrence of fires than those of M'Sila. In Beni Ouarssous, because of a strong greening of the Aleppo Pine, the trees are declining and the production of cork is weak and gives only a thin product type Q-C (washers).

In M'Sila, Q-B type cork is common in shallows on deep sandy loam soil. Conversely in the factorial plane (F2), whether in the mountains or on the coast, the suberaies of M'Sila and Hafir are the two major production areas in Oran. Q-A type cork with natural corks of choice is obviously Hafir while Q-B plugs are from M'Sila. Indeed, in northern Algeria, the quality of the cork takes a descending character from the Hafir-Zarieffet forest to all the other oak groves in Algeria (Lamey, 1893 and GGA, 1927). On the other hand, the type Q-D (refuse-grinding) does not seem to be related to any entity, it manifests itself in the four oak groves and it is more frequent in littoral. Thin Q-C type cork (washers) is not as specific for a single area, with a slight exception in Beni Ouarssous and Nesmoth.

Conclusion

Globally, we can say that the study of the impact of environmental parameters on the quality of cork remains a very complicated and controversial theme. The species is characterized by strong allogamy acting on forms of inter-specific and intra-specific hybridization where a large number of subjects per hectare do not have the same genetic characteristics resulting in a varied quality of cork., and a different form of adaptation. Indeed, in Algeria, *Quercus suber* covers 400000 ha and grows from a few meters of the coast to more than 1500 m in the mountains. This geographical distribution is characterized by very marked climatic, edaphic and silviculture contrasts. The obtained results, do not generally come out of those proposed by some cork authors and professionals (Lamey, 1893, Saccardy, 1937; Natividade, 1956; Montoya, 1988; Bossuet, 1988; Seigue, 1989 and Pereira, 2007). All these authors agree that the best quality improves with the increase in the operating cycle of cork (12 to 15 years instead of 9 to 10 years) where equilibrium cells formed in the spring and in autumn. This configuration is found more in the mountains, on the stony soils of the ridges and on the mature trees in full production, strewn with a vigorous sanitary state (crown and trunk). The cork forests management parameters are often quoted and on which the authors group together can improve the production mode of the stands. These are the silviculture operations (combating brushing, the space between trees, the quality of the exploitation and the respect of the rotations) and the plant health control against the defoliating and xylophagous insects and the pathogenic fungi, and also the fight against fires.

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CORK OAK RESISTANCE TO FOREST FIRES IN THE REGION OF TLEMCEN (NORTHWESTERN ALGERIA)

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Abstract

The fire resistance of cork oak has long been known. Through to the protection provided by its suberous bark and the many dormant buds located under it, can keep its trunk and restore a forest atmosphere in a few years. To confirm or deny this hypothesis, four stands of cork oak trees surviving two different fires were studied in 2018. In Hafir, two stands (H1 and H2) survived the 2005 fire and Zarieffet two others (Z1 and Z2) survived the 2007 fire. The measurements performed on the sample trees concerned dendrometric parameters and health status. On cork, we conducted an analysis of its growth and quality. The results revealed a major change in the structure of the four stands due to the dominance of the small wood species (Hafir: 14%, Zarieffet: 53%) and woodland (Hafir: 90.9%, Zarieffet: 42%). These traumatized subjects support verdant crowns with a sanitary state leading to a start of attrition (1.60 <IS <2.0). The percentage of "mother of cork" low damaged and very damaged is high in Hafir than Zarieffet, 57% against 40% of the total. The visual quality of cork produced by these trees carries the clues of a healthy cork since only the bark is soaring. Due to a high presence of waste cork, the quality indices is better in Hafir stands (H1: 10.20; H2: 6.69) than in Zarieffet (Z1: 5.92; Z2: 6.92). 66% of cork trees can produce stoppers cork in Hafir versus only 48% in Zarieffet.

Keywords: *Cork forest, fire, caliber, growth, quality.*

Introduction

The forest fire is an element of nature; it is an exteriorization of energy. This destructive element is, in forests of many parts of the world, a recurring phenomenon that recurs year after year with an apparently increasing intensity (Benyacoub, 1993). This disturbance is considered a natural ecological force against which certain plants have acquired adaptation mechanisms to survive and regenerate, mainly in the Mediterranean region (Pausas et al., 2004; Pausas and Verdu, 2005). Indeed, Mediterranean landscapes have been shaped from eternity by the action of recurrent fires, i. e. 2 million hectares were burned between 2006-2010 (FAO, 2013). According to Valette (1990), in this region, the absence of precipitation during the summer period, the air temperature, above 35°C under cover and 45°C in open ground level, and the atmospheric humidity, below 30%, modify not only the water status of dead plants and their debris (leaves or needles, twigs, pieces of bark), but also that of living plants. Among the typical Mediterranean species, *Quercus suber* L. is the best adapted to the structural phenomenon represented by the summer fire since its suberous protection allows it to resist the passage of fire.

In Algeria, forest fires are currently the most destructive factor of degradation. Algerian forest fire statistics are among the best archived in the world, covering a continuous period of 137 years (1876-2013) (Sari, 1976; Grim 1989). The colonial period was fatal for this heritage, from 1876 to 1962, the records of the French administration record a cumulative surface of about 3506 942 ha or an average of 40 778 ha/year. The catastrophic fires, of more than 100 000 ha/year very frequent during this period, mark several years sadly famous in the Algerian

statistics on this subject (Meddour et al., 2008). After independence, the Algerian forest experienced a relative calm, since the areas burned decreased compared to the colonial period, when the annual average was about 34254 ha (16% less). As an indication, this heritage lost 1162484 ha between 1979 and 2009, with a total number of fires declared of 41 644, (Bekdouche, 2010). The year 2012 remains the most catastrophic with a total of 90 061 ha (DGF, 2013). Currently, forest fires threaten 40% of the Algerian population, or 13 million inhabitants living in or around forest massifs (Bachouche, 2013). The Algerian oak cork stands did not escape this phenomenon, the fires started during the period 1993-2012 had unfortunate consequences on the area where 117317 ha were lost (Abbas, 2013). Cork production is currently at its lowest level at 8000 t/year. The resistance of cork oak to fire has been known for a long time, the bibliography joins all on the same observation: "Where other oaks, pines, arbutus, and all the different woody species of the Mediterranean maquis are forced to reject stump or rely on natural regeneration, cork oak, thanks to the protection provided by its suberose bark and the many dormant buds located under it, can keep its tree habit and restore a forest atmosphere in a few years (Elena Rossello, 2004, Piazzetta,2004). Our work was carried out in the southern part of the Tlemcen mountains, in two cork production areas, namely "Zarieffet" and "Hafir" with a sub-humid climate. Indeed, during the last twenty years, the forest massif Zarieffet-Hafir has been transformed from a dense forest to an open area overwhelmed by the massive degradation of its trees and the structural transformation of its stands; these two areas are very well known by the recurrence of fires. In this massif, nearly 228 ha of cork oak went up in smoke between 2005 and 2011 (C. F. W. T. ,2012). The objective of this work is to explain the behaviour of cork oak after a fire by a series of measurements in the field and laboratory. The vulnerability and post-fire resistance of the species were studied following the various modifications recorded on the structure of the stands, their sanitary state and their productive and qualitative potential.

Material and Methods

Four cork oak stands surviving two different fires were studied. In Hafir, two stands (H1 and H2) survived the 2005 fire and in Zarieffet two others (Z1 and Z2) survived the 2007 fire. The sample trees taken into consideration are living subjects of cork oak, particularly inscribed in the archives of the same fire and holding a flaming reproductive cork. The number of trees meeting these criteria totals 45 individuals in Zarieffet and 38 in Hafir. The measurements made on these trees concerned the following parameters: external circumference (SW: small wood, MW: medium wood and LW: large wood), debarking height, cork thickness and measurement of annual increases. The assessment of the health status of the surviving subjects is based on leaf deficiency. It is noted after examination of the tree branches according to its sanitary class (Bouhraoua & Villemant, 2002). 1: healthy (defoliation < 25%); 2: weakened (defoliation between 25-60%); 3: decaying (defoliation > 60%). This index allows expressing directly the general state of the population from a set of trees taken individually. It is calculated as follows: $$Is = \frac{\sum_{i=1}^{5} n_i P_i}{N}$$

where

ni: Number of trees of defoliation type i
Pi: Weight of type i (1 if i=1; 2 if i=2, etc.)
N: Observed total number of observed trees

The growth and quality of the cork were analysed by the assessment of the "mother" state of the cork and by the first use of Coveless CQ05. The quality class is established according to the IPROCOR method (1994). Thick : 19 plus 6ª plus; stopper cork bark (15-19 5ª more 13-15

5ª more, 11-13 5ª more); Thin (15-19 6ª; 13-15 6ª; 11-13 6ª); Low (4ªmore 8-11minus) and Refuse. The calculation of the quality index of each stand is obtained according to the formula proposed by IPROCOR (1994):

$$Q = (5/100) \times \Sigma i = 1\text{-}9\ (Ai \times Qi)$$

where:

Ai = number of trees of defoliation type I; Qi = weight of type i (1 if i = 1; 2 if = 2, etc.);

The chi-square test was used to compare certain parameters taken into consideration.

Results and discussion

All the indicators taken into account on the four stands are mentioned in Table 1.

Table 1. Average characteristics of sample trees and study area

Caractéristiques	**Forêt de Zarieffet**		**Forêt de Hafir**	
	Z1	**Z2**	**H1**	**H 2**
Geographic position (Plot center)	34°50'51.94''N 1°2'30'.19''O	34°50 '19.86''N 1°22'07.77''O	34°47'01.00''N 1°26'27.44''O	34°46'59.59''N 1°26'31.12''O
Place says	Forest division Zarieffet	Forest division Zarieffet	Forest division Oued El Fernane	Forest division Oued El Fernane
Average altitude (m)	Min: 1041 Ave: 1079 Max: 1117	Min : 1127 Ave : 1148 Max: 1151	Min : 1213 Ave : 1258 Max: 1288	Min : 1283 Ave : 1297 Max : 1313
Slope(%)	Max : 28.9 - 30.80 Ave : 7.4 -8.1	Max : 30.5-32.3 Ave : 9.7-9.5	Max: 47.6-35.4 Ave: 23.0-24.1	Max : 45.9-28.0 Ave : 18.6 -13.8
Perimeter (m)	2236	1057	643	402
Nature of the soil	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-loam
overgrown	Moderately	Strongly	Moderately	Moderately
Stand origin	Natural	Natural	Natural	Natural
Nature of the stand	Irregular	Irregular	Irregular	Irregular
Stand structure	Seedling of oak cork	Seedling of oak cork	Seedling of oak cork	mixed with holm oak
Average density (N tree / ha)	55	200	60	100
Height of trees (m)	6.47±1.03	5.80±0.90	5.89±0,97	7.88±0,97
Average circumference (m)	0.65±0.12	0.90±0.13	0.95±0.08	0.98±0.12
Average debarking height (m)	2.11±0.28	1.83±0.26	1.61±0.14	1.77±0.27
Last fire	2007	2007	2005	2005
Number of sample trees	24	21	22	16

The table shows that the trees that survived the 2005 and 2007 fires are generally natural, of varying ages, resting on sandy silty soil. Zarieffet and Hafir plots 1 appeared to be the least dense with 55 and 60 subjects/ha while Zarieffet and Hafir plots are the least dense with 55

and 60 subjects/ha. The altitude of 1313m is maximum at H2 (Hafir) while it is minimal at Z1 (Zarieffet) around 1041m.

Stand structure

One of the first negative consequences of forest fires is a marked reduction in overall stand density. The size distribution of the subjects is shown in Figure1.

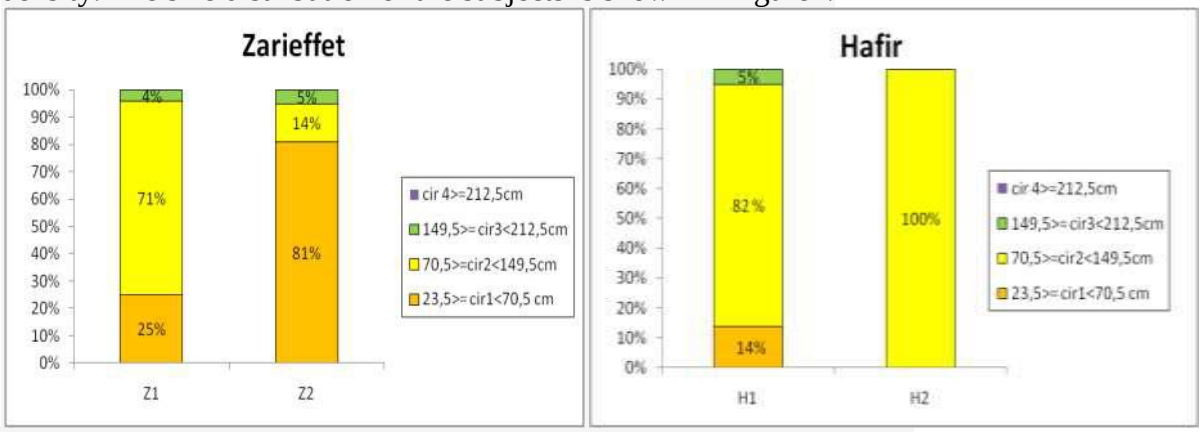

Figure1. Distribution of resistant trees according to their circumference

The fires of 2005 and 2007 directly affected the structure of the two forests. Only three categories of wood exist in the four stands (small, medium and large). The presence of small wood records a rate of around 14% (H1) and 25 to 80.9% (Z1,Z2). The average wood occupies simultaneously 81 to 100% (H1,H2) and 70.8 to 14. 29% (Z1,Z2). Finally, large wood has a low resistance with a percentage of 5% (H1) and 4. 76 to 4% (Z1,Z2).

According to Amandier (2004) and Bernal Chacón and Cardillo Amo (2005), it is in particular the old trees (very large wood) and the small poles (very small wood) that give way first to the flames. In the case of cork oak, this situation is dependent on the thickness of the cork on these subjects at the time of the fire. In this context, measurements made on cork increases revealed that the average age of the latter is around 9 years in Hafir and 11 years in Zarieffet. This is made by the suberous layer detached from the neo-formed layer after healing (Fig. 2).

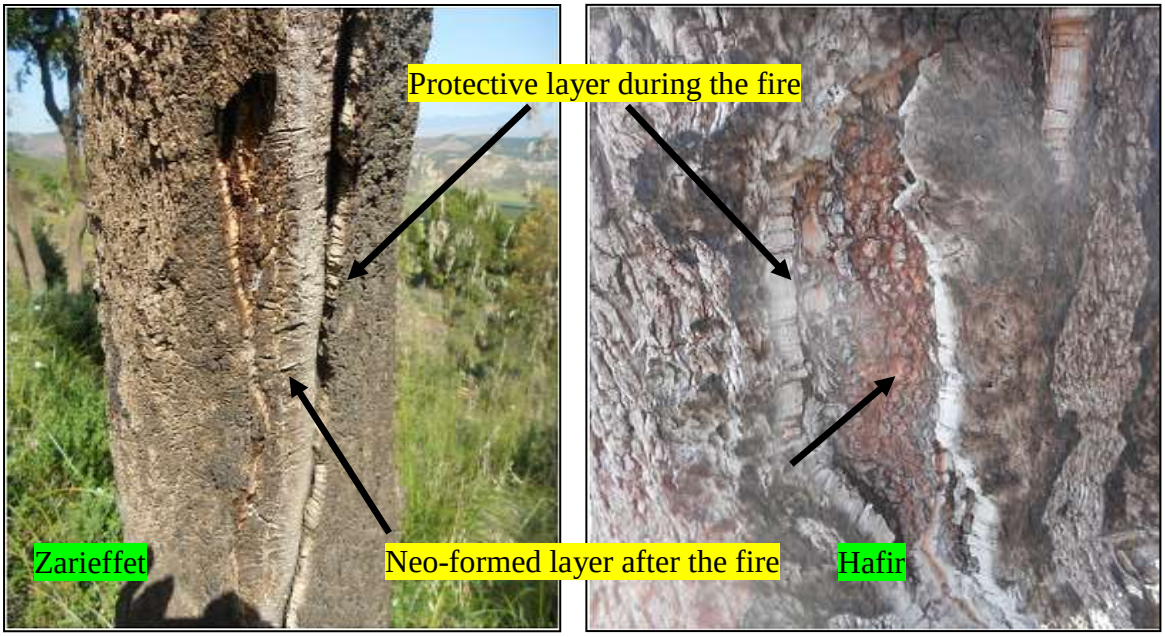

Figure 2: Bark resistance due to visible cork growth before and after the fire

State of the peaks

The foliar deficit (of the tops) recorded on the sample trees is illustrated in Figure 3.

According to the figure, we could note that the tops of the sample trees are generally green, i. e. a rate of 47.36% of healthy subjects in Hafir against 48.88% in Zarieffet. The Hafir plot1 has 59% vigorous individuals while they are 61.47% in Zarieffet (Pl2).

The average number of weakened cases is 39.47% in Hafir and 35.35% in Zarieffet. The Hafir H1 plot is individualized with 40.90% of cases against 54.16% in Z1 (Zarieffet). For decaying subjects exceeding 60% leaf deficit, this rate varies from 13.15% (Hafir) to 15.55% (Zarieffet). H2 trees total 31.5% while they are only 23.8% at Z2. The Chi-square test records a non-significant difference (χ^2 = 0.17; 2ddl).

Furthermore, the health indices seem balanced between the two entities: Hafir (IS = 1. 67) and Zarieffet (IS = 1. 62) and converge the four stands towards the beginning of decline (1. 60 < IS < 2.0) (Fig.3).

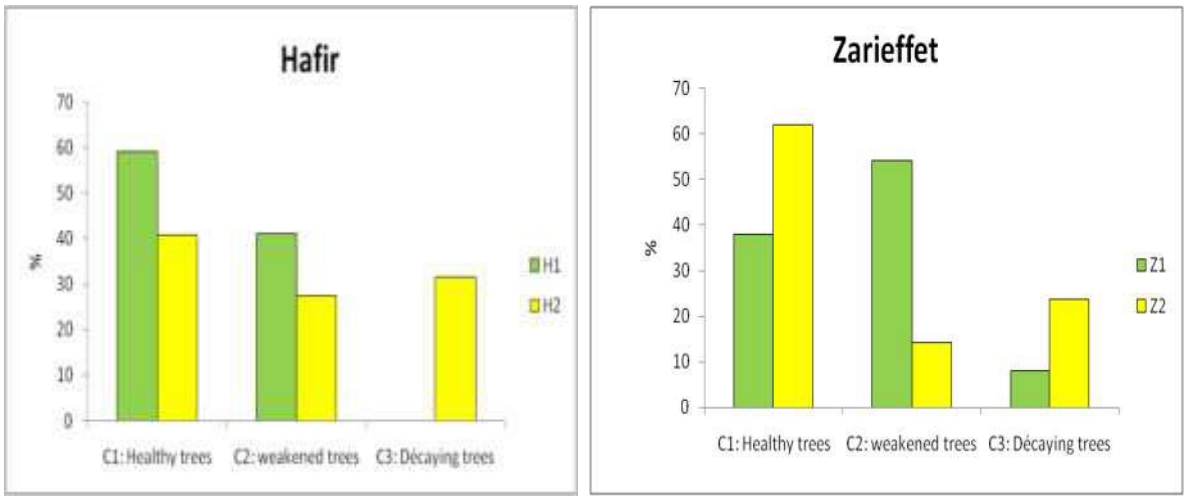

Figure3. Post-fire crown appearance on sample trees

Indeed, forest fires are among the factors that often trigger the description of the health deterioration of cork oak and correspond quite clearly to regions where low annual rainfall, high summer temperatures and a low useful soil water reserve are combined. The intensity of the fire firstly burns the soil and favours its exposure to sunlight and leads to its degradation; subsequently accelerates the phenomenon of erosion and consequently the disappearance of fertility which is essentially linked to organic matter and the biological activity of the soil (Dehane, 2012). This pattern creates a dry atmosphere unfavourable to cork oak. Trees directly affected by fire irreversibly lose their foliage and then die, the survivors regenerate it (dormant buds) with symptoms of twig alteration and a modified crown architecture, by the fall of the leaf mass (Nageleisen, 1999).

It has been found in several studies that repeated fires over a period of time and in the same area, without the possibility of sufficient healing, accelerate completion factors that are generally related to the presence of xylophagous and xylomycetophagous insects and pathogenic fungi of weakness and injury (Griffin et al. , 1992; Cherubini et al. , 2002). Some of these symptoms were encountered in Zarieffet plot 1 with respect to the mother's charcoal (Fig. 4).

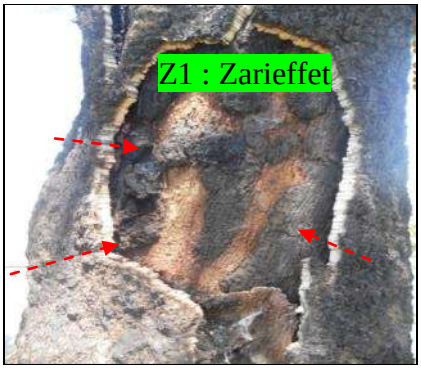

Figure 4.Mother of cork damaged by the fire and infested by mother's coal *Hypoxylon mediterraneum* (De Not) Mill.

State of mother of cork

The distribution of the appearance of "mother" on the sample trees is illustrated in Figure 5.

Legend: SW(small wood) ; MW(medium wood); LW(large wood)

Figure 5. Proportion of the state of "mother" of cork on the sample trees

Through the figure 5, we notice that in the plots of Hafir the aspect of the mother of the cork seems very damaged on the large circumferences is a rate of 100%. However, in Zarieffet, this rate does not exceed 4% respectively for small and medium wood (Z1) and 9% for PB (Z2). As regards the damaged states, they always remain within the individuals of Hafir (particularly MB) with a rate of about 42% against 22% in Zarieffet (PB+MB). The Chi-square test calculated between the two forests makes a significant difference (χ^2 = 8.36; 2ddl).

During the fire, the mother of cork (subero-phellodermic Assisi and libero-ligneous Assisi) very sensitive to flames can be destroyed by the heat which will completely consume it. This heat depends on the intensity of the fire and the adequate protection of the cork. The destruction of a localized cork mother surface involves the eradication of wood growths in this area so that they cannot create new conductive vessels and consequently the meristematic activity of bast. Indeed, when the sap rise is highest during spring and summer, this area (on trees surviving after the fire) appears dry and bare. The death of cork mother also means the end of its suberous production and the appearance of zones vulnerable to the penetration of fungi and xylophagous insects (Fig.6). If the destruction occurs over the entire circumference, the entire life of the tree will be compromised.

Bernal Chacón and Cardillo Amo (2005), state that the protective power of cork is proportional to its thickness. A cork thickness of 20 mm implies sufficient protection for the mother whatever the type of fire. In the case of low intensity lights, 10 mm seems sufficient to protect the mother. Indeed, on our sample trees, the rates of "mothers" damaged and very damaged (according to our observations) are more related to the quality of exploitation before the fire in both entities. Whether they act on young or old trees, the accumulation of injuries, the slow growth of wood and cork with age increase the deteriorated surfaces of the bunk during fire. This situation is more present in Hafir. According to Piazzetta (2011), the injuries caused as successive debarks are as many entry doors ("decay") for the fire inside the tree (Fig.6).

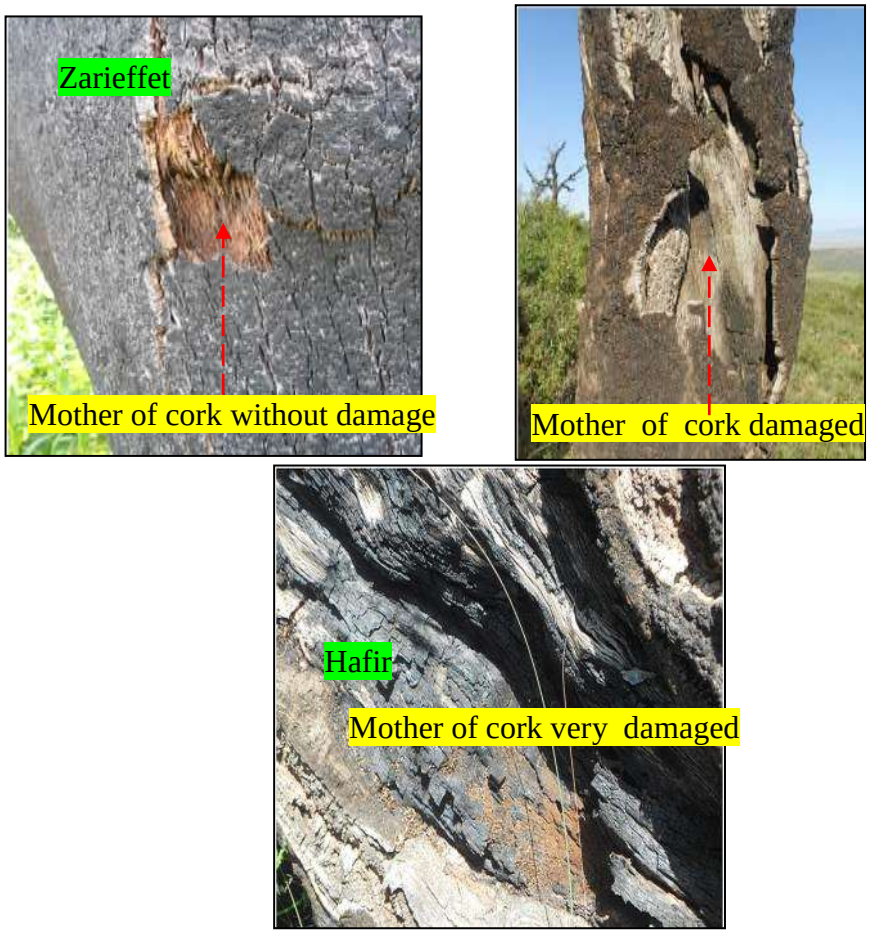

Figure 6. Observation made on the appearance of cork mother

Study the quality of cork

The results of cork quality sampling on the surviving fire trees are shown in Figures 7 and 8 according to the commercial and industrial classification:

-Extended classification: The extended classification merges the calibers and visual appearance to produce the quality class according to the IPROCOR method (1994) (Fig. 7).

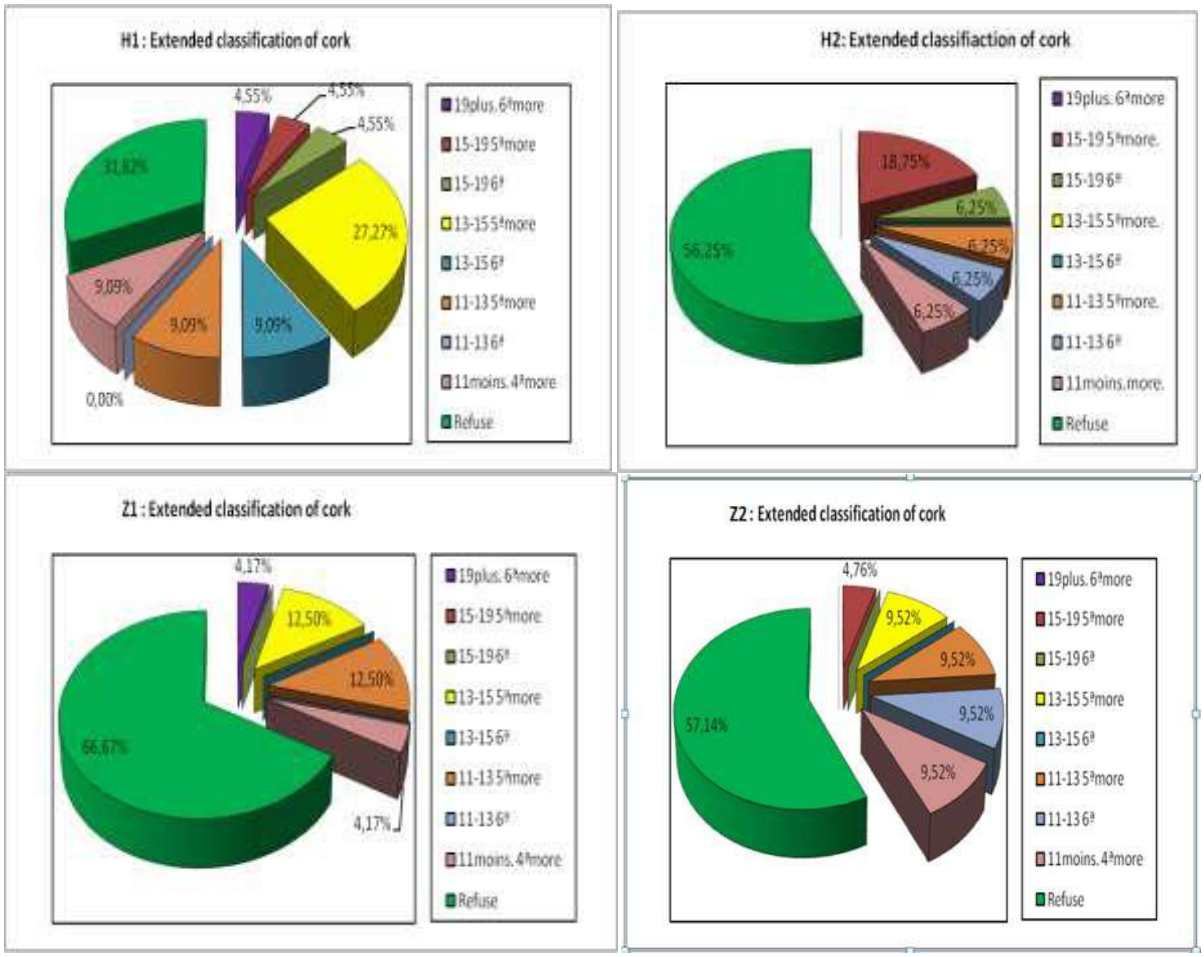

Figure 7.Commercial cork quality classification on the subjects of both forests

In Hafir, only H1 sample trees generate 4.55% thick cork (quality 19 plus. 6ªplus). The cork of the class 2 (15-19 5ª more) is imputed to H2 of the order of 18.75 % against 4.5 % with Pl1. At the same time, very poor quality waste cork (Refuse) is more present in H2 with a rate of 56.25% and in H1 with 31. 82%. Very thin cork of 4th choice and higher (11minus.4ª more) ranges from 9.09% (H1) to 6.25% (H2).

In Zarieffet, the proportion of thick cork in class 1 (19 plus. 6ªplus) is identified only in 4. 17% of the Z1 population. In plot Z2, class 8 (11 minus. 4aplus), synonymous with very thin cork, seems to be the most important, i. e. a rate of 9.52%. Refuse cork is generally higher in both plots, 66.67% (Z1) and 57.14% (Z2).

Summary classification

The summary classification gives us an idea of the industrial outlets of the product surveyed on the sample trees. Figure 8 summarizes the results of the extended classification.

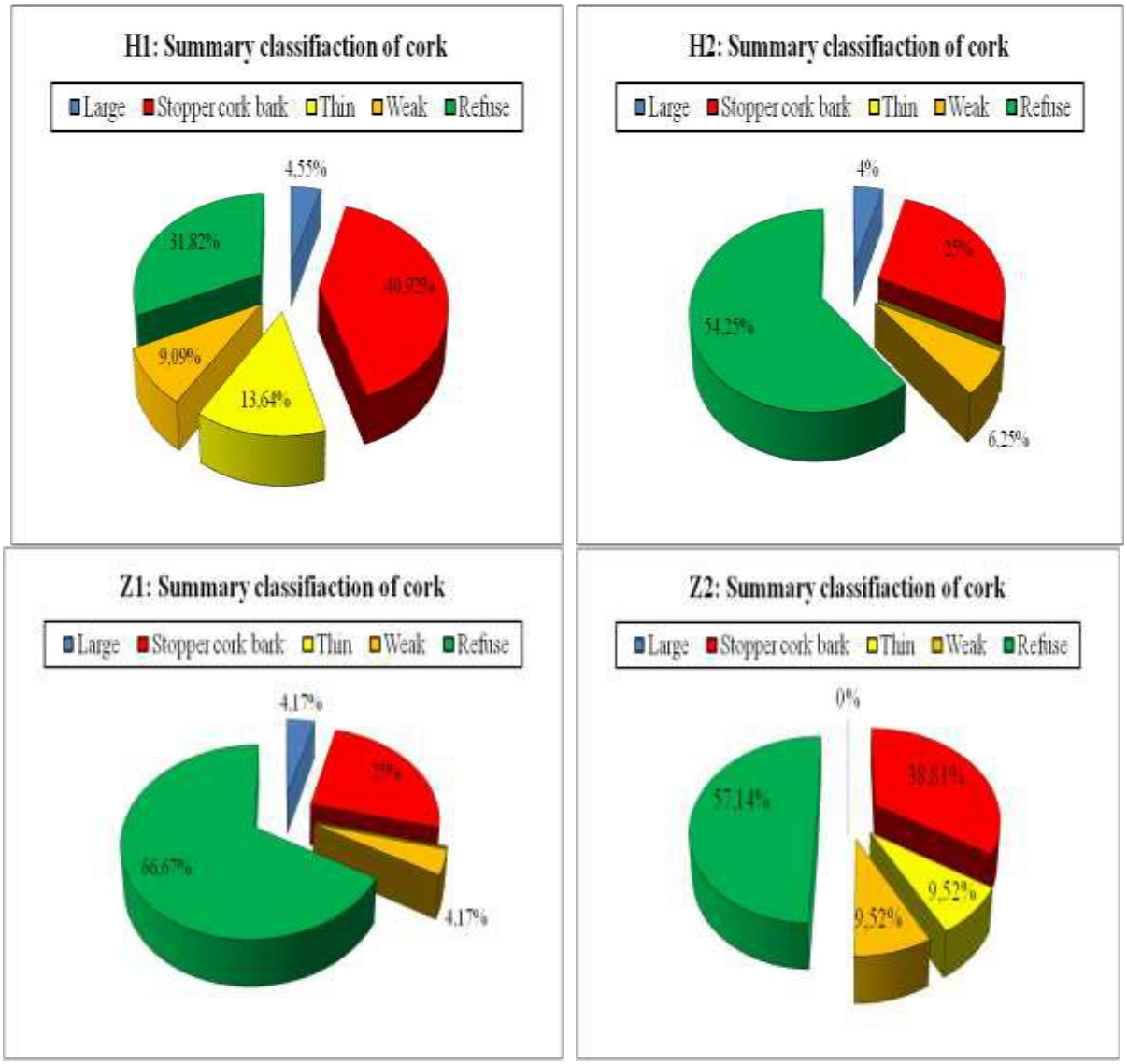

Figure 8.Industrial classification of cork quality on the subjects of the two forests

The summarized classification indicates that the stopper cork bark (Φ: 24mm) of Hafir groups together 3 classes (15-19 5ª more, 13-15 5a more, 11-13 5a more) of 49,91 % on H1 and 25% on H2. Thin cork concerns only 13.64% (H1) and 12.50% (H2) of cases. The weak cork for the confection of the discs is identified in the 6th choice (of the classes 15-19,13-15,11-13) with a percentage of 9,09%(H1) and 6,25%(H2). Refuse cork for crushing and other uses holds 31.82% and 52.25% respectively.

The Zarieffet workforce produces less cork (Φ : 24mm) than in Hafir, i. e. 25% (Z1) and 23. 81% (Z2) (Fig.9). Thin cork for 21 mm diameter corks is only present at Z1(9.52%). The weak cork for the washers varies from 4.17% (Z1) to 9.52% (Z2). Refuse cork is important, exceeding 55% in both plots.

Several authors agree that the decline in cork quality on trees is linked to excessive competition within the cork forests, which would lead to strong competition between trees, a weak vegetative state with a slight increase in wood and cork (Bossuet 1988 ; Montoya,1988). The passage of fire depreciates the quality of the cork only through the thickness of the flare recorded on the gauge of the boards (Fig. 9). The buyers decline this type of cork because of the flambéed bark and the additional work during the boiling and scraping of the crust. Dubois (1990) estimates the additional cost of exploiting burnt cork at 20%, and that of

transport at 12 to 15%. According to Bernal Chacón and Cardillo Amo (2005), whatever the intensity of the fire, the black band that appears on the transverse layer of the board rarely exceeds 2.5 mm for a thickness of 10 mm and 10 to 12 mm for an over-thick cork of 50 mm (Fig.9).

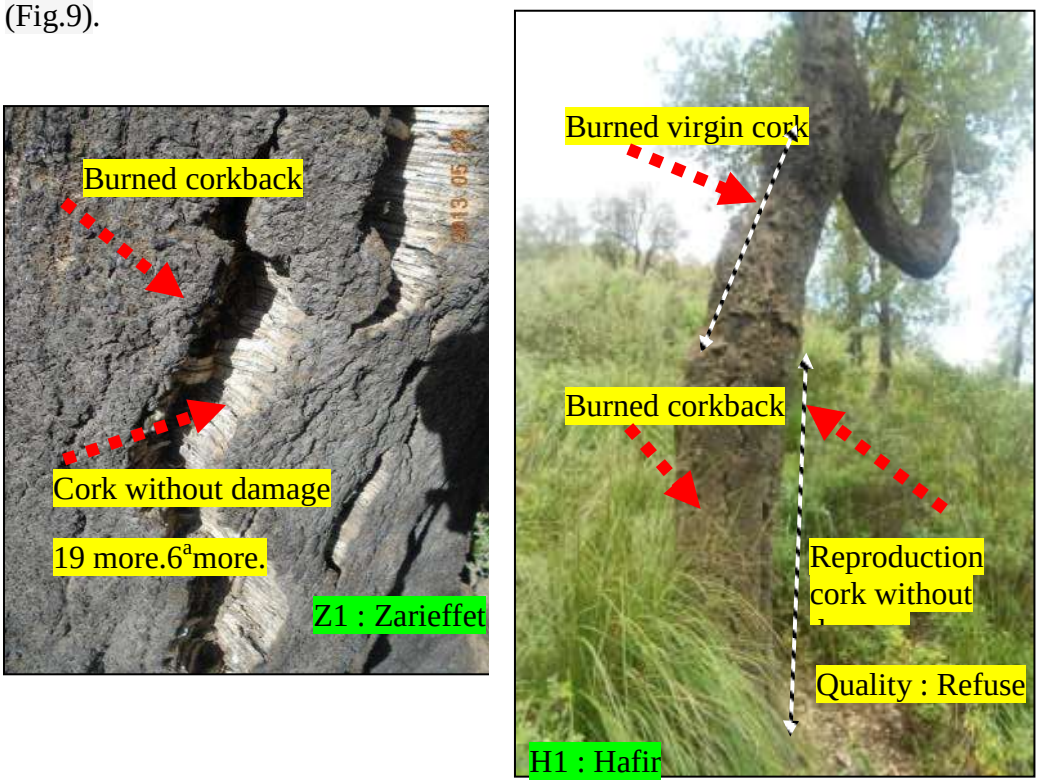

Figure 9.Quality of cork produced by burned trees

In this work, in spite of the healthy visual aspect of the cork which is characterized by a localized superficial flare at the level of the crust, the coveless probing showed that the quality of the cork is rather attenuated. This consequence is due in large part, to the strong presence of defects especially folded corkwood, vertical and horizontal lignification. Indeed, the quality indices were different from one plot to another. Only H1 subjects seem to stand out with a QI = 10.20 (Fig. 10).

Figure 10.Quality indices recorded in the plots of the two forests

Conclusion

The forest massif Zarieffet-Hafir represents the large area of the oak cork stands of north-west Algeria. These stands with regard to the entire subericolous Mediterranean are characterized by their resilience and their more or less marked resistance to fires. Through this work, it is found that fire influences certain aspects related to stand structure, the state of health of trees

and their productive potential in general. Repeated fires lead to clear cork oak stands that favour the development of the maquis, thus increasing the risk of future fire. The good management of these two entities must be based essentially on the notion of the omnipresent occurrence and intensity of fires according to a fire hazard map. Indeed, both forests have been subject to more than 4 fires in 50 years. Fuel in both forests must be located and mechanically controlled according to its structure and type (plant species, height, cover, dense scrub, clear scrub).

In Zarieffet, the landscape is dotted with gaps between the surviving trees and very colonized by a very flammable thorny bush.

In Hafir, the existence of a dense and high maquis based on rock rose and tree heather favours the vertical connection between the trees, thus allowing fire to spread to the top.

In our opinion, in both cases, the exaggerated presence of this forest fuel does not in any way help any attempt at natural or assisted regeneration. Even more, it acts negatively on the physiology and nutrition of trees and consequently on their radial and suberous growth.

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ESTIMATION OF BIOMASS AND CARBON STOCKS: ADAPTIVE MEASURES TOWARDS CLIMATE CHANGE AND FOREST MANAGEMENT IN RUSSIA

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Abstract

Current land use in Russia has an impact on the pools of carbon and biodiversity conservation in forest areas. Total forest area in Russia has exceeded 885 million hectares, that is around almost a half of the total area of the country. These areas play a major role in climate change, due to the carbon concentration. However, it is problematic to measure of total carbon stocks and biomass. The carbon storage estimation can be represented by three methods: an individual-based gap model of forest dynamics (FAREAST), mapping of distribution of living forest biomass (MODIS) and individual tree-based forest gap model University of Virginia Forest Model Enhanced (UVAFME). Due to the results of the Model estimations multiply scenarios could be implemented; for example, improvement of the stakeholder's network and land use control on the governmental level. At the same time, a complex of factors has an influence on future scenarios. For instance, permafrost affected soil and led to the stronger dependence on climatic conditions over the last 15 years in Russia, as well as on fire disturbance. To follow changes in soil and air temperature, in this paper was analyzed collected data from weather stations in Russia. Climate change influenced on agricultural land use. Traditional Siberian crops would be changed to new crop varieties with an extra irrigation. These adaptation measures would promote sustainability and food security in Siberia within the context of global warming. Meanwhile the new land use legislation does not rely on natural ecosystems and biodiversity conservation. Alternative policies will be reflected on through the framework of *Green economy*.

Key words: *Biomass, Biodiversity, Carbon stocks, Policy implementation.*

Introduction

The discourse about sustainable development began in the 1980s. During the last year policy has been improved in a response of forest goods, ecosystems and woodland landscape (e.g., Kennedy *et al.,* 2001; Campbell and Sayer, 2003; Innes and Hoen, 2005; Sastamoinen, 2005). To support *Green economy* policy implementation (Zaharova, 2015), several approaches such as "Priorities of the national environmental policy of Russia: from the federal center to the regions", the "Environmental and Climate Doctrine of the Russian Federation", "The Comprehensive Plan for the Implementation of the Climate Doctrine of the Russian Federation for the Period to 2020" will be introduced and discussed during the International conference "Basics of federal policy in field of ecological development of Russia till 2030". However, polices will be beneficial in case of appropriate observation and research methods. Hence analyzing the results of empirical studies and evaluation of climate change as well as their influence on sinks of carbon, biomass and biodiversity, are researched using the following three methods: an individual-based gap model of forest dynamics (FAREAST), mapping of distribution of living forest biomass (MODIS), and the individual tree-based forest gap model University of Virginia Forest Model Enhanced (UVAFME). The paper is aimed to address three specific questions. First, which strength and weaknesses have reviewed methods? Second, how disturbances and climate change influence on carbon stocks? Third, if

the forest management can be one of the adaptive practices to the climate change? The research aim of this paper is built around these questions. Thus, the author aims to identify the current state of research to depict how the scientists respond to problems regarding carbon stocks in Russia and adoptive measures towards the climate change. Thereby 2 main objectives are specified. The first objective is to summarize the evidence and provide a quantitative assessment concerning the recent research statement on carbon stocks in Russia. The second objective is to find out the adaptive management actions, for instance, possible initiative collaboration between neighboring countries.

In the Siberia changes in carbon stock was caused by burned part of the southern dark taiga aimed to convert those lands to the arable one (Baranchikov and Kondakov, 2002). An intensive land use for the agricultural purposes contributes to the biodiversity and biomass losses in Russia. It is important to promote future scenarios for the ecological, economic and sociocultural development by estimation of carbon and biomass content in different regions of Russia, to promote improvement of the regulation norms and the structural management in agricultural land and forests use.

Material and Methods

These paper is based on the literature review of secondary data published in research articles on the topic "Carbon stocks and biomass estimation methods in Russia" and aimed to compare the efficiency and output of the used methods. Selection of the articles have been done by the key words: "Land use policy in Russia", "Forestry in Russia", "Carbon stocks in Russia", "Climate change in Russia", "Green economy in Russia" in Google Scholar database and IOP Science online service.

Represented articles observe qualitative and quantitative methods. For the MODIS from polygons ranged in size from 0.7 to 1503 ha in 12 Russian geographical units (Krasnoyarsk Yart, Northern Khabarovsk, Karelia, Krasnoyarsk Usol, Kamchatka, Southern Khabarovsk, Magadan, Murmansk, Udmurtia, Novosibirsk, St. Petersburg, Kursk), with an average of 18 ha/polygon (Houghton *et al.*, 2007). Estimation air and soil temperature changes are represented by collected data from regular weather stations of permanent frost regions and samples from the locations over 100m x 100m (1 ha) and forest management enterprises (Leskhozes) or special stations for soil temperature estimation in framework of the Roshydromet network in Russia (Sherstiukov *et al.*, 2012). Russian territory based on all of the available daily soil temperature data was originally developed in the late 2000s (Sherstyukov, 2008). Linear regression allows to compare simulated and inventory measured biomass by the FAREAST model at around 2000 sites in Russia.

Sustainable Forest Management implementation provided by the survey (2010) on multi-stakeholder model forests (MFs) initiatives opinions in selected Russian regions (Komi MF and Pskov MF) and Sweden one (Vilhelmina MF and the Foundation Säfsen Forests in the Bergslagen region) (Elbakidze *et al.*, 2010). Research and clear vision on the present problematic zones in Russia can develop proper management and policy, which will correspond current climate changes.

Results and Discussion

Soil as natural resource and soil sciences play a major role towards achieving sustainable development goals (Keesstra *et al.*, 2016). Three soil functions are acting as carbon pool, biomass production, and biodiversity, have been assumed applying three methods mentioned above (MODIS, FAREAST and UVAFME). Data on change in forests dynamics (burned, deforestation, recovered) and volume of biomass are used for the carbon stocks measurements.

Each of the overviewed articles has an inaccuracy in the final estimation results. In the article of Houghton et al (2007) proposed MODIS database use was only partially successful. The analysis was performed with the random Forest package (Liaw and Wiener, 2002) and the calculation of weighted mean biomass was an aggregation process that often follows to assign a particular forest type to a MODIS cell. According to the analysis, ∼60% of the forest area in Russia had biomass values greater than 64 Mg ha^{-1}. However, there was a number of estimation limitations: the first was - in regression model, which did not include calculation option of the biomass in closed-canopy forests (the age was more than 20 years), as a result the error in final volume of biomass was around 39%. A second limitation to the prediction of above-ground biomass with MODIS data has been the extent to which the forest polygon data (difference in size of observed land by individual polygons) matched the spatial size criteria of MODIS cells. Disturbance effects of fire, insect damage and logging, are not explicitly included in these simulations using the FAREAST model. Therefore, the model can provide higher biomass estimation compare to the inventory-based estimation. FAREAST model can be improved by Dynamic global vegetation models (DGVMs), which focuses on type of vegetation and can cover large scales for stimulations (future scenarios) for carbon accounting (Shuman *et al.,* 2013). The simulation of all individual trees and their dynamics for around 30.000 plots can provide a complete carbon accounting in dynamics influenced by changes in the system. In the last years Eurasian forests were disturbed in the western part of Russia; however it was affected by intensification of land use and climate change that influenced on the pattern of carbon sources and sinks (Krankina *et al.,* 2002). The capacity for aboveground biomass growth is limited, as well as maintenance of the extensive landscape mature stage forest for the purpose of accumulating and accounting of low carbon stocks capacity across Russia during disturbances. Decreasing the rotation age for an even-aged distribution from 210 to 150 years does result in decreased biomass in all regions, but this decrease is only statistically significant in the east region. Where the simulated average total standing biomass (tC ha1) is 69.21 for the 150-year rotation which is significantly lower than the current simulated biomass of 83.42, whereas the 210-year rotation has a simulated biomass of 79.42 (Shuman *et al.,* 2013). This method does not consider the sink coming from build-up of downed and dead trees that one might expect in a mature forest mosaic landscape, weather it is an important component of the carbon content. Within the FAREAST model, individual trees competition in nutrient uptakes determines biomass accumulation, annual leaf and fine root renewal (Shuman *et al.,* 2013). The model allows to determinate in the Eurasian forests, as 87.4% of the forested areas in Russia are covered by *Pinus spp, Picea spp, Abies spp, Larix spp, Betula spp, and Populus ssp* (Shvidenko and Nilsson, 2003).

The alternative individual-based gap model has been proposed by University of Virginia Forest Model Enhanced (UVAFME). It is based on the systematical approach of the FAREAST (Yan and Shugart, 2005) and contributes to the Regionally-Adjusted MODIS Burned Area (RAMBA) method of mapping burned area (Loboda *et al.,* 2011). The model also allows to estimating future scenarios, formed by fire and climate disturbance (Shuman *et al.*, 2017). Fires effect on carbon and biomass, as well as biodiversity, seedbank, seeds can be destroyed or removed by other species seedlings. The results of the studies in the increase of aboveground carbon storage are familiar with previous boreal forest modeling work of Kasischke *et al.* (1995), which demonstrated the positive effect of biomass storage with warming and growth of fire frequency. In case reduced fire frequencies carbon will increase from 82.06 tC ha^{-1} to 86.11 tC ha^{-1} and within climate change and fire from 112 tC ha^{-1} to 119.17 tC ha^{-1}(Shuman *et al*., 2017). It is predicted that low to mid-severity fires will lead to a thin organic layer and changes in temperature of soil. The UVAFME model does not review results of relationship between vegetation and fire. However, predicted future scenarios

present changes in carbon and biomass dynamics and help to form relevant management according to the use of available natural resources.
The increase of soil temperature (around 0.4 °C/decade) in the Central Siberian Plateau (although observations are sparse) and in the southern mountainous regions (Altay, Sayan Mountains, Stanovoy Range) during the last 50 years resulted in thickening of the active layer and possibility to develop the residual thaw layer above the permafrost over the last decade (Streletskiy *et al.,* 2015). Changes in the ground thermal regime will afford the food security of indigenous communities, development of infrastructure and agricultural land use in geographical areas of the Russian Arctic (Streletskiy *et al.*, 2014). Scientists already predict about 50 to 85% of central Siberia areas to be climatically suitable for agriculture by the end of the century, however, soil fertility (carbon contain) can be a boarder for a crop cultivation (Tchebakova *et al.*, 2011). The previous experience without proper estimation of fertility limitations and establishment of regulations of intensive land use in the 1991 in the southern central Siberia, leads to the degradation of 1.5 million ha of an arable land. In Khakassia, in particular, 81% of the arable land was subject to Aeolian erosion (Lysanova, 2001).
Biomass estimations are diverse; therefore carbon stores and fluxes from Russian forest ecosystems are also vary. Those findings contribute mainly to the uncertainty of world estimates. Improved commitment of biomass and the results of management policy implementation is a base of future management formation in concordance with the Kyoto Protocol[28], signed by Russia in 2004. This document is just one of the 100 international agreements of Russia. Nowadays Russia started to follow in *Green economy* concept in order to minimize ecological risks and to achieve the basic sustainable goals. The main aims are sustainable consumption and production (food security), protect and restore sustainable use of terrestrial ecosystems, and sustainable manage of forests (Zaharova, 2015). Survey from the 2010 year analyzes the four selected Model Forest initiatives (Komi MF and Pskov MF in Russia and Vilhelmina MF and the Foundation Säfsen Forests in the Bergslagen region in Sweden) and the influence on decision-making and sustainable forest management (SFM) policies formation processes, as well as the motivations for their establishment (Elbakidze *et al.*, 2010). Fragile governance system at the national level in the Russian Federation does not have any NGO's influence, lack in the property rights, corruption and demonstrated low level of the social capital (Olsson *et al.*, 2006). However, in Sweden exists a rigid governance regulations at the national level. As an adaptive measure to the forest management and climate change – collaboration between neighboring countries, which aims to obtain the experience and exchange knowledge (Elbakidze *et al.*, 2010). Furthermore, initiative elaboration of approaches will lead to sustainable development by creation of projects between Sweden and Russian researchers. Those projects are often supported by international companies, organizations and NGO's, for instance, the World Wildlife Fund (WWF) submitted project to prevent exploitation of the large intact forest landscapes (Yaroshenko *et al.*, 2001). Collaboration initiatives can be implemented easily on the local, regional, national and international levels; however, modern Nordic approaches are often conflicting with the present Russian system of forestry norms and regulations (Elbakidze *et al.*, 2010). International collaborations will be successful when primary data from the local institutes will be analyzed in sufficient scientific way. Moreover, agricultural lands' biodiversity directly depends on the land use peculiarities. Soil fertility and land value elements comprise of current and former biodiversity (more than 6 000 species of plants, around 100 species of mammals, 180 species of birds and thousands of species of insects and other invertebrates),

[28] The Kyoto Protocol is an international treaty which extends the 1992 United Nations Framework Convention on Climate Change (UNFCCC) that commits State Parties to reduce greenhouse gas emissions, based on the scientific consensus that (a) global warming is occurring and (b) it is extremely likely that human-made CO2 emissions have predominantly caused it.

which also influence on carbon pools and biomass. This fact is forgotten by society and is not taken into account either by political and administrative decisions in regards to the Russian agricultural lands or while current use practices (Smelansky *et al.,* 2003).

Conclusions

The measuring of carbon and biomass is important in sense of eco-system dynamics and proper management implementations. Several models can provide such data and predict future scenarios. MODIS data showed a difference in biomass across the mixed types of forests in Russia (non-forests, open forests, young forests, and old forests). The maps of forest biomass described by this method will help to construct estimation of carbon emissions connected to changes in the land use, fire disturbances and changes in permanent frost period. However, better understanding of influence of disturbances on biomass and carbon can be assumed by UVAFME model, which tracks individual trees. This model is based on the FAREAST, which gives an opportunity to predict and analyze management influence on the aboveground carbon storage.

Sources and sinks of carbon are the outcome of movements in biomass volume (carbon stocks), which can be better measured by changes in carbon stocks directly, than by observing transitions across an arbitrary threshold of forest–non-forest. With the same trends towards climate change conditions, being the same one can say with a rather high degree of certainty that this accumulation is practically irreversible and unlimited in time. The adaptation measures to the climate change and loses in carbon stock are creation of proper managed forest condition even when if these forests is highly unlikely given the economic importance of wood harvest. The State have to allocate funds on the bases of *Green economy* policy implementation for that purpose. In fact, the regulation norms in land use and management proposed by government is weak, despite the formal compliance with extraordinary strict lands accounting and extremely complicated permission granting procedure required for introducing changes in the way of land use. It is important to underline that Russia has a rich biodiversity and natural resources, but still formal institutions are week because of gaps in knowledge. Hence, the third adaptation measure is collaboration initiatives on the regional, national and International levels by civil, private and public sectors. Further development of international Educational and Cooperative Programmes with neighboring countries on issues of land use, tools of carbon, and biodiversity conservation remain an important measure.

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COMPARISON OF CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY BETWEEN INDIAN STRAWBERRY (*Duchesnea indica* (Jacks.) Focke) AND OTHER SPECIES OF STRAWBERRIES

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Abstract

Indian strawberry (*Duchesnea indica* (Jacks.) Focke) comes from subcontinent of India and south China. It is unknown when exactly this species has arrived on our soil. The analyzed population of Indian strawberry from a forest garden in Banja Luka is relatively old. It was brought from Syria in the middle of the last century. Despite the fact that this species is considered invasive, its micro-population in this forest garden has not expands.

Indian strawberry is classified into strawberries, although in terms of the systematic it does not belong to the genus *Fragaria*, but to the genera *Duchesnea* or *Potentilla*. From the common forest strawberry (*Fragaria vesca L.*) it is distinguished by yellow flowers, top-fruits, which are almost tasteless and odourless, while the flowers of the forest strawberry are white, the fruits slightly hanging, sweet and fragrant.

Regarding the chemical composition, the content of dry matter (14.35 g/100g), total content of sugar (4.26 g/100g), vitamin C (5.96 mg/100g), total acidity (expressed as the content of citric acid) (0.25 g/100g) have been analyzed. Also, the content of some mineral elements in Indian strawberry have been determined, with following results: sodium (5.3 mg/100g), potassium (152.4 mg/100g), magnesium (17.7 mg/100g), calcium (26.6 mg/100g), iron (0.72 mg/100g), phosphorus (27.6 mg/100g), zinc (0.22 mg/100g), manganese (0.32 mg/100g), copper (0.06 mg/100g), selenium (0.16 μg/100g) and chrome (2.1 μg/100g). Content of total phenolic (4.985±0.204 mg GEA/g_{FW}), flavonoids (2.124±0.183 mg Qc/g_{FW}), flavonols (0.518±0.03 mg Qc/g_{FW}), total anthocyanins (1.274±0.001 mg/g_{FW}) and monomeric anthocyanins (1.05±0.08 mg/g_{FW}) have been determined as well. The results obtained for Indian strawberry are compared with the content of these components in other strawberries.

Key words: *Indian strawberry, chemical composition, mineral composition, antioxidant activity*

Introduction

A number of scientific names have been attributed to the Indian strawberry plant species. The first name of *Fragaria indica* was given by Andrews in 1807. In 1811, Smith extracted a new genus of *Duchesnea* from the *Fragaria* genus. This motivated Teschmscher to give the species the name *Duchesnea fragiformis*. This name was not widely accepted in the scientific community. Focke in 1888 introduced new name, *Duchesnea indica*. In 1904, Wolf included this species to the genus *Potentilla* and named it *Potentilla indica* (Ertter et al., 2015).

Duchesnea indica is a native species in southern China and the Indian subcontinent. All parts of this plant have been found in Chinese medicine for anti-cancer drug production. It is used to reduce swelling, also for skin treatment such as abscesses, eczema and ringworm, infections such as laryngitis and tonsillitis, as well as snake and insect bites, under Chinese species and drug name "*she mei*" (Umesh & Thoppil, 2014). The exact time when the species is brought to Europe and our regions is not known. In the flora of the former Yugoslavia, the

species was the first time mentioned by Trinajstic (1973), who found it in the village of Turcin in the vicinity of the city Varazdin (today in Croatia).
Although named strawberry, it does not belong to the genus *Fragaria*, but is classified in the genera *Duchesnea* or *Potentilla*. It is distinguished from the common wild strawberry by yellow flowers, topped fruits (Fig. 1 & 2), which are almost without flavour and odour, while the flowers of the wild (forest) strawberry (*Fragaria vesca* L.) are white, the fruits are slightly hanging, sweet and fragrant. The observed population of Indian strawberry in the city of Banja Luka is relatively old. It was brought from Syria in the middle of the last century. Even though this species is considered as a rather invasive, the footprint of its mico-population remained unchanged from the moment of introduction until today.


Figures 1 & 2: Flower and fruit of Indian strawberry (Photo: S. Ljubojevic)

Material and Methods

Fresh fruits of the Indian strawberry were collected in the summer of 2017. To determine the chemical composition of the components, the fruits are washed, cut into chops, chopped and homogenized in an stainless steel rotating knife homogenizer according to Vasilisin et al. (2015, 2016, 2017). Certain components of the chemical composition were determined by standard AOAC methods (AOAC, 2000). For each component of the chemical composition three parallel analyzes were performed, and the results are expressed as mean values. For the purposes of determining total and monomeric anthocyanins, 20g of the sample was extracted with 20 mL of anthocyanin extraction solution (85 mL of 95% ethanol solution and 15 mL of 1.5 mol/l HCl solution) at 24°C for 24 hours (Kukric et al. 2018), after standing, the resulting mixture was filtered through a paper filter and the filtrate was used for further analysis. The content of total phenols was determined by the modified Folin-Ciocalte method (Wolfe et al., 2003). As a standard compound, gallic acid was used and the results were expressed as equivalents of equivalent galenic acid (GAE), i.e. mg GAE/g_{FW}. Total flavonoids were determined by Kumaran method (Kumaran & Karunakaran, 2007), and the total flavonols by Ordonez method (Ordonez et al., 2006). Quercetin was used as the standard compound, and the results were expressed as mg quercetin (Qc)/g_{FW}. Antioxidant activity against the DPPH radical was determined by the Liyana-Pathiranana and Shahidi method (Liyana-Pathiranana & Shahidi, 2005), and for the ABTS radical the modified method of Re and associates was used (Re et al., 1999). As a standard compound, Trolox was used. The results were presented with the TEAC value (Trolox equivalent antioxidant activity), i.e. as μg Trolox-a/g_{FW}. Total and monomeric anthocyanins were determined by spectrophotometrically modified "*single*" pH and pH differential method (Sun et al., 2002). Experiments were made in three parallel repetitions, and the results are expressed as the mean ± standard deviation.

Plant material samples were manually cut and homogenized for digestion. After preparation samples are burned in a mixture of concentrated acids (nitrogen and perchloric acid) in the digestion thermoblock according to the prescribed temperature regime. After digestion, the solutes were filtered in 50 mL dilution flask, diluted with deionized water, with conductivity of 0.056 μS/cm and stored for analysis of mineral substances. Each sample was prepared in duplicate.

The obtained solutions were used to determine the concentration of the analyzed elements (Na, K, Ca, Mg, P, Fe, Mn, Cu, Zn, Se, Mo, Ni, Cd, Pb). Containers and equipment, used during ICP OES analysis, are made of plastic or stainless steel. Before contact with plant samples, dishes and equipment were washed with distilled water, then with 2% nitrogen solution and finally deionized water (0.056μS/cm). Nitrogen and argon used during analysis had a purity of 5.0 (99.999%).

Optical Emission Spectrophotometer (ICP OES) was used to determine the mineral content of samples of Indian strawberry *Duchesnea indica*. The sample solutions were pumped by a peristaltic pump from tubes arranged on a Perkin Elmer auto-sampler model 510. Certificate reference standard solution, instrument calibration standard , with concentration of elements 100 mg/L was used for calibration of spectrophotometer. The concentrations of the elements for the calibration direction were different, determined according to preliminary measurements for the specified metals (Vucic et al., 2018).

Linearity was performed by analysis of standard solutions. 20 standard solutions of five concentration levels depending on the analyse were determined. Limit of determination and quantification was performed by analysis of blanks. At least 21 blank samples after correction for typical sample weight were determined. Different concentration of CRM solution was analyzed as check on the accuracy of the analyzers. The LOD values (limit of detection) and LOQ values (limit of quantification) was calculated as 3 times and 10 times of STD (standard deviation) of the ten measured blank signals. The achieved results were compared with the values for the wild (forest) strawberry (*Fragaria vesca*) and for the cultivated (commercial) strawberries, which are cultivars (cultivated varieties) of *Fragaria* × *ananassa* Duch (Savic et al., 2007; Hossain et al., 2016; USDA, 2018).

Results and Discussion

The content of individual components of the chemical composition in fresh fruits of Indian strawberriy *Duchesnea indica* and their comparison with the literature data is presented in Table 1.

Table 1. Chemical composition of *Duchesnea indica*

	Duchesnea indica	*Fragaria ananassa*[*]	*Fragaria vesca*[**]	Strawberry raw in USA[***]
Dry matter (%)	14.35	7.81	19.98	9.15
Total sugars (%)	4.26	4.45	6.26	4.89
Total organic acids (like citric acid. %)	0.25	-	1.64	-
Vitamin C mg/100g	5.96	26.46	75.80	58.80

[*](Hossain et al., 2016); [**](Savic et al., 2007); [***](USDA,2018)

The content of dry matter in the Indian strawberries *Duchesnea indica* is higher than in cultivated strawberries, other strawberries, but lower in comparison with wild strawberry *Fragaria vesca*. The content of total sugars in Indian strawberry is approximately the same as for other strawberry species, while the content of vitamin C is considerably lower.

The total acidity expressed as citric acid in the Indian strawberry is almost seven times lower than in wild strawberries. Table 2 shows the results of measurements of secondary metabolic content and antioxidant activity of *Duchesnea indica*.

Table 2. Content of secondary metabolites and antioxidant activity of *Duchesnea indica*

Analyzed parameters	*Duchesnea indica*
Total phenolics mgGEA/g_{FW}	4.985±0.204
Flavonoids mgQc/g_{FW}	2.124±0.183
Flavonols mgQc/g_{FW}	0.518±0.003
Total antocyanins mg/g_{FW}	1.274±0.001
Monomeric antocyanins mg/g_{FW}	1.05±0.008
DPPH (IC_{50})* µg Trolox/mg_{FW}	2.32±0.027
ABTS (IC_{50})* µg Trolox/mg_{FW}	0.506±0.007

*IC_{50}-concentracion for 50% inhibition of DPPH radicals

The content of total phenolic in the Indian strawberry was significantly higher than the literature data for different strawberry varieties, ranging from 96-236 mg GEA/ 100g_{FW} (Toronnen & Maatta, 2002; Voca et al., 2008). The flavonoid and flavonol content according to literature data for different strawberry varieties ranges from 176-335 µgQc/g_{FW} (Kukric et al., 2016). Accordingly it can be stated that the amount of flavone and flavonoids in Indian strawberry is higher than other strawberry varieties. The content of total and monomeric anthocyanins is also several times higher compared to the literature data (Kukric et al., 2016). Results in Table 2 presents that the antioxidant activity of Indian strawberry is considerably higher in compared to literature sources. According to literature data, the antioxidant activity against the DPPH radical for different strain varieties was 6.11 mmolTrolox/kg_{FW} (Jakobek, 2007), 9.7 mmolTrolox/kg_{FW} (Hartman et al., 2008) and 8.29-11.15 mmolTrolox / kg_{FW} (Dragovic-Uzelac et al., 2007). Literature values for antioxidant activity against a stable ABTS radical were 9.70 mmolTrolox/kg_{FW} (Jakobek et al., 2007), 12.08 mmolTrolox/kg_{FW} (Hartman et al., 2008) and 26.92-30.06 mmolTrolox/kg_{FW} (Dragovic-Uzelac et al., 2007). Disclosed in Table 2, antioxidant activity values for a stable ABTS radical in Indian strawberry are higher than those of literature.

The mineral composition of Indian strawberry is presented in Table 3. In addition to certain macro and microelements, toxic metal concentrations (Pb, Cd, As) were also measured. Estimated toxic elements were below the detection level (LOD) and were labelled with ND (not detected). From the tables above, it is evident that the results, for virtually all metals, with the exception of Fe (0.72 mg/100g), are significantly different from the literary allegations. According to research by Ozean & Haciseferogullari (2007), the content of Fe in the strawberry varieties surveyed was around 1.22 mg/100g, while Serce et al. (2016) reported the Fe content of 0.264 mg/100g. The content of Ca (26.6 mg/100) is considerably lower than the literary allegations of some authors. Ozean & Haciseferogullari (2007) and Serce et al. (2016) were determinate values for calcium in strawberries of 459.2 to 4202 mg/100g respectively.

Table 3. Mineral content of *Duchesnea indica* in comparison with some other strawberry varieties (literary citations)

Mineral content			**Literature sources**					
Minerals	Content (mg/100g)	Mineral content in *Duchesnea indica*	Content (mg/100g)	Ozean& Haciseferogullari (2007)	Content (mg/100g)	Serce et al. (2016)	Content (mg/100g)	Vanessa Rios de Souza et al.(2014)
Al	-		2.011		-		-	
As	ND		1.06		-		-	
Ca	26.6		459.2		420.2		2.20	
Cd	ND		0.019		-		-	
Cr (µg/100g)	2.1		0.241		-		-	
Cu	0.06		0.165		0.043		-	
Fe	0.72		1.22		0.264		1.00	
K	152.4		1490		9700		51.24	
Mg	17.7		131.5		1695		8.78	
Mn	0.32		0.44		0.052		-	
Na	5.3		70.1		-		-	
Ni	ND		0.013		-		-	
P	27.6		366.9		646		6.59	
Pb	ND		0.051		-		-	
Zn	0.22		0.82		0.153		0.13	
Se (µg/100g)	0.16		-		-		-	

Conclusions

The content of individual components of the chemical composition of Indian strawberry *Duchesnea indica* is approximately the same as in other strawberries species. The exception is the content of vitamin C, which is considerably lower than. Antioxidant activity in Indian strawberries is considerably higher than in other species. These facts clarify the usage of *Duchesnea indica* in Chinese traditional medicine for cancer treatment. Toxic elements have not been detected in the analyzed samples. The content of certain macroelements is significantly lower compared with literature data.

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CONSERVATION AND MANAGEMENT OF GENETIC POTENTIAL OF SESSILE OAK (*Quercus petraea* /Matt./Libl.) IN SERBIA

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Abstract

In Serbia, there are 10 native species of oaks, of which three species in the sessile oak aggregate hold a significant position: Central European sessile oak (*Quercus petraea* /Matt./Libl.), Balkan sessile oak (*Quercus dalechampii* Ten.) and cluster-fruited oak (*Quercus polycarpa* Schur.) Sessile oak in Serbia makes 13 different associations and numerous sub-associations on different bedrocks which is manifested by the great phyto-coeno-diversity of this species. This paper deals with sessile oak variability and breeding in Serbia. Thanks to a great heterogeneity of site conditions of this polymorphic oak species, there is a significant intra- and inter-provenance variation of morphological and physiological properties. The research of sessile oak genetic variation by molecular markers, cp DNA, mt DNA and micro-satellite methods, point to the need for further analyses of sessile oak genetics diversity. These results will be practically applicable in the identification and selection of seed stands with the best-quality autochthonous populations, with the suitable desirable quantitative and qualitative characteristics. Further efforts on breeding of this polymorphic and polyvalent species, require the application of the methods of mass and individual selection, by which the already registered seed forests will be revised, the new ones will be selected and test trees will be selected in all regions of sessile oak native range. In this way, a wide sessile oak genetic potential will be archived in the established seed orchards and progeny tests.

Key words: *sessile oak, conservation, management, Serbia*

Introduction

In the last 13 thousand years, *Quercus* L. species have been undergoing constant dynamic shifts caused by dramatic climate change, *i.e.* migrations of its populations in the south of Europe towards central, western, and Eastern Europe. Previous studies of the dynamics and directions of these migrations were based on the analysis of fossil pollen. Recent research studies have been using methods of molecular genetics with chloroplast (cp) DNA, mitochondrial (mt) DNA and microsatellites to confirm and complete data on the postglacial migration of oaks and the specificities of their populations. The research of Petit *et al*., 1993, Petit *et al*., 2002 confirmed earlier assumptions on the differentiation of the haplotype variability of oaks caused by the shifts in the range of distribution of species belonging to *Quercus* L. towards central and northern Europe following the last Ice Age.

Of numerous botanical synonyms for sessile oak, the best-known ones are *Quercus sessilis* Ehrh.1789 and *Quercus sessiliflora* Salisb. 1796. This species includes a large number of subspecies since natural habitats of sessile oak are quite heterogeneous in terms of climatic and edaphic conditions while the established populations have different gene pools. Comparative morphological and physiological analyses of the sessile oak variability, as well as the statistical analysis of the habitats of autochthonous sessile oak and related oaks, have shown that *Quercus petraea* Liebl is an aggregate of several species. The differences between the species of *Quercus petraea* agg, namely between the sessile oak /*Quercus petraea*/, Transylvanian sessile oak /*Quercus polycarpa* Schur/ and Balkan sessile oak /*Q.dalchampii* Ten/ have been determined for the habitus, bark, leaves – hairy underside and fruit – the shape

and the color of stipules on the cupules. The genetic bases of the differences between the species that form the aggregate have not been sufficiently studied and defined. These studies have been greatly hampered by a large number of spontaneously formed hybrids, as well as their backcross with each other and with parental species. Further application of molecular studies with cpDNA, mtDNA, microsatellitec, *etc*., will help define species, intraspecific differences and hybrid characteristics more precisely.

In order to achieve closer determination and targeted use of intra- and inter-population variability of sessile oak, provenance tests have been established and analyzed in several European countries. These tests are experimental polygons in which the methods of tree improvement are used to study the effects of the interaction between the ecological and the genetic components on the extent of variation of phenotypic properties of vegetative and generative organs of the sessile oak. However, morphological traits are highly susceptible to the influence of changing environmental conditions, thereby reducing the expression of the genotype itself. More accurate studies of the genetic variability of sessile oak of different provenances involve molecular genetics methods with the use of molecular (genetic) markers, which considerably reduce the effects of environmental factors, and thus the variability that is primarily and directly under genetic control can be observed. It has been determined the shape and the straightness of the stem are the properties strongly controlled by genetics, which can be significantly improved by applying special tree improvement programes. The study of the morphological variability included several quantitative parameters, while the molecular variability included chloroplast DNA and microsatellites, Jan Svejgaard Jensen (2000), Bruschi, P. *et al*., 2003, Graham M *et al*., 2004. The results of the conducted analyses point to significant genetic variability between the studied populations. However, the scope of the variation of morphological properties was not completely confirmed at the level of microsatellite differentiation. The relatively high genetic diversity in the studied populations of sessile oak suggests that their evolutionary potential can be preserved if the size of the population is maintained or increased with time. The results of the conducted analyses have shown that these populations have very pronounced genetic variability which is similar to that found in most European countries despite the facts that sessile oak forests occur only in refugia and that they suffered severe destruction in the past. In addition, the present genetic structure of sessile oak forests does not show any undesirable founder effects.

A programe for the protection and targeted use of sessile oak in Serbia

The results of the analyses carried out in the provenance and progeny tests indicate that sessile oak is an extremely variable species. The differences were observed in the tree growth stem formation and in the attacks of mildew and insects. Furthermore, biochemical studies of the fruit of different provenances revealed significant differences in the content of agglutinin, which could contribute to an easier identification of oak provenances, Jovanović and Tucović, 1973, 1975. Apart from the inter-provenance differences, there are significant differences within provenances, *i.e.* within individual stands in terms of the height increment, onset of certain phenophases, crown shape, branching, trunk characteristics, morphometric properties of the acorn, *etc*. (Figure 1). Within a natural sessile oak stand, trees clearly differentiate into the following phenotypes: monopodial, forked and broomed.

Figure 1. Acorn from different trees from seed stand 02.02.02.07 in MU «Cer-Vidojevica», 186b

The progeny tests revealed that the phenotypic characteristics of the trees and the acorn were under significant genetic control. The literature on dendrology contains extensive data on the distribution of spontaneous hybrids of: *Quercus petraea* and *Quercus robur*; *Quercus petraea* and *Q. dalechampii*; *Quercus petraea* and *Q.polycarpa*, described under different names. The hybrid progeny of these parental species is characterized by extreme variability of quantitative and qualitative characters. The great variability of morphological and physiological characters of spontaneous hybrids causes great difficulties in the taxonomic, sociological and ecological research of species belonging to the genus of *Quercus*, as well as in the organization and realization of the production of raw materials for the needs of forest seed and seedling production.

New program for the establishment of sessile oak provenance tests in Serbia include: (a) the selection of provenances to be tested, (b) the locations where the tests will be set up, and (c) the selection of control provenance(s). The implementation of this program is based on the past and future activities, such as:

- study of climatic and edaphic similarities and differences between the areas belonging to the belt of sessile oak forests in the selected regions of Serbia;
- carrying out biological and economic analyses of sessile oak forest communities, especially those where it is the dominant species, regardless of the status and degree of the site and (or) stand degradation;
- application of the methods of classical and molecular genetics to determine and measure genetic variability within and/or between sessile oak provenances, populations and genotypes;
- application of mass and individual selection to quantitative and qualitative characters within natural sessile oak populations;
- permanent monitoring of the sessile oak reproduction cycle at population and individual levels in order to gain a deeper insight into gene-environment interactions that affect the frequency, abundance and quality of seed production;
- securing abundant seed crop and production of seedlings of half and full-sib lines, scions, cuttings and rooted cuttings with the aim of increasing the number of experimental and productive seed orchards of sessile oak.

Further long-term research within the framework of sessile oak provenance tests will provide a higher degree of genetic determination of the production potential of certain provenances, *i.e.* accurate information on the characteristics and variability of sessile oak within and between the selected provenances in the network of experimental objects in Serbia. By gaining an insight into the genetic potential and degree of determination of the adaptability, productivity and quality of different sessile oak populations, we will obtain the basis for the zonation of seed stands on the basis of gene-environment characteristics. Investigations conducted within the network of provenance tests play an important role in the forest

profession because they facilitate the selection of provenances or groups of provenances suitable for specific sites. The selection and cultivation of the reproductive material of sessile oak from the provenances in which the range of variation in the adaptability and production capacity is determined will help accomplish the established objectives by carrying out the planned works in the areas with defined general ecological and production characteristics Isajev et all. (2005, 2006).

Further improvement of the sessile oak in Serbia is based on individual level by selection of test trees in seed stands of this species. There are nine seed stands of sessile oak in Serbia, Table 1, and ninety test trees have been selected – ten in every seed stand.

The variability of the continuous and discontinuous traits of test («plus») - seed trees is analyzed based on the variability of several characters

- leaf phenophase;
- good growth;
- good trunk and crown shape;
- absence of spiral grain;
- straight and monopodial trunk;
- slight tendency towards the formation of water sprouts;
- frost resistance;
- resistance to diseases and pests;

Table 1. Sessile oak seed stands in Serbia

	Seed object register number	Management unit (MU) title, section numbera	Area (ha)	Organization title or owner's name
1.	S 02.02.02.01	GJ «Rudnik I», 105b	12,56	MU «Kragujevac» - Kragujevac
2.	S 02.02.02.02	GJ «Crna Reka», 104b	6,33	Forestry Facult, Belgrad
3.	S 02.02.02.03	GJ «Vrdnik – Morintovo», 49a	7,71	JP «National Park Fruška gora» - Sremska Kamenica
4.	S 02.02.02.04	GJ «Juhor I», 82g.	1,49	MU «Južni Kučaj» - Despotovac
5.	S 02.02.02.05	KO Rokci, MZ «Milosavljevići»	3,00	MU «Golija», Ivanjica
6.	S 02.02.02.06	GJ «Zajčevac-Ajkobila-Šajići», 11c	12,18	MU «Šuma» - Leskovac
7.	S 02.02.02.07	GJ «Cer-Vidojevica», 186b	7,20	MU «Boranja» - Loznica
8.	S 02.02.02.08	GJ «Crni Vrh», 91a		MU « Timočke šume» - Boljevac
9.	S 02.02.02.09	GJ «Vršački breg», 131b	2,75	MU «Banat» - Pančevo

The results of the analysis will direct further work towards the selection of the best sessile oak genotypes so that a certain genetic improvement can be expected in the next generation. If the parent population has a poor phenotype, *i.e.* if there is a high percentage of minus trees and a small or insufficient percentage of above-average or plus trees, this tree improvement method cannot yield positive results. If the work is directed towards the production of new species and hybrids of sessile oak, the improvement will last longer, but it will produce a higher genetic gain.

The Program for the establishment of sessile oak seed orchards in Serbia

In addition to preserving, testing and directed use of the genetic potential of sessile oak in natural populations in different sites, the establishment of purposeful orchards - seed orchards of this species, will create conditions for:

- the improved production of selected seed;
- checking the combining ability and the heritability of the selected test trees;
- determining the value of the potential variability of sessile oak within the same and different provenance regions;
- biodiversity testing and preservation;
- production of prospective hybrids.
-

Seed orchards and pilot objects, as specialized cultures, not only contribute to the translation of the potential genetic variability into the free variability, which is the basis for the targeted use of the sessile oak gene pool, but also create polygons for testing and preservationing its biodiversity. Starting from the floristic, genetic and production potential of sessile oak, it is not possible to establish seed orchards of this species using the same model.

Different models of clonal and generative seed sessile oak orchards will ensure a more reliable determination and targeted use of heritability and combining ability of the selected genotypes of sessile oak. The quality and regularity of the seed crop in clonal seed orchards are primarily determined by the genetic potential of vegetative copies - ramets – of selected genotypes and ecological characteristics of the sites where they are established. The selection of genotypes to be incorporated into seed orchards should be preceded by long-term analyses of the degree of genetic control of their reproductive functionality and the variation of properties, Isajev *et al*., 2012. The application of controlled hybridization and the testing of the obtained hybrids through progeny tests will determine the heritability of characters from parents to hybrids. This procedure will determine potentially valuable genotypes which should be cloned to be incorporated into the seed orchard.

The selection of parents, *i.e.* their combinations, with good general and specific combining ability and heritability, is the starting point for the establishment of clonal seed orchards for the production of intraspecific hybrids of sessile oak. Which of the genetic compositions of the hybrids obtained will be the focus of further interest will primarily depend on the biotechnical conditions for which the reproductive material is produced and on the market demands. These factors must be taken into account in the future mass production of seed, which means that the type of genotype we want to create should be clearly determined in the initial stages of work. Multiline sessile oak varieties to be obtained as a result of controlled hybridization and the composition of the seed orchard should provide higher yield stability and adaptability.

Further work on the conservation and targeted use of sessile oak in Serbia

In Serbia, forest phytocoenoses of sessile oak belong to *Quercion petraeae-cerris* Lakš- et Jov. 1980. association, in which there are pure and mixed communities Table 2.

Table 2. Main sessile oak communities in Serbia, according to Tomić Z 2004

Sessile oak and Turkey oak forests	-	***Quercetum patreaeae-ceris* Jov. 1979**.
Hop-hornbeam and sessile oak forests	-	***Helleboro – Ostryo-Quercetum* Tom.1980.**
Pure sessile oak forests	-	***Qurecetum montanum* Čer.et Jov. 1953.s.l.**
Forests of sessile oak with fescue	-	***Festuco drymeiae-Qurcetum petraeae* Jang. 1968.**
Forests of sessile oak with Oriental hornbeam	-	***Carpino orientalis – Qurcetum montanum* Jov. 1979.**
Forests of sessile oak with flowering ash	-	***Orno-Quercetum petraeae* Miš. 1972.**
Forests of Transylvanian sessile oak	-	***Poa nemoralis – Qurcetum polycarpae* Jov. 1979.**

General environmental characteristics of the areas in Serbia where pure and (or) mixed populations of sessile oak spontaneously occur in interaction with their gene pool have caused expressed variability in a large number of its morphological and genetic-physiological traits. Considering the fact that *Quercus petraea* is characterized by great individual and group variability and includes plethora of intraspecific taxa - varieties, biotypes and ecotypes, this calls for a multidisciplinary and complex approach to its further improvement. Protection and targeted use of the most valuable genetic resources of sessile oak will be mostly realized: ***in situ*** – by selecting the best natural sessile oak populations and revising the existing ones as well as establishing new seed stands, groups and individual trees, and ***ex situ*** – by establishing provenance tests, live archives, clonal and generative seed orchards.

According to the needs of the forestry profession and the state of its forests, further work on the improvement of sessile oak trees in Serbia should be carried out in two directions. It is necessary to continue and modernise activities aimed at improving the stability, productivity and genetic richness of natural sessile oak populations, and then to intensify improvement methods in order to obtain new selected genotypes and hybrids that will be better according to the values of target characteristics. In the first stage of work which is based on the application of the mass selection method, it is necessary to revise the selected seed populations of sessile oak and, if necessary, to determine the new ones in order to cover its vertical and horizontal distribution in Serbia. Using the method of individual selection, a large number of test trees should be continually specified in the selected populations. Subpopulations, or provenances from Serbia, should be tested in a network of provenance field experiments. Selection of genotypes - test trees - should be done in all the regions of sessile oak provenances in order to get a deeper insight into its ecological, sociological and genetic diversity. Further research work in specialized plantations and seed orchards of second and subsequent generations should create conditions for long-term production of sessile oak variety seed.

Conclusions

Thanks to a great heterogeneity of site conditions in sessile oak natural forests in Serbia there is a significant intra- and inter-provenance variation of morphological and physiological properties within populations of this species. The comparative analyses of provenance tests performed in several West European countries point to a rich gene pool of sessile oak, resulting from the action of complex genetic factors, ecological factors and different management procedures in sessile oak natural forests. The research of sessile oak genetic variation by molecular markers, cp DNA, mt DNA and micro-satellite methods, point to the need of future analyses of sessile oak genetic diversity. These results will be practically

applicable in the identification and selection of seed stands with the best-quality autochthonous populations, with the suitable, desirable quantitative and qualitative characteristics. Further efforts on breeding of this polymorphic and polyvalent species, taking into account the current states of its forests and the need for increasing the areas under this species, require the application of the methods of mass and individual selection, by which the already registered seed forests will be revised, the new ones will be selected and test trees will be selected in all regions of sessile oak native range, In this way, a wide sessile oak genetic potential will be archived in the established seed orchards and progeny tests.

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CHEMICAL COMPOSITION, NUTRITIONAL VALUE AND ANTIOXIDANT PROPERTIES OF CRABAPPLES

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Abstract

The aim of this study was to determine the chemical composition, nutritional value and antioxidant properties of three crabapples: *Malus* `Pink Perfection` *Malus* `Royalti` and *Malus* x *zumi* `Golden Hornet`, with distinctive appearance characteristics. Subjects of the analysis were fresh fruits and juice obtained from the last one by steam extractor. Crabapples were taken from an urban garden. Chemical analysis includes the following parameters: macro- and microelements (As, Ca, Cu, Fe, K, Mg, Mn, N, Na, P, Se, Zn), water, dry matter, ash, proteins, sugars, total acidity, pectin, minerals, pH, vitamin C, anthocyanins, phenolic compounds, nonflavonoids, flavonols and free radical scavenging capacity (applying DPPH and ABTS tests). Basic phenotypic and morphometric characteristics of the fruits were observed as well. Results obtained from the research have shown that *M.* 'Pink Prefection' and *M.* x *zumi* 'Golden Hornet' have a fairly similar chemical composition. *M.* `Royalti contains significantly more vitamin C and a little more sugar, but is also poorer in pectin and has much greater acidity. Compared to commercial apples, crabapples do not lag behind in sugar content, but they have significantly higher acidity and half the amount of vitamins C. The elemental composition of crabapples is also fairly uniform. When it comes to phytochemicals, *M.* 'Royalti is particularly prominent by the high content of anthocyanins, flavonols and phenols. The ABTS test showed that *M.* x *zumi* 'Golden Hornet' has the strongest antioxidant capacity. We have reached the same result by applying the DPPH method.

Key words: *crabapples, chemical composition, nutritional value, antioxidant properties*

Introduction

Crabapple is rather loosely defined term in a different connotation. Its origin was believed to come from Scandinavia at the beginning of the 18th century, with meaning - "fruit of the wild apple tree" (Anon., 2017). Jefferson (1970) indicates that any tree or shrub of genus *Malus* whose fruit diameter does not exceed 5.2 cm (2 inches), whether it is ornamental or economical plant implies under crabapple. From our point of view this term implies decorative (horticultural) apples, which are introduced into urban and peri-urban gardens and other horticultural facilities primarily due to their aesthetic values: the color of leaves, flowers and fruits, the abundance and duration of flowering, the abundance of fruiting and prolonged fruits retention. These characteristics do not exclude or neglect their other useful properties, such as: pollinizers in apple orchards, raw materials for the production of vinegar, jams, juices, then rootstocks in the production of grafted seedlings, bonsai cultures, etc., not to forget their role as a habitat and a shelter for wildlife, especially birds.

About the names of crabapples there are certain dilemmas and doubts, most often when it comes to the categories hybrid and cultivar. The assignment of names within the first category is governed by the International Code of Nomenclature for algae fungi and plants (earlier International Code of Botanical Nomenclature) and within the second category through the

International Code of Nomenclature for Cultivated Plants. According to Jefferson (1970), referring to Wyman (1955), cit.: "Crab apples hybridize very freely, and because of this, much controversy has resulted in their proper identification. Seed has been gathered in large collections, grown and seedlings were named after the trees from which the seed was collected. Frequently, such seed has produced plants with totally different characteristics from the parent plant, and once this has become evident, it has caused much confusion." The native (indigenous) apple in Europe is considered a wild (forest) apple *Malus sylvestris* (L.) Mill., although many orchard apples actually derived from Asian apple *M. pumila* (Mabberley *et al*., 2001). This paper analyzes the chemical composition, the nutritional value and the antioxidant properties of three taxa of crabapples, one hybrid and two cultivars. The obtained results were compared with the values of the most common sort of apple (*Malus domestica* Borkh.) in South East Serbia: `Fuji` (hybrid), `Golden Delicious` (cultivar), `Granny Smith` (cultivar), `Idared` (cultivar) and `Mutsu` (cultivar), presented by Stojanović (2014); it is undivided opinion that apples are delicious and nutritous fruits, which, in addition, possess a high antioxidative capacity (Lotito *et* Frei, 2004). Morphometric parameters of the fruit, such as diameter and weight (*id est* mass), are also measured.

Material and method

Material

The subject of our research are three taxa of crabapples that grow in an urban garden in the city of Banja Luka in Bosnia and Herzegovina. These are two cultivated varieties: *Malus* `Pink Perfection` and *Malus* `Royalty`, and hybrid cultivar *Malus* x *zumi* `Golden Hornet`. The trees were purchased from garden center "Flora" in Busovača (B & H), previously imported from Italy. At the time of planting, in the spring of 2013, each tree was five years old. Material for analysis was collected during year 2017.

Malus 'Pink Perfection' was selected by W. Flemer, III, 1960, at Princeton Nurseries, USA. Seed was taken from *Malus* 'Katherine' and the male parent was *Malus* 'Almey' (Hebb, 1970). The main characteristic of this crabapple is blossom. It is made of clusters of lightly fragrant, double, light pink and white flowers (Fig. 1). Fruits from this apple also have a beautiful appearance and may be used in nutrition (Fig. 2). Research by Matsumoto *et al*. (2015) have shown that *Malus* 'Pink Perfection' is among the most superior pollinators and can be a useful pollinator for all domestic cultivars.

Malus 'Royalty' was selected by W. L. Kerr in 1958 in Canada (Saskatchewan), using a large number of open pollinated rosy bloom seedlings he found growing there. As a rootstock he used wild apples of small fruits - *M. baccata* (L.) Borkh. Crabapple *M*. 'Royalty' is recognizable by the purple-red pigmentation of foliage, flowers and fruits (Fig. 3 & 4), and was the first crabapple with this feature, which was until then unique to plums. Purple pigmentation comes from the presence of anthocyanins. These compounds are powerful antioxidants in vitro, and are

Figures 1 & 2: *M.* 'Pink Perfection' in blossom and fruits (photo: S. Ljubojević)

widely believed to contribute to human health (Van Nocker *et al*., 2012). However, due to very low mass and hardness, the fruits are not yet used in the nutrition.
Malus x *zumi* 'Golden Hornet' originates from the United Kingdom, created in the first half of the 20th century. It is thought to be a natural cross hybrid between two other crabapple species, *Malus sieboldii calocarpa* and *Malus prunifolia coccinea*. Its main characteristic is lush pink-white flowers, also from which numerous butter yellow fruits occur, which remain on the tree for long (Fig. 5). The leaves also turn yellow and become attractive in autumn. Unlike the previous taxon, the fruits are used in culinary art, preparation of jams and jellies, juices and vinegar.

Figures 3 & 4: Flowers and fruits of *M.* 'Royalty' (photo: S. LJubojević)

Figure 5: Fruits of x *zumi* 'Golden Hornet' (photo: S. LJubojević)

Thus, all three observed trees are of the same age and grow under the same agro-environmental regime. This prevents bias in terms of fruit acidity and some other features, considering that many agronomic studies have shown the impact of cultural practices on these characteristics (Etienne *et al*., 2013).

Method

Fresh samples of crabapples were collected at the moment of optimal ripeness. This stage was visually evaluated on the basis of daily monitoring. The harvest was carried out at the time when participation of the immature or overripe fruits compared to mature ones, was minimal. Namely, it has been known for a long time that the total acid content of apples is directly related to the ripening processes (Nybom, 1959). In addition to fresh fruits, a sample of *Malus* x *zum* 'Golden Hornet' juice was taken, using a steam extractor, simple household utensil, without any additives (sugar, preservatives, etc.).

Before harvesting and homogenization on the mill "Triple Spindle Mixer, Hamilton Beach", according to Vasilišin *et al*. (2017), the seeds were extracted from the fruits. It is known that seeds of crabapples contain trace amounts of amygdalin, which is under stomach acid transformed into toxic compound. At the same time, it is known that apple seed is a rich source of selenium, which is an antioxidant and important element for enzymatic activity of liver (Stojanović, 2014). From the homogenised material, 5 g of fruits of each taxon was extracted with 80% ethanol, twice at 25 ml. The solutions were refilled with 80 % ethanol up to 50 mL. Thus, 100 mg/mL concentrations were obtained and further used to determine total phenols, flavonoids, flavonols, neutralization of 2.2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS +) radicals. To determine a total and monomeric anthocyanins, 20 g of samples was extracted with a 20 mL solution (85 ml of 95 % ethanol solution in 15 ml of 1.5 mol/L HCl solution) at 0^o C for 24 hours. After being left to stand, resulting mixture was filtered through a filter paper, and the filtrates were used for further analysis. The content of dry matter, ash, total acidity and vitamin C were determined by standard AOAC methods (Anon., 2000). Total phenol content was determined by the modified Folin-Ciocalteu method (Wolfe, 2003). Gallic acid was used as the standard compound and the results were expressed as phenol equivalent to gallic acid (GAE), i.e. μg GAE/g_{FW}. The total flavonoids are determined by the method of Kumaran and Karunakaran (Kumaran, 2007), and the total flavonols according to the method of Ordoñez *et al*. (2006). Quercetin was used as the standard compound, and the results were expressed as μg Quercetine (Qc) /g_{FW}. The antioxidant activity in relation to the DPPH radical was determined by the method of Liyana-Pathirana and Shahidi (Liyana-Pathirana, 2005). The modified method of Re *et al*. (1999) was used fot the ABTS radical. The results were presented with the TEAC value (Trolox equivalent of antioxidant activity), i.e. as μg Trolox/g_{FW}. Total and monomeric anthocyanins were determined by the spectrophotometrically modified "single" pH and by the pH differential method. The overall experiments were performed in three parallel repetitions.

Five hundred healthy, undamaged fruits were collected from each taxon. In a random manner 120 fruits were further taken from each set. They are given an average diameter in millimeters, as the mean of two cross-measurements. Also, their weight (mass) was measured with accuracy of 0.01 g. In this way, a better insight into the morphometric characteristics of the observed fruits was obtained.

Results and discussion

The chemical composition of *Malus* 'Pink Prefection' and *M*. x *zumi* 'Golden Hornet' is quite similar (Tab. 1). *M*. 'Royalti' contains considerably more vitamin C and a little more sugars, but is also poorer in pectin and has much higher acidity. The relationship between increased vitamin C concentration and total acidity, among the first was observed by Nybom (1959), in soft apples. However, the same author finds that sometimes sweet fruits have a rather high vitamin C content.

Table 1: Chemical composition of fresh fruit and juice of crabapples

Components	Unit	*M.* 'Pink Prefection'	*M.* 'Royalti '	*M.* x *zumi* 'Golden Hornet'	Crabapple juice
		Mean value			
Invert sugars	%	9.99	11.19	9.17	3.71
Pectin	%	0.48	0.35	0.56	0.43
Proteins	%	0.37	0.37	0.39	-
Total acidity (as malic acid)	%	1.11	2.75	1.12	0.56
Total ash	%	0.48	0.78	0.35	-
Vitamin C (Tillmans)	mg/100 g	2.45	5.94	1.19	2.07
pH		3.28	3.02	3.17	-
Sugar-acid ratio	Invert sugars / Total acidity	9.0	4.33	8.19	6.62

Compared to commercial apples (Campeanu *et al*., 2009; Stojanović, 2014), crabapples do not lag behind sugar content, but they have significantly higher acidity and double less vitamin C (Tab. 2). Difference in malic acid concentration between cultivated and wild apple fruits was also noted by Ma *et al*. (2015). The same source emphasizes that the major components of soluble sugars (fructose, glucose and sucrose) are different between cultivated and wild fruits.

Table 2: Comparative overview of fresh fruits of crabapples and commercial apples

Components	Unit	Crabapples	Comercial apples (Stojanović, 2014)	Statistical differences
		Mean value & range		
Sugars content	%	10.12 (9.17 – 11.19)	10.62 (9.53 – 12.34)	statistically random
Water content	%	78.27 (70.97 – 82.269	83.75 (76.69- 88.37)	statistically random
Vitamin C	mg/100 g	3.19 (2.45 – 5.94)	7.51 (7.19 – 7.89)	statistically significant
Ash content	%	1.61 (0.35 – 0.78)	2.18 (1.63 – 2.77)	statistically significant
Total acidity	%	1.66 (1.11 – 2.75)	0.20 (0.127- 0.345)	statistically highly significant

By variance analysis, a statistically significant difference was found between Ca, P, Mn and Se content in crabapples fruits and juice (Tab. 3). Crabapples on average have significantly lower Na and Fe content compared to the average for five varieties of commercial apples from Southeast Serbia - 11.31 mg / 100 g and 0.41 mg / 100 g, respectively, and slightly lower content of Ca - 87.62 mg / 100 g (Stojanovic, 2014). However, crabapples have significantly higher content of K and Mn compared to orchard apples from Southeast Serbia, where K takes values from 57.25 to 98.23 mg / 100 g, Mn - 0.05 mg / 100 g, then Mg - 6.44 mg / 100,

Cu - 0.09 mg / 100 g and Zn - 0.04 mg / 100 g (Stojanović, 2014). The average selenium content in apple peel is also lower in relation to fresh fruits of crabapples - 0.62 μg / 100g (Stojanović, 2014), taking note that peel is considered as a better source of minerals in comparison to apple flesh.

Table 3: Macro- and microelements in fresh fruits and juice of crabapples

Elements	Unit	*M.* 'Pink Prefection'	*M.* 'Royalti '	*M.* x *zumi* 'Golden Hornet'	Crabapple juice
		Mean value			
Na	mg/100 g	1.15	1.11	1.07	1.07
K	mg/100 g	161.92	161	160.45	160.45
Ca	mg/100 g	8.28	8.27	8.35	10.03
Mg	mg/100 g	10.05	9.99	9.58	9.83
P	mg/100 g	17.02	17.42	17.67	13.42
Fe	mg/100 g	0.21	0.20	0.20	0.19
Cu	mg/100 g	0.11	0.11	0.12	0.09
Mn	mg/100 g	0.39	0.38	0.34	0.46
Zn	mg/100 g	0.13	0.12	0.12	0.10
Se	μg/100g	0.73	0.68	0.62	0.82

When it comes to phytochemicals, *M.* 'Royalti is particularly prominent in the high content of anthocyanins, flavonols and phenols, compared to the other two taxa. The ABTS test showed that the strongest antioxidant capacity has *M.* x *zum*i 'Golden Hornet'. We obtained the same results using the DPPH method (Tab. 4).

Table 4: Phytochemicals and antioxidant activity of fresh fruits and juice of crabapples

Component	Unit	*M.* 'Pink Prefection'	*M.* 'Royalti '	*M.* x *zumi* 'Golden Hornet'	Crabapple juice
		Mean value			
Anthocyanins	mg/L	0.09	2.77	0.04	0.02
Monomeric anthocyanins	mg/L	0.02	2.01	-	-
Flavonols	mgQcE/g	0.65	1.21	0.62	0.24
Phenols	mg GAE /g	7.99	42.46	5.86	4.21
Flavonoids	mgQcE/g	7.39	42.12	5.36	3.74
Nonflavonoids	mgQcE/g	0.60	0.34	0.50	0.47
ABTS	mg Trolox /mL	0.08	0.04	0.13	0.39
DPPH	mg Trolox /mL	0.46	0.18	0.70	1.21

Among the observed crabapples *Malus* x *zumi* 'Golden Hornet' has the largest and heaviest fruits (Tab. 5). An ocular estimation suggests that this apple yields the highest fruit yield per tree, under the same other conditions.

All observed species possess high aesthetic properties (Fig. 1-5) throughout the vegetation period and even afterwards in the first winter months. In this context, the crabapple *M.* x *zumi* 'Golden Hornet' is particularly remarkable, which keeps the fruits many months during the winter and can serve as Christmas or New Year decoration.

Table 5. Morphometric features of crabapple fruit

Crabapple	Diameter (mm)			Weight (g)			$r_{d.w}$
	M	S_x	Range	M	S_x	Range	
*M*alus 'Pink Prefection'	22.87	± 2.23	14 - 33	7.03	± 1.23	4.8 – 9.5	0.54
Malus 'Royalti'	17.19	± 1.58	14 - 20	2.97	± 0.67	1.7 – 4.5	0.82
Malus x *zumi* 'Golden Hornet'	24.92	± 3.17	15 - 30	9.06	± 2.00	3.4 – 13.8	0.52

Conclusions

The analyzed crabapples have a fairly similar chemical composition, with *M.* 'Royalti' predominating in the vitamin C content and hold a slightly higher sugar content but are also poorer in pectin content and contain much greater acidity.

Compared to commercial apples, crabapples do not lag behind in sugar content but are inferior to vitamin C and have significantly higher acidity.

The elemental composition of crabapples is also fairly uniform. Compared to commercial apples, they have lower sodium and iron content and a partially lower amount of calcium. On the other hand, they have significantly higher quantity of potassium and manganese, and some higher content of magnesium, copper, zinc and selenium.

Crabapple *Malu*s 'Royalti' predominates in the amount of anthocyanins, flavonols and phenols versus *M.* 'Pink Prefection' and *M.* x *zumi* 'Golden Hornet', while the ABTS test showed that the strongest antioxidant capacity has *M.* x *zum*i 'Golden Hornet'. We came to the same conclusion by applying the DPPH method.

Crabapple *Malus* x *zumi* 'Golden Hornet' has the largest and the heaviest fruits and gives the highest fruit yield per tree, under the same other conditions.

All observed species have high aesthetic properties throughout the vegetation period and even during the winter .

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FOREST CONTRIBUTION IN VEGETATION AND FLORA IN A SPECIAL PROTECTED AREA OF MOUNT TAYGETOS (SOUTHERN GREECE)

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Abstract

Greece has various climatic and biological conditions which favors the development and spread of forests with rich biodiversity (flora and fauna species). The present study was conducted at the Special Protected Area of Mount Taygetos, Southern Greece (*Site of Community Importance: GR2550006*), during 2016 and 2017. This study presents the species richness, relative abundance (%) and alpha diversity of flora and vegetative species in burned and unburned areas in forest ecosystems of *Fraxinus ornus* L. The sampling of flora was carried out in randomly selected plots of 0.25 m^2 and vegetation was recorded in randomly selected sampling plots of 100 m^2. The highest relative abundances of flora and vegetative species were *Bromus rubens* L. (21%) and *Fraxinus ornus* L. (27%), respectively, in forest burned areas. Also, *Prunella vulgaris* L. (32%) and *Fraxinus ornus* L. (61%) have the highest relative abundances in unburned areas. It is noteworthy that two important endemic species (*Abies cephalonica* Loudon and *Origanum scabrum* Boiss. & Heldr.) were recorded in the study area. These species have high ecological value and may attract researchers and nature enthusiasts. Moreover, the data revealed significantly higher vegetation diversity in the forest burned areas whereas the forest unburned areas had the lowest vegetation diversity. It is important that the study areas are appearing to have evidence supporting the fact that Mount Taygetos is a self-healing ecosystem with very high rates of regeneration. This contribution will be utilized as a valuable tool to the scientific community and providing baseline information for further research.

Keywords: *Native flora, regeneration, monitoring, utilization, Natura 2000.*

Introduction

Greece with lengthiness 132.000 km^2 is located in the southeast region of Balkan Peninsula and it belongs in Mediterranean zone of paleoarctical biogeographic region. More specifically, it is located in the limits of three continents (European, Asian and African), consisted by huge topographical variety (big number of islands, rivers, valleys, gorges, etc.). Also, it demonstrates large varieties of geomorphological formations (caves and other underground formations), and stones (pro-alpine, alpine and after alpine).

Greece is characterized by high values of flora and fauna, with big numbers of macro-fungi, a variety of ecosystems and landscapes, as well as big numbers of endemism in plant and animal kingdom. The flora of Greece is consisted approximately of 5.652 species and 1.893 subspecies of angiosperm plants, which represent approximately 6.600 taxa which are belonging to 1.072 genus and 185 families. As far as it concerns fauna, by this day, are recorded about 23.130 species of animals, inland and fresh waters, noting that the 3.956 are endemic as other 3.500 sea species (Strid and Tan, 1997; 2002; Ministry of Environment, Energy and Climatic Change, 2014).

The vegetation of an area is the result of interactions of a number of factors such as climate, geomorphology, human impact, etc. Regarding the vegetation zones identified in Taygetos, decisive shapes of plant communities' physique are the geographical position, altitude, soil and bioclimatic characteristics (Legakis and Maragou, 2009). According to the distribution of vegetation zones of Dafis (1973), which is based mainly in the Braun-Blanquet system, in the region of Taygetos divided into three different zones vegetation: the eu-mediterranean, the para-mediterranean and Alpine- subalpine zones (Biosfaira, 2011). Hence, the main purpose of the present study is to research the flora and vegetation between burned and unburned areas in forest ecosystems mainly inhabited by ash trees (*Fraxinus ornus* L.), in the Special Protected Area, with rare species of plants, landscapes and ecosystems of Mount Taygetos (Site of Community Importance: GR2550006).

Material and methods

Study area

The present study is conducted at the Special Protected Area of Mount Taygetos, Southern Greece (*Site of Community Importance: GR2550006*) ($37^0$13′19,898′′N, $22^0$12′21,663′′E), during 2016 and 2017 (Figures 1, 2, 3). Mount Taygetos (2.407 m.) is the highest mountain range in the Peloponnese peninsula in Southern Greece.

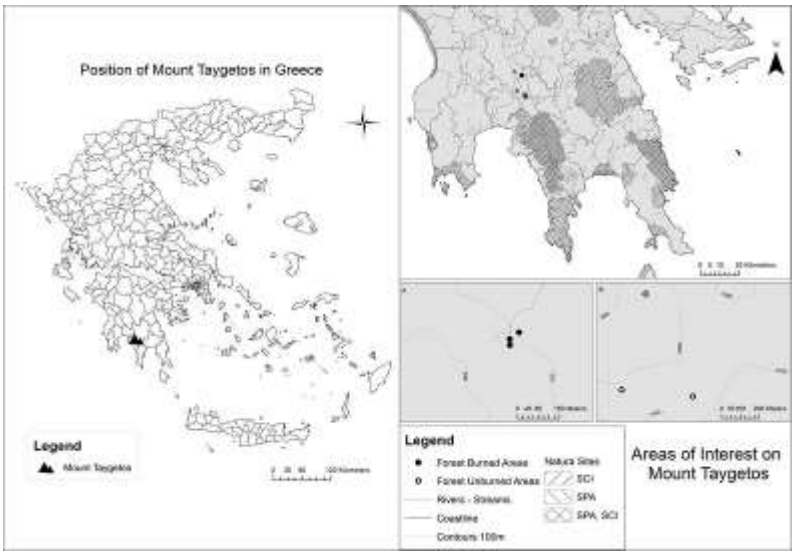

Figure 1. Study area.

Figure 2. Mount Taygetos.

Figure 3. Forest view (*Fraxinus ornus* L.).

Geologically, it is a continuation of the Gavrovo-Tripoli zone composed of phyllites, plated limestones and flysch. Its length is about 40 km and its southern limits touch Mt. Sangias

while the northern one is the Langada gorge. The forest ecosystems of the mountain are dominated by the Greek endemic, Greek Fir (*Abies cephalonica* Loud.) and excellent clusters of Black Pine (*Pinus nigra* Arnold). Also, Mount Taygetos constituted by *Fraxinus ornus* L. which is a small-medium deciduous tree which occurs typically in Mediterranean temperate colline-mountain forests. The mean annual precipitation needed is between 500 and 650 mm, although it has a good drought resistance, storing water in densely branched roots, and reducing transpiration. The root system is widely developed, requiring gravelly, well-aerated and drained soils. Moreover, this is an important site for breeding raptors and species associated with open montane habitats. Breeding birds include some of the species in Europe that are restricted (when breeding) to the Mediterranean biome. Species of concern include *Aquila chrysaetos*, *Hieraaetus fasciatus*, *Falco naumanni*, *Sylvia rueppelli* and *Pyrrhocorax pyrrhocorax*. Also, the low altitude gorges at the peripheral of the mountain are of great importance for birds of prey. Unfortunately, wildfires in 2005 and 2007 spent much of the forests on the central west slopes. In accordance with the Forest Service, the overall burned area of Taygetos is calculated almost 113,000 acres (Biosfaira, 2011). In total, within the protected area were burned 86, 542 hectares, i.e. 16.3% of the total forest areas (WWF, 2007).

Flora and vegetation survey

The sampling of flora was carried out in spring of 2016 and 2017 in randomly selected plots of 0.25 m^2. Species richness and relative abundance were estimated (Theodorakis, 1995). Vegetation was recorded in randomly selected sampling plots of 100 m^2. Species richness and relative abundance were measured (Koutsidou, 1995).

Statistical analysis

Flora and vegetation were evaluated for normality and homogeneity with the Kolmogorov–Smirnov and Shapiro–Wilk tests. This data was transformed using log (x+1) when necessary to meet normality assumptions. General Linear Model (GLM, Type III Sum of Squares) (One-way ANOVA) was used for the analysis of the above data. All statistical analyses for the flora and vegetation data were performed using the software package IBM SPSS Statistics ver. 23.0 for Windows (IBM, 2015).

Results and discussion

Flora and vegetation species richness and relative abundance

In total, in the forest ecosystems, 18 species of flora (burned areas: 9, unburned areas: 9) and 14 vegetative species (burned areas: 8, unburned areas: 6) were detected in the study area. The highest relative abundances of flora and vegetative species were *Bromus rubens* L. (21%) (Status: Native, Chorology: Mediterranean-SW Asian, Life-form: Therophyte, Habitat: Coastal habitats) and *Fraxinus ornus* L. (27%) (Status: Native, Chorology: Mediterranean-European, Life-form: Phanerophyte, Habitat: Woodlands and scrubs), respectively, in forest burned areas. Moreover, *Prunella vulgaris* L. (32%) (Status: Native, Chorology: European-SW Asian, Life-form: Hemicryptophyte, Habitat: Temperate and submediterranean grasslands, agricultural and ruderal habitats, woodlands and scrubs) (Dimopoulos et al., 2013) and *Fraxinus ornus* L. (61%) (Status, etc. see above) have the highest relative abundances in forest unburned areas (Figure 4, 5, 6).

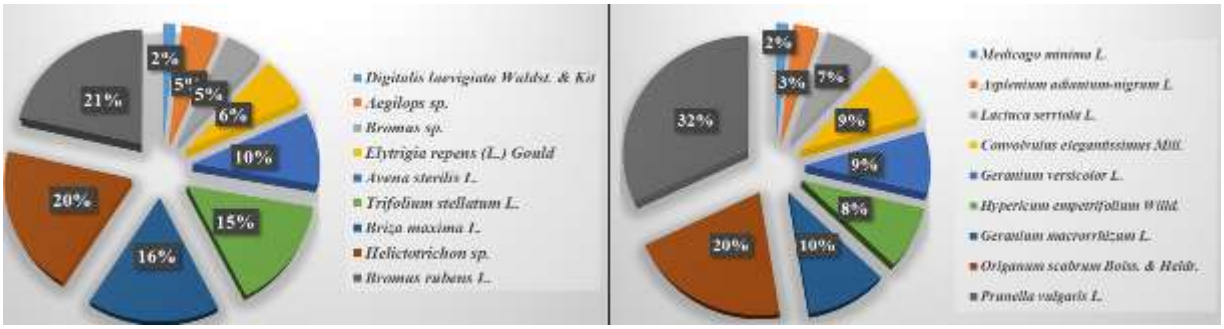

Figure 4. Relative abundance (%) of flora species in burned and unburned areas.

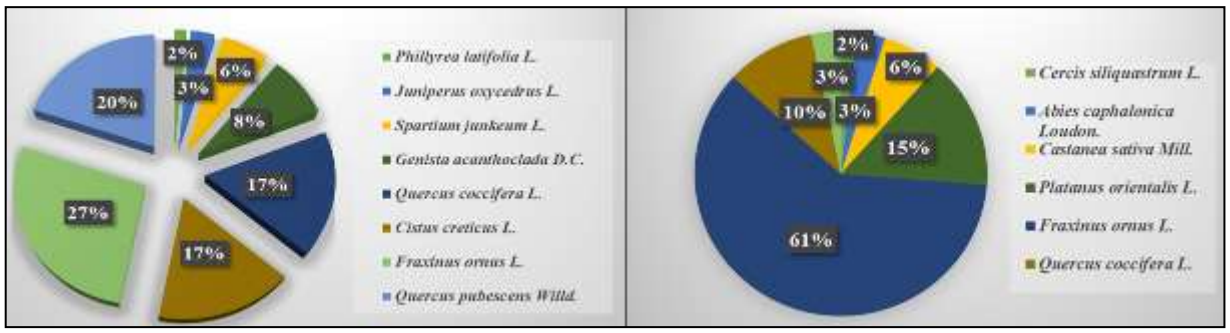

Figure 5. Relative abundance (%) of vegetative species in burned and unburned areas.

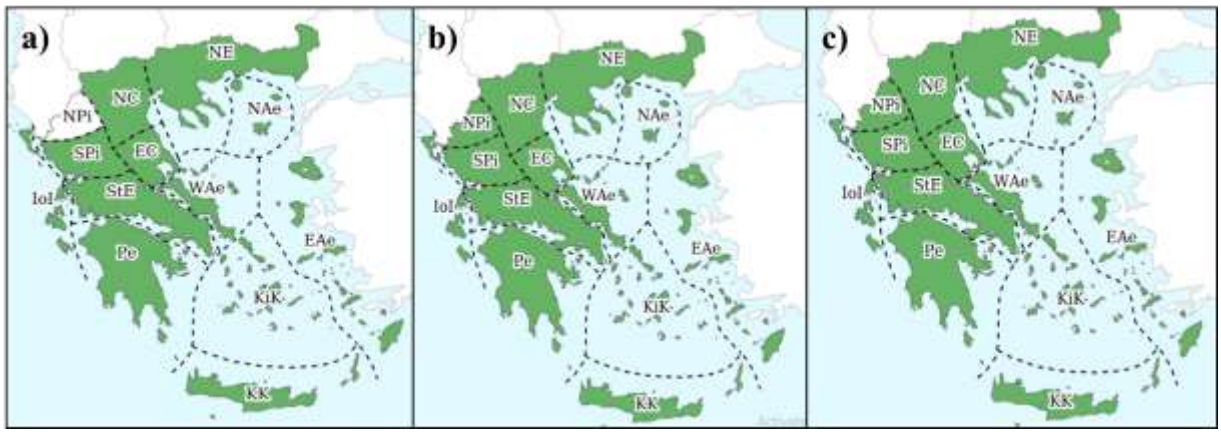

Figure 6. Distribution of highest relative abundance plant species in Greece: a) *Bromus rubens* L., b) *Fraxinus ornus* L., c) *Prunella vulgaris* L. (IoI: Ionian islands, NPi: North Pindos, SPi: South Pindos, Pe: Peloponnisos, StE: Sterea Ellas, EC: East Central Greece, NC: North Central Greece, NE: North-East Greece, NAe: North Aegean islands, WAe: West Aegean islands, Kik: Kiklades, KK: Kriti and Karpathos, EAe: East Aegean islands) (Dimopoulos et al., 2013).

Alpha diversity in burned and unburned areas

According to Table 1, the results didn't show higher alpha flora diversity between forests burned areas and unburned areas. Also, the forest burned areas exhibited the highest values of alpha vegetation diversity, whereas the forest unburned areas had the lowest values (Table 2). Disturbances such as fire play a role in the maintenance of species diversity that has become well recognized in ecological theory (Mackey and Currie, 2000). Even if a fire had occurred, the flora diversity between burned and unburned forest areas is at a relative balance. The most feasible explanation for this result is that the fire was not absolute and most of the trees have survived. These referred burned areas of this study happen to be in the exact limits which the

fire had stopped. For example, in this mountain, a previous research about burned and unburned forest areas showed that higher alpha flora diversity in Forests-Unburned areas, Natural Ecosystems-Unburned areas, and Forests-Burned areas than in Natural Ecosystems-Burned areas have occurred (Solomou and Tsipas, 2017).

According to literature, fire frequency determines the floristic composition of an area by selecting species, which will continue to occupy a site. A plant species may eclipse depending on the time that a fire occurs, e.g. late, early or after the plant's life cycle (Chandler et al., 1983). However, species have many reactions to fire in order to survive. There are sprouts which can endure repeated fires while those that produce seed are favored by infrequent fire (Keeley, 1981).

Table 1. Alpha diversity indexes of flora in forests (burned and unburned areas).

Alpha diversity indices	**Forests-Burned areas**	**Forests-Unburned areas**
Shannon-Wiener	1.99a*	1.9a
Simpson	7.26a	5.92a
Fisher's alpha	2.9a	2.95a
Eveness	0.69a	0.66a

*Different letters show statistical significances.

Table 2. Alpha diversity indexes of vegetation in forests (burned and unburned areas).

Alpha diversity indices	**Forests-Burned areas**	**Forests-Unburned areas**
Shannon-Wiener	1.82a*	1.28b
Simpson	5.65a	2.52b
Fisher's alpha	2.03a	1.87b
Eveness	0.67a	0.47b

*Different letters show statistical significances.

Conclusions

Mount Taygetos is the highest and most impressive mountain not only of Messenia but of all Peloponnese. This ecosystem is a real botanical paradise, preserving an ecological balance as well as providing different biotic components. On the basis of the above, it is important to estimate and monitor the populations' size of flora and fauna dynamics, all seasons of the year in order to be effectively protected and utilized from the scientific community and wider audiences. It is known that the forest-based sector plays a significant role in Bioeconomy. Forests supply and contribute to bioenergy with wood and non-wood materials forming the basis for many management, regulating and ecosystem services. Hence, management of forest biomass in Mount Taygetos can be succeeded within the Bioeconomy concepts, while this high-value diversity can be protected and utilized through these concepts, projects and monitoring.

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POTENTIAL ENERGY WOOD RESOURCES IN UNDERSTOREY OF *MYRTILLOSA MEL.* FOREST SITE TYPE STANDS IN LATVIA

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Abstract

Forest resources are the most significant natural resources of the state of Latvia. According to the second stage data of Forest Resource Monitoring, in 2014, Latvia has 3575 thousand hectares of forest land, which accounts for approximately 55.3 percent of the total territory of Latvia, while the total wood stock is estimated at 668 million cubic meters. A considerable part of it can be used for energy production, thus practically providing most of the necessary heat energy, a large part of the amount of electricity, as well as the amount of energy needed to provide transport movement. In recent years, the consumption of certain woody biomass products, such as chips and pellets, has a tendency to grow, due to a number of factors: the opportunity to use its own resources, export them, the increase in international financing for renewable energy projects etc. The fossil fuel price increases and the development of strategic and regulatory decisions contribute to this.The forest undergrowth and understorey which according to the Rules for cutting trees in forest lands are suggested partially to be preserved, contain a considerable amount of potential energy wood.In the forthcoming study on the assessment of energy wood resources, a sufficient number of plots to be established in drained forests are foreseen, in order to ascertain the exact potential of their energy extraction in the drained forest site types in the country, as they reach or are close to the cutting age.The results obtained in Myrtillosa mel. forest site type suggest that the biomass of naturally humid wood of shrubs of understorey comprises of 22 665 kg per hectare but calculated the dry mass - 12 590 kg per hectare. The results suggest that this biomass is considerable and it is reasonable to use it for energy wood extraction simultaneously with final felling.

Keywords: *energy wood, understorey, Myrtillosa mel. forest site type*

Introduction

Forest resources are the most significant natural wealth of the state of Latvia. According to the data of the 2nd stage of the forest resource monitoring for 2014, Latvia has 3575 thousand hectares of forest land comprising 55.3 percent of the total territory of Latvia, while the total timber stock was estimated at 668 million cubic meters (Bumanis *et al*., 2014). With economically justified solutions of forestry issues, a significant part of it can be used for energy production, thus practically providing most of the required amount of heat energy, a large part of electric power, as well as of the required energy for transport access. In recent years, the consumption of definite wood biomass products, like wood chips and pellets, tends to increase due to several factors, and it is clear that the possibility to use own resources and to export them, the increase of international financial support for projects anticipating usage of renewable energy, the price increase of fossil fuel and the development of strategic and regulatory decisions are promoting it. In the last decade, with the financial support of EU Funds, a substantial amount of cogeneration plants and boiler houses have started their work, replacing imported fossil fuel with fuelwood. During the EU fund programming period 2007-2013, over 84 million Euros worth co-financing was invested in increased energy efficiency of the centralized heat supply. While carrying out131 projects, an appreciable increase of power capacity due to the renewable energy resources was achieved. Although in recent years the consumption growth of wood biomass has halted, starting the implementation of the new

projects of the EU fund programming period of 2014-2020, there will be a need for additional supply of wood biomass. In March 2017 the Cabinet of Ministers of the Republic of Latvia approved a new program supporting the improvement of the energy efficiency of the centralized heat supply systems and the usage of local renewable energy resources. Within the framework of the first project call, the support of these activities reached 35 million Euros. For these reasons, the usage of wood chips, pellets and briquettes fruitfully increases in recent years, especially in comparative perspective, contrary to the use of firewood (Table 1).

Table 1. Wood biomass products consumed in Latvia in the period from 2005 to 2013 (thous. m^3thous. t^{-1}) (Bumanis *et al*., 2014).

Year	Firewood thous. m^3	Woodchips thous. m^3, loose volume	Sliver thous. m^3, loose volume	Pellets, briquettes thous. t
2005	5127	1804	3142	28
2006	5113	2039	3023	26
2007	5046	2165	2616	30
2008	4880	1961	2287	30
2009	5426	2386	2868	26
2010	5073	2528	2951	31
2011	4439	2418	2988	33
2012	4573	2474	2956	136
2013	4237	4171	3120	96

However, from the available forest resources data it is only theoretically and hypothetically possible to define what proportion of these resources would be useful and economically justified for energy supply. Each stand has a certain amount of undergrowth resources, which need not necessarily be left for further provision of its vitality, and there are felling residues in any final cutting where the economic justification of usage would undoubtedly be that of the source of energy (Ohman *et al*., 1976). The most precise determination of the amount of wood energy in Latvian forests, the creation of mathematical models for its determination, as well as economic substantiation of the usage of forest resources for energy production, as opposed to importing energy resources, would be of great benefit to Latvian economy.

The object of the study is the timber resources in drained forest conditions available in the country and potentially to be used for energy production in the near future. Forests on drained peat soils occupy 14%, and forests on drained mineral soils occupy 19% of the total forest area (Libiete, 2008).

The subject of the study is the stands of felling age in drained forest conditions, in addition, starting the research with the class of the seasoning stand and estimating the potential 20-year period of wood resources available for energy production. The research reveals the volume of wood energy resources in undergrowth and its potentially available volume from the felling residues. Research hypothesis – the use of undergrowth and felling residues in the production of wood energy is profitable, and therefore, is also economically viable, and also corresponding to silvicultural and ecological substantiations.

The study aims to clarify precisely as possible the mass and volume of energy-producing mechanical pulp in the stands of felling age (and also seasoning stands) in drained forest conditions in Latvia.

The research objectives are to develop the methodology of the assessment of wood energy resources (development of mathematical models), as well as to provide a silvicultural and economical estimation of the usage of wood energy resources grown in drained forest conditions for energy production.

Material and Methods

The research data were collected in forests of Jelgava Forest District of Agency "Forest Research Station". The territory is located in the Cenu Parish of Ozolnieki District, in the northern part of Zemgale Plain.

The research was carried out in two forest subquarters of forest site type *Myrtillosa mel.* The *Myrtillosa mel*. forest site type belongs to the edaphic row of drained hydromorphic mineral soils. The site conditions here are excellent to grow up a high yield tree stands, as well having a dense floor of undergrowth and understorey. Taking in to account the potential richness of woody plants biomass eight circle shaped sample plots with radius of 2.82 m were established. The area of each single plot is 25 m^2.

The data were collected in area of 1st and 4th Subquarters of the 37th Forest Quarter. The area of 1^{st}subquarter comprises 0.6 hectares, the stand composition is 10Pine66 years old, stand density 0.7. The average tree height of dominant stand in this subquarter is 27 m and average diameter 25 cm. The stand volume is 365 m^3 ha^{-1} and site index Ia. The area of 4^{th}subquarter reaches 0.9 hectares, the stand composition is 9Pine1Birch88 years old, stand density 0.7. The average height of trees is 26 m, the average diameter – 29 cm. It is aIst site index stand with volume of 352 m^3 ha^{-1}.

The sample plot method is the method used for obtaining the figures of wood resources, which means that there is a characteristic sample plots created in forest subquarter (four sample plots per hectare) and the results obtained there are applied to the entire stand (Kangas and Maltamo, 2006). The chosen forest parcel has its randomly defined centre. At its ninety-degree angle diagonals are drawn in different directions and circular plots are established on each side of the diagonal, equidistant from the centre and the border of the sample plot.

In the sample plots, the understorey and undergrowth trees were surveyed. The treeswere cut into the root neck. Using the measuring tape there was the height of trees measured and the diameter in height of 1.3 m. For trees which had not reached the height of 1.3 m, the diameter was determined at the root neck. The mass was determined with or without leafs. Leafs were also weighted separately.

From each tree harvested, there was also a sample prepared which was then sent for drying. The mass, length, and diameter were determined for this sample. The length was determined in three places, in the lower half, in the middle and in the upper half.

The wood samples for moisture determination were transferred to Ltd. "Forest and wood products research and development institute", where the total moisture content of the wood sample is determined according to the standards of LVS EN ISO 18134-2: 2016. The Laboratory uses the simple method ISO 18134 - 2: 2015 (LVS EN ISO 18134-2: 2016, 2016).

The wood samples for drying are initially weighed using Li - 86 Weights. After weighing, the samples are placed in a Drying Oven Li-77, where they are dried at 105 ± 2ºC. After 60 minutes of drying, an intermediate check is carried out, during which time the mass change percentage is determined. Drying is completely completed after 48 hours when mass changes are no longer observed. After drying the trees, they are weighed again and the moisture content of the sample is determined as a percentage. The standard deviation of the moisture value and the coefficient of variation are also calculated in the Laboratory.

Dry mass rate is calculated using the results obtained from the drying of wood samples. To calculate the dry matter content of 100, the percentage moisture content of the wood sample is subtracted. For example, if the wood moisture after drying is 36%, then the dry matter content is 64%.The obtained mass of energy producing wood is attributed to the whole plot and hectare of forest.

In the research there is a wood mass of undergrowth and understorey separated. The undergrowth is formed by tree species which are able to reach the dimensions of dominant stand by suitable circumstances, e.g., improvement of lighting or by cutting of mother stand

trees. Such species in Latvian conditions are, e.g., Norway spruce (*Picea abies* (L.) Karsten), Scots pine *(Pinus sylvestris* L.), English oak (*Quercus robur* L.) and common ash (*Fraxinus excelsior* L.). The understorey consists of trees and shrubs which are not able to reach the size of dominant stand. There belongs such species as rowan (*Sorbus aucuparia* L.), alder buckthorn (*Frangula alnus* Mill.), bird cherry (*Padus avium* Mill.), common hazel (*Corylus avellana* L.), plum (*Prunus domestica*L.) and other species.

Several species like silver birch (*Betula pendula* Roth), pubescent birch (*Betula pubescens* Ehrh.), Norway maple (*Acer platanoides* L.), small-leaved lime (*Tilia cordata* Mill.), common aspen (*Populus tremula* L.) and gray alder (*Alnus incana* (L.) Moench) act uncertain: theoretically they can reach the dominant stand tree dimensions but as they are light demanding, they do not survive long time under the canopies of mother stand. Growing long time in suppressed conditions they form a weak crown, the stems are sinuous or inclined – not being able to take a vertical position any more. Taking in to account mentioned arguments, these tree species are considered as understorey.

Results and Discussion

Together, 10 different species of woody plants – birch (both species - silver and pubescent birch counted together), English oak, Norway maple, rowan, common aspen, gray alder, alder buckthorn, bird cherry, common hazel and plum - were measured in undergrowth and understorey of both forest subquarters.

In the first sample plot of the forest subquarter with tree stand composition 10Pine 66 years old, the mass of separate woody plants in naturally conditions varies between 5 and 4235 grams. The average mass before drying is 982.18 g. The largest mass before drying is for buckthorn, but with the lowest weight are both the rowan and the oak. Absolutely dry mass of wood varies from 3.59 to 2275.31 g, the average mass is 605.94 g. The average mass difference between the raw and absolutely dry biomass in this plot is 376.24 g. The wood moisture here varies from 56.33 to 65.42%. The highest moisture content of wood was for plum tree - 65.42%.

In the 2nd plot, the mass of the trees before drying varies from 50 – 26080 g, the average mass is 3601.58 g. Absolutely dry mass of wood varies from 29.92 to 14403.09 g, the average absolutely dry mass is 1967.65 g. The biggest loss in weight is for buckthorn with an initial mass of 2300 g, but after drying its weight decreased by 1765.52 g.The highest moisture content in the 2nd plot is for birch - 57.00%. The lowest moisture content is for the buckthorn - 41.97%. The average moisture content in plot 2 is 51.80%.

In the 3rd plot, the average weight of the trees before drying is 3643 g, but the average dry weight of the wood is 2066.85 g. The largest mass before drying was for oak - 14220 g, but after drying oak wood mass decreased by 7006.74 g. The moisture content of the wood here varies from 53.86 to 75.99%. The highest moisture content of wood is for hazel 75.99%, but the lowest moisture content is for oak - 53.86%.

Of the trees in the 4th plot considerably superior is rowan which mass before drying is 21170 g and dry mass is 11125.83 g. The average mass of woody plants in naturally moist conditions is 1215.51 g, after drying – 653.15 g. The average moisture content in plot 4 varies from 53.24 to 62.44%. The average moisture content is 56.66%. The highest moisture content of wood is for oak, the smallest – for rowan.

In the first sample plot of the forest subquarter with tree stand composition 9Pine1Birch 88 years old, the woody plants mass before drying varies from 5 to 2660 g, the average mass is 290.32 g. The absolute dry mass of wood varies from 3.85 to 1648.2 g, the average mass is 179.16 g.In the first plot of the given subquarter, the lowest moisture content of the wood is for buckthorn - 61.66%, the largest - 62.52% for rowan. However, the difference between the moisture content of both species here is small - 0.86%.

In the 2nd plot of subquarter, the wood mass varied from 2 to 2858 grams before drying. The absolute dry mass of wood varied from 1.43 to 1609.3 g. The largest changes in mass are observed for rowan with an initial weight of 34 g, but after drying it has a mass of 18.37 g. The highest moisture content here is for rowan - 67.25%, the lowest is 56.47% for the buckthorn. The difference between the highest moisture content and the smallest is 10.78%.
In the 3rd plot, the average weight of wood before drying is 635.90 g. Absolutely dry wood has an average mass of 338.88 g. Consequently, the difference between the weight before drying and after the average is 297.02 g. The largest mass of wood before drying is for maple - 7730 g. The highest moisture content of wood is oak - 56.53%. The lowest moisture content of the wood is for the buckthorn - 4.41%. The difference between these two indicators is high - 52.12%.
In the 4th plot, the wood mass varies from 40 to 49140 g before drying, with an average weight of 6538.64 g. Absolutely dry mass of wood varies from 21.23 to 23559.2 g. The largest and smallest mass before drying is for the rowan. The largest loss of weight is for buckthorn with an initial mass of 260 g. After drying, its weight has decreased by 163.86 g. The highest moisture content of wood here is for gray alder - 63.01% and the smallest for oak - 40.74%. The average moisture content of wood in plot is 49.60%.
The average biomass of undergrowth and understorey in Subquarters 1 and 4 of Quarter 37 is shown in the Table 2.

Table 2. Amount of biomass in inventoried sample plots.

Research object	Biomass per sample plot, t	Biomass, t ha^{-1}
1^{st}subquarter 1^{st} plot	0.027	10.68
1^{st}subquarter 2^{nd} plot	0.031	12.4
1^{st}subquarter 3^{rd} plot	0.047	18.84
1^{st}subquarter 4^{th} plot	0.019	7.56
Average in 1^{st}subquarter	0.03	12.37
4^{th}subquarter 1^{st} plot	0.005	2
4^{th}subquarter 2^{nd} plot	0.011	4.28
4^{th}subquarter 3^{rd} plot	0.014	5.68
4^{th}subquarter 4^{th} plot	0.073	29
Average in 4^{th}subquarter	0.03	10.24

According to previous researches, in Sweden obtains 7-20 t ha^{-1} of dry mass, in Poland -7 to 12 t ha^{-1}, in Germany, from 6 to 14 t ha^{-1} and in Latvia 8 to 12 t ha^{-1} (Lazdina *et al*., 2010).There are also several researches that describe biomass from young hardwood stands on abandoned agricultural lands in Canada: the values varies between 0.6 t ha^{-1} and 82.6 t ha^{-1} (Lupi *et al*., 2017). Consequently, a sufficient amount of biomass is obtained in forest subquarters investigated in our research, which fits with the results of other studies carried out.

Conclusions

The diameter of the woody plants in undergrowth and understorey of the inventoried *Myrtillosa mel*. site type forest stands varied between 0.2 and 8 cm, the height – between 0.2 and 9.5 m, the average naturally moist wood mass is 813.68 g. The dimensions of trees are big enough to get the sufficient amount of biomass.
In the forest subquarter with stand composition of 10Pine 66 years old the sum of total biomass of undergrowth and understorey comprises 177.91 kg per sample plot. In the forest

subquarter with the stand composition of 9Pine1Birch 88 years old there is the sum of understorey tree mass 180.9 kg but of undergrowth – 16.17 kg per plot. It means that in understorey is the more biomass as in undergrowth in the site investigated.

In conditions of tree stand 10Pine 66 years old the amount of dry matter to be extracted in average from all sample plots is 12.37 t ha^{-1}. In the forest subquarter with stand composition of 9Pine1Birch 88 years old the amount of dry mass in average is 10.24 t ha^{-1}. In Latvia there the average amount of dry biomass obtained in other investigations is between 8 and 12 t ha^{-1}. This allows to conclude that *Myrtillosa mel*. forest site type is appropriate for the use of undergrowth and understorey biomass for production of energy wood.

The amount of the energy producing wood growing in drained forest conditions, adding also the felling residues and the potential energy producing wood of the undergrowth and understorey, comprises almost half of the wood potentially and justifiably used for energy production in Latvia.

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SOIL/PLANT RELATIONSHIPS IN ARCTIC AND ALPINE AREAS A LITERATURE SURVEY.

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Abstract

A literature survey on the issue of soil/plant relationships at low temperatures showed that plant growth at low temperatures are controlled by incomplete seed maturation because of too low summer temperatures (1), winter damage on needles and bark tissue above snow cover, as a result of freezing and drying stress, due to temporarily frozen soil in spring, leading to photochemical damage on chloroplasts (2), and growth limitation at low soil temperatures because of slow uptake rates of nutrients and decomposition rates of organic nitrogen (3). This last process is particularly important because it is directly linked to growth and uptake of nutrients through the dark respiration process. In most plants the uptake of nitrate and phosphate from soil takes place against a concentration gradient and is dependent on energy from respiration, and the growth limitation is probably through the active uptake of nutrients (mostly nitrogen) in roots. The process is further dependent on nutrient content in soil and decomposition rates of organic matter. In accordance with this, a strong response in root nitrogen uptake rates have been found in birch seedlings on soil nitrogen level and temperature, and the effect of soil temperature is further amplified by its effect on microbial activity and decomposition rates. The mycorrhiza in soil is in this respect functioning as an extended root system, supplying the plants with nitrogen, in exchange with carbohydrates.

Keywords: *nutrient uptake, temperature, respiration, soil, decomposition, mycorrhiza.*

Introduction

The treeline ecotone in alpine and arctic areas is an important climatic indicator. The growth and survival of trees in these areas is mainly controlled by temperature (e.g. Kupfer & Cairns 1996) through various mechanisms like winter desiccation and freezing stress (Tranquillini 1979, Taulavuori et al. (2004)), incomplete seed maturation and regeneration (Juntunen & Neuvonen 2006) and limitation of nutrient uptake (e.g. Karlsson & Nordell 1987), where the last mentioned process seems to be the most important. A close relationship has been found between soil temperature, nitrogen accumulation and winter survival in birch seedlings (Weih & Karlsson 1999). In addition, Körner & Paulsen (2004) found support for a general world-wide tree-line related mean ground temperature limit of 6.7^{o}C, regardless of winter conditions.

The basic process behind this temperature-limited nutrient uptake at low temperatures, is probably the active uptake of nitrogen and phosphorus through the root membranes, against a concentration gradient (Chapin 1979, Karlsson & Nordell 1996). This process is mediated through the dark respiration, which is strongly temperature-dependent with exponential increase (e.g. Skre & Laine 2007).

Materials and methods

The energy kcal/g (R) from dark respiration is released in small steps through energy-rich nucleotides, and in addition to the active uptake of nutrients, it is used for growth, maintenance and transport of photosynthates, in accordance with the following relationship (Lambers 1998, Skre & Laine 2007), where carbon exchange units are given relative to leaf area:

$R = r_m + c_g \times RGR + c_t \times TR$ where

r_m = maintenance respiration $gCm^{-2}yr^{-1}$
RGR = relative growth rate mg g^{-1} yr^{-1}
TR = transport across membranes (including active uptake in roots and photosynthates) $gCm^{-2}yr^{-1}$
c_g - kcal g^{-1} /mg g^{-1} yr^{-1} = kcal/mg yr^{-1}
c_t - kcal $g^{-1}/gCm^{-2}yr^{-1}$

According to Lambers (1998) the balance between growth and maintenance respiration is dependent on the availability of nutrients and the genetic background of the plants. Plants with free nutrient availability have a tendency of using the available energy for biomass growth in order to increase their root uptake capacity, while plants with limited nutrient supply tend to put their energy into root respiration and active uptake of nutrients, which would increase the photosynthetic capacity in leaves (Thornley 1972). This is a useful adaptation, formulated as the "balanced growth" model. Seedling growth rates in birch has been found to be controlled by nitrogen concentration, which is dependent on N uptake rate (summer + winter) and N loss in the autumn. Simulation experiments showed that increased net uptake of nitrogen during autumn increased growth the following season by the same magnitude as a 1-2^oC increase in soil temperatures (Weih 2000a).

Results and discussion

Nutrient uptake and balanced growth. In accordance with Hagedorn et al (2010) the soil respiration and decomposition rates are much more temperature dependent than the uptake rates through root respiration. However, Kabeya (2010) found that the growth limitation was a two-step process, i.e. the relative growth rates were limited by (1) nitrogen uptake rates in roots and (2) by availability of non-structural carbohydrates from photosynthesis. In arctic soils the uptake and decomposition rates of nitrogen and phosphorus are low because of the low soil temperatures and have been shown to be limiting factors by Chapin (1979, 1980) and by Karlsson & Nordell (1996) and Weih & Karlsson (1999) in experiments on *Betula pubescens* seedlings. Further evidence was found by Aphalo et al. (2010) on *Betula pendula*, where relative growth rates (RGR) and stomatal conductance were almost completely inhibited by low soil temperature through limitation of nitrogen uptake in roots. However, these relationships were not found in slow-growing conifers with evergreen leaves. According to e.g. Goh et al (2012) photo-inhibition of photosynthesis may occur as a result of formation of harmful by-products from the light reaction or other stress factors, e.g. freezing and drying stress. Plants have developed mechanisms e.g. CO_2 acclimation or alternative respiration to avoid photo-inhibition (cf. Skre 2001).

Table 1
Root nitrogen uptake rates (mmol N $g^{-1}week^{-1}$) as a function of soil temperature (^oC) and nutrient level for mountain birch seedlings grown under experimental conditions for one season in Abisko, northern Sweden. Data is taken from Karlsson & Nordell (1996), see also Skre & Laine (2007).

Soil temperature (^oC)	**Nitrogen uptake rates (mmol N $g^{-1}week^{-1}$)**		
	Low fertilization level	**High fertilization level**	**Organic soil (humus)**
5	0.00	0.04	0.27
10	0.02	0.20	0.75
15	0.03	0.45	1.47

Repo et al (2004) found that low soil temperatures would lead to more starch accumulaton in spruce needles and delayed loss of starch in fall, because of higher C/N ratio, due to reduced N uptake in roots. In accordance with this, Karlsson & Nordell (1996) found a strong response in nitrogen uptake rates in fast-growing birch seedlings to soil nitrogen level and temperature, and the effect of soil temperature was further amplified by its effect on microbial activity and decomposition rates (Table 1). The much higher nutrient uptake rates from organic soil than from artificial soil with fertilization indicates this strong effect of soil temperature on decomposition rates, making nutrients more available for root and mycorrhiza uptake. In accordance, Sveinbjørnsson et al. (1992) found a strong increase in shoot elongation rates in mountain birch (*Betula pubescens*) grown at treeline in Abisko, northern Sweden, in response to N and P, but the response to P was particularly strong at treeline altitudes. P has been shown to be limiting factor for growth in arbuscular mycorrhiza (Valentine & Kleinert 2007) and it has been shown that mycorrhizal symbiosis increases P uptake at low and medium soil temperatures (Kytöviita & Ruotsalainen 2007). Plants growing close to their distribution limits often show strong responses to N and P, mostly because of low soil temperatures and low concentrations of available nitrogen and (partly) phosphorus in soil. Hence mycorrhizal is functioning as an extended root system in nutrient uptake.
The nitrogen/carbon balance in plants is affecting shoot and root growth in the following manner, according to the balanced growth model (Thornley 1972) where > means "leading to":

N > Leaf protein > Photosynthesis > C > Root growth > N uptake

The significance of this relationship is that at high temperatures and low light intensity, excess nitrogen is transported from roots to shoots for leaf protein production, increasing photosynthetic efficiency. On the opposite, high light intensity and low temperatures would lead to formation of excess carbohydrates, that is transported from leaves to roots in order to increase root growth and the uptake capacity of nitrogen at low soil temperatures (cf. Skre & Laine 2007, Lambers 1998). In this connection, arbuscular mycorrhiza (Valentine & Kleinert 2007) is functioning as an extended root system.

Adaptation to temperature changes. Arctic and alpine plants that are adapted to a nutrient-poor environment without competition and where low temperatures are limiting factor, tend to avoid growth rather than producing nutrient-deficient tissue (Chapin 1979). Instead they are using excess carbohydrates to put out more root growth in order to increase their nitrogen uptake capacity. In addition, northern plants have been found to have slightly higher photosynthetic efficiency than southern relatives (Weih & Karlsson 1999). This is called "metabolic compensation", because it partly compensates for the shorter growing season in northern areas (Billings et al. 1971). Stronger root growth and related increased nutrient uptake is a precondition for this process. In experiments with birch seedlings, Weih (2000b) found that reduced soil temperatures and/or nutrient supply caused delayed growth response, e.g. the plants maintained a high root/growth ratio (RGR) during the next season, at the

expense of plant nitrogen concentration (PNC). This situation is relevant because great year-to-year climatic variations are common in the Arctic (e.g. Skre et al 2002).

The raised temperature impact on sensitive stages like seed reproduction, winter dormancy, migration rates and soil conditions (Callaghan 1993, Heide 1993, Huntley 1997) is expected to affect subarctic plant species. Increased CO_2 level will normally cause increased growth rates due to a combination of increased photosynthetic rates and increased water use efficiency (Hall et al. 1993). In most deciduous trees, e.g. birch, shoot growth will take place over the whole season ("free growth") and there will be no sink limitation on photosynthesis. According to the "balanced growth model" (Thornley 1972), the expected CO_2 increase would lead to higher C/N ratio, that would have to be balanced by increased root growth. The most pronounced effect of increased CO_2 content is therefore increase in root biomass (Mousseau 1993). In Norway spruce (*Picea abies*) Lahti et al. (2005) found that there was a certain depression of root growth during the period of strongest shoot growth. In another study on *Betula pubescens,* Weih & Karlsson (2002) found that root damages due to freezing soil temperatures would be compensated by new root formation the following season, but at the expense of shoot growth. The results agree well with previous studies (e.g. Murray et al. 1989), indicating that ecotypes and species that are competetive and adapted to a high-growth strategy may take advantage of the expected climate change, because they would tend to put more of their carbon and nutrients into new growth, while the northern populations tend to use their resources for storage and defence against climatic and herbivore stress (e.g. Skre & Naess 1999), which seems to be more important as selective factors in northern areas than competition (Chapin 1980, Ågren 1985).

Conclusion

The active uptake of nitrogen and phosphorus in roots has been found to be the probably most important limiting factor for growth and survival in arctic and alpine plants at low temperatures. This process is mediated through the respiration and is strongly temperature-dependent. In accordance with this, a strong response in root nitrogen uptake rates has been found in birch seedlings on soil nitrogen level and temperature, and the effect of soil temperature is further amplified by its effect on microbial activity and decomposition rates. The mycorrhiza in soil is in this respect functioning as an extended root system, supplying the plants with nitrogen, in exchange with carbohydrates. In agreement with these relationships, plants from northern and alpine populations have been found to have higher photosynthesis rates than southern and lowland relatives, this so-called «metabolic compensation» is looked at as a compensation for the short growing season in cold areas.

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DETERMINATION OF VARIOUS SPECIES OF THE GENUS *POPULUS* TRUNK DIAMETER BASED ON THE STUMP DIAMETER: CASE STUDY PERM, RUSSIA

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Abstract

The poplar is widely used in protective and recreational plantings due to the ease of reproduction, the rate of increase and decorativeness. Often poplar trees become the object of unauthorized destruction, due to its negative qualities: brittle wood, insect and fungus infestation, annual and abundant fuzz. The destruction investigator must determine the damage cost based on the tree species and trunk diameter. On the locale, the trunk diameter will be determined by the stump diameter. This calculation is made by using a specific table. Nevertheless, there are not poplar species in that table, except the aspen. The article presents the results of a study on the poplar trunk diameter dependence on the stembase diameter. The research was done in a specially protected natural area called the «Chernyayevsky forest» in Perm. The purpose of the study was to improve the methodology for determining damage caused by illegal logging of trees (poplar) in forest and urban areas. The study on the poplar stembase formation was carried out in September 2017 in artificial plantations of the «Chernyayevsky forest». The plantation age was 60 years. The forest type of the research plantation was a pine pleurocarpous moss forest. The forest site was fresh subor (B_2) according to the Pogrebniak classification. The research data showed the trunk diameter calculation error attained one calliper scale.

Key words*: Berlin poplar, aspen, breast height diameter, stump diameter, correlation, illegal logging.*

Introduction

Poplar is a genus of deciduous fast-growing trees in the family *Salicaceae* (Willow), widely used in protective, landscaping and recreational plantings, due to the ease of reproduction, high performance and decorative qualities. A significant disadvantage of poplars are: brittle wood, short-lived, damage by pests and diseases, annual and abundant fuzz (Redko, 1975; Tsarev, 1985; Plotnikova, 1994; Isebrands et al., 2014). Therefore, poplar is often the subject of unauthorized destruction of city residents. Calculation of damage in the case of unauthorized felling of wood in the city of Perm is carried out in accordance with the document on approval of "procedure for calculating the replacement value of green spaces demolished in the city of Perm" (About the statement…, 2015), where the cost of restoration of planting increases depending on the type of wood and its diameter at breast height. In forestry of the Perm Krai in the investigation of cases of illegal logging of aspen (*Populus tremula* L.) using a translation table developed by A. M. Mezhibovski (Zagreev et al., 1992). For other types of poplar, there are no table data. At the same time, analyzing the "Sorting and commodity tables" (Verkhunov et al., 2002), it is possible to observe differences in the stock of the same thickness of the tree between aspen and poplar (aspen). Thus indicating differences in the formation of the trunks of different species of poplar.

The aim of the study was to improve the methodology of determining the damage caused by illegal tree logging (poplar) in forest and urban areas. Objectives: 1) to determine the dependence of the trunk diameter based on the stump diameter, for the subsequent

determination of the stock; 2) to create a translation table from the diameter of the stump to the diameter of the trunk for use in practice.

Material and Methods

In September 2017, on the territory of the protected area called the "Chernyaevsky forest", studies were conducted out on the formation of the butt part of poplars growing in artificial plantations of the 6th class of age. The investigated stratums belongs to the type of forest – pine pleurocarpous moss forest. The survey of plantings was conducted by the method of sub-tree inventory (Regulations on works…, 2007); the species belonging of poplar was determined by a number of sources (Redko, 1975; Tsvelev, 2000; Ovesnov et al., 2007 Molganova et al., 2016), the height of each tree was measured. As every tree was measured, the diameters of the trunk at a height of 10 cm (d10), 20 cm (d20) and 1.3 m (d1,3) from the surface of the earth or the presence of roots from the root collar, up to 0.5 cm (in this case also determined the height of the root collar above the ground). Trees with a diameter of more than 64 cm were measured along the circumference using tape measure. Trunk volume in the bark was determined on the basis of state-approved structures of assortment and commodity tables (Verkhunov et al., 2002). A total of 446 trees of Berlin poplar were examined.

Results and Discussion

Due to the fact that in the literature devoted to the study of compliance of stump diameter to diameter at a height of 1.3 m from the root collar, often missing the height of the diameter measurement of the stump, were analyzed two ratios: measuring at a height of 10 cm and 20 cm. Plots of the diameters of the stump and the diameter of the trunk is shown in Figure 1. As control was used the dependence developed by A. M. Mezhibovski (for aspen).

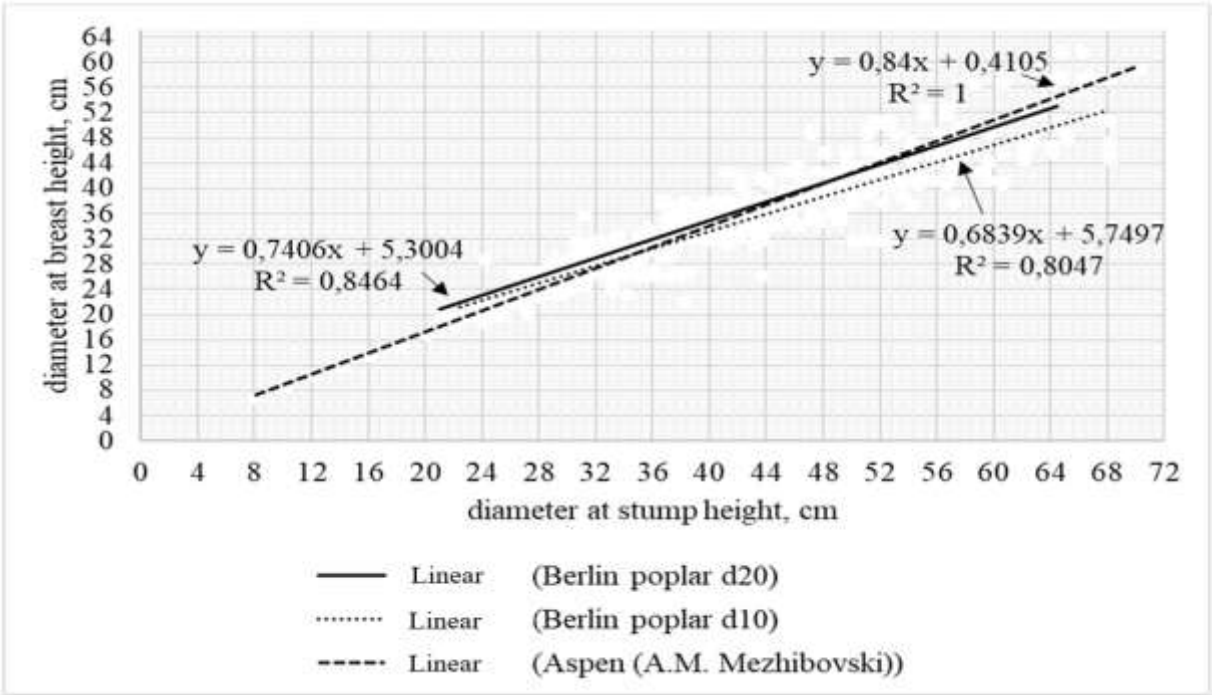

Figure 1. Graphs and linear equations based on diameter at breast height from stump diameter at different heights

Graphics matching the diameter of the stump at different heights to a diameter at 1.3 m to the Berlin poplar indicate that the thinner trunks are in the form of a butt close to the cylindrical. With the increase in the thickness of the trunk, the butt become more fleeting. The dependence of the diameter of the trunk from stump diameter at a height of 20 cm from the

ground to the tree somewhat reminiscent of a similar relationship with aspen but doesn't allow to fully use the data of A. M. Mezhibovski in relation to the poplar. The table shows the error values when using different diameter conversion techniques

Table 1. Comparison of the established D1,3 dependencies on stump with the data of A. M. Mezhibovski

calliper scales diameter of stump, cm	Diameter Aspen at 1.3 m heigh,cm (data from A. M. Mezhibovski)	Trunk volume in the bark, m^3	Berlin poplar, the diameter of the stump at a height of 10 cm (D10)			Trunk volume in the bark, m^3	Berlin poplar, the diameter of the stump at a height of 20 cm (D20)			Trunk volume in the bark, m^3
			diameter at 1.3 m heigh, cm	deviations			diameter at 1.3 m heigh, cm	deviations		
				cm	%			cm	%	
22	18.9	0.31	21.0	-2.1	-10.0	0.31	22	-3.1	-14.1	0.31
24	20.6	0.31	22	-1.4	-6.4	0.31	23	-2.4	-10.4	0.48
26	22.2	0.31	23.5	-1.3	-5.5	0.48	24.5	-2.3	-9.4	0.48
28	23.9	0.48	25	-1.1	-4.4	0.48	26	-2.1	-8.1	0.48
30	25.6	0.48	26.5	-0.9	-3.4	0.69	27.5	-1.9	-6.9	0.69
32	**27.3**	**0.69**	**27.5**	**-0.2**	**-0.7**	**0.69**	29	-1.7	-5.9	0.69
34	**28.9**	**0.69**	**29**	**-0.1**	**-0.3**	**0.69**	30.5	-1.6	-5.2	0.98
36	**30.6**	**0.98**	**30.5**	**+0.1**	**+0.3**	**0.98**	32	-1.4	-4.4	0.98
38	**32.7**	**0.98**	**31.5**	**+1.2**	**+3.8**	**0.98**	33.5	-0.8	-2.4	0.98
40	**34**	**0.98**	**33**	**+1.0**	**+3.0**	**0.98**	35	-1.0	-2.9	1.29
42	**35.7**	**1.29**	**34.5**	**+1.2**	**+3.5**	**1.29**	**36.5**	**-0.8**	**-2.2**	**1.29**
44	**37.4**	**1.29**	**36**	**+1.4**	**+3.9**	**1.29**	**38**	**-0.6**	**-1.6**	**1.29**
46	**39**	**1.6**	37	+2.0	+5.4	1.29	**39.5**	**-0.5**	**-1.3**	**1.6**
48	**40.7**	**1.6**	38.5	+2.2	+5.7	1.6	**41**	**-0.3**	**-0.7**	**1.6**
50	**42.4**	**1.6**	40	+2.4	+6.0	1.6	**42**	**+0.4**	**+1.0**	**1.6**
52	**44.1**	**2.01**	41	+3.1	+7.6	1.6	**44**	**+0.1**	**+0.2**	**2.01**
54	**45,8**	**2.01**	42.5	+3.3	+7.8	2,01	**45.5**	**+0.3**	**+0.7**	**2.01**
56	**47.4**	**2.47**	44	+3.4	+7.7	2.01	**46.5**	**+0.9**	**+1.9**	**2.47**
58	**49.1**	**2.47**	45.5	+3.6	+7.9	2.01	**48**	**+1.1**	**+2.3**	**2.47**
60	50.8	2.91	47	+3.8	+8.1	2.47	49.5	+1.3	+2.6	2.47
62	52.5	2.91	48	+4.5	+9.4	2.47	51	+1.5	+2.9	2.91
64	54.2	2.91	49.5	+4.7	+9.5	2.47	52.5	+1.7	+3.2	2.91

In the study of dependencies (see table 1) d1,3 of diameter stump of Berlin poplar with the data of A. M. Mezhibovski shows that, with d10 values ranging from of -2. 1 (-10%) to +4.7 in (9,5%), while d20 from -3,1 (-14,1%) +1,7 (3,2%). Translation table for aspen can be used in the calculation of damage from the illegal logging of poplar, with the following amendment: at the diameter of the stump 32 to 44 cm, measure the diameter of the stump at a height of 10 cm from ground level (root collar); when the stump diameter from 42 to 58 cm, measure the diameter at a height of 20 cm, considering the use of the calliper scale of 4 cm.

Conclusions

1) Comparison of the graphs of dependence of the barrel diameter on the diameter of the stump Berlin poplar and aspen showed differences for both thin and thick trunks.

2) Recommended to use the translation table (Mezhibovski) can be used to calculate the damage from the illegal logging of poplars as amended: when the thickness of the stump 32 to 44 cm, measure the diameter of the stump at a height of 10 cm from ground level, with a thickness of

42 to 58 cm, measure the diameter at a height of 20 cm, considering the use of the calliper scale 4 cm. However, for a more accurate calculation of appropriate tabulations for each poplar separately.

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FOREST MANAGEMENT IN THE FIRST PROTECTION REGIME OF SPECIAL NATURE RESERVE "KOVILJ-PETROVARADIN MARSHES" – ANALYSIS AND IMPROVEMENT OPTIONS

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Abstract

Forest management in a protected area, such as the Special Nature Reserve "Kovilj-Petrovaradin marshes" is determined by the rules and regulations that require nature protection, as well as forestry. All actions to be taken must strive to achieve the objectives of both professions. But, most importantly, it is necessary to manage the forest, this complex and very valuable natural resource, in the way that is most appropriate to the natural conditions on the ground. This paper presents an analysis of the natural conditions, bioecological characteristics of the forest habitats in the first protection regime. Pedological analyses are given, data about the plant cover on the studied area are given and finally, the characteristics of the forests are shown. Forest stands are also analysed from the aspect of classification into high conservation value forests, together with the classification and management process of these forest types, all in terms of the protection provided in this regime.

The aim of this paper is to examine and analyse the natural characteristics of forests and forest habitats in the first protection regime, as well as the management of this natural resource in the past. Eventually, the most important goal is to provide potential improvement options based on the greatest challenges during the past period.

Keywords: *Special nature reserve, Forest management, I protection regime, HCV forests*

Introduction

Forest management presents a set of harmonised technical, technical-technological, economic, organisational and social activities taken to protect, maintain, improve and use the forests (Forest Law, OG of RS 30/10, 93/12 and 89/15). On the other hand, nature protection presents a multidisciplinary approach of managing natural resources, created in order to solve different questions of nature management in a sustainable manner (Panjković *et al.*, 2016). Forest management in Serbia is defined by the Forest Law. The nature protection is defined by the Law on Nature Protection, which is determined as "lex specialis", considering specific function of forests and forest sites having the conservation function prioritized (Tarjan Tobolka, Stevanov, 2016). Common forest management measures cannot be applied in protected areas, they should rather be harmonised with protection goals. Forest management in the Special Nature Reserve "Kovilj-Petrovaradin Marshes" should be in accordance with the nature protection plans, which leaves very little space for forest management goals, in the first protection regime almost none. In the first protection regime there is neither forest management, nor nature resource management, in classical terms. In fact, only survey and research of spontaneous processes are allowed (Law on Nature Protection, OG of RS, 14/16). The aim of this paper is to provide analyses of the nature resource conditions in the first protection regime of the selected area by crossing the relevant data from forest management and nature protection. The final goal is to contribute to better understanding of the specialities and possible improvement of forest management in protected areas.

Materials and methods

The present work is done by document analysis, an iterative process of skimming, reading and interpretation of relevant data. According to Bowen (2009), combination of content and thematic analysis leads to new discoveries about the central questions of the research.

Materials for the analyses were the Study for the Protection, Forest Management Plans, revision documents, publications about the protected area and about other protected areas or nature protection both in Vojvodina province and Serbia. The legal framework of nature protection in Serbia has been included in the examinations. The analysis has been done in combination with the authors' personal studies and experience altogether with numerous contacts and interviews with relevant professionals. The analytic processing of relevant data has made it possible to provide a general overview of the environmental conditions and forest management in the first protection regime of the Special Nature Reserve Kovilj-Petrovaradin Marshes and to formulate possible suggestions for the future protection and forest management.

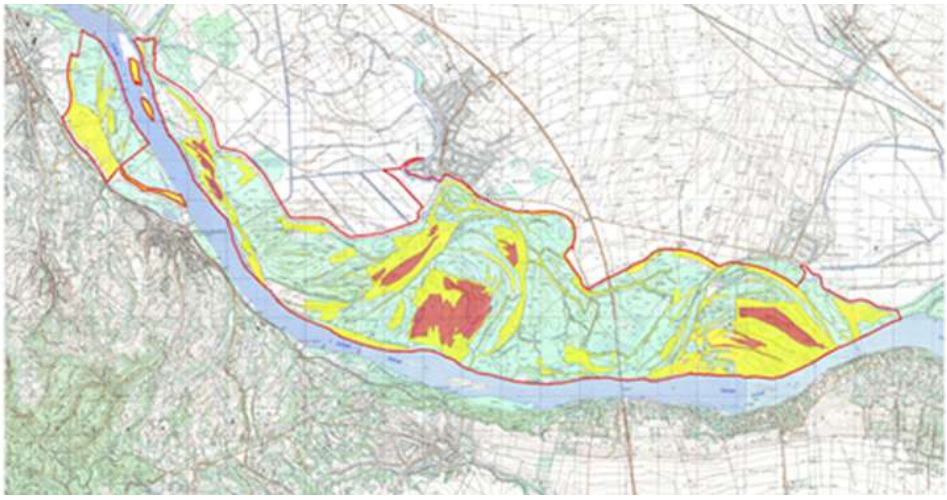
First protection regime

The first regime of protection (strict protection) is established on 373 ha (6%) of the total of 5.895 ha. It includes parts of the protected area with original or slightly modified ecosystems of exceptional scientific and practical significance (Law on Nature Protection, OG of RS", 14/16). In the first protection regime focus is put on the preservation of habitats and on enabling the processes of natural succession, being defined in the Law. The first protection regime is prescribed for the localities where natural or slightly modified forests of white willow (*Salix alba*), black and white poplars (*Populus nigra and P. alba*), pedunculate oak (*Quercus robur*), narrow-leafed ash (*Fraxinus angustifolia*), smoothleaf elm (*Ulmus carpinifolia*), and European white elm (*Ulmus effusa*) can be found, and also for the larger complexes of swamps and damp meadows that are isolated and difficult to access (Kurjacka greda: Ada Jamina I, Ada Jamina II; Kozjak: Veliki pesak, Kotao, Kozarnica; Carski Prud, swamps: Agla, Tonja, Zatonja, Barka, Provalija). From the aspect of forestry, the most important are the white and black poplar stands and the natural willow stands.

Characteristics of the vegetation

The whole area of the Kovilj-Petrovaradin Marshes lies by the river Danube and is in direct influence of the river's water regime. The vegetation is also hydrologically conditioned. The water regime of the habitats (high groundwater level and surface waters that regularly float them) has been the main ecological factor in the formation of the vegetation. Stevanović (1995) classes this area into the Pannonian-Danubian subregion of the Pontic-South Siberian floristic region.

The potential natural vegetation would be the community of lowland autochthonous forests of willows and poplars (*Ass. Salici-Populetum sensu lato*) and community of pedunculate oak (*Ass. Genisto-Quercetum roboris* Horv. 1938) (Parabućski *et* Janković, 1978). The associations of poplars and willows are formed on recent soils with longer remaining of floods, with groundwater on around 150 cm, the only few fragments of pedunculate oak communities are formed on older, stabilized or genetically developed alluvial soils (Panjković *et al.*, 2010).



Picture 1 SNR Kovilj-Petrovaradin Marshes - protection regimes
(I-red, II-yellow, III-light blue colors)[29]

Forest vegetation

The actual forest management plan (2012) classifies the forests of the first protection regime into two major groups: the forests formed in inundation, under influence of the water regime of the Danube river, and the forests formed by intensive human influence (plantations of poplar and willow clones) under influence of water management facilities such as embankments, canals, conduits, etc.

The forest management plan recognises seven groups of forests in the first protection regime:

1. High willow stands on wet recent alluvial deposits and gleyed soils;
2. Devastated willow stands on wet recent alluvial deposits and gleyed soils;
3. High stands of other poplars on mosaic of different alluvial soils;
4. High devastated poplar stands on mosaic of different alluvial soils;
5. High stands of green ash on mosaic of different alluvial soils;
6. Willow plantations on wet recent alluvial deposits and gleyed soils;
7. Plantations of euroamerican poplar clones on mosaic of different alluvial soils.

Natural stands of white and black poplars (on 30.60 ha) are classified into the third group, other poplars, with the aim to divide them of the economically managed plantations. Even in the first protection regime we can find clonal plantations, euroamerican poplars on 1.55 and willow on 18.19 ha. The green ash (4.33 ha) presents an introduced, invasive species, its presence testify about the forest management in the past period. However, one of the main goals is to replace it with native species, but only after the period of renewal (80 years for green ash).

Regarding the soil types, the management plans mention just two groups of soils, the wet recent alluvial deposits and gleyed soils and the mosaic of different alluvial soils. The Study of protection states that soils are formed on alluvial deposits, and that they are in permanent influence of the floods and groundwater. The soils are generally undeveloped, because of the oscillations of the groundwater, which transports elements with water, and because of the floods which removes the few formed soil parts. The mechanical composition of soils alters by moving from the river banks: sand dominates near the riverbed, and in farther parts dominate the sludge, with different combinations of deposited layers between them. The chemical characteristics are favourable for forest vegetation, they are rich in carbonates, neutral to slightly alkali reaction. Humification and mineralisation is well, except on swamped

[29] Source: Panjković *et al*., 2010.

soils. Beginning of real pedogenetical processes can be found only on higher positions with minimal deposition and removing of substrates (Panjković *et al.*, 2010).
The available reference data about the soils suggests, that further research, particularly for smaller sites, would describe better the site characteristics, which could lead to better understanding of the natural conditions and processes, convenient for each tree species, and finally help in deciding for future planning in forestry.

Results and discussion

Analysis of the improvement options

The guidelines for nature protection suggest the monitoring of populations of autochthonous flora and natural habitats along with the education and popularisation of preserved values of the entire biological diversity of the reserve, as well as fostering development of naturally emerging forests on recent Danube deposits (Panjković *et al*., 2010). On the other hand, the same Study of protection and the Protection act (Panjković *et al.*, 2010, SG of RS 27/98, 91/06, 81/08) limits the actions in the first, and in all, protection regimes, but also prescribes some measures to be taken. The question is whether or not these actions support the natural succession of forest vegetation in the strict zone of protection?
Since the autochthonous forests of willows and poplars, and also the communities of pedunculate oak are really rare, and remained only in fragments or very small areas, they should also be protected. Dredging of the canals is a highlighted activity since the protection was proclaimed (Puzović *et al.*, 2015). But, on the other hand, dredging of canals and other linear wetland depressions change the actual natural transport and deposition of flooded material, the soil formation is also altered, and finally formation of higher sites is questioned. Dredging of meanders of the old Danube, ecological restauration of wet habitats, revitalization of wet and swamp habitats is favourable for some ecosystems (Stojnić *et al.*, 2015, Puzović *et al.*, 2015), but their impact on forest ecosystems and further formation of forests of autochthonous tree species along the excavation is questioned.
Reviewing reference data and contacting the relevant professionals hasn't been found any concrete taken or planned activity with the aim of restoration of natural, autochthonous forests. The Study of protection, the forest management plans, management program of the protected area all recognise the importance of protection of natural forests. Particularly, the Study of protection prescribes filling the secondarily opened sites with autochthonous tree species, but none of the documents prescribes real measures with the aim of enabling the natural processes form the soils and micro habitats convenient for native forest vegetation of the area.
The Study of protection claims that regular removing of deposits and maintenance of water courses in the canals significantly slows raising of the ground and succession of vegetation, which contributes to the preserving of natural values of the area (Panjković *et al.*, 2010). In contrary, selecting parts of the first protection regime and creating favourable conditions for natural forest formation would improve and accelerate the restoration of autochthonous forests, which are also natural values of the area. It would be necessary to leave the processes of deposition and soil formation flow undisturbedly. The invasive and alochthonous tree species should be removed, leaving the space for autochthonous tree species. Permanent monitoring and acting accurately if needed would obviate disturbing the natural processes of forest restoration, and also obviate disturbing the preservation of other natural habitats and ecosystems in the close sites.
The Study of protection (Panjković *et al.*, 2010) recognises the importance of the preservation of forests and prescribes measures mostly oriented towards the protection and reintroduction of autochthonous tree species. It highlights that the species of pedunculate oak, narrow-leafed ash, smoothleaf elm, European white elm and black poplar should be preserved, because of

their rare prevalence, even if they are native in alluvial forests. The Study also suggests the consideration of the possibility of planting these species on higher sites or places where euroamerican hybrid poplars have taken their place.

Revising the forestry and the protected area management plans and the abovementioned documentation, it has not been found any exact locations of the mentioned species. Neither the exact locations of the trees, nor the ecological - pedological conditions of the sites are known. This suggests, that further analysis of the natural spread, the ecological characteristics of the site conditions are needed to be discovered. Mapping of the tree spread, together with soil characteristics, a database with exact GPS coordinates of the remaining trees of these species, would give an exact data convenient for further management. It would also enable us the monitoring of the situation in the field which would answer to the demand defined in the "Possible perspectives of sustainable development" of the Study of protection that "sustainable management of forests and development of forestry as part of economy is possible to achieve only by constant monitoring and determination of the state of forest fund, professional planning for long and short term and effective realisation of plans".

Forests of High Conservation Value (HCV forests) are proclaimed when some of their environmental or social values are dominatingly important on global, regional or local level (Laketić *et al*, 2012). Management should be oriented towards the conservation and promotion of their specific values. Review of the relevant documentation has shown, that in the process of forest certification all forests in the first protection regime are proclaimed as HCV forests (PE Vojvodinašume archive, 2018). On the other hand the forest management plans in the Republic of Serbia doesn't recognise HCV forests as management class, and does not classify them by this name (OG of RS 122/03, 35/10, 30/10, 93/12 and 89/15). Revision of the management plans actually shows their similar management status, but by other name. The data revealed shows the need of harmonisation of the terminology and management options in forest management with the international standards of certification.

Conclusions

Selecting sites and creating favourable conditions for natural forest formation in the first protection regime would possibly lead to natural formation of communities of pedunculate oak and other autochthonous species. Monitoring of the forest formation processes would give valuable data regarding the really rare appearance of such natural forests. The actions would be as prerequisite need either the change of the protection zone (to one where land management is allowed) or amendments in the higher policy.

Creating a database with GPS coordinates of the spread of foundation species like pedunculate oak, narrow-leafed ash, smoothleaf elm, European white elm and black poplar could contribute to further management planning and monitoring of the situation in the field.

All the forests in the first protection regime are classified as HCV forests, management options are actually accurate to the demands of the specific needs, but the forest management plans doesn't recognise this management category.

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COMPARATIVE EFFECTIVENESS OF SOME INSECTICIDES IN CONTROLLING *IPS TYPOGRAPHUS*

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Abstract

In the field (Golija Mountain, spruce forest approximately 80 years old, outbreak of *Ips typographus*) and semi-controlled laboratory conditions (Institute of Forestry in Belgrade), studies of the biological efficacy in the control of *I. typographus* adults, two concentrations (2% and 3%) of selected chemical (Fastac®Forst – active ingredient Alpha-cypermethrin) and biological insecticides (Naturalis Biogard® – active ingredient spore of *Beauveria bassiana*), were conducted. On June 13th, 2017, five TRIPODs in three repetitions, were installed along the edge of a forest stand, alternating in a single line with 10 m spacing. TRIPODs comprised of three spruce logs, 1.5 m in length and minimum 19 cm in diameter. Logs were taken from freshly cut healthy spruces. Trap logs were treated with insecticides diluted in water. The TRIPODs were baited with aggregation pheromone dispenser IT Ecolure that was attached to the top. After two months, bark beetles were sampled together with bark. Three samples approximately 34 x 13 cm in size, were taken from the upper, central and lower part of each log. The samples were placed in plastic boxes, stored in the laboratory and controlled at seven-day intervals. Experiments was established in the complete random block pattern in three repetitions, where the blocks for each variant present 3 samples. Fastac®Forst, in accordance with its functioning, caused 100% mortality of the adult *I. typographus* before their boring in the logs. No evidence of *B. bassiana* infection was reported in the collected individuals. The percentage of *I. typographus* individuals infected with *B. bassiana* on logs treated with Naturalis Biogard® 2% was 45.9%, and for Naturalis Biogard® 3% - 39.3%.

Key words: *Fastac®Forst, Naturalis Biogard® biological efficacy, Ips typographus*

Introduction

The epidemic desiccation of mainly autochthonous spruce stands, the uniform and mixed ones, as the modern phenomenon, is present in the most countries of Southeastern and Central Europe, and in the Republic of Serbia, due to the very unfavorable, extreme climatic conditions over the period 2011-2012, gained unprecedented scope in the forest areas which are under the special regime of protection, such as the Nature Park and the "Golija-Studenica" Biosphere Reserve. The changes in the trees induced by the desiccation, the reduction of the transpiration, of the carbon assimilation, have significantly reduced their natural, mainly chemical resistance to the attacks of the various xylophagous insects, so the population size of *Ips typographus* L. (Coleoptera: Curculionidae) was extremely increased over the period 2015-2017 (Tabaković-Tošić & Milosavljević, 2016, 2018).

In the Serbian forestry the certification based on the principles of the sustainable forest management was adopted in 2006. During the preparation of it, the FSC Criteria (FSC-STD-01-001, 2004), relating to pesticide use (Criterion 6.6, 10.7, 10.8), proved to be particularly interesting. FSC has a list of chemicals that are prohibited, and almost all previously registered and applied chemical insecticides are on this list. A company applying for certification would normally have to stop using these chemicals before it can receive an FSC certificate. One of the steps on the path towards obtaining the permission for putting into circulation and for application of some insecticide is the study of the biological efficacy of it,

i.e. of its ability to reduce a number of economically harmful insects to the normal level, which is considered to be safe.

The main aim of this research was to compare the biological efficacy of two insecticides (chemical and biological), both in two concentrations (2 and 3%) in the control of adults of *I. typographus*.

Material and method

In the field (Golija Mountain, spruce forest approximately 80 years old, outbreak of *I. typographus*) and semi-controlled laboratory conditions (Institute of Forestry in Belgrade), studies of the biological efficacy in the control of *I. typographus* adults, two concentrations (2% and 3%) of insecticides Fastac®Forst and Naturalis Biogard®, were conducted. Based on the technical documentations, Fastac®Forst (active ingredient - 1.5% Alpha-cypermethrin) is non-systemic chemical insecticide with contact and digestive action, in the form of a liquid concentrated suspension (SC). The formulated product, bionisecticide Naturalis Biogard® is a concentrated suspension of at least 2.3 x 10^7 spores/ml of *Beauveria bassiana* (Balsamo) Vuillemin strain ATTC 74040. It is a suspension of conidiospores in vegetal oil, which improves spore germination and UV protection, enhancing the efficacy of the antagonist in the field. *B. bassiana* acts primarily by contact. According to technical documentation for Naturalis Biogard®, once attached to the insect's cuticle, its conidia germinate producing hyphae, which penetrate the cuticle and proliferate inside the insect's body and causes their death within 3-5 days. After the host's death, new conidia may be produced and released on the outside of the insect cadaver. *B. bassiana* strain ATTC 74040 does not produce any toxins: the infected host dies due to dehydration and/or depletion of nutrients. On June 13th, 2017, five TRIPODs in three repetitions, were installed along the edge of a forest stand, alternating in a single line with 10 m spacing. TRIPODs comprised of three spruce logs, 1.5 m in length and minimum 19 cm in diameter. Logs were taken from freshly cut healthy spruces. Trap logs were treated with insecticides diluted in water. The TRIPODs were baited with aggregation pheromone dispenser IT Ecolure that was attached to the top and covered with spruce branches (Figures 1-3).

Figures 1-3: Treated TRIPODs (Original)

After two months, bark beetles were sampled together with bark. Three samples approximately 34 x 13 cm in size, were taken from the upper, central and lower part of each log. The samples were placed in plastic boxes, stored in the laboratory (Figures 4-6) and controlled at seven-day intervals.



Figures 4-6: Samples and second part of experiment in semi-controlled laboratory conditions (Original)

Table 1. Scheme of the experiment in the field - the complete random block pattern in three repetitions.

BLOCK I			**BLOCK II**			**BLOCK III**		
Fastac®Forst 2%			Naturalis Biogard® 2%			Naturalis Biogard® 3%		
12	25	41	16	32	33	5	21	36
Naturalis Biogard® 2%			Naturalis Biogard® 3%			Fastac®Forst 3%		
1	2	31	19	20	35	23	24	39
Fastac®Forst 3%			Distilled water - control			Fastac®Forst 2%		
37	38	22	14	28	29	27	40	42
Distilled water - control			Fastac®Forst 3%			Distilled water - control		
15	30	45	7	8	9	13	43	44
Naturalis Biogard® 3%			Fastac®Forst 2%			Naturalis Biogard® 2%		
4	6	34	10	11	26	3	17	18

Experiments was established in the complete random block pattern with five treatments in three repetitions (Table 1), where the blocks for each variant present 3 samples. Mean daily temperature and relative humidity for the June and July 2017 [data of the Republic Hydrometeorological Service of Serbia, Zlatibor (α: 43°44' N; δ: 19°43' E; the altitude of 1029 m)] are presented in the Fig. 7 & 8.

Figures 7-8: Mean daily air temperature (7) and relative humidity (8) for the June and July 2017 (Zlatibor Meteorological station).

Results and discussion

After 40 days of exposure, Fastac®Forst, concentration 3%, in accordance with its mode of action, caused 100% mortality of the adult *I. typographus* before their boring in the logs (Table 2, Fig. 9&10). No evidence of *B. bassiana* infection was reported in the collected individuals.

Table 2. Average number of the entrance holes of overwintering generation of *Ips typographus* per dm^2 of the treated spruce logs in TRIPODs.

Insecticide concentration dose	Average circumference of spruce logs in TRIPOD (cm)			Average number of entrance holes/dm^2 of log					
	Block			Block					
	I	II	III	I		II		III	
				1	2	1	2	1	2
Naturalis Biogard® 2% 2 liter	45.0	69.7	73.0	0.2	0.7	0.0	0.5	0.6	0.9
	64.0	46.7	79.3	0.4	0.9	0.3	0.7	0.9	1.0
	68.7	53.7	65.0	0.4	0.7	0.2	0.6	0.4	0.7
Naturalis Biogard® 3% 2 liter	57.7	58.0	73.0	0.7	1.0	0.3	0.5	0.8	1.2
	53.7	53.0	72.7	0.4	0.9	0.5	1.2	0.6	0.9
	48.7	57.0	70.7	0.3	0.6	0.7	1.1	0.5	0.9
Fastac Forst® 2% 1,33 liter	60.0	56.0	76.0	0.0	0.1	0.0	0.0	0.0	0.0
	64.3	50.3	75.0	0.0	0.0	0.1	0.1	0.0	0.1
	47.7	54.3	76.7	0.0	0.2	0.0	0.0	0.0	0.2
Fastac Forst® 3% 1,33 liter	61.0	48.3	72.0	0.0	0.0	0.0	0.1	0.0	0.0
	50.7	59.3	66.3	0.0	0.0	0.0	0.0	0.0	0.0
	53.3	46.3	60.3	0.0	0.0	0.0	0.0	0.0	0.0
Destiled water 2 liter	70.0	64.7	57.3	0.7	1.2	0.7	1.5	1.1	1.6
	43.7	45.0	50.3	0.5	1.3	1.0	1.4	1.6	2.1
	61.3	60.3	53.7	0.9	1.7	0.9	1.3	3.0	3.1

1 – First evaluation after 15 days; 2 – Second evaluation after 40 days

Figures 9 & 10: Dead adults of *Ips typographus* on the spruce log treated with insecticide Fastac Forst® (First evaluation) (Original)

All the samples taken from logs treated with *B. bassiana*, manifested occurrence of the pathogen in direct association with adults of *I. typographus* at frequencies higher than in samples taken from untreated logs. The percentage of *I. typographus* dead and alive individuals infected with entomopathogenic fungus *B. bassiana* on logs treated with Naturalis Biogard®, concentracion 2%, was 45.9%, and for Naturalis Biogard®, concentracion 3% - 39.3%, and it were significantly higher than in the case of control (4.0%). Logs treated with

insecticide Fastac®Forst, concentrations 2% and 3%, were without attack of *I. typographus* (Table 3).

Table 3. The results of the second part of the experiment (laboratory conditions).

Treatment	Block	Sample of bark (cm^2)	*Ips typographus*						
			Exit holes Average		Adults (N)				
			Σ	/dm^2	Dead	Alive	Σ	Infected with *B. bassiana*	
Naturalis Biogard® 2%	I	415.5	48.7	1.2	20	147	167	67 (40.1%)	350 (45.9%)
	II	425.5	33.7	0.8	27	184	211	87 (41.2%)	
	III	434.6	63.6	1.5	17	368	385	196 (50.9%)	
Naturalis Biogard® 3%	I	432.4	68.5	1.6	29	151	180	89 (49.4%)	349 (39.3%)
	II	438.8	90.0	2.0	38	324	362	145 (40.1%)	
	III	426.2	66.6	1.6	43	302	345	115 (33.3%)	
Fastac Forst® 2%	I	412.4	0.0	0.0	0	0	0	0	0
	II	432.1	0.4	0.0	0	0	0	0	
	III	441.2	0.3	0.0	0	0	0	0	
Fastac Forst® 2%	I	413.3	0.1	0.0	0	0	0	0	0
	II	442.2	0.0	0.0	0	0	0	0	
	III	441.9	0.0	0.0	0	0	0	0	
Distilled water	I	421.2	33.7	0.8	12	106	126	3 (2.4%)	16 (4.0%)
	II	404.6	38.3	0.95	3	140	143	5 (3.5%)	
	III	459.4	25.7	0.6	6	126	132	8 (6.1%)	

Fungal activity is strongly influenced by the biotic and abiotic environment. The unfavorable condition (temperature, relative air humidity, precipitation…) hindering the pathogen to develop as saprophyte on host cadavers. These aspects must be considered in evaluation of results. During the experiment, the mean daily air temperatures and the mean daily values of relative humidity were mostly found below the optimum values, which contributed to the reduction of the biological efficiency of insecticides Naturalis Biogard® (Fig. 7&8).

In the conditions of high humidity, the mortality strongly depends on the temperature and the *B. bassiana* dosage (Wegensteiner, 1992). Kreutz (2001) have applied biopreparation of *B. bassiana* and insecticidae Fastac®Forst on spruce logs just after finishing of their colonization by *Ips typographus* an achieved 100% mortality in the case of insecticide and 93% mortality in case of biopreparate. Vaupel and Zimmermann (1996) and Jakuš & Blaženec (2011) in the similary experiment achived the lower mortality. Their results are more similar to mortality achieved in our experiment. The timing of biopreparation application, before or after colonization of *Ips typographus* is probably the critical factor.

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CHARACTERISTICS AND INITIAL EFFECTS OF THINNING IN A POPLAR PLANTATION OF THE PANNONIA CLONE

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Abstract

Research was performed in a plantation of the poplar clone Pannonia on alluvial soil of the Danube River, with a planting distance of 4.25×4.25 m. At the age of 10 years a field experiment was established comprising two experimental plots with moderate and heavy thinning and one control plot in three replications within a randomized block design, each area of 0.1 ha. The mean height grew to 26.2 m, and breast height diameters from 22 to 22.2 cm. On the experimental plots were recorded from 522 to 542 trees per hectare, i.e. the survival amounted from 94.1 to 97.7%, with a basal area of 19.9-20.7 $m^2 \cdot ha^{-1}$. On the experimental plots with moderate thinning an average of 130 trees per hectare (24.0%) were felled in total with the basal area of 3.61 $m^2 \cdot ha^{-1}$ (17.4%), and on the experimental plot with heavy thinning an average of 247 trees per hectare (47.3%) were felled with the basal area of 8.21 $m^2 \cdot ha^{-1}$ (41.2%). The diameters of all trees were measured after 11 years to determine the initial effects of thinning. The trees showed higher diameter increment by 4-11% and basal area by 11-21% on the thinned experimental plots compared to the control plot, but the differences were not significant. The growth of the basal area per hectare was highest in the control plot, 1.66 $m^2 \cdot ha^{-1} \cdot year^{-1}$, while in the experimental plot with moderate thinning it was 1.51 $m^2 \cdot ha^{-1} \cdot year^{-1}$, and the differences were not significant. The increment of the basal area per hectare was lowest in the experimental plot with heavy thinning, 1.06 $m^2 \cdot ha^{-1} \cdot year^{-1}$, and the differences were significant in relation to the control plot.

Key words: *Clone Pannonia, alluvium of the Danube River, thinning, diameter increment, basal area increment*

Introduction

Poplars are fast-growing and light-demanding tree species whose growth significantly determines the density of plantations, i.e. the space for tree growth. This allows establishment of plantations with different densities, depending of the purpose, such as plantations for the production of logs, plantations for the production of cellulose wood and energy plantations (Marković *et al.*, 1997). In the practice today, the plantations for the production of technical wood are dominant, which is a consequence of the market demands for more valuable assortments.

Thinning in poplar plantations are not mandatory silvicultural measures and are implemented in case where the plantations are established in denser spacing with the aim that at a certain age the tree number, which carry the production and its value by the end of the production cycle, are decreased. They are mostly schematic and are performed by removing of every second rows (Marković *et al.*, 1994). However, recent research highlights the importance of a selective approach in the thinning, because in this way the collective of the remaining trees, which carry the production and value of the plantation until the end of the production cycle, is homogenized (Andrašev *et al.*, 2011; 2012; 2015).

Thinning, as very sensitive silvicultural measures in poplar plantations, are defined by the knowledge of the biological characteristics of tree growth and the maximum reaction of the

trees to thinning can be expected in the period when the trees are in the phase of intensive height increment (Marković *et al.*, 1994; Andrašev *et al.*, 2011; 2015).
Previous research, as well as experience gained in practice, suggests that in poplar plantations should be used several registered varieties (clones) with the permanent change of existing clones susceptible to pathogens with newly registered clones which should be established in polyclonal plantations or mixtures of several monoclonal plantations on smaller areas (Andrašev, 2008).
The objective of this study is to show characteristics of moderate and heavy thinnings and their initial effects after one year in a poplar plantation of the Pannonia clone having in mind that this clone has a higher representation and the tendency of expansion in practice in Serbia.

Materials and methods

The research was carried out in the experimental trial of the Pannonia clone (*Populus* × *euramericana* Dode Guinier), the working name M-1, established on the planting distance of 4.25×4.25 m. The trial is located in the area of Public company Vojvodinašume, Forest holding Sombor, Management Unit „Kamarište", department 5, section a (latitude 45°29', longitude 19°03'). At the age of 10 years an experimental trial in randomized block design with three repetition was established. Each block consists of two experimental and one control plot (CP) with an area of 0.10-0.12 ha separated from each other by a single row. On the plots, selective moderate (EP1) and heavy (EP2) thinnings were performed. All trees were numbered and their breast height diameter was measured. The heights of the trees were measured with the Vertex III hypsometer, as well as with tape on all felled trees in the thinning, on at least 10 trees in each diameters class of 5 cm wide, and used to construct a height curve. In order to examine the initial effects of performed thinnings on diameter and basal area increment all trees were measured in the diameter at the breast height at the age of 11 years. To reconstruct the height growth, the height (length) of each whorl of branches were measured with tape on three mean trees felled in the thinning, which correspond to heights of these trees in each previous years.
Data processing consisted of a standard procedure for determining the elements of growth of mean and dominant trees, where the height curve was previously constructed. The volume of trees was obtained using volume table by Pantić *et al.* (2009), while the basal area and volume per hectare were obtained as the sum of the basal areas and the volumes of the trees on the experimental plots and multiplying with the corresponding coefficient of the area of each plot. For the objective comparison of growth elements, the ANOVA and $LSD_{0.05}$ test were used.

Results and discussion

Trees of poplar clone Pannonia in the researched conditions after 10 years of development achieved uniform growth in height on all plots. The dominant heights are 27.2-27.3 m, and the mean heights are the same, 26.2 m. Similar to heights, the mean and dominant breast height diameters are uniform in the plots and range from 22.0-22.2 cm (d_g) to 25.1-25.5 cm (D_{100}). In the plantation were found 522-542 trees per hectare, i.e. the survival was 94.1-97.7%. The total basal area was 19.9-20.7 $m^2 \cdot ha^{-1}$, and the total volume was 225.2-233.7 $m^3 \cdot ha^{-1}$. The ANOVA test did not determine the significant variation of the elements of growth of trees and plantations between experimental plots at the age of 10 years (Table 1).
The high intensity of height increment continued to 7 years of planting age, after which height increment decreased, which is expected biological characteristics of poplar clones (Marković *et al*, 1994, Andrašev, 2008). The growth of trees in height was intense in the period from 3 to 5 year, and in the age of 6 years a significant decrease was caused by exogenous influence (Figure 1).

Table 1. Growth elements of trees and plantations before the thinning at the age of 10 years and results of ANOVA and $LSD_{0.05}$ test.

Experimental plot	h_L [m]	H_{100} [m]	d_g [cm]	D_{100} [cm]	N [trees·ha^{-1}]	G [m^2·ha^{-1}]	V [m^3·ha^{-1}]
CP	26.2 a*	27.2 a	22.2 a	25.1 a	525 a	20.28 a	229.41 a
EP1	26.2 a	27.3 a	22.0 a	25.5 a	542 a	20.70 a	233.68 a
EP2	26.2 a	27.2 a	22.0 a	25.1 a	522 a	19.94 a	225.20 a
F test	0.02	0	0.02	0	0.02	0.05	0.05
p value	0.8813	0.9804	0.9746	0.9744	0.8834	0.8274	0.8333

*Letters indicate different homogeneity groups according to the $LSD_{0.05}$ test.

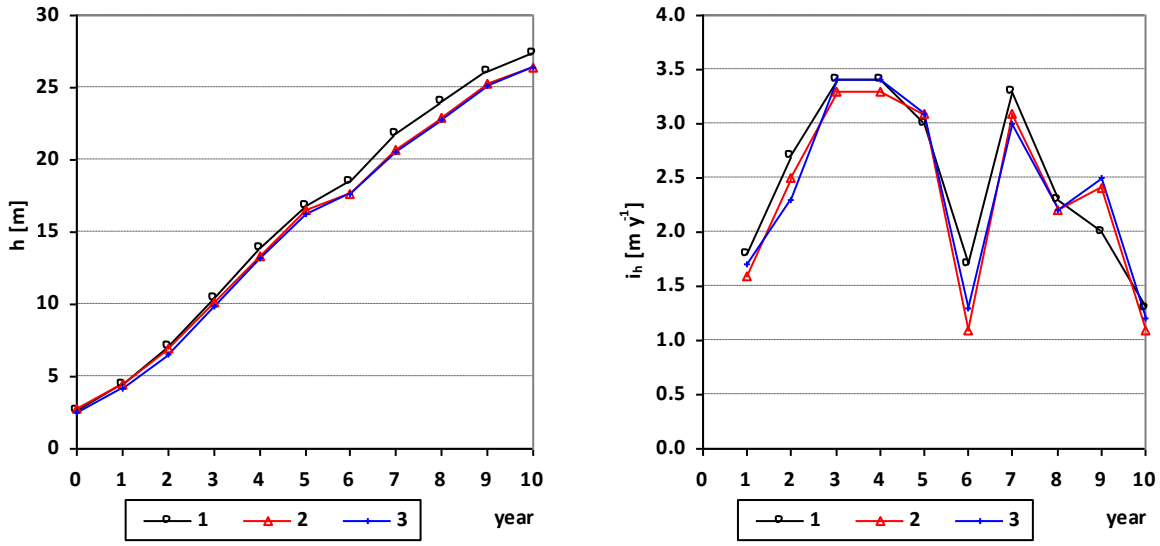

Figure 1. Height growth (left) and current height increment (right) of three mean trees.

The plantations had a good growth and the achieved mean height of 26.2 m at the age of 10 years is by 5.8 m higher than the same clones on a medium favorable habitat in the middle Danube region established at the spacing of 5×5 m (Andrašev, 2008). Also, the achieved mean heights of the Pannonia clone is higher by 1.9 m from the heights of clone I-214 at the same planting space on a favorable habitat (Pudar, 1982), and by 3.7 m on medium favorable, or by 6.3 m to less favorable habitat (Pudar, 1986). This suggests that the researched habitat belongs to the category of favorable habitats for poplar growth. However, the mean diameter of 22.1 cm at the age of 10 years is less than 0.5 cm from the mean diameter of the same clone at a medium favorable habitat in the middle Danube region established at the 5×5 m planting distance (Andrašev, 2008), as well as from clone I-214 at the same planting distance at a favorable habitat where is less 6.3 cm (Pudar, 1982) and on medium favorable where is less 1.4 cm, while they are 0.9 cm higher in a less favorable habitat (Pudar, 1986). The different dynamics of the height and diameter growth of the clone Pannonia is due to in part the different growth characteristics of poplar clones (Andrašev, 2008), but also the influence of density, i.e. the competition between trees in the zone of crowns. The decrease in current height increment after 7 years, and especially at the age of 10 years can be related to the impact of the density of the researched plantation. This indicates that the thinning was to be carried out at the age of 8-9 years, when the current height increment was still high and when the trees are expected to best respond to increase of growing space.

On the plots with moderate thinning (EP1) an average of 130 trees per hectare were felled, with total basal area of 3.6 m^2·ha^{-1} and total volume of 38.7 m^3·ha^{-1}, i.e. the harvest was 24%

by number of trees, 17,4% per basal area and 16.6% by volume. On the plots with heavy thinning (EP2) an average of 247 trees per hectare were felled, with total basal area of 8.2 $m^2 \cdot ha^{-1}$ and total volume of 90.9 $m^3 \cdot ha^{-1}$, i.e. the harvest was 47.3% by number of trees, 41.2% per basal area and 40.4% by volume. The mean diameter and the mean height of the allowable cut are smaller than the mean height and mean diameter of plantation before the thinning, which indicate that the thinnings on both experimental plots (EP1 and EP2) had the character of a low thinning. The ANOVA test did not confirm a significant difference between average diameters and heights of the allowable cuts on the plots, while the total number of trees harvested per hectare, as well as their total basal area and volume, are significantly higher on the EP2 plots (Table 2).

Table 2. Growth elements of felled trees, characteristics of allowable cut at the age of 10 years, and results of ANOVA test.

Exper.	h_L	d_g	N	G	V	Percentage of the initial state				
plot	[m]	[cm]	[trees·ha^{-1}]	[$m^2 \cdot ha^{-1}$]	[$m^3 \cdot ha^{-1}$]	h_L	d_g	N	G	V
EP1	24.5	18.8	130	3.61	38.73	93.5	85.5	24.0	17.4	16.6
EP2	25.4	20.5	247	8.21	90.88	96.9	93.2	47.3	41.2	40.4
F test	1.49	1.87	72.36	15.41	13					
p value	0.2893	0.2431	0.001	0.0172	0.0226					

The intensity of thinning at the age of 10 years in EP2 of 47% by number of trees is a procedure that is usually carried out in plantations with schematic thinning. The intensity of thinning by volume is lower (40%) and indicates that it has the character of a low thinning and differs from the typical schematic thinning. The achieved allowable cut on EP1 of 91 $m^3 \cdot ha^{-1}$ is smaller than allowable cut of the clone I-214 on a favorable habitat conducted after 6 (Marković *et al.*, 1994) and 8 years (Pudar, 1986) at the same planting distance, and higher than allowable cut of the clone I-214 on medium and less favorable habitat after 8 years (Pudar, 1986). In the researched plantation allowable cut is also higher than that obtained in the thinning of the clone I-214 with wide spacing (6×6 m) after 11 years (Andrašev *et al.*, 2012), as well as in the thinning after 8 years in the plantation of the clone B-229 established at a distance of 5×5 m on a medium favorable habitat (Andrašev *et al.*, 2011).

The implemented silvicultural treatment of moderate thinning is rarely applied in the practice. The obtained allowable cut of 39 $m^3 \cdot ha^{-1}$ on EP1 is twice lower than that obtained in the moderate thinning of the clone I-214 after 9 years established on a favorable habitat at a wide spacing of 6.5×5.63 m (Novaković, 1981).

After thinnings, which were performed on the plots EP1 and EP2, mean heights and mean diameters are slightly larger than these values befores the thinnings, while the dominant heights and the dominant diameters remained almost unchanged. The ANOVA test did not reveal significant differences between mean and dominant diameters and heights on plots. However, a significant difference was found in growth elements of plantations per hectare (N, G, V) (Table 3).

At the age of 11 years the mean diameters were the smallest on the control plots, on average 23.1 cm, they were somewhat larger on plots with moderate thinning (EP1), on average 24 cm, and the highest on plots with heavy thinning (EP2), on average 24.3 cm. The dominant diameters were less variable compared to the mean diameters. The ANOVA test did not confirm the significant difference between the mean and dominant diameters on the plots at the age of 11 years. The number of trees per hectare decreased on control plots, due to exogenous influence, and on plots it remained unchanged. The total basal area is the highest on the control plots, on average 21.9 $m^2 \cdot ha^{-1}$, on experimental plots it is significantly lower, and on average amounted 18.6 $m^2 \cdot ha^{-1}$ in EP1, and in EP2 amounted 12.8 $m^2 \cdot ha^{-1}$ (Table 4).

Table 3. Growth elements of trees and plantations after the thinning at the age of 10 years and results of ANOVA and $LSD_{0.05}$ test.

Experimen. plot	h_L [m]	H_{100} [m]	d_g [cm]	D_{100} [cm]	N [trees·ha^{-1}]	G [m^2·ha^{-1}]	V [m^3·ha^{-1}]
CP	26.2 a*	27.2 a	22.2 a	25.1 a	525 a	20.28 a	229.41 a
EP1	26.5 a	27.3 a	23.0 a	25.5 a	412 b	17.08 b	194.95 b
EP2	26.6 a	27.2 a	23.3 a	25.0 a	276 c	11.73 c	134.32 c
F test	1.83	0	1.77	0	335.79	57.8	43.16
p value	0.2183	0.99	0.2255	0.9735	0	0.0001	0.0003

*Letters indicate different homogeneity groups according to the $LSD_{0.05}$ test.

Table 4. Elements of growth and increments of trees and plantations at the age of 11 years and results of ANOVA and $LSD_{0.05}$ tests.

Exper. plot	d_g [cm]	D_{100} [cm]	N [trees·ha^{-1}]	G [m^2·ha^{-1}]	i_{dg} [cm·y^{-1}]	i_{D100} [cm·y^{-1}]	i_g [cm^2·y^{-1}]	i_{G100} [cm^2·y^{-1}]	I_G [m^2·ha^{-1}·y^{-1}]
CP	23.1 a*	26.3 a	522 a	21.89 a	0.93 a	1.20 a	31.85 a	48.72 a	1.61 a
EP1	24.0 a	26.8 a	412 b	18.59 b	0.97 a	1.33 a	35.48 a	54.84 a	1.51 ab
EP2	24.3 a	26.2 a	276 c	12.79 c	1.03 a	1.13 a	38.69 a	45.78 a	1.06 b
F test	1.85	0.01	335.32	48.95	0.48	0.13	2.42	0.11	6.55
p value	0.2159	0.9291	0	0.0002	0.5124	0.7254	0.1639	0.7463	0.0376

*Letters indicate different homogeneity groups according to the $LSD_{0.05}$ test.

At the age of 11 years the current diameter increment of mean trees was approximately 1 cm on all experimental plots, or from 0.93 cm (CP) to 1.03 cm (EP2). The differences are not statistically significant, nor are the differences in the increment of dominant trees (i_{D100}). Basal area increment of the mean trees (i_g) were uniform, from 31.8 cm^2·year^{-1} (CP) to 38.7 cm^2·year^{-1} (EP2), as well as the basal area increment of the dominant trees (i_{G100}). However, the basal area increment per hectare (I_G), on average 1.61 m^2·ha^{-1}·year^{-1}, is the highest on the control plots and significantly differs from the basal area increment on experimental plots with a heavy thinning, where is on average 1.06 m^2·ha^{-1}·year^{-1} (Table 4).

The annual effects of the thinnings expressed by a tree are positive in comparison with the control plot. Diameter increment of the mean tree is larger by 7-15%, and the basal area increment by 11-21%, indicating that the remaining trees reacted to the increased growing space. On the reaction of the remaining trees after the thinning clearly indicates the amount of basal area increment per hectare being higher by 51% on the control plot than on the plot with heavy thinning, while the number of trees on the control plot is higher by 89%.

The obtained results are in agreement with previously published results that poplar clones strongly respond to increased growing space already after 3 years (Andrašev *et al.*, 2015), and 5 years after the conducted thinning (Andrašev *et al.*, 2012), under conditions that the remaining trees were in the stage of intensive height and diameter increment.

Conclusions

In the plantation of poplar clone Pannonia, established on the planting distance of 4.25 × 4.25 m, at the age of 10 years the mean height was 26.2 m indicating favourable habitat conditions for the poplar growth. The mean diameter was 22.1 cm and is the result of the influence of tree competition that shows a clear influence on the current height increment of mean trees at the age of 8 years when from biological aspect was supposed to make a thinning in order to achieve the maximum effects.

Realized low thinnings with moderate and heavy intensity were achieved the allowable cut of 39 $m^3 \cdot ha^{-1}$ and 91 $m^3 \cdot ha^{-1}$ which are within the limits of the realized allowable cuts in poplar plantations of other clones.

The initial effects of thinning after a year are reflected in the intensification of the diameter and basal area increment of mean tree by 4-11%, ie by 11-21%, compared to the control plot, and the differences are not significant. However, the basal area increment per hectare of 1.61 $m^2 \cdot ha^{-1}$ is the highest on the control plot and is significantly different from the basal area increment on the plot with a heavy low thinning where it was 1.06 $m^2 \cdot ha^{-1}$.

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CHARACTERISTICS OF THE INCREMENTS OF SPRUCE TREES IN THE PERIOD FROM 32 TO 50 YEARS AFTER THE APPLICATION OF TWO HEAVY SELECTIVE THINNINGS

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Abstract

The paper presents the characteristics of the increment of tree growth elements (height, diameter, basal area and volume) in the monoculture of the spruce (*Picea abies* Karst.) in the period of 33-40 and 41-50 years, after the application of heavy selective thinnings. In both periods, the trees with larger dimensions, i.e., breast height diameter, had greater increments of growth elements compared to the smaller trees. By grouping trees in the group of 200 trees per hectare sorted by their diameters showed that after the first thinning the 600 thickest trees per hectare had uniform increments of diameter at breast height, basal area and volume being significantly larger than the next group of thinner trees. In the period after the second thinning, the groups of the 200 thickest trees per hectare had significantly higher increments of diameter, basal area and volume in relation to the next groups of 201 to 600 thickest trees that had a uniform and significantly greater increments from the following groups of the thinner trees. The results indicate that in the studied spruce plantation only 600 thickest trees per hectare singled out according to the size of the increment in the period after the first thinning. In the period after the second thinning, only the 200 thickest trees were singled out according to the size of the increment in diameter at breast height, basal area and volume, which indicates to intensive differentiation of trees according to the increment potential under the influence of heavy selective thinning.

Key words: *Picea abies* Karst., increment of growth elements, differentiation of trees, increment potential.

Introduction

In Serbia excluding Kosovo, Norway spruce cultures cover an area of 32,400 ha (Banković et al, 2009). A large number of these cultures typically had a spontaneous development in the first decades after the establishment. The first thinnings were usually carried out at the age when economically viable assortments were most likely to be obtained. Such a trend has also been a characteristic of other conifer cultures throughout Europe because late crown thinnings provide higher and more valuable cutting yields (Slodičak et al, 2005).

The results of long-term research of the effects of thinnings in spruce cultures generally indicate that the stands have a positive reaction to the thinnings, regardless of the age when commercial thinnings are performed (Mäkinen and Isomäki 2004a, 2004b). Based on the results of the research on the application of two heavy selective thinnings on a permanent experimental plot in Serbia, a positive reaction of the tree for tending, *i.e*. the future trees, was determined at the age from 32 to 50 years (Bobinac et al., 2017). However, under conditions of ice and snow breaks to which the researched stand was exposed, a permanent selection of trees for tending based on the approach introduced by Schedelin (1934) was applied, and the reaction of trees depending on their dimensions was not clearly defined.

The aim of the paper is to point out to the characteristics of the increment of the growth elements (height, diameter, basal area and volume) of trees under the influence of two heavy selective thinnings depending on their dimensions, *i.e.* their breast height diameters, and to

define groups or the number of trees in certain stages of development with a similar reaction of the increment of growth elements to thinnings.

Materials and methods

The research was conducted in a Norway spruce culture on Velika Brezovica of the Kučaj mountain range in northeastern Serbia (MU Bogovina I, compartment 87a) which was established by afforestation of pasture with dense planting (2×1 *m*), and according to the available data from the management records it had not been thinned before the age of 32 years. A permanent experimental plot was established in the culture in 1994 when it was at the age of 32 years and in which strong heavy selective thinning was carried out. The second thinning, which was also a heavy selective thinning, was carried out when the culture was 40 years old. In the first thinning 1,378 trees were cut per hectare (35.2%), with a volume of 115.1 $m^3 \cdot ha^{-1}$, while in the second thinning 911 trees per hectare (46.1%) were felled down with the volume of 142.3 $m^3 \cdot ha^{-1}$ (33.7%) (Bobinac *et al*, 2017). All trees on the experimental plot were numbered and at the age of 32, 40 and 50 years two cross diameters at breast height, with an accuracy of 1 mm, were measured. For the construction of the height curve, the heights of the trees are measured with a Vertex III type hypsometer.

The influence of the thinnings on the current (average periodic) height increment (i_h), diameter increment (i_d), basal area increment (i_g) and volume increment (i_v) was analyzed with an identical group of trees that grew after the thinning in the period from 33 to 40 years and in the period from 41 to 50 years.

Testing the differences between mean increments of growth elements among the observed two periods was done with a t-test. The ANOVA and least significant difference ($LSD_{0.05}$) tests were used for testing differences between the average size of the increment of growth elements of trees grouped by the size of their breast height diameters in groups of 200 trees per hectare.

Results and discussion

The mean value of the current height increment of 52 $cm \cdot year^{-1}$ was significantly higher in the period from 33 to 40 years, compared to 42 $cm \cdot year^{-1}$ in the period from 41 to 50 years. However, the mean value of diameter increment (5.1 $mm \cdot year^{-1}$), as well as basal area (18.98 $cm^2 \cdot year^{-1}$) and volume increment (24.12 $dm^3 \cdot year^{-1}$) per tree, was significantly higher in the period from 41 to 50 years in relation to the mean value of diameter (3.1 $mm \cdot year^{-1}$), as well as basal area (8.92 $cm^2 \cdot year^{-1}$) and the volume increments (11.23 $dm^3 \cdot year^{-1}$) per tree in the period from 33 to 40 years (Figure 1).

Increments of growth elements grouped by the size of their breast height diameters into groups of 200 trees per hectare significantly differ in all increments and in both researched periods (Table 1).

The mean values of current height increments by groups of 200 trees per hectare, sorted by its diameters, show that the thickest trees have the highest current height increments in both periods and that increments are decreased in the thinner trees. The height increments of 600 thickest trees per hectare are uniform in both periods, and there are no significant differences between them. The mean values of current diameter increments, as well as basal area and volume increments per tree, are the highest in the thickest trees and are reduced in the thinner trees in both researched periods after the implementation of a heavy thinning. However, in the period after the first thinning a group of 600 thickest trees per hectare had a uniform diameter increment of 4.9-5.2 $mm \cdot year^{-1}$, a basal area of 15.5-17.4 $cm^2 \cdot year^{-1}$ and a volume of 18.5-23.4 $dm^3 \cdot year^{-1}$, as opposed to the period after second thinning, in which a group of 200 thickest trees per hectare with a current diameter increment of 7.5 $mm \cdot year^{-1}$, a basal area of 33.5 $cm^2 \cdot year^{-1}$ and a volume of 44.3 $dm^3 \cdot year^{-1}$, are separated. A group of 201-600 trees per hectare, sorted by their diameters, has significantly less current increment compared to the

group of 200 thickest trees per hectare, but also significantly higher than the next groups of thinner trees (Figure 2).

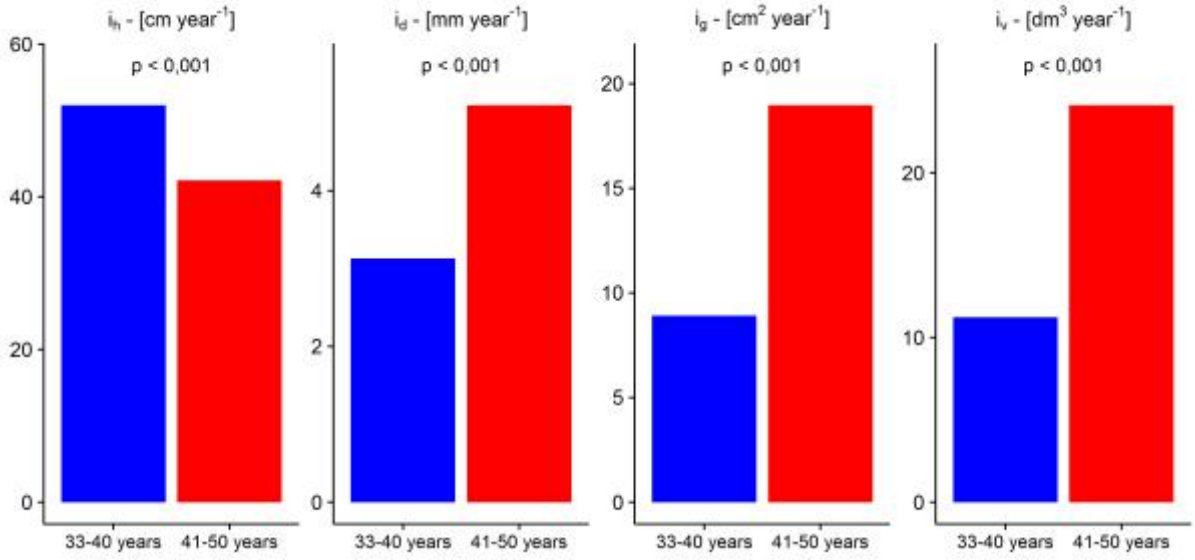

Figure 1. Average values of current increments of height, diameter, basal area and volume per tree in periods 33-40 and 41-50 years.

Table 1. Results of the ANOVA test (F coefficient) of the comparison of height, diameter, basal area and volume per tree of different breast height diameters by groups of 200 trees per hectare.

Period	Current increment of the growth elements			
	i_h	i_d	i_g	i_v
33-40 god.	595.68***	217.16***	294.58***	413.20***
41-50 god.	88.35***	108.30***	148.57***	164.22***

The thinning made in 32 years was the first intervention in the researched stand and was carried out in a period that does not significantly differ from the period when the first commercial thinnings in Europe were performed (Slodičak, Novak, 2003). Based on the characteristics of increments of the diameter and height of the dominant trees, the thinning is characterized as a delayed tending measure (Bobinac, 2004). However, the dominant trees, in particular 400 thickest trees per hectare, were in the phase of significant height increments at the time of the thinning, which conditioned the maintenance of high height increments in the period from 33 to 40 years. The mean height increment of 0.5 m·year^{-1} is in agreement with the growth characteristics of spruce trees at a similar age when the thinnings begin (Stojanović and Krstić, 1984, Orlić, 1991). However, the current height increment of the mean tree in the researched culture is higher compared to the height increment of the trees after the application of different thinnings on several series of experimental trials in the Czech Republic at a similar age (Slodičak, Novak, 2003). Makinen and Isomaki (2004a) state that the height increment does not depend on the intensity of the thinning and decreases with the age of trees, which is in line with our results.

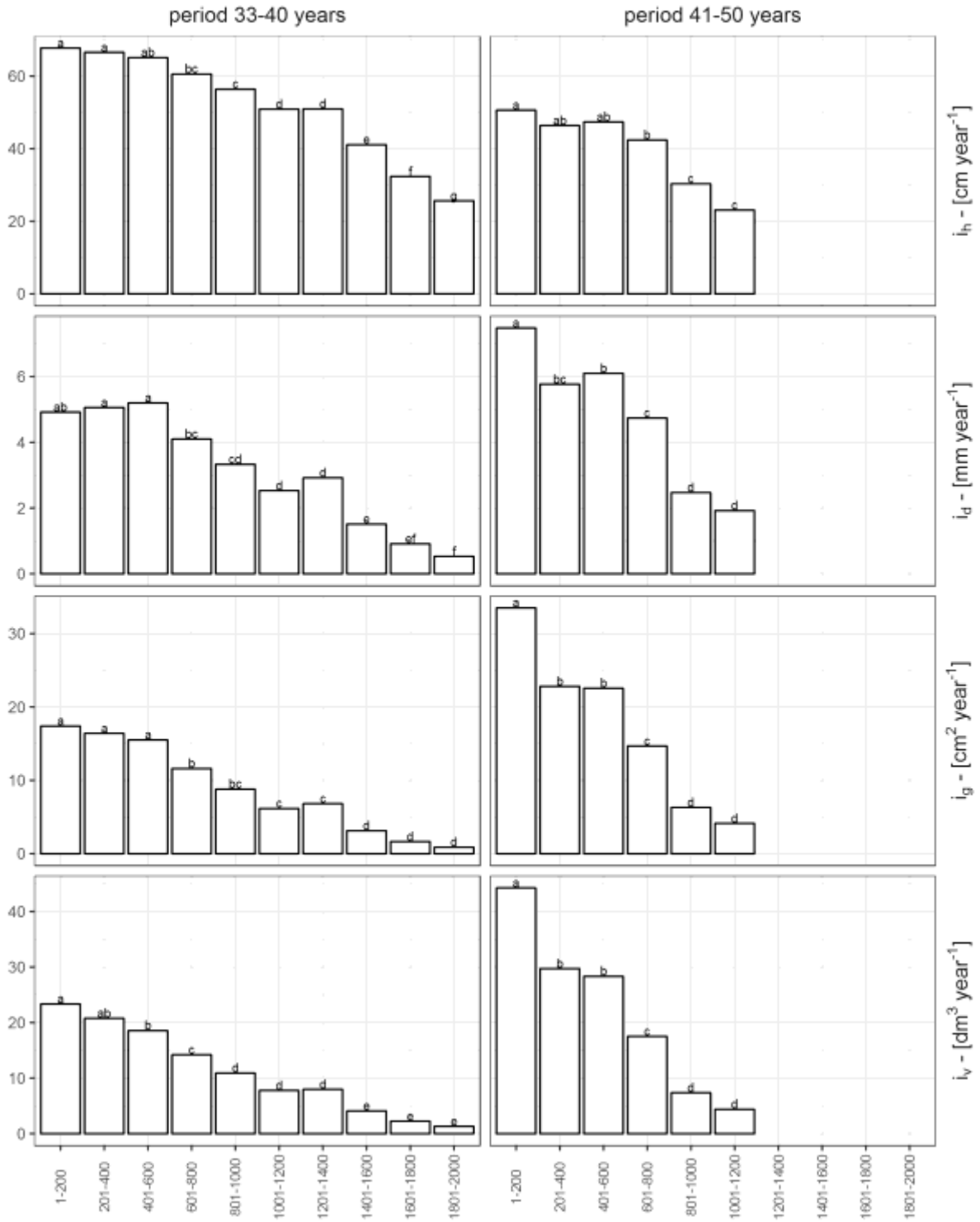

Figure 2. Current increments of height, diameter, basal area and volume of trees grouped according to the size of their breast height diameters in groups of 200 trees per hectare in the periods 33-40 and 41-50 of culture age.

During the period from 33 to 40 years, there was noted a weak reaction of diameter increment on the remaining trees, which is a consequence of a large number of trees, i.e., the absence of thinnings in the previous period. The current diameter increment of the remaining trees after thinning in the researched culture (0.31 cm·year^{-1}), compared with the results of Stojanović and Krstić (1984), is similar to the diameter increment on the control plot in two five-year

periods (0.34 cm·year^{-1}) with a 30% higher number of trees per hectare in the period from 32 to 42 years.
The second thinning at the age of 40 years caused a significantly higher diameter increment, i.e. a better reaction of diameter increment on the remaining trees compared to the thinning in the age of 32 years. The difference in the diameter increments between the two observed periods in the examined stand agrees with the results of other authors that spruce trees in the stands at favorable habitats and with a number of trees that are not too large for the appropriate age positively respond to thinnings and in the later period (Mäkinen and Isomäki, 2004a; 2004b; Preuhsler and Schmidt, 1989).
The increments of different elements of tree growth (height, diameter, basal area and volume) are reduced with decreasing breast height diameter of trees that are in line with previous research (Mäkinen & Isomäki, 2004b). This means that the effects of the thinnings are greatest in the trees of larger dimensions in the stand, that is, smaller trees are not able to react to the increasing space for growth as strongly as the larger trees can do.
The diameter increments of 200 and 400 of the thickest trees in the examined stand from 4.9 to 5.2 mm·year^{-1} in the period after the first thinning are somewhat higher (5-20 %) than the increment of the same group of trees in several series of experimental plots with a heavy crown thinnings in Sweden (Karlsson, 2006). Compared to the above results, *i.e.* diameter increments of the same collective of trees after the application of heavy low thinnings, the diameter increments at the experimental plot after the first thinning are smaller by 50% and are in the range with the obtained diameter increments at the control plots. Also, the diameter increments of 200 and 400 thickest trees per hectare in the period after the second thinning on the series of experimental plots where low thinnings were performed, as well as on the control plots, are smaller than in the period after the first thinning, and are larger only on the experimental plots with a heavy crown thinning, which is in line with our research.
Diameter increment of the 600 thickest trees per hectare in the examined culture in the period of 33-40 years was significantly smaller than the increment of the similar number of future trees per hectare when the first thinning is carried out at the age of 20 years (Štefančik, 2012). However, the diameter increment of 200 thickest trees per hectare of 7.5 mm·year^{-1} on our examined plot in the period after the second thinning is in agreement with the results stated by Štefančik (2012).
Basal area increment per tree of the 600 thickest trees in the stand after the thinning in the year of 32, from 15.5 to 17.4 cm^2·year^{-1}, is in the ranking with the increment of the basal area of the 300 thickest trees per hectare in the spruce stands in Finland in which heavy low thinnings were carried out in the early period of development, *i.e.*, they are in the ranks with increments of the 100-150 thickest trees when heavy low thinnings performed in the later period (Mäkinen and Isomäki, 2004b).

Conclusions

The results of the study show that the characteristics of increments of the growth elements (height, diameter, basal area and volume) of the spruce trees vary depending on the condition and intensity of the thinnings. In the period after the first thinning in the age of 32 years compared to the period after the second thinning in the age of 40 years, it is a significantly higher increment of height, which is a consequence of high height increments after culmination. In contrast, diameter increment, as well as basal area and volume increments, are significantly smaller, which is a consequence of a large number of trees and the absence of thinnings in the earlier period.
By grouping the trees into groups of 200 trees per hectare sorted by their breast height diameter after the first thinning was shown that the 600 thickest trees per hectare have a uniform reaction of diameter, basal area and volume increments, that is, those trees that had

the greatest increment potential. The second thinning allowed a more intense reaction of all the remaining trees, but the highest diameter, basal area and volume increment had only the 200 thickest trees per hectares. This indicates to intensive tree differentiation according to the increment potential under the influence of heavy selective thinning and that the increment potential of the tree is related to their dimensions.

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VARIABILITY OF MORPHOMETRIC TRAITS OF SEED AND SEEDLINGS OF DIFFERENT GENOTYPES OF PEDUNCULATE OAK (*Quercus robur* L.)

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Abstract

This paper presents the results of the morphometric analysis of seed and one-year-old seedlings of different genotypes of Pedunculate oak (*Quercus robur* L.). The seed was collected in the pure stand of Pedunculate oak with 10 mother trees which are minimum 50 meters away from each other. The length, width and weight of the acorn have been measured on the sample of 50 acorns per tree and based on the measured values of the length and the width the volume and the shape index of the acorn have been calculated. The collected seed was planted in the seedling nursery of the Institute of Forestry in Belgrade (Serbia) and the seedlings were produced in equable environmental conditions. At the end of the first vegetation season the root collar diameter and the height of the seedlings were measured on the random sample of 50 seedlings per mother tree, and on the basis of the measured values the ratio height : root collar diameter was calculated. The detailed data on morphometry and variability of several traits of seed and seedlings have been presented using the comparative analysis of 10 mother trees. On the basis of the obtained statistical parameters it can be concluded that the genetic variability exists both within and between analyzed mother trees. The analysis of variance for a single factor (One-Way ANOVA) was performed for the purpose of determination of existence of the statistically significant differences for all observed traits. The results of the analysis of variance showed that there are statistically significant differences between studied mother trees for the observed morphometric traits.
The obtained results contribute to acquiring knowledge on analyzed traits, give preliminary assessment of the genetic variability of the studied mother trees and represent a good start point for future researches of the species breeding; the results can also be used for the improvement of the production of good quality raw material of Pedunculate oak.

Keywords: *Pedunculate oak, seed, seedlings, morphometry, variability*.

Introduction

Pedunculate oak (*Quercus robur* L.) is one of the most valuable and most important tree species in the growing stock of the Republic of Serbia. The Pedunculate oak belongs to the genus *Quercus* and Fagaceae family (Nixon, 1993). It is a long-lived species, which can reach an age of more than 1000 years, reaching a height of over 40 m and a diameter at breast height of 2-2.5 m. It starts to produce fruit at age between 40 and 100 years. The seed quantity varies at the level of individual trees, populations and seed yield on harvest year (Ducousso and Bordacs, 2004). The Pedunculate oak is one of the most valuable species of forest trees due to its high quality wood. Specifically because of this characteristic, as well as the fact that it grows on very quality types of soil ideal for agricultural purposes, the oak forests have been significantly reduced in the last century (Yakovlev and Kleinschmidt, 2002; Thomas *et al.*, 2003; Balboa- Murias *et al.*, 2006; Kutnar, 2006; Helama *et al.*, 2009; Tikvić, 2011). It occurs in the moderate zone of the Northern Hemisphere, in almost all European countries, from northern Spain to southern Scandinavia and from Ireland to Eastern Europe. Environmentally, the species is adapted to both continental and forest-steppe climate as well as to mediterranean and submediterranean climate in the south of its distribution area. In relation to the soil, the Pedunculate oak is tolerant, but it prefers deep and fertile soil influenced by

groundwater, which is occasionally flooded. Large variability within species is a consequence of the wide distribution area of this species, as well as the occurrence of spontaneous hybridization with Sessile oak (*Q. petraea* L.), which greatly complicates its determination in the stand conditions (Ducousso and Bordacs, 2004). In Serbia, the largest areas under the Pedunculate oak forests are in the valleys of large rivers: Sava, Danube and Great Morava (Velika Morava).

An increasing number of researchers in the country and abroad study the quality and quantity of forest tree yield and the quality of seedlings. The degree of study of the genetic variability of the quantity and quality of seeds of different forest tree species and the possibility of their use in our conditions is still under real needs and it is not in accordance with the economic significance that this scientific field can provide (Popović *et al.,* 2015). The researches of the following authors: Tucović, (1975), Tucović and Stilinović, (1982), Lucic *et al.,* (2011), Popovic *et al.,* (2012, 2015), Nonic *et al.,* (2012) etc. contributed to gradual completion of knowledge related to the variability within and between populations of different forest tree species at the level of seed and juvenile stage of development.

The aim of the research in this paper is to determine the variability within population of Pedunculate oak in terms of morphometric traits of seed and one-year-old seedlings. The morphometry and variability of seed and seedlings are of a great importance for understanding the variability and taxonomy of species, production of seedlings and cultivation in stand conditions. The obtained results can serve for acquiring knowledge on the genetic variability of the studied population and for the improvement of the production of high quality seed and planting material of the Pedunculate oak in Serbia.

Material and Method

The seed intended for the examination of traits and variability of seeds and one-year-old seedlings of different genotypes of the Pedunculate oak (*Quercus robur* L.) was collected in a pure Pedunculate oak stand in the memorial park Šumarice in city of Kragujevac (Serbia). 10 mother trees which are at least 50 meters away from each other were selected for the test trees using individual selection. The seed was collected from each tree for the purpose of breeding half-sib lines where mother is known and the other parent is not, according to the method of the genetic analysis of trees (Isajev and Mančić, 2001). About 5 kg of ocularly healthy and undamaged acorns were collected as per tree, regardless of the acorn size. After collection, the acorn was dried to a moisture content of 35% and stored at a temperature of 3-5º C. The collected seed separated by mother trees was sown in April 2017 in the seedling nursery of the Institute of Forestry in Belgrade (Serbia) and the seedlings were produced in the equable environmental conditions in a hotbed size of 1x10 meters. The row spacing is 15 cm, the rows are parallel with the shorter side of the hotbed.

The length, width and weight of an acorn were measured on the sample of 50 acorns per test tree, and based on the measured values of the length and the width the volume and the shape index of the acorn were calculated. The length and the width of the acorns were measured with a vernier calliper with an accuracy of 0.01 mm and the acorn weight with an electronic scale with an accuracy of 0.01 g. The following traits were calculated based on the measured values: shape index, volume of acorn and average number of acorns per kilogram. The shape index of the acorn was calculated as a quotient of the acorn length and width. The volume of the acorn was calculated using formula for calculating volume of a cylinder (the shape of the acorn is approximated as a cylinder).

The root collar diameter and the seedling height were measured at the end of the first vegetation season on the random sample of 50 seedlings per mother tree, and based on measured values the ratio height : root collar diameter was calculated. The root collar

diameter was measured with the vernier calliper with an accuracy of 0.01 mm and the seedling height with a ruler with an accuracy of 0.1 cm.
The morphometric traits of the acorns and the one-year-old seedlings were described using descriptive statistical indicators: arithmetic mean (x), standard deviation (SD), coefficient of variability (CV %). The analysis of variance (ANOVA) was used for the purpose of determining variability within population. The analyzed variability factor was a tree. All statistical analyses were performed using the statistical software package STATISTICA 7.0 (StatSoft Inc. 2004).

Results and Discussion

The average value of the acorn width on its widest part at the level of the studied genotypes is 16.42 mm, with a range from 15.02 mm (test tree 9) to 17.72 mm (test tree 3) and the standard deviation from 0.78 mm to 1.25 mm (Table 1). Nikolić and Orlović, (2002) determined the average acorn width from 13.9 mm to 18.0 mm. Ivanković *et al.,* (2011) determined the average acorn width ranged from 14.1 mm to 17.5 mm. In the studies of Franjić *et al.,* (2001) the results of the average width from 13.0 mm to 17.8 mm were obtained. The average acorn width on the sample from the recognized and selected stands in Croatia ranged from 16.6 mm to 18.4 mm (Roth, 1999). The acorns originated from seed stands in Serbia have the average width of 17.4 mm (Popović *et al.,* 2016).
The average value of the acorn length at the level of the studied genotypes is 30.68 mm and ranged from 28.65 mm (test tree 5) to 33.20 mm (test tree 6) and the standard deviation from 1.21 mm to 3.22 mm (Table 1). The acorns of the Pedunculate oak are 20-40 mm long, rarely longer (Jovanović and Vukićević, 1983). The average acorn length on the sample from the seed orchard Banov Brod ranged 23.8 - 32.3 mm (Nikolić and Orlović, 2002). The average value of the acorn length of 1.2 - 3.6 cm can be found in the paper on importance of morphometric characteristics for identification of oak species (Smole *et al.,* 1992). The average length of the acorns originated from seed stands in Croatia ranged from 26.8 to 32.4 mm (Ivanković *et al.,* 2011). In the paper about variability of the Pedunculate oak acorn shape in Croatia the obtained average values of the acorn length were 26.2-31.9 mm (Franjić *et al.,* 2001). In the researches of the Pedunculate oak acorn morphology from different seed stands in Croatia, the measured average values of the acorn length ranged 30.5-37.0 mm (Roth, 1999). The average length of acorns from seed stands in Serbia ranged from 15.02 mm to 17.72 mm (Popović *et al.,* 2016).
The greatest average value of the acorn weight is 7.05 g (test tree 1), and the lowest is 4.38 g (test tree 6), while the average value at the level of the studied genotypes is 5.52 g with the standard deviation from 0.68 g to 1.19 g (Table 1). The average weight of an acorn in the research of Ivanković *et al.,* (2011) ranged from 3.89 g to 6.11 g. Nikolić and Orlović, (2002) indicated that in their research the average value of the acorn weight ranged from 2.8 g to 6.1 g. In the studies of Popović *et al.,* (2016) the determined average acorn weight ranged from 3.6 g to 7.5 g.
The shape index of the acorns ranged from 1.63 (test tree 10) to 2.14 (test tree 6) with the average value of 1.88 at the level of the studied genotypes and the standard deviation from 0.07 to 0.15 (Table 1). Krstinić, (1996) in his researches indicated that the shape index of the Pedunculate oak acorn is greater than 1.6. The researches of Ivanković *et al.,* (2011) showed that the shape index ranged from 1.74 to 2.12. The average values of the shape index from 1.68 to 2.35 were determined in the studies of Franjić *et al.,* (2001). Roth, (1999) indicated that in his researches the average values of the shape index ranged from 1.7 to 2.2. Popović *et al.,* (2016) determined the average shape index of 1.9.
The average value of the acorn volume at the level of seed stand was 6.56 cm^3 and ranged from 5.31 cm^3 (test tree 5) to 7.96 cm^3 (test tree 1) with the standard deviation from 0.78 cm^3

to 1.28 cm^3 (Table 1). The average value of the acorn volume in the studies of Ivanković *et al.*, (2011) ranged from 4.4 cm^3 to 7.8 cm^3, while in the studies of Popović *et al.*, (2016) the average value of the acorn volume ranged from 4.8 cm^3 to 9.7 cm^3.
The obtained results of the averages values of the morphometric traits of acorns that were determined in this paper are to a large extent similar to the mentioned literature data, which was expected because a similar work methodology was used.

Table 1: Descriptive statistics of the studied acorn traits

Tree	Wd (mm)			L (mm)			W (g)			SI			V (cm^3)		
	x	SD	CV	x	SD	CV	x	SD	CV	x	SD	CV	x	SD	CV
1	17.46	0.81	4.66	33.10	2.31	6.97	7.05	1.06	15.03	1.90	0.11	5.95	7.96	1.11	13.99
2	15.81	0.82	5.21	28.78	1.39	4.82	4.82	0.68	14.06	1.82	0.09	5.19	5.67	0.78	13.72
3	17.72	0.89	5.01	30.20	1.91	6.31	6.28	0.92	14.60	1.71	0.10	5.86	7.48	1.06	14.19
4	16.78	1.12	6.69	30.14	1.85	6.15	5.76	1.10	19.11	1.80	0.08	4.28	6.73	1.23	18.31
5	15.31	0.95	6.18	28.65	1.61	5.63	4.38	0.75	17.06	1.87	0.09	4.87	5.31	0.86	16.15
6	15.57	0.78	5.03	33.20	1.21	3.65	5.49	0.74	13.58	2.14	0.09	4.02	6.35	0.81	12.79
7	17.00	0.88	5.17	31.67	1.64	5.17	6.02	0.89	14.70	1.87	0.07	3.75	7.23	1.06	14.70
8	15.90	1.06	6.68	31.77	2.88	9.06	4.86	1.18	24.30	2.00	0.15	7.25	6.37	1.23	19.31
9	15.02	1.25	8.33	30.50	1.59	5.21	4.46	0.98	22.04	2.04	0.14	7.00	5.46	1.07	19.59
10	17.63	0.86	4.90	28.78	3.22	11.20	6.07	1.19	19.65	1.63	0.17	10.28	7.07	1.28	18.13
Average	16.42	0.94	5.79	30.68	1.96	6.42	5.52	0.95	17.41	1.88	0.11	5.85	6.56	1.05	16.09

Note: Wd-width; L-length; W-weight; SI-shape index; V-volume

Table 2 shows the results of the analysis of variance where the studied source of variability was the tree (family) within the population. Statistically significant differences caused by the observed source of variability were determined for all the studied morphometric traits of the acorn, and that confirmed the assumption of significant differentiation within population. High statistically significant differences between the studied genotypes indicate a high level of genetic diversity within population. The occurrence of a high degree of variability within population is characteristic for most species of forest trees and can be explained by the process of migration of genes (change of genes between different populations through natural processes, but also by human action) and low degree of local adaptation (Bogdan, 2009).

Table 2: The analysis of variance for the measured morphometric traits of acorns

	SS Effect	df Effect	MS Effect	F	p
Wd	277.82	9	30.87	33.89	0.0000
L	789.20	9	87.70	20.76	0.0000
W	205.74	9	22.86	24.55	0.0000
SI	6.20	9	0.69	53.94	0.0000
V	2.16E+08	9	2.40E+07	21.19	0.0000

The average value of the root collar diameter of the studied half-sib lines was 3.4 mm and ranged from 3.1 mm (half-sib line 5) to 3.7 mm (half-sib lines 3 and 10) with the standard deviation from 0.4 mm to 0.7 mm. According to the root collar diameter the half-sib line 3 is the most homogeneous (13.1%) and the most heterogeneous is the half-sib line 7 (21.9%). The average value of the seedling height at the level of the studied half-sib lines is 27.5 cm and ranged from 20 cm (half-sib line 6) to 33.8 cm (half-sib line 3) with the standard deviation from 4.4 to 8.4. According to the seedling height the half-sib line 10 is the most homogeneous (16.9%) and the most heterogeneous is the half-sib line 7 (27.8%). The average value of the seedling sturdiness coefficient is 8.1 and ranged from 6.1 (half-sib line 6) to 9.2 (half-sib line 3) with the standard deviation from 1.2 to 2. According to the seedling sturdiness coefficient the half-sib line 2 is the most homogeneous (15.3%) and the most heterogeneous is the half-sib line 1 (22.1%).

Table 3: Descriptive statistics for the measured morphometric traits of seedlings

Half-sib line	d (mm)			h (cm)			h/d		
	x	SD	CV	x	SD	CV	x	SD	CV
1	3.4	0.7	19.9	28.4	8.3	29.1	8.4	1.9	22.1
2	3.3	0.7	20.7	27.9	7.6	27.3	8.4	1.3	15.3
3	3.7	0.5	13.1	33.8	8.4	24.9	9.2	2.0	21.4
4	3.6	0.5	15.1	30.7	7.7	25.1	8.5	1.7	20.4
5	3.1	0.6	19.3	25.5	5.4	21.2	8.3	1.5	18.0
6	3.3	0.4	11.3	20.0	4.4	21.8	6.1	1.2	18.9
7	3.3	0.7	21.9	24.5	6.8	27.8	7.4	1.6	21.5
8	3.3	0.5	16.4	26.3	6.1	23.4	7.9	1.6	19.8
9	3.2	0.7	21.5	28.6	5.1	17.9	9.1	1.5	16.2
10	3.7	0.7	18.4	29.1	4.9	16.9	8.1	1.4	16.7
Average	3.4	0.6	18.5	27.5	7.4	27.0	8.1	1.8	21.6

Note: **d**- Root collar diameter; **h**- Seedling height; **h/d**- Ratio Height: Root collar diameter

Table 4: The analysis of variance for the measured morphometric traits of seedlings

	SS Effect	df Effect	MS Effect	F	p
d	9.59	9	1.07	2.88	0.0028
h	3756.20	9	417.40	9.49	0.0000
h/d	210.95	9	23.44	9.53	0.0000

In order to determine the existence of the statistically significant differences for all measured traits of seedlings, the analysis of variance for a single factor was performed (Table 4). The results of the analysis of variance showed the existence of the statistically significant differences between 10 half-sib lines for all measured morphometric traits of seedlings.

Conclusion

The obtained results contribute to the knowledge of the analyzed traits, the preliminary assessment of the genetic variability of the studied test trees and represent a good starting point for future researches in the breeding of the species.

The existence of the statistically significant differences between the studied test trees and the half-sib lines for all the analyzed morphometric traits of the seed and the seedlings clearly indicate a genetic differentiation and a high degree of variability within population. Based on the obtained statistical parameters, it can be concluded that there is a genetic variability both within and between the analyzed test trees and half-sib lines. The analyzed traits of the seed and the seedlings have a quantitative character and they are controlled by polymeric genes whose effects are summed up. The variability of the quantitative traits is wide and has a continuous character, and it is conditioned by the interaction of polymeric genes and environmental factors.

For preservation and directed use of the available gene pool of the studied Pedunculate oak test trees it is necessary to know their genetic variability. The determined variability of the morphometric traits of the seed and the seedlings can serve as an indicator of the further development of the seedlings of the selected half-sib lines, and can also serve for improvement the production of high-quality raw material of the Pedunculate oak.

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PERCEPTIONS ON CONSTRAINTS TO AGROFORESTRY COMPETITIVENESS: A CASE STUDY OF SMALL HOLDER FARMERS IN LIMPOPO PROVINCE, SOUTH AFRICA

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Abstract

Agroforestry is a land use system that includes the use of woody perennial, agricultural crops and animals in combination to achieve beneficial ecological and economical interactions for food, fiber and livestock production. However limited understanding, incorrect information and a negative mindset could hinder the competitiveness of this practice. This case study of smallholder farmers in Limpopo Province attempted to explain the farmers' constraints to agroforestry competiveness by analysing their perception on agroforestry. The survey was conducted in Limpopo Province by the Agricultural Research Council, University of Venda and Water Research Commission. The research is fully funded by the Water Research Commission. The aims of the survey were to document smallholder farmer's perception on constraints to agroforestry competiveness in Limpopo Province and to review the current knowledge on Agroforestry in the province. A total of 65 smallholder farmers participated in the study and were spread in districts as follows: Vhembe (40), Capricorn (21) and Mopani (4). Quantitative and qualitative designs were used as a questionnaire, stakeholder's discussion and field observations were part of the data collection. A purposive sampling technique was used to select 65 potential agroforestry farmers from the list provided by the Department of Agriculture, Forestry and Fisheries and Forestry South Africa Limpopo. Data was coded, captured, and analysed using SPSS. The results indicated the following as the most important constraints identified by farmers: cost of production (13.92%); labour (13.92%); distance to the market (10.7%); cost to the market (11.36%); financial institutions (12.81%); suppliers (11.8%); indirect support (12.04%), fiscal policy (9.8%) and labour policy (12.6%).The identified farmer's perceptions were in line with some of the researcher field observations. It is thus recommended that stakeholders should take note of the constraints identified by farmers in an attempt to increase agroforestry competitiveness.

Keywords: *Smallholder Farmers, Agroforestry, Perceptions, Limpopo Province and South Africa.*

Introduction

Results from several studies have indicated that agroforestry practices are perceived in different ways. According to (Lundgren and Raintree 1982) agroforestry is viewed as the set of land-use practices which involves the combination of trees, agricultural crops and/or animals on the same land management unit. Nair (1993) emphasized that although cultivating trees in combination with crops and livestock is considered an ancient practice, factors such as the deteriorating economic situation in many parts of the developing world, increased tropical deforestation; incorrect agricultural practices; degradation and scarcity of land because of population pressures; and growing interest in farming systems, intercropping and the environment have contributed to a rising interest in agroforestry since the 1970s. Based on the above mentioned factors (Mercer and Miller 1998) further acknowledged that most research

on agroforestry has been conducted from the biophysical perspective, but socio-economic aspects in relation to perception of farmers should be given much attention.

According to Combe (1982) classified agroforestry systems into three broad groups, namely agrosilvicultural (mixing trees and crops), silvopastoralism (mixing trees, pastures and animals) and agrosilvopastoralism (mixing trees with crops and animals). According to Rethman *et al.,* (2007) these groups can further be subdivided as either simultaneous (where trees and crops are grown simultaneously), or sequential (where trees and crops are grown separately, temporally, over a number of seasons, as with improved fallows). According to DAFF (2017) Agroforestry is a land use system that includes the use of woody perennial and agricultural crops and animals in combination to achieve beneficial ecological and economical interactions for food, fiber and livestock production. It is further emphasized that properly managed Agroforestry system provides multiple benefits and contribute to improved livelihoods and income generation (DAFF, 2017). It is against the above background that a comprehensive survey was conducted in Limpopo Province to identify farmer's perception on agroforestry competitiveness. The aims of the survey were to document smallholder farmer's perception on constraints to agroforestry competiveness and to review the current knowledge on Agroforestry in the Limpopo Province of South Africa.

Materials and Methods

All research done so far with partners is focused on achieving or working towards a participatory research approach since the researcher, collaborators, extension officers, farmers and funder were actively involved in all phases. According to Backeberg and Sanewe (2010) the method of participatory action research is most appropriate since people especially farmers benefit while the research is ongoing. The participatory action approach was also recommended by various researchers who emphasised that the participatory action approach is a good alternative to the traditional "transfer of technology" or" top - down approach" to agricultural research and extension. It is against this background that the approach was used to achieve the research aims, deliverables and phases. The research used quantitative and qualitative methods. A detailed questionnaire written in English was developed as a quantitative data collection method and data was collected from 04th to 15th September 2017 in Vhembe, Capricorn and Mopani Districts. The questionnaire used both open and closed ended questions. Qualitative data collection method included focus group discussions and field observations. The two methods were also used to identify the smallholder farmers and potential agroforestry systems in Limpopo Province as indicated in Figure 1. A purposive sampling technique was used to select 65 potential agroforestry farmers from the list provided by the Department of Agriculture, Forestry and Fisheries and Forestry South Africa Limpopo. As indicated in Figure 1, Vhembe district is dominant as a location for smallholder farmers with interest in agroforestry systems establishment. The same trend is observed at a local municipality level where Thulamela local municipality in Vhembe district had the most potential agroforestry systems followed by Molemole local municipality in Capricorn district. Data collected was analysed quantitatively using the Statistical Package for Social Sciences (IBM SPSS Statistics) Windows version. The top three farmers perceptions on agroforestry competitiveness were calculated as follows: (Agree x Total Average) + (Strongly Agree x Total Average).

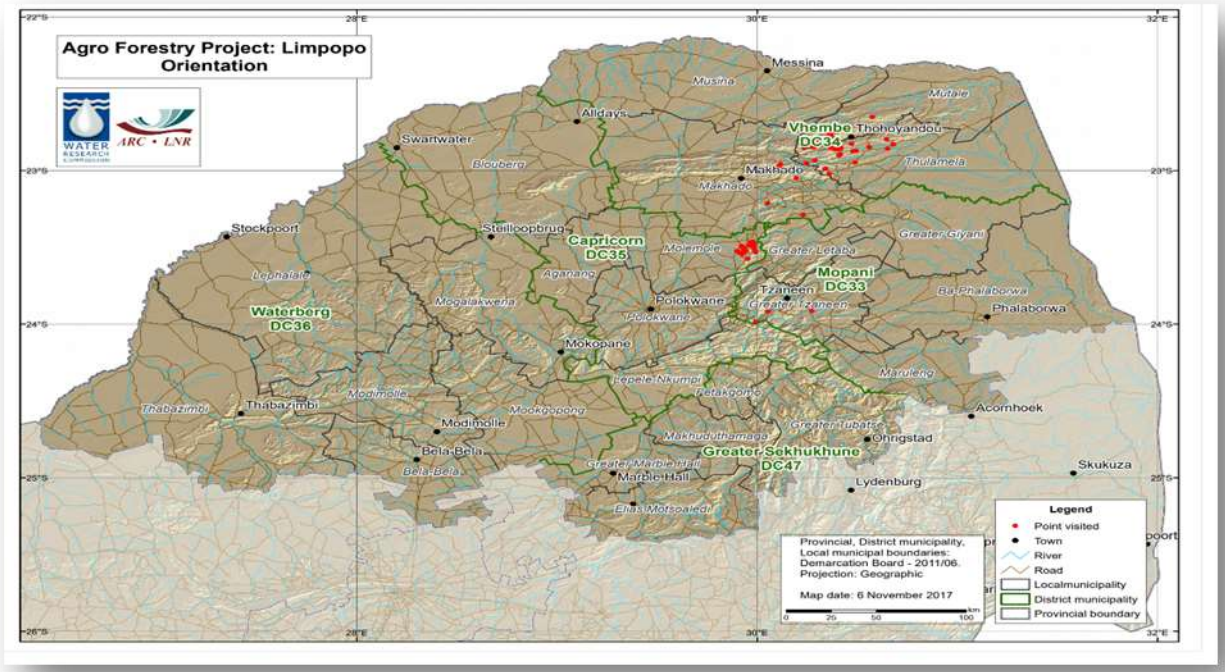

Figure 1: Limpopo Province Orientation (ARC ISCW, 2017)

Results and Discussions

The results from Figure 2 showed different ages of beneficiaries. As indicated in Figure 2, the average age of the beneficiaries was 52 years, which indicated the need for youth involvement in agroforestry business as any future agricultural development in the identified districts should be tailor made to attract youth. There's a need to formulate strategy in order to involve youth on agroforestry issues, various market incentives need to propose by all key role players within the agroforestry sector as a way of attracting young people.

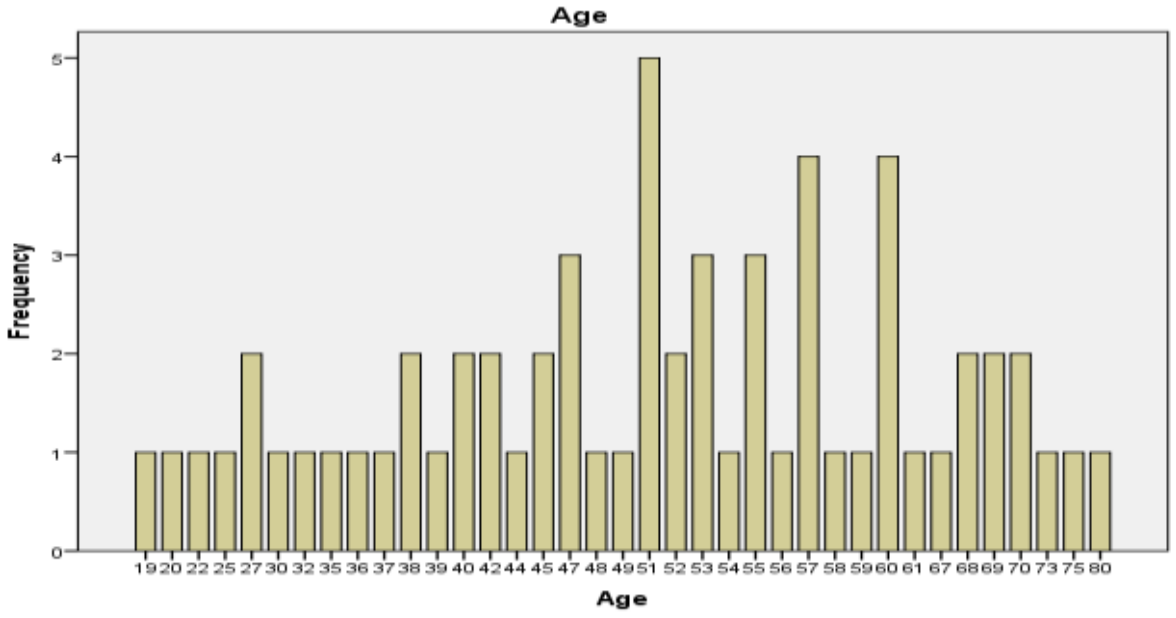

Figure 2: Age Categories of Farmers

The majority of farmers interviewed were men. According to Table 1, fifty one men were interviewed as compared to fourteen females. As shown in Table 1 there is a huge difference between the number of female and male farmers, which implied that any developmental strategy for the farmers in the areas will benefit more males than females. All farmers interviewed were black South African citizens. In terms of educational attainment (Table 1), 9 farmers had no education, 20 of the farmers completed primary education, 27 secondary education and 9 tertiary education. The educational levels of the farmers was generally good. Almost all farmers attended secondary and tertiary education level. These results indicated that educational level of the selected farmers is generally adequate to enable interpretation and understanding of basic farming activities. It is expected of farmers with tertiary education to interpret, understand different farming principles to make informed decisions on general farming operations and to be able to negotiable contracts better than beneficiaries with lower education levels. The lower educational levels among the farmers implied that written information might be of minimal benefit to such farmers and alternatives will have to be developed.

According to Maponya *et al.,* (2016) training and education plays an important role in smallholder farmer development. Failure to address some of the training needs has led to constrained agricultural growth in some districts in South Africa (Maponya *et al.,* 2014 and Maponya *et al.,* 2015). As indicated in Table 1; only 24 farmers received training and whereas the majority of farmers did not receive any training. The results showed farmers variation in terms of potential agroforestry experience acquired over time (Table 1). The majority of farmers (30) had more than ten years' experience while 21 and 14 farmers had less than five years and six to ten years' experience respectively. Results on land acquisition (Table 1) indicated that the majority of farmers got land through Permission to Occupy (PTO) (25) while others received land through the following: government (14), leased (11), renting (6) and inheritance (2). The role of traditional leaders must be applauded as the majority of farmers had PTO.

Table 1: Limpopo Province Farmers Demographics

Variables		**Respondents**	**% of Respondents**
Gender			
	Female	14	21.5
	Male	51	78.5
	Total	65	100
Level of Education			
	No Education	9	13.8
	Primary Education	20	30.8
	Secondary Education	27	41.5
	Tertiary Education	9	13.8
	Total	65	100
Training Skills Acquired			
	Yes	24	40
	No	41	60
	Total	65	100
Potential Agroforestry Experience			
	Less than Five Years	21	32
	Six to Ten Years	14	22
	More than Ten Years	30	46
	Total	65	100

Land Acquisition			
	Bought	9	14
	Leased	11	17
	Inherited	2	3
	Government	14	22
	Permission to Occupy	23	35
	Renting	6	9
Total		**65**	**100**

Perceptions on constraints to competitiveness to Agroforestry

Table 2 indicates that an average of 42% farmers strongly agreed/agreed that production factors were causing a decrease in agroforestry competitiveness. Among factors of production: cost of production (13.92%), labour (13.92%) and availability of skilled labour (13.32%) were perceived as the most important factors causing decrease in agroforestry competiveness. However, 36% of farmers perceived production factors as not causing any decrease in agroforestry competiveness and 22% of farmers were not sure.

Table 2: Production factors causing decrease in agroforestry competitiveness

Views The following production factors are causing the decrease in competitiveness of Agroforestry	**Responses (65)**				
	Strongly disagree	Disagree	Not sure	Agree	Strongly agree
Cost of production	17%	15%	15%	11%	42%
Labour	11%	22%	15%	11%	42%
Cost of unskilled labour	20%	14%	23%	9%	34%
Quality of unskilled labour	9%	29%	23%	11%	29%
Availability of unskilled labour	6%	23%	31%	14%	26%
Cost of skilled labour	9%	20%	20%	14%	37%
Availability of skilled labour	5%	31%	14%	11%	40%
Administration cost associated with labour matters	11%	25%	23%	8%	34%
Insufficient source of water	11%	29%	23%	9%	28%
Infrastructure	20%	23%	19%	8%	31%
Lack of knowledge	6%	29%	23%	15%	26%
Lack of Technology	11%	26%	31%	9%	23%
Capital / Finance	14%	20%	22%	19%	26%
Average Total	**12%**	**24%**	**22%**	**12%**	**30%**

Table 3 indicates that an average of 40% farmers strongly agreed/agreed that demand factors were causing a decrease in agroforestry competitiveness. Among demand factors: distance to market (10.7%), cost to the market (11.36%) and quality of products (9.19%) were perceived as the demand factors causing decrease in agroforestry competiveness. However 35% of farmers perceived demand factors as not causing any decrease in agroforestry competiveness and 25% of farmers were not sure. It was highlighted several times that market access, distance from the agroforestry site to market and quality of the products were cited as some of the key factors hindering the development of the agroforestry sector across the country (DAFF, 2017).

Table 3: Demand factors causing decrease in agroforestry competiveness

Views The following demand conditions are causing the decrease in competitiveness of Agroforestry	**Responses (65)**				
	Strongly disagree	Disagree	Not sure	Agree	Strongly agree
Distance to market	12%	25%	23%	5%	35%
Market information	22%	17%	23%	9%	29%
Cost to the market	8%	20%	26%	11%	35%
Quality of products	9%	22%	28%	15%	26%
Market for Agroforestry	12%	25%	25%	14%	19%
Average Total	**13%**	**22%**	**25%**	**11%**	**29%**

Table 4 indicates that an average of 45% farmers strongly agreed/agreed that related and supporting industries were causing a decrease in agroforestry competitiveness. Among related and supporting industries: financial institutions (12.81%), research institutions (11.28%) and suppliers (11.8%) were perceived as related and supporting industries causing decrease in agroforestry competiveness. However 35% of farmers perceived related and supporting industries as not causing any decrease in agroforestry competiveness and 20% of farmers were not sure.

Table 4: Related and supporting industries causing decrease in agroforestry competitiveness

Views The following Related and supporting industries are causing the decrease in competitiveness of Agroforestry	**Responses (65)**				
	Strongly disagree	Disagree	Not sure	Agree	Strongly agree
Financial institutions	9%	25%	17%	14%	35%
Research institutions	17%	15%	19%	23%	26%
Suppliers	12%	22%	23%	9%	34%
Electricity suppliers	17%	23%	23%	9%	28%
Average Total	**14%**	**21%**	**20%**	**14%**	**31%**

Table 5 indicates that an average of 45% farmers strongly agreed/agreed that government support were causing a decrease in agroforestry competitiveness. Among government support: labour policy (12.6%), fiscal policy (9.9%) and indirect support (12.04%) were perceived as government support causing decrease in agroforestry competiveness. However 36% of farmers perceived government support as not causing any decrease in agroforestry competiveness and 19% of farmers were not sure.

Table 5: Government support causing decrease in agroforestry competitiveness

Views The following production factors are causing the decrease in competitiveness of Agroforestry	**Responses**				
	Strongly disagree	Disagree	Not sure	Agree	Strongly agree
Poor interaction and support between Government	17%	25%	17%	9%	32%
Indirect support	23%	14%	17%	11%	35%
Trade policy	9%	26%	19%	12%	34%
Land reform policy	9%	28%	14%	20%	29%

Labour policy	9%	22%	17%	22%	31%
Fiscal policy	2%	28%	31%	14%	26%
Average Total	**12%**	**24%**	**19%**	**15%**	**30%**

Conclusions

This research evaluated selected potential agroforestry farmers' perceptions on constraints to agroforestry competitiveness in the Limpopo Province. Based on the findings of the research it can be concluded that most of the farmers in the study area agreed that production factors, demand conditions, related and supporting industries and government support were causing decrease in agroforestry competitiveness. The most important constraints identified by farmers include: (a) cost of production; labour; (b) distance to the market; (c) cost to the market; (d) financial institutions; suppliers; (e) indirect support; (f) labour policy and (g) fiscal policy. The identified farmer's perceptions were in line with some of the researcher field observations. It is thus recommended that stakeholders should take note of the constraints identified by farmers in an attempt to increase agroforestry competitiveness.

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RARE MACROMYCETES OF THE REPUBLIC OF ABKHAZIA (RUSSIA)

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Abstract

A great relevance of the problem of studying and conservation of biological diversity of the Earth is reflected in International Convention on Biological Diversity signed at the United Nations (UN) conference in Rio de Janeiro in 1992 by representatives of 180 countries. An important direction of solving this problem is monographic study of taxa of living organisms, the identification of globally and regionally endangered and vulnerable species and their inclusion in the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species, national and regional Red Books. Fungi as a heterotrophic component of ecosystems present an important component of global biota. In nature, the fungi decompose plant debris and are included into the humification processes. The purpose of the present paper is a survey of macromycetes in forest ecosystems of specially protected natural areas of Abkhazia. The research was carried out in broad-leaved forests, pine forests, fir-beech forests and sub-alpine birch forest, the height interval was 5 to 1980 m above sea level. In the examined forests, 237 species of fungi belonging to 139 genera, 53 families, 21 orders and 6 classes were identified. The task of the present work includes an identification of rare species of fungi, with the aim to organize further protection in conjunction with their habitat. In our studies, 62 species fall under Rare category. The list of rare species recommended for inclusion in the Red Book of the Republic of Abkhazia when preparing its publication is given in this paper. The list includes such species as *Amanita caesarea, Clathrus ruber, Pseudocolus fusiformis, Geastrum triplex, Geastrum fimbriatum, Cortinarius violaceus*.

Keywords: *Rare macromycetes, Republic of Abkhazia, Forest ecosystems, Red Book.*

Introduction

The biodiversity conservation belongs to one of basic directions of environmental policy on global and regional scale. This is also an important task of the World Conservation Strategy adopted by International Conference in Rio de Janeiro in 1992. A sufficient aspect of this activity is study of regularities in development and distribution of various taxa of living world, whose representatives play an important role in the processes of recycling in nature ecosystems. A biodiversity conservation practice includes a foundation of networks of nature reserves and specially protected natural areas.

The Republic of Abkhazia is located in South-Western part of the Greater Caucasus and situated between 43°35'–42°27'N and 40–42°08' E. The total area of the republic consist 8665 km^2. Due to its unique geographical position on the border of temperate and subtropical zones and rather diverse relief varying from 0 to 4000 m above sea level, this area is characterized by local climates echoes all the zonal ones of the Northern Hemisphere, excluding tropical. Being a part of Colchis floristic refugium, the Abkhazia Republic is rich by various types of vegetation and a relic and endemic plants (Adzinba, 2011). On the Abkhazia territory there are three large areas having status of specially protected natural areas – the Pitsunda-Musser Scout Reserve, the Pskhu-Gumista Reserve, and the Ritza Relic National Park (RRNP).

Material and Methods

The main subject of the present study is macromycetes. This is a non-formal group of higher fungi that develop a huge mycelium biomass in the substrate and form macroscopic fruiting bodies as a rule more than 1 mm large. In nature ecosystems, the macromycetes are the main regulator of recycling processes (Burova, 1986). These fungi regulate a wide range of important ecosystem functions as primary and secondary production of ecosystems, regeneration of biogenic elements, wood humification and related aspects of forest environment formation (Bondartseva, 2001; Mukhin et al., 2000). The macromycetes is an integral component of forest ecosystems playing a key role in enzymatic degradation of the wood and herbaceous plants with formation of forest litter and underlying soil strata on the one hand and involved to mineral nutrition of forest plants through mycorrhizas on the other (Burova, 1986). The studied forest ecosystems in the Musser Scout Reserve and RRNP occupy heights varying between 5–134 and 376–1817 m, being presented by pine forests, deciduous mixed forests, hornbeam-box forests, chestnut-hornbeam forests, fir (or beech-fir) forests, and birch woodland. Radial routes and stationary search methods were used by us. Stationary plots measure 25 × 25 (625 m^2) and 50 × 50 (2500 m^2). A fungal diversity and ecology monitoring was carried out during all the vegetation season (Khacheva, Dbar, 2013; Khacheva, 2015; Khacheva, 2018; Yupina et al., 2011).

The material collected was identified using basic key-books for some groups of macromycetes (Perevedentseva, 2015; Eriksson, Ryvarden, 1973, 1975, 1976; Eriksson et al., 1978, 1981, 1984; Bondartseva, Parmasto, 1986; Kõljalg, 1996; Hansen, Knudsen, 1997; Bondartseva, 1998; Zmitrovich, 2008). The taxonomy on generic level corresponds to 10th edition of the Ainsworth and Bisby's Dictionary of the Fungi (Kirk et al., 2008). The species names are giving according to the "Index Fungorum" nomenclatural database (http://www.indexfungorum.org).

Results and Discussion

A total of 237 species of macromycetes belonging to 139 genera, 53 families, 21 orders, and 6 classes were revealed in the forests under study. It was established that the group of highly adaptive and widely distributed species have a dominant position in studied forest communities. Also, were found sporadically distributed species, represented by single finds or found constantly, but in a limited specimens number (Mukhin, 1993).

In our studies, a total of 62 macromycetes species seem to be having a rare status. In establishing a rare status, such attributes adopted to conservation status scale of Red Book of the Russian Federation are taken into account as geographical distribution, habitat specificity, sizes of local population. Species can have a local, regional, national and international rarity status (IUCN Red List Categories and Criteria, Version 3.1, 2001).

When analyzing a rare species of the Musser Scout Reserve and RRNP, the distribution of species throughout whole area, the rarity of species in studying area, and the inclusion of species to international, national and regional Red Data Books were taken into account. The list of rare species of macromycetes of the protected natural areas of the Republic of Abkhazia is given in Table 1.

Table 1. Rare species of macromycetes of the protected natural areas of the Republic of Abkhazia.

Species	Conservation status (category)	
	Abkhazia	IUCN or regional status
Amanita caesarea (Scop.) Pers.	3	EN (IUCN)
Amyloporia crassa (P. Karst.) Bondartsev et Singer	2	VU
Amylocorticium subincarnatum (Peck) Pouzar	3	EN
Antrodia malicola (Berk. et M. A. Curtis) Donk	3	
A. gossypium (Speg.) Ryvarden	2	CR (IUCN)
A. romellii (Donk) Niemelä	3	
Antrodiella fissiliformis (Pilát) Gilb. et Ryvarden	2	
Ceriporia excelsa S. Lundell ex Parmasto	3	NT
C. purpurea (Fr.) Donk	3	
C. tarda (Berk.) Ginns	3	
C. viridans (Berk. et Broome) Donk	3	
Ceriporiopsis mucida (Pers.) Gilb. et Ryvarden	3	
Clathrus ruber P. Micheli ex Pers.	3	NT (IUCN)
Climacodon pulcherrimus (Berk. et M. A. Curtis) Nikol.	3	
Cortinarius violaceus (L.) Gray	3	
Crustoderma dryinum (Berk. et M. A. Curtis) Parmasto	3	VU
Crustomyces expallens (Bres.) Hjortstam	3	
C. subabruptus (Bourdot et Galzin) Jülich	3	
Cystostereum murrayi (Berk. et M. A. Curtis) Pouzar	3	
Elmerina caryae (Schwein.) D. A. Reid	2	
Emmia latemarginata (Durieu et Mont.) Zmitr., Spirin et Malysheva	3	
Erastia salmonicolor (Berk. et M. A. Curtis) Niemelä et Kinnunen	3	CR (IUCN)
Fibroporia vaillantii (DC.) Parmasto	4	
Geastrum fimbriatum Fr.	4	
G. triplex Jungh.	4	
Gloeophyllum protractum (Fr.) Imazeki	3	
G. trabeum (Pers.) Murrill	3	
Gloiothele citrina (Pers.) Ginns et G. W. Freeman	4	
Hapalopilus ochraceolateritius (Bondartsev) Bondartsev et Singer	3	
Hericium alpestre Pers.	2	
H. cirrhatum (Pers.) Nikol.	3	
H. coralloides (Scop.) Pers.	3	NT (IUCN)
Hymenochaete cruenta (Pers.) Donk	2	VU (IUCN)
Inonotus dryadeus (Pers.) Murrill	2	CR
Irpex litschaueri (Bourdot et Galzin) Kotir. et Saaren.	3	NT (IUCN)
Loweomyces wynneae (Berk. et Broome) Jülich	3	EN

Oxyporus obducens (Pers.) Donk	3	
Perenniporia subacida (Peck) Donk	3	VU
Phellinus lundellii Niemelä	3	
Ph. viticola (Schwein.) Donk	2	
Pycnoporellus alboluteus (Ellis et Everh.) Kotl. et Pouzar	2	EN
Phlebia centrifuga P. Karst.	3	VU
Phylloporia ribis (Schumach.) Ryvarden	2	NT
Physisporinus vitreus (Pers.) P. Karst.	3	
Polyporus tuberaster (Jacq. ex Pers.) Fr.	3	NT
Porodaedalea chrysoloma (Fr.) Fiasson et Niemelä	2	
Postia floriformis (Quél.) Jülich	3	NT
P. hibernica (Berk. et Broome) Jülich	2	
P. rennyi (Berk. et Broome) Rajchenb.	2	
P. ptychogaster (F. Ludw.) Vesterh.	3	
Pseudocolus fusiformis (E. Fisch.) Lloyd	3	
Pseudohydnum gelatinosum (Scop.) P. Karst.	3	
Pseudomerulius aureus (Fr.) Jülich	3	DD (IUCN)
Radulodon licentii (Pilát) Ryvarden	2	
Rigidoporus crocatus (Pat.) Ryvarden	3	EN
R. sanguinolentus (Alb. et Schwein.) Donk	3	
Skeletocutis alutacea (J. Lowe) Jean Keller	2	DD (IUCN)
S. carneogrisea A. David	3	
Sparassis crispa (Wulfen) Fr.	3	
S. laminosa Fr.	2	
Trametopsis cervina (Schwein.) Tomšovský	3	
Tyromyces kmetii (Bres.) Bondartsev et Singer	3	NT

Note. Conservation status according to the Red Data Book of the Republic of Abkhazia: 2 – endangered, 3 – rare, 4 – having indefinite status. Conservation status according to IUCN: CR – critically endangered, EN – endangered, VU – vulnerable, NT – near threatened, DD – data deficient.

Conclusion

The data obtained show a necessity for further comprehensive study of macromycetes and protection of both individual species and entire natural complexes of the Republic of Abkhazia. The macromycetes biota of area under study is characterized by a high level of biological diversity and the presence of species confined to specific habitats. The old-growth beech and fir forests, where a significant number of rare species (69.4%) was identified, present a great conservation value. To date, the transformation of natural complexes is intensifying, since an irrational nature management leads to reduction in species diversity of native mycobiota and complexity of ecosystem structure. The main threatening factors for the macromycetes of Abkhazia are the recreational load on biotopes, the intensive exploitation of forest ecosystems and the uncontrolled forest cutting. The presented list of species is recommended for inclusion in the Red Data Book of Abkhazia. This list reflects the rarity categories, the basis for special protection measures, and the population state due to their vulnerability. Also, it is necessary to compile a complete list of rare species on the basis of expanding studies on the Abkhazia territory, since the presented list is limited to protected areas of the republic, only.

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