IX INTERNATIONAL SYMPOSIUM ON AGRICULTURAL SCIENCIES

24th September 2020
Banja Luka
Bosnia and Herzegovina

PROCEEDINGS

AGRORES 2020

IX International Symposium on Agricultural Sciences "AgroReS 2020"

24 September, 2020

Banja Luka, Bosnia and Herzegovina

Book of proceedings

Publisher

University of Banja Luka
Faculty of Agriculture
University City
Bulevar vojvode Petra Bojovića 1A
78000 Banja Luka, The Republic of Srpska, B&H

Editor in Chief

Željko Vaško

Technical Editor

Biljana Rogić

Language

English

Circulation

online on the website https://agrores.net/en/proceedings/

CIP - Каталогизација у публикацији

Народна и универзитетска библиотека

Републике Српске, Бања Лука

631(082)(0.034.2)

INTERNATIONAL Symposium on Agricultural Sciences (9 ; Banja Luka ; 2020)

Proceedings [Elektronski izvor] / IX International Symposium on Agricultural Sciences "AgroReS 2020", 24 September, 2020 Banja Luka, Bosnia and Herzegovina ; [organizer University of Banjaluka, Faculty of Agriculture ; editor in chief Željko Vaško]. - Onlajn izd. - El. zbornik. - Banja Luka : Faculty of Agriculture = Poljoprivredni fakultet, 2020. - Ilustr.

Sistemski zahtjevi: Nisu navedeni. - Način pristupa (URL): https://agrores.net/zbornici/. - El. publikacija u PDF formatu opsega 229 str. - Nasl. sa naslovnog ekrana. - Opis izvora dana 24.9.2020. - Bibliografija uz svaki rad.

ISBN 978-99938-93-65-3

COBISS.RS-ID 129502465

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PREFACE

This Proceedings contains 24 papers presented at IX International Symposium on Agricultural Sciences "AgroReS 2020". According to the original plan, the symposium was supposed to be held in May in Trebinje, but due to the pandemic caused by the corona virus, it was postponed for September and finally held online from Banja Luka on 24th September 2020.

The Proceedings includes those papers for which their authors, participants in the AgroReS 2020 symposium, decided to publish them in this way. All papers were the subject of double-blind peer review by two reviewers who determined the category of papers, which is indicated at the top of each paper.

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Original scientific paper

Common wild oat (*Avena fatua* L.) spikelet and seed morphology variation in Bosnia and Herzegovina

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Abstract

Intraspecific variation in spikelet and seed morphology was assessed for eleven populations of common wild oat (*Avena fatua* L.). Spikelet lengths varied between 1.13 and 1.81 cm, with highly significant differences being recorded between most analyzed populations. Lemma color varied between ocher, light-brown, brown and black, with the majority of analyzed populations (63.64 %) having predominately (≥50%) brown lemma color. Percent of lemma hairiness was also assessed and yielded some highly significant differences between the populations. Length of the awn varied between 2.34 and 4.74 cm, with differences in mean values of awn length per population being statistically significant between several analyzed populations. The angle between the awn and the dorsal surface of lemma was also studied and varied between 85.76° and 140.71°. Analysis of grain length has shown highly significant differences only between the populations from Bačevci (BIH02) and Aleksandrovac (BIH03), when compared with other nine analyzed wild oat populations, while contrary to this, grain mass was shown to be a highly significant factor when comparing wild oat populations. Consequently, it can be theorized that the wide range of variation documented for certain seed morphology traits results from a high local differentiation potential of wild oat populations.

Key words: genus *Avena*, wild oat, *Avena fatua*, morphological parameters, morphological variability

Introduction

Weeds present an important barrier in achieving high yields in agricultural production. The genus *Avena* is comprised of 30 species (Loskutov and Rines, 2011; Fu et al., 2019), displaying a high morphological and ecological diversity across their distribution range (Liu et al., 2017). *Avena fatua* L. is considered to be among the worst annual weeds in cereals in the temperate zone (Li et al. 2007), causing greater problems in agriculture, when compared to its closely related congener *Avena sterilis* subsp. *ludoviciana* (Durieu) Gillet & Magne (Bajwa et al., 2017; Jäck et al., 2017). Although the genus *Avena* has most likely originated in the western part of the Mediterranean region (Loskutov, 2008), the hexaploid group, including *Avena fatua* and common oat (*Avena sativa* L.), is thought to have formed in central Asia, within the centre of crop origin (Thurston and Phillipson, 1976; Loskutov, 2008).

Avena fatua is an annual grass, up to 90 (120) cm high. Its stems are either solitary or tufted, with linear, hairless leaves. The inflorescence is a loose panicle and the fruit is an oblong spikelet (Botha, 2001; DiTomaso and Healy, 2007). It is a wind-pollinated species, propagated by seeds (DiTomaso and Healy, 2007) and one plant (with 20 tufted stems), growing in favorable conditions, can produce up to 1500 seeds (Morrow and Gealy, 1982). The optimal temperature of wild oat germination is 15-18 ^{0}C (Božić and Stevanović, 2012; Saulić et al., 2015) and its seeds can maintain their viability in the soil for up to 10 years (DiTomaso and Healy, 2007).

Bearing in mind the high morphological and ecological variability characteristic for the entire genus *Avena*, the aim of this study was to evaluate the morphological variability of wild oat spikelets and seeds in the territory of Bosnia and Herzegovina.

Material and Methods

Wild oat seeds were collected from 11 populations of wild oat in wheat and rye fields at the beginning of summer in 2015 in Bosnia and Herzegovina. The populations were separated by at least 25 km, and the location of each sampling site was recorded using a hand-held Garmin GPS device.

Information on the sampling sites is given in Table 1. A total of 330 samples (30 samples per population) were analyzed.

Table 1. Sampling sites of the selected wild oat populations

Population name	Locality	Latitude	Longitude	Altitude (m)
BIH01	Bijeljina	+44.770327	+19.234344	88
BIH02	Bačevci	+44.090345	+19.507983	204
BIH03	Aleksandrovac	+44.9662778	+17.325666	107
BIH04	Patkovača	+44.731119	+19.22426	94
BIH05	Priboj	+44.603235	+18.942976	241
BIH06	Glavičica	+44.600266	+19.176308	150
BIH07	Ugljevička obrežina	+44.696203	+19.036466	163
BIH08	Ročević	+44.534486	+19.147164	123
BIH09	Čelopek	+44.44049	+19.136439	131
BIH10	Banjaluka	+44.78838	+17.20330	154
BIH11	Hrvaćani	+44.85787	+17.45322	185

Based on relevant morphological parameters, all collected wild oat samples were determined to belong to *Avena fatua*. A total of eight morphological features were chosen for this study: eight quantitative and one qualitative. Wild oat spikelets were photographed on a stereo trinocular microscope (Micro-SC2 EUinstruments). The measurements of spikelet length and width, awn length and the angle between the awn and the dorsal lemma surface and grain length were done subsequently using ImageJ software (Abramoff et al. 2004). Grain mass was also measured using an Analytical Balance (CANBEC120, COLO LabExperts). Lemma hairiness was assessed visually and graded on a scale of 0-100% and lemma color was assessed and assigned to each sample following a scale: white - golden - yellow - ocher - crème - copper – light brown - brown - black. Variability of the assessed morphological traits was analyzed using basic statistical analysis (Descriptive statistics), one-way analysis of variance (ANOVA) and a t-test for the comparison between means of the selected parameters in the studied populations in Statistica 7.0 (StatSoft Inc., Tulsa, USA).

Results and Discussion

Eight morphological traits of wild oat spikelets and seeds were analyzed on a total of 330 samples, from eleven populations. Results of descriptive statistics point to a low to moderate level of variation in these traits within the analyzed populations, which is evident in the Box and Whisker plot diagrams (Figures 1, 2 and 3). Analysis of variance (ANOVA) was used to test the significance of differences in mean values for all the studied morphological parameters (Table 2). Given that p values were below the threshold for extreme statistical significance (p

< 0.001) for all the chosen morphological traits, they can be considered as valuable in the study of morphological variability of the selected wild oat populations.

Table 2. Results of ANOVA analysis of mean values of eight selected morphological traits between 11 populations of wild oat in Bosnia and Herzegovina

Character name and unit	N	F	p
Spikelet length (cm)	11	27.72575	0.000000**
Spikelet width (cm)	11	7.55608	0.000000**
Color of the lemma	11	14.14534	0.000000**
Lemma hairiness (%)	11	26.54991	0.000000**
Awn length (cm)	11	8.66624	0.000000**
Angle of the awn (°)	11	12.24237	0.000000**
Grain length (mm)	11	23.67234	0.000000**
Grain mass (g)	11	12.09875	0.000000**

N – number of analyzed populations; F – Fisher's coefficient;
p – level of significance

Length of wild oat spikelets in the total sample of 330 spikelets from 11 populations varied between 1.13 cm as its minimum value and 3.38 cm as its maximum value, with population BIH09, from Čelopek (BIH09), having the lowest mean value (1.53 ± 0.17 cm) and BIH02 (Bačevci) the highest mean value at 2.07 ± 0.22 cm (Figure 1). The spikelet width varied between 0.12 and 0.57 cm. As expected, the populations from Čelopek and Bačevci were characterized by the lowest (0.20 ± 0.02 cm) and highest (0.26 ± 0.02 cm) mean values of spikelet width, respectively, which is in line with their mean spikelet length values.

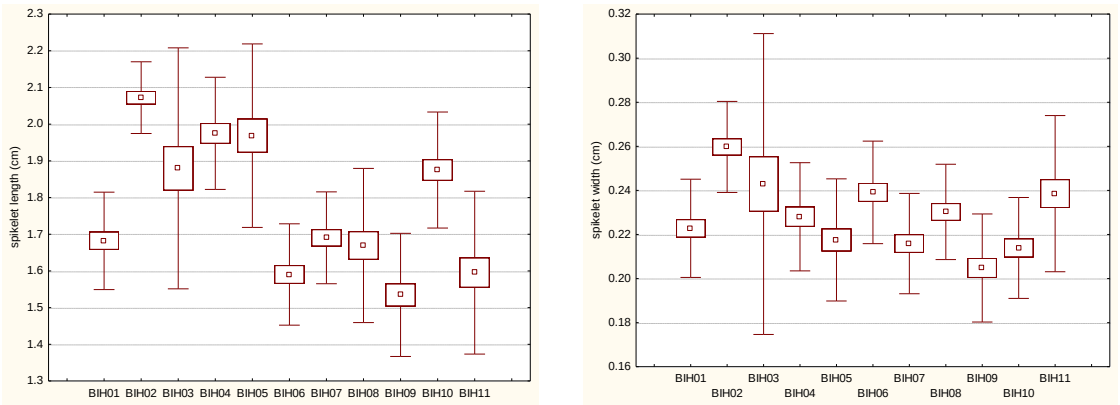

Figure 1. Box and Whisker plot of basic statistical parameters of the total spikelet length (a) and width (b) trait in wild oat populations from Bosnia and Herzegovina (middle point – Mean value; box – Mean ± SE; whisker – Mean ± SD)

The differences in mean spikelet length between most analyzed populations were highly significant (Table 3). Contrary to this, mean spikelet width proved to be a less variable morphological character between the selected populations, being highly significant ($p \leq 0.01$) in 40% cases (Table 3).

Based on spikelet length, populations Bačevci (BIH02), Patkovača (BIH04) and Priboj (BIH05) can be highlighted, as extreme statistical significance ($p<0.001$) is recorded in the differences of mean values between these three and a number of other studied populations (BIH02 / BIH06-11; BIH04 and BIH05 / BIH06-09 and BIH11, Table 3).

Table 3. T-test comparing the means of total spikelet length (white fields) and width (grey fields) between the studied wild oat populations in Bosnia and Herzegovina

	BIH01	BIH02	BIH03	BIH04	BIH05	BIH06	BIH07	BIH08	BIH09	BIH10	BIH11
BIH01	1	0.000000**	ns	ns	ns	0.007484**	ns	ns	0.004247**	ns	0.043990*
BIH02	0.000000**	1	ns	0.000001**	0.000000**	0.000595**	0.000000**	0.000001**	0.000000**	0.000000**	0.006358**
BIH03	0.003384**	0.003165**	1	ns	ns	ns	0.044112*	ns	0.005602**	0.031333*	ns
BIH04	0.000000**	0.004734**	ns	1	ns	ns	ns	ns	0.000531**	0.024652*	ns
BIH05	0.000001**	0.038779*	ns	ns	1	0.001837**	ns	ns	ns	ns	0.013177*
BIH06	0.011184*	0.000000**	0.00004**	0.00000**	0.00000**	1	0.000245**	ns	0.000001**	0.000084**	ns
BIH07	ns	0.000000**	0.00457**	0.00000**	0.00000**	0.00473**	1	0.015060*	ns	ns	0.004605**
BIH08	ns	0.000000**	0.004514**	0.00000**	0.00001**	ns	ns	1	0.000076**	0.006202**	ns
BIH09	0.000384**	0.000000**	0.000004**	0.00000**	0.00000**	ns	0.000143**	0.008091**	1	ns	0.000069**
BIH10	0.000004**	0.000000**	ns	0.015651*	ns	0.00000**	0.000005**	0.00007**	0.00000**	1	0.002238**
BIH11	ns	0.000000**	0.00023**	0.00000**	0.00000**	ns	0.045687*	ns	ns	0.00000**	1

**- highly statistically significant ($p < 0.01$), * statistically significant ($p < 0.05$); ns- not significant

Lemma color was assessed, with crème and copper being recorded in only one population each (Glavičica and Banjaluka, respectively). Three populations – Patkovača (BIH04), Priboj (BIH05) and Ročević (BIH08), had ocher lemma color, in 3.33%, 20% and 3.33% of samples, respectively. Light brown, brown and black lemma colors were most frequent among the samples, recorded in eight, ten and seven populations, respectively. Light brown color of lemma was documented in eight populations, with the highest proportion of samples of this color being characteristic for populations from Bijeljina (BIH01) (46.67%) and Patkovača (BIH04) (50%). Brown lemma color was recorded in a majority of analyzed populations (63.64%), where spikelets are predominately (≥50%) of this color. Black color of lemma was recorded with the highest value (96.67%) in the population BIH02, from Bačevci.

Lemma hairiness was also assessed and varied between 0 and 95% in some populations. Population from Bačevci had the lowest mean hairiness percentage ($8 \pm 7\%$), while BIH03 was

characterized by the highest mean hairiness percentage: 60 ± 21%. However, high values of standard deviation (Figure 2) in all the analyzed populations point to the fact that this morphological trait has proven to also be variable within the same population, therefore making it less informative in the study of interpopulation variation of the studied wild oat populations.

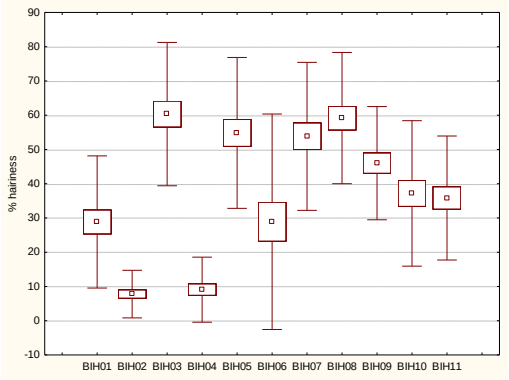

Figure 2. Box and Whisker plot of basic statistical parameters of lemma hairiness (in %) in wild oat populations from Bosnia and Herzegovina (middle point – Mean value; box – Mean ± SE; whisker – Mean ± SD)

Length and angle of the awn were also studied. Length of the awn varied between 2.34 and 4.74 cm, while the angle of the awn and the dorsal lemma surface varied between 85.76° and 140.71°. The lowest mean values of awn length (3.15 ± 0.39 cm) and the angle of the awn (98.87 ± 5.41°) were recorded for wild oat population from Čelopek (BIH09). On the other hand, the highest mean value of awn length (3.82 ± 0.50 cm) was characteristic for the population from Priboj (BIH05) and awn angle (118.2 ± 10.13°) in the population from Bijeljina (BIH01).

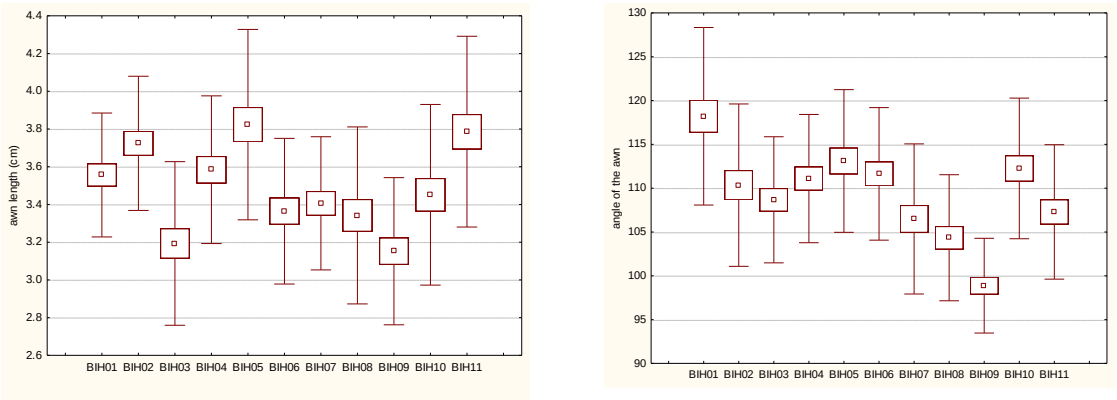

Figure 3. Box and Whisker plot of basic statistical parameters of awn length (a) and angle of the awn (b) in wild oat populations from Bosnia and Herzegovina (middle point – Mean value; box – Mean ± SE; whisker – Mean ± SD)

Statistical significance of the interpopulation differences for these two morphological traits are shown in Table 4, where it can be observed that both morphological traits act as strong parameters of interpopulation variation in wild oat. Based on statistical analysis of the awn length parameter it has been shown that highly significant differences are present between population BIH02 and populations BIH06-09, population BIH05 and BIH11 and populations BIH06-10 (Table 4).

Meanwhile, statistical analysis based on the angle between the awn and the dorsal lemma surface has shown that the most significant differences are present between populations BIH01, BIH08 and BIH09 and the remaining eight wild oat populations (Table 4).

Table 4. T-test comparing the means of awn length (white fields) and angle between the awn and the dorsal lemma surface (grey fields) of the studied wild oat populations in Bosnia and Herzegovina

	BIH01	BIH02	BIH03	BIH04	BIH05	BIH06	BIH07	BIH08	BIH09	BIH10	BIH11
BIH01	1	0.002700**	0.000093**	0.002857**	0.035769*	0.006069**	0.000010**	0.000000**	0.000000**	0.014480*	0.000016**
BIH02	ns	1	ns	ns	ns	ns	ns	0.006878**	0.000000**	ns	ns
BIH03	0.000557**	0.000003**	1	ns	0.029353*	ns	ns	0.023285*	0.000000**	ns	ns
BIH04	ns	ns	0.000538**	1	ns	ns	0.029072*	0.000655**	0.000000**	ns	ns
BIH05	0.018301*	ns	0.000003**	0.044993*	1	ns	0.003301**	0.000044**	0.000000**	ns	0.006117**
BIH06	0.042516*	0.000407**	ns	0.032484*	0.000210**	1	0.016772*	0.000325**	0.000000**	ns	0.031434*
BIH07	ns	0.000973**	0.041681*	ns	0.000465**	ns	1	ns	0.000122**	0.009325**	ns
BIH08	0.044972*	0.000764**	ns	0.033990*	0.000322**	ns	ns	1	0.001520**	0.000170**	ns
BIH09	0.000057**	0.000000**	ns	0.000070 **	0.000000**	0.038416*	0.010520*	ns	1	0.000000**	0.000008**
BIH10	ns	0.015155*	0.032805*	ns	0.004868**	ns	ns	ns	0.010256*	1	0.017399*
BIH11	0.041387*	ns	0.000009**	ns	ns	0.000601**	0.001322**	0.000834**	0.000001**	0.010868*	1

**- highly statistically significant ($p < 0.01$), * statistically significant ($p < 0.05$); ns- not significant

Grain length and mass of the collected samples were also analyzed. The length of grain varied between 5 and 11 mm, with population from Priboj (BIH05) having the lowest average grain length (6.53 ± 0.90 mm) and population from Bačevci (BIH02) having the highest average grain length (8.93 ± 0.61 mm).

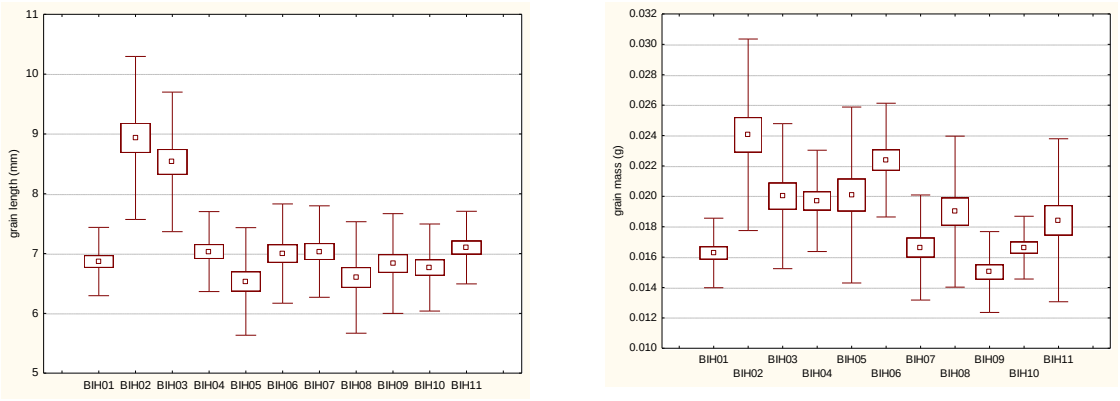

Figure 4. Box and Whisker plot of basic statistical parameters of grain length (a) and width (b) in wild oat populations from Bosnia and Herzegovina (middle point – Mean value; box – Mean ± SE; whisker – Mean ± SD)

Analysis of grain length has shown extremely significant differences (p = 0.00000) only between the populations from Bačevci (BIH02) and Aleksandrovac (BIH03), when compared with other nine analyzed wild oat populations, while contrary to this, grain mass was shown to be a highly significant factor in 55% of cases, when comparing the analyzed wild oat populations (Table 5). Grain mass varied between 0.001 and 0.037 g (data not shown), which is in line with the morphological characteristics of *A. fatua* in the literature. According to Фисюнов (1984) grain size in *A. fatua* is expected to be 8-16 x 1,75-2,50 x 1,25-2,25 mm, with an average mass of 1000 seed of 15-25 g.

Table 5. T-test comparing the means of grain length (white fields) and grain weight (grey fields) between the studied wild oat populations in Bosnia and Herzegovina

	BIH01	BIH02	BIH03	BIH04	BIH05	BIH06	BIH07	BIH08	BIH09	BIH10	BIH11
BIH01	1	0.000000**	0.000281**	0.000020**	0.001400**	0.000000**	ns	0.008517**	ns	ns	0.048077*
BIH02	0.000000**	1	0.006938**	0.001461**	0.013885*	ns	0.000001**	0.001046**	0.000000**	0.000000**	0.000445**
BIH03	0.000000**	ns	1	ns	ns	0.036278*	0.002644**	ns	0.000006**	0.000730**	ns
BIH04	ns	0.000000**	0.000000**	1	ns	0.004818**	0.000900**	ns	0.000000**	0.000067**	ns
BIH05	ns	0.000000**	0.000000**	0.017618*	1	ns	0.006775**	ns	0.000054**	0.003098**	ns
BIH06	ns	0.000000**	0.000000**	ns	0.041218*	1	0.000000**	0.004146**	0.000000**	0.000000**	0.001578**
BIH07	ns	0.000000**	0.000000**	ns	0.023921*	ns	1	0.036690*	0.047862*	ns	ns
BIH08	ns	0.000000**	0.000000**	0.043022*	ns	ns	ns	1	0.000285**	0.019006*	ns
BIH09	ns	0.000000**	0.000000**	ns	ns	ns	ns	ns	1	0.011558*	0.002867**
BIH10	ns	0.000000**	0.000000**	ns	ns	ns	ns	ns	ns	1	ns
BIH11	ns	0.000000**	0.000000**	ns	0.005888**	ns	ns	0.016835**	ns	ns	1

**- highly statistically significant (p < 0.01), * statistically significant (p < 0.05); ns- not significant

In general, high interpopulation morphological variation of the studied populations of wild oat, despite their relatively close proximity, can be explained by the fact that morphological variability is highly characteristic for the representatives of this genus. Furthermore, *A. fatua* is a widely distributed weed species, which can adapt to different soil types and environmental conditions (e.g. soil pH below 4,5, Holm et al., 1977; on alkaline soils, Korniak, 1996; Korniak et al., 2000), and therefore it is expected to exhibit high variations in morphology.

Acknowledgements

The authors are grateful for the financial support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Projects No. TR31018 and III 46008).

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Original scientific paper

ZP Admiral- facultative triticale variety

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Abstract

The present study displays the most important agronomic and technological traits of the first Serbian facultative triticale variety, ZP Admiral, developed at the Maize Research Institute, Zemun Polje. Grain yield of the ZP Admiral in the two-year trails of the Serbian Committee for the Release of Varieties as a spring type amounted to 6185 kg ha-1, and as a winter type 8398 kg ha-1. ZP Admiral is an early triticale variety, three days earlier than standard check, with excellent resistance to lodging. The variety is characterized with leaf and spike waxiness, while the surface of stem neck is hairy. The protein content of ZP Admiral as a winter crop amounted 12,9% comparing to 12.7% of standard variety Odisej, and had a slightly lower protein content 12% than in a spring sowing. Quality parameter ash content, a measure of the total amount of minerals, of the spring ZP Admiral was higher than in winter sowing 2.4% and 1.9%, respectively. Volume weight was 81.5 kg hl-1 and 78.2 kg hl-1 in spring and winter sowing respectively, and 1000 kernel weight 36g and 38.7g, respectively. Because of its adaptability, yield stability and good level of drought tolerance new facultative variety ZP Admiral has good potential for production in different agro-ecological environments.

Key words: triticale, facultative type, variety, breeding

Introduction

Triticale (×Triticosecale Wittmack) is a synthetic species developed by crossing wheat (Triticum spp.) and rye (Secale cereale L.), where favorable alleles from both progenitor species are incorporated; high yield potential and good grain quality of wheat with disease and environment tolerance of rye. That enable its adaptation to environments that are less favorable, providing better biomass yield and forage quality. Triticale is, therefore, a crop which is particularly suited for marginal environments (acid- or drought- prone soils) or where disease pressure is high (Mergoum et al., 2009). Beside, triticale is known to have lower production costs in comparison to other crops with high grain yield and large biomass production even in

marginal environments not usable for food crops (Cantale et al., 2016). High ability of nitrogen accumulation makes triticale more appropriate culture for growing grain on nitrogen poor soils and leads to decreased requirements for nitrogen fertilizers which is important for many developing countries.

Triticale can be classified as spring, winter and facultative types depending on vernalization requirements (Mergoum et al., 2009; McGoverin et al., 2011). Late planting of small grains varieties has become very actual in recent years due to climate changes, cause in many regions, in autumn there is not enough moisture for seed emergence, due to drought. In such cases, using facultative varieties is beneficial.

Triticale is used for food, feed (monogastrics and ruminants), grazed or stored forage and fodder, silage, green-feed, and hay (Mergoum et al., 2009). Triticale is the most implemented as feed in non-ruminant animals nutrition, especially in pigs and poultry nutrition (Đekic et al., 2009), but because of its higher starch digestibility, triticale is a better ruminant feed than other cereals (Mergoum et al., 2009). In recent years, triticale is recognized as an energy crop and its used in bioethanol production.

For last few decades of intensive triticale breeding, the genetic gain in yield potential was mainly due to a substantial increase in harvest index, grains/m2, spikes/m2, test weight, and a decrease in plant height (Zakir and Belete, 2019). However, because of its wide utilization, triticale breeding program in Maize Research Institute Zemun Polje will be continued especially towards creating new varieties with better drought adaptation.

Material and Methods

The first Serbian facultative triticale variety ZP Admiral was created by crossing varieties Kendo x Korm, and hybrid material is grown following the pedigree method. In the experimental field of Maize Research Institute Zemun Polje, during preliminary trials, the most important traits were observed. ZP Admiral, as a spring variety was tested at six locations in Serbia (Kikinda, Kruševac, Novi Sad, Pančevo, Sremska Mitrovica, Sombor) during 2015/2016 and 2015/2016 and finally recognized based on obtained results in 2017. As a winter variety ZP Admiral, was tested at seven locations (Kinkinda, Kruševac, Novi Sad, Pančevo, Požarevac, Sremska Mitrovica, Sombor) during 2017/2018 and 2018/2019 and was recognized as facultative type in 2019. The variety ZP Admiral was tested in those two trials with complete random block design, 5m2 plots on each location. In the trial for winter type recognition, standard variety was Odisej, while in spring type trials standard wasn't sown because none spring triticale variety has been recognized in Serbia until 2019. Analyzed traits were: yield,

resistance to lodging, plant height, test weight, 1000 kernel weight and some technological parameters.

Results and Discussion

According to the results of the Commission for variety testing (Table 1.), the variety ZP Admiral as a winter crop, for all sites, achieved the average grain yield of 8398 kg ha-1, 581 kg more than the standard Odisej, which is statistically significant difference. The highest yield of the ZP Admiral was achieved at the location of Novi Sad 10273 kg ha-1. As a spring crop, after two years of testing, ZP Admiral achieved averaged grain yield of 6185 kg ha-1, with the highest yield at the location of Novi Sad 8131 kg ha-1.

Table 1. Average two-year grain yield of variety ZP Admiral (in spring and winter sowing) and standard Odisej (kg ha-1)

Variety	*Sites*							
	KI	*KR*	*NS*	*PA*	*SM*	*SO*		*Aver.*
Spring ZP Admiral	*4419*	*3832*	*8131*	*6539*	*7294*	*6898*		***6185***
	KI	*KR*	*NS*	*PA*	*SM*	*SO*	*PO*	*Aver.*
Winter ZP Admiral	*6433*	*7818*	*10273*	*9830*	*8988*	*7911*	*7242*	***8398*****
Odisej	*5860*	*6667*	*10456*	*8936*	*8438*	*7101*	*6677*	***7816***

KI- Kikinda, KR- Kruševac, NS- Novi Sad, PA- Pančevo, SM- Sremska Mitrovica, SO- Sombor, PO- Požarevac

ZP Admiral is an early triticale variety, three days earlier than standard check, with excellent resistance to lodging throughout whole vegetation period until full maturity. In winter sowing practice stem height was 107.3 cm and as a spring crop ZP Admiral reached 94cm. The variety is characterized with leaf and spike waxiness, while the surface of stem neck is hairy. These traits are related with stress resistance, they form a physical barrier to pathogen penetration and also act as photoprotectants for ultraviolet light (Krauss et al., 1997). Several studies showed that, waxiness correlate with glaucousness, a bluish-green appearance of the plant cuticle that is associated with drought tolerance (Hen-Avivi et al., 2016; Wang et al., 2015). The ear is white colored with awns and large amber grains.

The physical properties such as volume weight, 1000 kernel weight and grain size are still the important characteristics that determine the price of triticale on the market. Volume weight of ZP Admiral was 81.5 kg hl-1 and 78.2 kg hl-1 in spring and winter sowing respectively, and

1000 kernel weight 36g and 38.7g, respectively. In the both sowing terms content of grains above 2.5mm and 2.2mm was similar.

High quality grain and its nutritional value, determines the use of triticale for consumption so beside high yield more attention is paid in triticale breeding for its quality (Gil, 2002). The protein content of ZP Admiral as a winter crop amounted 12.9% comparing to 12.7% of standard variety Odisej.

Grown like a spring crop ZP Admiral had a slightly lower protein content 12% than as a winter crop, although other authors reported that grain protein content was higher in the spring triticales than in the winter types (Royo and Pares 2006). Quality parameter ash content, a measure of the total amount of minerals, of the spring ZP Admiral was higher than in winter sowing 2.4% and 1.9%, respectively.

Table 2. Grain characteristics of ZP Admiral compared to standard Odisej

Traits	*Spring ZP Admiral*	*Winter ZP Admiral*	*Odisej*
Volume weight	*81.5*	*78.2*	*79.5*
1000 kernel weight	*36*	*38.7*	*40.2*
Content of grains (>2.5mm)	*17*	*17.5*	*23*
Content of grains (>2.2mm)	*5*	*4*	*5*
Protein content (%)	*12*	*12.9*	*12.7*
Ash content	*2.4*	*1.9*	*1.9*
Hagberg Falling	*133*	*77*	*81*

The third quality parameter tested in this trial was the Hagberg Falling Number, important indicator because triticale is a crop with relatively high alfa- amylase activity.

Hagberg Falling Number is an indicator of the pre-sprouting process and of high pregermination alfa-amylase activity potentially interacting with dough properties (Jestin and Bonhomme, 1996). Although Tupits et al., 1999 reported that requirements for food crops with regard to the falling number are often too high for growing triticale in the local climate, value of this parameter for winter ZP Admiral was 77, slightly lower than for standard variety Odisej 81. However, Hagberg Falling Number for ZP Admiral as a spring crop amounted 133.

Conclusions

ZP Admiral is the first Serbian facultative triticale variety, developed at the Maize Research Institute, Zemun Polje. Facultative varieties becoming more important in the recent years, because beside during grain filling time, drought occurs often in the fall, during optimal sowing time. Another advantage of ZP Admiral is its earliness, which is important trait for skipping drought, common in South East Europe during grain filling process.

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Original scientific paper

Evaluation of grain yield and its components of some experimental, registered and commercial ZP maize (*Zea mays* L.) hybrids

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Abstract

The grain yield and its components of eighteen experimental, one registered and two commercial maize hybrids were examined in six different environments. The main objectives were to identify the best performing experimental and registered hybrids and to select appropriate hybrids for approving and commercialization. Based on the results, many decisions are made. Experimental hybrid Exp.15 is submitted for testing to the Variety Commission of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. This hybrid, as a high-yielding and a stable one, could enrich the assortment of registered medium-late maize hybrids in Serbia. First year of its testing for the purpose of being registered is going to be 2020. Further, registered hybrid ZP 685 is suggested for commercialization as one of the best performing in present study. In addition, the goal of this study was also identification of the superior inbred lines for use in future breeding programs. Inbred lines L1 (BSSS) and L4 (Independent heterotic group) are chosen for crossing with an aim to create a new origin population - source for deriving new recombinant inbred lines. Cross L1 x L4 will be a part of maize breeding program at Maize Research Institute "Zemun Polje" in 2020. Lines L1 and L4 are also suggested for crossing with all others Lancaster lines from MRIZP (which are not parental components of tested hybrids) for deriving new experimental F1 hybrids. As well, Lancaster lines L5, L6 and L7 should be crossed between themselves for creating new origin populations for deriving new recombinant Lancaster inbred lines. Hybrid Sister 1 had very high and stable grain yield and as such it is the most cost effective for seed production.

Key words: maize, experimental hybrids, grain yield, yield components, variety registration

Introduction

The main task of maize breeders is constant development of new maize hybrids more productive and more adaptable than all hybrids created so far. In order to examine their value, phenotypes of newly developed hybrids must be compared with registered hybrids in multi-environments. Multi-locational and multi-year field trials are required for this purpose. Only the best experimental hybrids are submitted for testing for the purpose of being registered by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. After registration, more extensive field trials follow in order to commercialize some of the registered hybrids. According to Čamdžija et al. (2012), it is important for commercial hybrid that a high yield is accompanied by maximum stability in favorable and unfavorable conditions. Such hybrids must be superior in all environments, i.e. capable of accomplishing stable and high yield under various environmental conditions (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966). Only genotypes with a minimal variance for yield across environments are considered stable (Mohammadi et al., 2009). For development of superior hybrids and lines it is important to have a good knowledge of traits which have significant association with grain yield as the most important goal of all breeding programs (Malik et al., 2005). For that reason, it is necessary for breeders to take into consideration these traits when they create new origin population for development of new inbred lines (Ojo et al., 2006). As the grain yield is highly influenced by environmental factors, in order to develop high-yielding desirable maize genotypes, yield components with stable expression and highly correlated with grain yield could be useful (Panwar et al., 2013; Stanković, 2016). Filipović et al. (2014) also state that those yield components as agronomically important traits that mostly determine the yield can be used as important breeding criteria. During inbred lines development breeders pay attention to their combining ability and yield components of their hybrids and themselves per se. The correct choice of initial material having a high frequency of desirable alleles, good agronomic traits and good combining abilities is crucial for the success of any breeding program (Mišević, 1996). Since for future breeding programs inbred lines are selected based on the performance of their hybrids, elite inbred lines are the best source material for developing of new superior inbred lines (Hallauer, 2000). Maize breeders at Maize Research Institute "Zemun Polje" work hard on development of new high-yielding and adaptable maize hybrids. Out of the thousands of newly created hybrids, only a few dozens of the best apply for approving and registration. This study was undertaken to assess grain yield and its components of some experimental, registered and commercial maize hybrids. The objectives were (i) to identify the best

performing experimental and registered hybrids, (ii) to select appropriate hybrids for approving (registration) and for commercialization, (iii) to choose some parental inbred lines which should be crossed in order to create a new origin population for development of new inbred lines.

Material and Methods

Twenty-one single-cross maize hybrids from Maize Research Institute "Zemun Polje" (MRIZP) were chosen as a material for this experiment (Table 1). Out of these twenty-one, fifteen hybrids are experimental (Exp.1 – Exp.15) and are currently in the process of being examined in order to make a decision to initiate a process of their possible registration. Hybrids ZP 606 and ZP 666 are commercial and already have been widely grown in Serbia. They were used as standard checks in this study. ZP 606 is the best-selling hybrid of the MRIZP. Hybrid ZP 685 is registered but it has not yet been commercialized. The other three hybrids, Sister 1, Sister 2 and Sister 3 are female parental components (mothers) of many registered and commercial three-way cross hybrids of MRIZP. All hybrids have the same kernel type – yellow dent and belong to FAO 500 and FAO 600 maturity groups.

Table 1. Twenty-one maize hybrids and their status, formula, pedigree and maturity group

No.	Hybrid	Status	Formula	Pedigree data	Maturity group
1	Exp.1	Experimental	L1 x L2	BSSS x Iodent	FAO 600
2	Exp.2	Experimental	L1 x L3	BSSS x BSSS	FAO 600
3	Exp.3	Experimental	L1 x L4	BSSS x Independent	FAO 600
4	ZP 606	Registered - Commercial	L1 x L5	BSSS x Lancaster	FAO 600
5	ZP 666	Registered - Commercial	L1 x L6	BSSS x Lancaster	FAO 600
6	Exp.4	Experimental	L1 x L7	BSSS x Lancaster	FAO 500
7	Exp.5	Experimental	L2 x L3	Iodent x BSSS	FAO 500
8	Exp.6	Experimental	L2 x L4	Iodent x Independent	FAO 600
9	Exp.7	Experimental	L2 x L5	Iodent x Lancaster	FAO 500
10	Exp.8	Experimental	L2 x L6	Iodent x Lancaster	FAO 500
11	Exp.9	Experimental	L2 x L7	Iodent x Lancaster	FAO 500
12	Exp.10	Experimental	L3 x L4	BSSS x Independent	FAO 600
13	Exp.11	Experimental	L3 x L5	BSSS x Lancaster	FAO 600
14	Exp.12	Experimental	L3 x L6	BSSS x Lancaster	FAO 500
15	Exp.13	Experimental	L3 x L7	BSSS x Lancaster	FAO 500
16	ZP 685	Registered (Not Commercial)	L4 x L5	Independent x Lancaster	FAO 600
17	Exp.14	Experimental	L4 x L6	Independent x Lancaster	FAO 500
18	Exp.15	Experimental	L4 x L7	Independent x Lancaster	FAO 500
19	Sister 1	Mother of TWC hybrids	L5 x L6	Lancaster x Lancaster	FAO 600
20	Sister 2	Mother of TWC hybrids	L5 x L7	Lancaster x Lancaster	FAO 500
21	Sister 3	Mother of TWC hybrids	L6 x L7	Lancaster x Lancaster	FAO 500

All twenty-one hybrids used in this study were derived from seven inbred lines crossed according to incomplete diallel method (without reciprocal hybrids) (7x6/2=21). These seven parental lines were developed in breeding programs at Maize Research Institute "Zemun Polje" and belong to different heterotic and maturity groups (Table 2).

The phenotypes of the selected hybrids were tested at three locations in Serbia (Pančevo, Bečej and Zemun Polje) in 2018 and 2019 (total six environments). At all three locations the same soil type – chernozem was present. Field trials were set up according to the randomized complete block design (RCBD) with two replications. Mechanical planting of the trials was done in the two-rowed plots. The length of the plot was 5 m and the inter-row distance amounted to 75 cm forming a plot area of 7.5 m^2. Sowing density was 67000 plants per hectares for all hybrids. Standard maize production technology was used. Harvesting of the trials was done by hand.

Table 2. Heterotic groups, origins and maturity groups of the parental lines of the tested hybrids

Line	Heterotic group	Origin/Source	Maturity group
L1	BSSS – *Iowa Stiff Stalk Synthetic*	MRIZP	FAO 600
L2	Iodent	MRIZP	FAO 500
L3	BSSS – *Iowa Stiff Stalk Synthetic*	MRIZP	FAO 600
L4	Independent (Unknown)	MRIZP	FAO 600
L5	Lancaster - *Lancaster Sure Crop*	MRIZP	FAO 600
L6	Lancaster - *Lancaster Sure Crop*	MRIZP	FAO 500
L7	Lancaster - *Lancaster Sure Crop*	MRIZP	FAO 500

Grain yield and following yield components were examined: ear length (cm), kernel depth (cm), number of kernel-rows, number of kernels per row and 1000-kernel weight (kg). The grain yield is converted to tones per hectares (t/ha) at 14 % moisture level. The yield of all ears per every plot was measured immediately after harvesting. Moisture measurement was done immediately after harvesting, as well, on the samples of five ears taken from each plot. Observations of the yield components were recorded from ten representative ears selected from each plot. Recorded data from the trials were statistically analyzed by one-factorial analysis of variance (ANOVA) for all six traits. After that, data were further analyzed using Least Significant Difference (LSD) test (student's t-test) at 99% and 95% probability levels. Pearson's simple correlation coefficients among all examined traits were also calculated. Statistical processing was done by Microsoft Office Excel 2016.

Results and Discussion

Grain yield of tested hybrids in 2018 and 2019

The analysis of variance (ANOVA) showed a statistically significant influence ($p<0.01$) of genotype on grain yield in both years (Table 3).

Table 3. One-factorial analysis of variance (ANOVA) – significance of factor genotype influence on all investigated traits in both years

GY18	GY19	KRN 18	KRN 19	KD18	KD19	KW18	KW19	NKPR18	NKPR19	EL18	EL19
**	**	**	**	**	**	**	**	**	**	**	**

(GY= grain yield; KRN= kernel-rows number; KD= kernel depth; KW= 1000-kernel weight; NKPR= number of kernels per row; EL= ear length; 18= year 2018; 19= year 2019; ns- no significant; *-significant at p=0.05; ** -significant at p=0.01)

Environmental conditions in 2018 were almost ideal for maize production in Serbia, while they were less favorable in 2019 thanks to drought and strong winds (Republic Hydrometeorological Service of Serbia, 2020). As a consequence, average grain yields of all tested hybrids in our experiment was higher in 2018 than in 2019 (Table 4). In 2018 grain yield ranged from 7.569 t/ha (Sister 3) to 16.776 t/ha (ZP 606) (average in 2018 was 13.453 t/ha), and in 2019 from 6.204 t/ha (Sister 3) to 14.770 t/ha (ZP 606) (average in 2019 was 11.375 t/ha).

Table 4. Average grain yields of tested hybrids and significance of differences (t-test) in grain yield of each hybrid between two years

Hybrid	Grain yield (t/ha)		Significance of differences between 2018 and 2019	Pedigree data
	2018	2019		
Exp.1	14.324	13.130	*	BSSS x Iodent
Exp.2	14.136	12.112	**	BSSS x BSSS
Exp.3	14.562	12.551	**	BSSS x Independent
ZP 606	16.776	14.770	**	BSSS x Lancaster
ZP 666	16.169	13.887	**	BSSS x Lancaster
Exp.4	14.073	12.945	*	BSSS x Lancaster
Exp.5	12.757	8.772	**	Iodent x BSSS
Exp.6	13.889	9.351	**	Iodent x Independent
Exp.7	13.991	11.339	**	Iodent x Lancaster
Exp.8	13.267	11.258	**	Iodent x Lancaster
Exp.9	12.408	11.908	ns	Iodent x Lancaster
Exp.10	13.905	9.917	**	BSSS x Independent
Exp.11	13.915	10.995	**	BSSS x Lancaster
Exp.12	14.779	11.932	**	BSSS x Lancaster
Exp.13	13.764	10.368	**	BSSS x Lancaster
ZP 685	15.515	14.332	ns	Independent x Lancaster

Hybrid	Grain yield (t/ha)		Significance of differences between 2018 and 2019	Pedigree data
	2018	2019		
Exp.14	14.999	13.622	*	Independent x Lancaster
Exp.15	15.301	14.420	ns	Independent x Lancaster
Sister 1	8.279	8.046	ns	Lancaster x Lancaster
Sister 2	8.135	7.014	*	Lancaster x Lancaster
Sister 3	7.569	6.204	**	Lancaster x Lancaster
LSD (0.05)	0.654	0.723		
LSD (0.01)	0.827	0.921		

(ns- no significant; *-significant at p=0.05; ** -significant at p=0.01)

The most yielding tested hybrid in both years was commercial ZP 606 which had 16.776 t/ha and 14.770 t/ha, in 2018 and 2019, respectively (Table 4). The second highest grain yield in 2018 (16.169 t/ha) and the forth in 2019 (13.887 t/ha) showed commercial hybrid ZP 666. These two hybrids have the same female parent line which belongs to BSSS heterotic group (L1) and two Lancaster male parents (L5 and L6). This is an evidence supporting the claim that a long-established heterotic couple BSSS x Lancaster is still widely used in selection and it represents the basis for planning maize breeding programs (Čamdžija, 2014). ZP 606 and ZP 666 showed a statistically significantly higher grain yield in 2018 than in 2019, which means that they can only reach their genetic potential under the best conditions which were characteristic for year 2018. However, their results of grain yield in 2019 also showed they were superior in different environments. These results confirmed the statement of Čamdžija et al. (2012) that only high-yielding and stable hybrids should be commercialized. Crevar et al. (2011) also detected highest average grain yield in ZP 606 and highest adaptation to more favorable growing conditions in ZP 666.

Registered hybrid ZP 685 was in the third place in both years with 15.515 t/ha in 2018 and 14.332 t/ha in 2019 (Table 4). This indicates it showed very high yield and yield stability (no significant difference was observed between 2018 and 2019). Based on that, it will be proposed for commercialization.

Of all experimental hybrids, Exp.14 and Exp.15 performed best in 2018, with 14.999 t/ha and 15.301 t/ha, respectively, by taking forth (Exp.15) and fifth (Exp.14) place (Table 4). In 2019, which was a less favorable year for maize production, experimental hybrid Exp.15 had higher grain yield than ZP 666 and ZP 685 (14.420 t/ha vs. 13.887 t/ha and 14.332 t/ha, respectively)

and found itself on the second position. ZP 606 only showed higher grain yield than Exp.15 in 2019, but not statistically significantly higher (0.350 is less than LSD at 0.05 probability level in 2019). Not ideal environmental conditions – such as the ones in 2019 – are common in Serbia. Hybrids like Exp.15, which could show high grain yield in a year like 2019, are needed for Serbians farmers. Beside good performances shown in 2019, this experimental hybrid (Exp.15) did not show significantly lower yield in 2019 compared to 2018 which indicates its high yield stability. Based on these results, hybrid Exp.15 will be signed up for testing by Serbian Variety Commission in the two-year trial starting from 2020. This will initiate a process of possible future approving and registration by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. Also, based on the results shown, experimental hybrid Exp.14 (in both years it was rated fifth) is suggested for further evaluation. All six hybrids (Exp.1, Exp.2, Exp.3, ZP 606, ZP 666, Exp.4) with L1 female parent line (BSSS) were in the top ten in terms of grain yield in both years (Table 4). Further, Line L1 crossed as mother with line L5 as father give the most yielding and the best-selling hybrid of MRIZP ZP 606. This indicates that L1 is a maize inbred line with the best combining ability of the Maize Research Institute "Zemun Polje". Hybrids ZP 685, Exp.15 (signed up for registration) and Exp.14 (chosen for further evaluation) have the same female parent line (L4) which belongs to unknown heterotic group (Independent). These two lines (L1 and L4) should be crossed for creating a new origin population (new source) for quickly deriving new inbred lines via double haploid method (Vančetović et al., 2004). Some of these recombinant inbred lines which are going to be originated from this source (L1 x L4) will possibly have better combining ability, adaptability and stability than their parents (L1 and L4). They are expected to produce better hybrids with Lancaster lines in future breeding programs. L1 and L4 are also suggested for crossing with all others Lancaster lines from MRIZP (which are not parental components of tested hybrids in this study) for deriving new hybrids.

As well, Lancaster lines L5, L6 and L7 are all male parents of the most yielding hybrids (ZP 606, ZP 666, ZP 685, Exp.15 and Exp.14). It is concluded that L5, L6 and L7 should be crossed between themselves for creating new origin populations (F2 sources) for deriving new recombinant Lancaster inbred lines.

As expected, the lowest grain yield was recorded in hybrids Sister 1, Sister 2 and Sister 3 (from 6.204 t/ha to 8.279 t/ha) (Table 4). These hybrids are female components of many three-way cross hybrids and they all derive from three Lancaster lines (L5, L6 and L7). Their low yield is a consequence of a small heterosis which occurs due to genetic similarity of the parents of these hybrids (Shull, 1908). However, these hybrids were high yielding and based on that very

cost effective for seed production (as female parents-mothers). This is also confirmed by statement of Čamdžija (2014). He states that for profitable seed production female parents must be characterized by high yield (minimum 3 t/ha) and tolerance to both abiotic and biotic stresses. Sister 1 had the highest (8.279 t/ha and 8.046 t/ha, in 2018 and 2019, respectively) and the most stable grain yield (no significant difference was observed between 2018 and 2019) and as such it is the most cost effective for seed production.

Yield components of tested hybrids in 2018 and 2019

The analysis of variance showed a statistically significant influence of genotype factor on all yield components in both years (2018 and 2019) at 0.01 level of probability (Table 3). It is well-known that all yield components contribute to greater grain yield (Hallauer et al., 2010). The kernel-rows number of the tested hybrids varied from 12.85 to 17.85 in 2018, and from 12.7 to 17.8 in 2019 (Table 5). Experimental hybrids Exp.1 and Exp.3 showed the largest values of this trait in 2018 (17.85 and 17.35, respectively) and in 2019 (17.8 and 17.3, respectively). These two hybrids have the same female parent line, L1 (BSSS). It is noted that hybrids which had Lancaster as male parental components showed a smaller number of kernel-rows then hybrids with both non-Lancaster parental components. The smallest number of kernel rows in both years was showed by hybrids Sister 1, Sister 2 and Sister 3 whose parental components were both from Lancaster heterotic group. These results confirmed the claims of Hallauer et al. (2004) that Lancaster heterotic group had fewer kernel rows as opposed to genotypes originating from Reid Yellow Dent (from which some progenitors of BSSS population were originated). It is also noted that the most yielding hybrids (ZP 606, ZP 666, ZP 685, Exp.15) had the medium number of kernel rows. There is a general stance that this trait has high heritability and positive correlation with grain yield (Singhal et al., 2006). Hence, inbred lines which derive hybrids with high number of kernel-rows should be widely used in breeding programs.

Table 5. Average values of yield components of tested hybrids in both 2018 and 2019

Hybrid	KRN		KD (cm)		KW (kg)		NKPR		EL (cm)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Exp.1	17.85	17.8	1.244	1.089	0.396	0.373	42.91	41.79	23.32	21.59
Exp.2	16.5	16.55	1.183	1.151	0.391	0.379	44.45	41.2	23.24	22.08
Exp.3	17.35	17.3	1.286	1.265	0.385	0.375	43.54	41.58	22.20	21.41
ZP 606	14.55	14.55	1.274	1.249	0.433	0.431	47.64	45.88	23.87	21.91
ZP 666	15.15	14.85	1.306	1.217	0.432	0.438	49.40	49.41	24.02	23.37
Exp.4	16.15	16.25	1.279	1.254	0.383	0.387	49.06	48.46	23.24	22.17
Exp.5	16.15	17	1.097	1.000	0.364	0.342	41.35	40.9	21.75	21.77

Hybrid	KRN		KD (cm)		KW (kg)		NKPR		EL (cm)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Exp.6	16.6	16.5	1.235	1.155	0.373	0.369	42.75	41.13	20.90	19.79
Exp.7	14.9	14.85	1.256	1.156	0.416	0.403	47.73	44.3	22.25	20.89
Exp.8	15.4	15.75	1.195	1.150	0.383	0.377	47.86	45.21	23.49	21.52
Exp.9	15.75	15.7	1.163	1.149	0.390	0.396	46.65	44.13	23.16	20.77
Exp.10	15.6	15.3	1.129	1.098	0.364	0.359	45.46	45.19	21.80	21.75
Exp.11	14.75	14.05	1.122	1.102	0.388	0.397	51.14	50.49	25.10	23.40
Exp.12	15.45	15.65	1.135	1.155	0.355	0.363	51.04	50.74	25.11	24.34
Exp.13	15.2	15.2	1.096	1.090	0.322	0.325	50.73	50.18	23.89	23.32
ZP 685	14.9	14.95	1.260	1.245	0.425	0.418	49.98	49.91	23.14	22.85
Exp.14	14.95	15.15	1.245	1.174	0.407	0.410	47.66	47.28	23.22	22.52
Exp.15	15.45	15.35	1.219	1.206	0.368	0.374	47.73	47.85	22.19	21.81
Sister 1	12.9	13.2	1.079	1.084	0.346	0.343	45.35	44.15	21.18	19.59
Sister 2	12.85	12.7	1.070	0.996	0.301	0.302	45.39	45.19	19.91	19.64
Sister 3	13.55	13.35	1.020	1.001	0.297	0.305	44.48	43.86	19.80	19.75
LSD 0.01	0.843	0.818	0.049	0.059	0.016	0.016	2.417	1.789	0.858	0.680
LSD 0.05	0.641	0.622	0.037	0.045	0.012	0.012	1.838	1.361	0.653	0.517

(KRN= kernel-rows number; KD= kernel depth; KW= 1000-kernel weight; NKPR= number of kernels per row; EL= ear length)

Hybrids Exp.3, Exp.4, ZP 606, ZP 666 and ZP 685, all with the same female parent (L1) except ZP 685 (L4 is female parent) showed the greatest value of kernel depth in both years (2018 and 2019) (Table 5). Between this trait and grain yield there is significant positive correlation (Rafiq et al., 2010) suggesting the importance of this trait in maize breeding. These results of kernel depth are confirmation of high performances of ZP 606, ZP 666 and ZP 685, and indicate that L1 and L4 might be potential donors of desirable alleles for improving kernel depth of breeding material.

Results showed that hybrids ZP 606, ZP 666 and ZP 685 had the highest 1000-kernel weight of all tested hybrids in 2018 (0.433 kg, 0.432 kg and 0.425 kg, respectively) and in 2019 (0.431 kg, 0.438 kg and 0.418 kg, respectively) (Table 5). All male parents of these hybrids belong to Lancaster heterotic group. Nonetheless, L1 (mother of ZP 606 and ZP 666) and L4 (mother of ZP 685) are carriers of desirable alleles for high values of kernel weight. This is important to know because grain yield is positively and significantly correlated with 1000-kernel weight (Reddy et al., 2012) which indicates that this trait is important trait for improving grain yield.

Experimental hybrids Exp.11 and Exp.12 showed the highest values of ear length and number of kernels per row in both 2018 and 2019 (Table 5). These results are in agreement with Begum et al. (2016) and many other researchers who found significant positive correlation between ear length and number of kernel per row. Both of these hybrids (Exp.11 and Exp.12) have the

same female parent, line L3 belonging to BSSS heterotic group. It may be concluded that this line is a source of desirable alleles for these two traits.

There is a general agreement that yield components should be used as target traits to improve maize grain yield i.e. they should be breeding criteria within phenotypic selection during development of inbred lines (Rafiq et al., 2010). The results of the yield components confirmed the best performances of the hybrids designated as the most yielding in this study. As well, these results serve as an evidence that the lines previously selected for further use in breeding programs have good performance when it comes to yield components. This goes in favor of earlier made decision that parental components (inbred lines) of best performing hybrids tested in this study should be crossed with both related and unrelated inbred lines with an aim to create new origin populations (F2 sources) or new hybrids, respectively.

Correlation among investigated traits

In addition, simple correlation coefficients among all tested traits were calculated (Table 6). The highest positive and statistically significant (at p=0.01) correlations were found between grain yield and ear length (0.712), grain yield and 1000 kernel weight (0.817), grain yield and kernel depth (0.829), ear length and number of kernels per row (0.707) and kernel depth and 1000-kernel weight (0.818). Similar results were reported by many researchers (Begum et al., 2016; Čamdžija et al., 2011). Between kernel-rows number and grain yield a positive statistically significant (at p=0.05) correlation (0.512) was also found. Negative correlation (-0.357) showed only number of kernel per row and kernel-rows number, but it was not statistically significant, which was in accordance with result obtained by Čamdžija et al. (2011). All these results confirm many widely accepted claims that yield components could be important selection criteria in maize breeding.

Table 6. Pearson's correlation coefficients between investigated traits

	KRN	KD	KW	NKPR	EL	Grain Yield
KRN	1					
KD	0.345^{ns}	1				
KW	0.264^{ns}	0.818^{**}	1			
NKPR	-0.357^{ns}	0.098^{ns}	0.29^{ns}	1		
EL	0.259^{ns}	0.283^{ns}	0.43^{ns}	0.707^{**}	1	
Grain Yield	0.512^{*}	0.829^{**}	0.817^{**}	0.323^{ns}	0.712^{**}	1

(KRN= kernel-rows number; KD= kernel depth; KW= 1000-kernel weight; NKPR= number of kernels per row; EL= ear length; ns- no significant; *-significant at p=0.05; ** -significant at p=0.01)

Conclusion

This research was conducted to provide important information in the breeding process. Based on the results of this study, some steps are suggested and some important decisions are made:

- Experimental hybrid Exp.15 is submitted for testing to the Variety Commission of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. This hybrid, as a high-yielding and a stable one, could enrich the assortment of registered medium-late maize hybrids in Serbia. First year of its testing for the purpose of being registered is going to be 2020.
- Registered hybrid ZP 685 is suggested for commercialization as one of the best performing in the present study. Results showed that this hybrid isn't only high yield, but stable as well.
- The goal of this study was also identification of superior inbred lines for use in future breeding programs. Based on the results, lines L1 (BSSS) and L4 (Independent heterotic group) are marked as carriers of the most desirable alleles for grain yield and its components examined in this study. These two lines are female parental components of the most yielding hybrids and they showed the best combining ability for grain yield and its components. Hence, L1 and L4 are chosen for crossing with an aim to create a new origin population (source) for deriving new recombinant inbred lines. Cross L1 x L4 will be a part of maize breeding program at Maize Research Institute "Zemun Polje" in 2020. Inbred lines which are going to be originated from this source are expected to produce better hybrids with Lancaster lines in future crosses.
- Inbred lines L1 and L4 are also suggested for crossing with all others Lancaster lines from MRIZP (which are not parental components of tested hybrids in this study) for deriving new experimental F1 hybrids.
- Lancaster lines L5, L6 and L7 as male parents of the most yielding hybrids (ZP 606, ZP 666, ZP 685, Exp.15 and Exp.14) should be crossed between themselves for creating new origin populations (sources) for deriving new recombinant Lancaster inbred lines.
- Hybrid Sister 1, single cross female parental component of many registered and commercial three-way cross hybrids of MRIZP, had very high and stable grain yield and as such it is the most cost effective for seed production.

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Original scientific paper

Bioecological analysis of city block greenery in Novi Sad (Serbia)

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Abstract

The aim of this paper is analysis of existing greenery and making plant inventory list for the area of the city block of Liman I in Novi Sad. In the studied area flora is mainly consisted of native deciduous trees which are common for the southeast Europe and a small percentage of introduced species. Coniferous trees are less present, and most of them belong to the mountain and humid climate belt, so that they demonstrate lower adaptability to the existing environmental conditions. The most numerous species are field elm (*Ulmus minor* Mill.) and small-leaved linden (*Tilia cordata* Mill.). The species that have shown the best adaptability to urban condition and usually urban stress are *Tilia cordata* Mill. and *Tilia tomentosa* Mnch. This kind of analysis are important for creation of the realistic inventory database which can be used for the rational green management and planning.

Key words: Key words: urban greenery, plant inventory list, city block

Introduction

Green infrastructure is a very important part of the urban ecosystem and provides many benefits to residents of populated areas (Li et al., 2016). In ever expanding communities and where urbanization is on the rise, public green spaces are of strategic importance for raising the quality of life (Chiesura, 2004). This has been especially pronounced in the last ten years, when architects and urban planners use greenery to emphasize objects, while objects emphasize the architectural features of greenery, so that together they form a unique composition. According to Olmsted (1870), no park like itself, no matter how large and well designed, provides citizens with beneficial environmental impacts, but they must already be interconnected with the surrounding housing estates. Vegetation and buildings have always had a special relationship as they represent two standards for emphasizing landscapes and as such, they have found a way to coexist (Kalen, 2007). The importance of urban greenery was recognized as early as the 19th

century, when the first benefits of greenery in conditions of high pollution were noticed due to the rapid industrialization and urbanization of cities. This has led to an increase in interest from the public and the whole society, both for the qualitative and quantitative characteristics of public green (Swanwick et al., 2003). Nevertheless, Leigh-Rutt et al. (2016) state that the functional value of greenery is more significant and greater than the decorative value itself. Green spaces within a city represent the most flexible element of the physical structure of a populated place. In order to fulfill their basic functions, green spaces are organized into a system of greenery. Block greenery plays an important role in the functioning of the block itself, and making the system of urban greenery plays an important role in the functioning of the entire city. In addition to influencing environmental conditions and creating a more favorable microclimate within the city, it effectively mitigates negative urban influences, influences visual experience and stimulates people's life activities. In the last decade, there has been an expansion of various studies into the impact of greenery on the functioning of the city and on people's lives. One such study was conducted in a laboratory to test the impact of greenery on human health at the University of Illinois. Test results show that the presence of vegetation within apartment blocks can drastically reduce crime rates. The number of thefts is 48% lower in green blocks, while the incidence of violence is reduced by 56% (Kuo et al., 2001a; 2001b). According to Van den Berg et al. (2016) residents of green areas of the city have better physical and mental health than those living in less green spaces. Greenery also significantly reduces the negative impact of urbanization, such as air pollution, increasing air temperature and noise (Kabisch et al., 2015).

Materials and Methods

Prior to the field survey, data preparation was carried out to facilitate the insertion and positioning of the objects being analyzed. The datas were taken from the GeoSrbija portal in the form of orthophoto images. Field data collection involves determination of species present, as well as the determination of biometric data and the determination of the quality of plant material. For flowering species, analysis and recording of species was also performed, as well as their representation expressed in the number of pieces per square meter. The method used to measure biometric data is according to the Anastasijević (2007). The measured parameters are: plant height, height to first branches, diameter to the brest hight, crown width, area covered and number of specimens per surface. After biometric data were collected, condition assessments were determined based on the presence of mechanical damage, which include broken branches in the canopy, dryness or truncated trunk branches, the presence of trunk rot

and thicker branches, and finally the presence of entomological damage. On the basis of all the previously determined elements that directly affect the fitness of the individual and reflect the conditions of the environment in which the plant lives, each individual is assigned a vitality score ranging from 1 to 5, followed by a decorativeness rating, which is a phenotype-based assessment and depends on the condition of the individual.

All the data obtained are entered into special Excel spreadsheets that provide objectivity in the evaluation, as well as successful comparison with other types. The Microsoft Excel spreadsheet contains biometric data and status estimates for each recorded plant. Data collection was performed in the period May-July 2019.

Figure 1. a) Location of Liman 1 in relation to the center (yellow) of Novi Sad, b) the boundaries of the tested city block

Liman I is located in the southeast of Novi Sad, on the left bank of the Danube River (Figure 1a). The residential block under study is located in the southwest of Liman I. On the north side, the block is bounded by Draga Spasić Street, the eastern boundary is Strumička Street, and in the south, it is restricted by parking along the Sunčani Quay promenade, while in the west it extends to Fruškogorska Street (Figure 1b).

In the past, the Liman was exposed to frequent flooding and deposition of river sediment composed of fine particles of sand and clay, resulting in the formation of hydromorphic soil. In the second half of the 20th century, the rapid urbanization and construction of Liman began, and a specific anthropogenic soil layer, “urbisol”, was formed by embankment. The altitude of the Liman I is 76-78 m.

Results and Discussion

Liman I is one of the settlements with increased intensity of road traffic. The block under study in the west is bounded by Fruškogorska Street, which is one of the busiest roads on Liman I. This part of the city is exposed to the effects of urban stress, and therefore there is great pressure on the surrounding urban greenery and the greenery within the block to fulfill its basic functions. Tree canopy plays a very significant role in reducing the negative impact of harmful gases, noise and wind (McGovern et al., 2016). A total of 151 tree trunks, 400 shrubs and 30 flowering plants were recorded and determined during the bioecological basis. Tables 1, 2 and 3 give the representation of all plant species. Based on analyzes of plant species representation, the relationship of individual plant groups is observed. Indigenous species with 116 trees is predominant in the trees compared to the introduced ones, of which 35 are present. In the shrub species, allochthonous species with 366 shrubs are far more prevalent, while there are 34 indigenous ones is it a good solution to limit the plant stock to native plant species only. In their study by Sjöman et al. (2016) consider the question of the sustainability of the urban ecosystem should the selection of plant material be restricted to native species. Using examples from Northern and Central Europe, they concluded that, except that the complete exclusion of allochthonous species would be difficult from a financial point of view, such a restriction threatens to disrupt the urban ecosystem, since indigenous species alone cannot meet all the requirements especially in extreme environmental conditions. As mentioned above, a total of 151 trees were recorded in the block. Of the total number of trees taken, 130 are deciduous trees, while 21 are conifers.

Table 1. Representation of deciduous and coniferous trees

Decidous /Coniferous	Species	No.	Decidous /Coniferous	Species	No.
D	*Aesculus hippocastanum* L	6	D	*Populus nigra* 'Italica'	4
D	*Acer pseudoplatanus* L.	7	D	*Robinia pseudoaccacia* L.	4
D	*Acer platanoides* L.	7	D	*Celtis occidentalis L.*	1
D	*Morus alba* L.	2	D	*Fraxinus angustifolia* Vahl.	3
D	*Betula pendula* Roth.	1	D	*Tilia platyphyllos* Scop.	4
D	*Juglans nigra* L.	2	D	*Populus alba* L.	4
D	*Tilia tomentosa* L.	7	D	*Salix alba* L.	2
D	*Quercus rubra* L.	1	D	*Quercus cerris* L.	1
D	*Ulmus pumila* L.	2	D	*Malus silvestris* (L.) Mill	1
D	*Prunus cerasifera* Ehrh. var Pissardi	8	D	*Morus nigra* L.	3
D	*Ulmus minor* Mill.	21	D	*Prunus cerasifera* Ehrh.	5
D	*Tilia cordata* Mill.	22	D	*Platanus x acerifolia* (Ait.) Wild.	3
D	*Prunus cerasus* L.	2	D	*Corylus avellana* L.	2
D	*Sophora japonica* L.	5	C	*Cupressus sempervirens* L.	2
C	*Picea abies* (L.) Karst.	5	C	*Taxus baccata* L.	6
C	*Pinus nigra* Arn.	1	C	*Abies concolor* (Gord.) Engelm.	1
C	*Pseudotsuga menziesii* Fran.	6			

A total of 400 shrubs were recorded in the analyzed block, of which 274 shrubs, 5 conifers and 121 evergreens. In addition to the listed shrubs, the block also contains a hedge formed of the kalina (*Ligustrum vulgare* L.) species, with a total length of 58.7m.

Table 2. Representation of shrub species

Decidous /Coniferous	Species	No.	Decidous /Coniferous	Species	No.
D	*Philadelphus coronarius* L.	51	D	*Forsythia x intermedia* Zab.	7
D	*Hibiscus syriacus* L.	42	D	*Deutzia scabra* Thunb.	31
D	*Spiraea x vanhouttei* (Briot) Zbl.	106	D	*Symphoricarpus albus* (L) Blake	15
D	*Syringa vulgaris* L.	3	D	*Ligustrum vulgare* L.	3
D	*Tamarix tetrandra* Pall.	16	D	*Mahonia aquifolium* (Pur) Nutt	109
C	*Juniperus horizontalis* Mnch.	5	D	*Prunus laurocerasus* L.	12

A small number of flowering species are present in the block area. The largest areas are covered by Creeping phlox (*Phlox subulata* L.), common hollyhock (*Althaea rosea* L.) and hosta (*Hosta fortunei* L.).

Table 3. Representation of flowering species

Annaul/ Biannual /Perennial	Species	No.	Annaul/ Biannual /Perennial	Species	No.
P	*Hosta fortunei* L.	6	B	*Althaea rosea* L.	1
P	*Phlox paniculata* L.	2	P	*Phlox subulata* L.	16
P	*Iris germanica* L.	5			

After recording biometric data and assessing the condition, a vitality and decorative evaluation was performed. The vitality score is determined descriptively by a score of 1 to 5 (1-poor to 5-excellent). For the most part, this grade (1-poor to 5-excellent, high decorative) depends on the condition of the individual. High decorativeness is crucial for the health of the plant individual. Analyzing the vitality of deciduous and coniferous trees, it is concluded that most species have a vitality rating of 3 and 4 (Graf. 1), while the highest number of deciduous and coniferous trees have a decorative score of 5 (Graf. 2). The trees with the highest prevalence of high decorativeness and vitality ratings are *Tilia cordata* Mill. and *Tilia tomentosa* Mnch. In the study of Thomsen et al. (2016), *Tilia cordata* Mill. is one of the most represented species in Danish cities.

Graph. 1. i 2. Graphical representation of the percentage share of the vitality and decorativeness of deciduous and coniferous trees

The analysis of the vitality of the deciduous, conifer and evergreen shrubs concludes that most species have a vitality rating of 4, while the complete absence of shrubs with grades 1 and 5 occurs. Analyzes of decorative grades lead to the result that the highest number has a rating of 3. The species with the highest prevalence of high decorative and vitality ratings are English dogwood (*Philadelphus coronarius* L.), Vanhut sparrow (*Spiraea x vanhouttei* (Briot) Zbl.) and English laurel (*Prunus laurocerasus* L.). Based on the analyzes of vitality and decorativeness, it was concluded that all flowering species have high ratings. No drastic interventions are needed in the studied area for the revitalization of the plant stock, because, based on the analysis, a large part of the plant material is in good condition. The suggestion of woody and shrubby species for replacement and removal was made on the basis of the state of vitality and decorativeness. Among the species with low vitality and decorative characteristics, the most commonly encountered are the elm trees (*Ulmus minor* Mill.) and the cherry plum (*Prunus cerasifera* Ehrh. Var Pissardi). Shrub species, with excelent vitality are English dogwood (*Philadelphus coronarius* L.), tamarix (*Tamarix tetrandra* Pall.) and the Syrian rose (*Hibiscus syriacus* L.) so that their numbers can be increased. With floral species, no removal or replacement is required as all individuals are in good condition and with a high degree of decorativeness. The greenery within the blocks is intended primarily for the tenants of the block, but the circle of users is usually expanded. Apart from being intended for recreation, social gathering, human interaction with nature, the importance of these surfaces for the health of the users is of great importance.

Numerous laboratory and field tests have shown that human contact with greens has a positive effect on mood and reducing stress levels. The results show that staying in a green space has both a short-term and a long-term positive outcome (Barton et al., 2010). Special experimental methods have shown that staying in a green space helps in recovery from psychic trauma and stress much more efficiently than in a green one. Greenery has the effect of accelerating positive emotions and reducing negative ones (Berto, 2014). In addition to encouraging faster

recovery, exposure to greens affects the concentration and intensification of the thought process, the rejuvenation of the organism, and the creation of a sense of pleasure and serenity (Kaplan, 1985). In research and practice, many plant species have been shown to possess gaseous active substances - phytoncides, which destroy pathogenic microorganisms or prevent their development. That is why such plants are called phytocidal plants or natural antibiotics (Jovanovic, 2007). Attention to this characteristic of plants was first drawn by Tokin (1951). His research has shown that 1 ha of coniferous forests excrete about 4 kilograms during the day, while the same area under the leaves about 2 kilograms of volatile organic matter with phytoncide. Due to the effect of phytoncid in $1m^3$ of forest air there are only 200-300 bacteria, while in the air of bigger cities there are 200-250 times more. Gorshenin and Shvidenko (1977) give the following groups of plants in relation to phytocides: very phytoncid, strongly phytoncid, medium phytoncid, poorly phytoncid and least phytoncid species. In the area of the studied block, species from the following groups are represented: from the group of very phytoncid plants there is one species - Norway maple (*Acer platanoides* L.), from the group of strongly phytoncid species there are silver birch (*Betula pendula* Roth.), spruce (*Picea abies* (L .) Karst.), creeping juniper (*Juniperus horizontalis* Mnch.) and common hazel (*Corylus avellana* L.), while small-leaved linden (*Tilia cordata* Mill.), lilac (*Syringa vulgaris* L.), white fir (*Abies concolor* (Gord.) Engelm.), London plane tree (*Platanus x acerifolia* (Ait.) Wild.) and Horse chestnut (*Aesculus hippocastanum* L.). Some plants in the group of flowering species also have a devastating effect on bacteria and fungi, reflecting their particular importance. In addition to the aforementioned positive effects that greenery has on the quality of life in the city, there are many benefits that green spaces offer to their customers. That is why it is necessary to plan and design green spaces with special care, and to provide the existing ones with adequate care and to manage them rationally.

Conclusion

The area of the apartment block on Liman I belongs to the habitat of the willow and poplar blue forests. Today, in the area of the studied block, the most prevalent taxa are characteristic of temperate continental climate, with some specifics that are borne by the proximity of the Danube and Fruška Gora. Much of the dendrological material is made up of native foliage. The most numerous species are field elm (*Ulmus minor* Mill.), small-leaved linden (*Tilia cordata* Mill.), endemite of the Balkan Peninsula, Horse chestnut (*Aesculus hippocastanum* L.), and cherry plum (*Prunus cerasifera* Ehrh.). The species that have shown the best results in response

to urban stress are *Tilia cordata* Mill. and *Tilia tomentosa* Mnch. Most of these trees are located in a tree line along Fruška Gora Street.

The coniferous trees are less present, which generally shows a lesser degree of adaptation to the existing ecological conditions in the block, above all showing intolerance towards urban conditions. This applies primarily to the coniferous Douglas-fir (*Pseudotsuga menziesii* Fran.), which belongs to the wetter Pacific zone, and to the Norwey spruce (*Picea abies* L.), which belongs to the colder, mountain climate and is resistant to aerosol pollution. Of the shrub plants, mostly allochthonous species are present, which are fully adapted to our climate. The most abundant deciduous species are English dogwood (*Philadelphus coronarius* L.), Syrian rose (*Hibiscus syriacus* L.) and Vanhut sparrow (*Spiraea x vanhouttei* Briot.), while conifer and evergreen shrubs are significantly less prevalent. The decorative and vitality of some shrubs is impaired due to the difficult care and maintenance in inaccessible parts. Floral species occur most commonly in the form of flower spots or as solitaires and are located in front of the objects themselves. They are taken care of by the residents of the block, which shows the citizens' interest in preserving and maintaining plant material and the need of the population for these urban oases. It is necessary to enrich the plant stock, both indigenous and introduced species, as there is a risk that the existing floral elements may not meet all the requirements required by the city. By introducing evergreen and conifers, seasonal dynamics will be achieved. The analysis revealed that the plant material reached its biological optimum, with predominantly high grades of vitality and decorativeness. Poor results are shown by species that have not fully adapted to the existing conditions prevailing within the bloc. In certain parts of the block, due to the closed type of building construction, shadows appear throughout most of the day, and some plants need to be replaced with species adapted to such conditions. Entomological and phytopathological lesions have been reported in small numbers and smaller plant areas have been affected, and the affected plant parts need to be treated or removed to prevent further spread.

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Original scientific paper

The presence of leafminers on different apple varieties in the location Kula (East Sarajevo)

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Abstract

Lepidopteran leaf miners are economically important pests in areas where apple are grown. The damage are caused by larvae that are feed on the leaf parenchyma below the epidermis, leaving empty couloirs, or "mine" of different shapes and sizes. The presence of mines on leaves reduces assimilation and transpiration surface, which adversely affects the yield, fruit quality and, in general, physiological condition of plants.

The presence of leafminers on different apple varieties (Idared, Jonagold, Golden Delicious, Gloster, Melrose, Elstar and Granny Smith) was examined in 2018 in intensive plantations in the location Kula. Six species of leafminers from four families was determined.

Family Lithocolletidae is presented with three species: *Lithocoletis blancardella* Fabricius, *Lithocoletis corylifoliella* Haworth, *Callisto denticulella* Thunberg and family Lyonetiidae with one species: *Lyonetia clerkella* Linne. Also, one species was belong family Leucopteridae: *Leucoptera malifoliella* (Costa (1836)) and *Stigmella malella* Stainton from family Nepticulidae.

Six species of leafminers were determined. The highest number of damaged leaves was found on the cultivar Idared, and the smallest number was found on the cultivar Gloster. The most numerous species was *L. malifoliella*, and the least numerous was *L. corylifoliella.*

Keywords: leafminers, apple, East Sarajevo.

Introduction

Apple is one of the most important fruit crop that is exposed to the attack of harmful insects throughout the vegetation, among the order Lepidoptera leafminers have a great significant. Importance of these pests and their negative impact on fruit production, started to increase

during end of the 60´s in the XX century. Leafminers became a serious problem, particulary in plantations in most European countries (Dimić, 1964, 1968; Ciglar, 1988; Kereši and all., 2016). The presence and importance of species isn't the same in all areas of their distribution. In Europe and Asia, the most harmful is *Leucoptera malifoliella* (Grichanov et al., 1994). In Spain, the most prevalent is *Lithocolletis blancardella* (40,7%), then following *Leucoptera malifoliella* (17,3%), *Stigmela malella* (15,3%), *Lyonetia clerkella* (13,8%), *Lithocolletis corylifoliella* (11,4%) and *Callisto denticulella* (1,5%) (Minarro et al., 1988). In recent years, the significant damages in Romania's orchards have caused *L. corylifoliella,* while in Bulgaria, *Leucoptera malifoliella* is dominant and the most important species of leafminers on apple (Andreev et al., 2001). In the former Yugoslavia, a mass phenomenon leafminers occurred in the late 70´s in XX century, when they became a serious problem. In Serbia, leaf miners, the most prominent of which is *Leucoptera malifoliella,* have become an economically important pest of apple in recent years. Distribution and harmfulness caused by leafminers were studied in Bosnia and Herzegovina during the 60´s, when a rapid increase in population density and damages were registered. In that period, a total of 45 species of leafminers were found, out of which in Sarajevo and its wider area 5 species was recorded on the apple from families Lithocolletidae and Coleophoridae (*Lyonetia clerkella, Leucotera scitella, Lithocolletis blancardella pomifoliella, Coleophora prunifoliae* and *C. hemerobiell*a (Dimić, 1964). In the area of East Sarajevo, the presence of six species of leaf miners has been identified: *Leucoptera malifoliella, Lithocolletis blancardella, Lithocolletis corylifoliella, Lyonetia clerkella, Stigmella malella, Callisto denticulella* (Tešanović and Spasić, 2007).

Having in mind that leafminers are economically important pests of apple, the aim of this study was to register their presence, to detect the most numerous species on different varieties, as well differences in their number.

Materials and Methods

The study was conducted in 2018. in the field, in intensive plantations in the location Kula on seven apple cultivars (Idared, Jonagold, Golden Delicious, Gloster, Melrose, Elstar and Granny Smith) and in the laboratory of the Agricultural faculty in East Sarajevo. Samples of apple leaves were taken ten times from May to the end of October for leafiminers presence in all studied varieties. Each time 100 randomly sampled leaves were taken from each apple cultivars (total of 7000 leaves were inspected during research). All sampled leaves were observed in laboratory for presence of mines as well the type of mines. Number of leaves with mines was

calculated. Preadult stages of insects who were located in the mines, were reared to adult stadium. The rearing was in plastic containers and Petri dishes with a layer of cellulose wadding on the bottom of which is occasionally added yeast in order to maintain the freshness of the leaf . Identification of species was based on morphological characteristics of the adults and shapes of mines, followed by keys and appropriate entomological literature (Balachowsky 1966, Hering, 1957).

Results and discussions

Six species of the Lepidopteran leaf miners belong to four family were identified in association with seven apple cultivars. These results according to the literature data about presence leafminers on apple (Almaši, 2004). From family Lithocolletidae, three species were found: *Lithocoletis blancardella* Fabricius, *Lithocoletis corylifoliella* Haworth and *Callisto denticulella* Thunberg. From family Lyonetidae results of our examination showed presence of *Lyonetia clerkella* Linne. From family Leucopteridae, it was determined *Leucoptera malifoliella* (Costa (1836)), while *Stigmella malella* Stainton belongs to family Nepticulidae. All six species of leafminers were found on all apple varieties. The number of damaged leaves, varied by the species of leafminers and inspected varieties (Table 1).

Table 1. The number of mined leaves on apple by species of leafminers

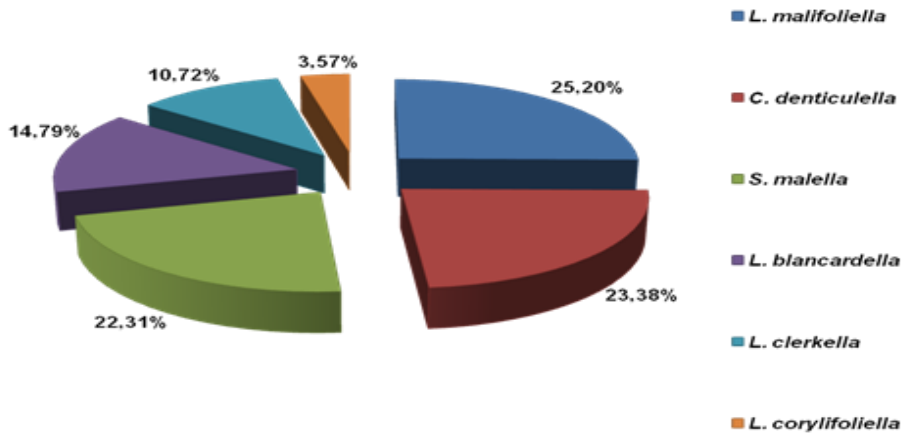
Species of leafminers		Total number of mines leaves	The number of mines leaves on varieties						
			Idared	Jonag.	G.del.	Mel.	G.Smith	Gloster	Elstar
1.	*Leucoptera malifoliella*	402	75	53	43	58	49	43	81
2.	*Lyonetia clerkella*	171	30	25	17	30	28	20	21
3.	*Lithocolletis blancardella*	236	54	31	29	37	21	31	33
4.	*Lithocolletis corylifoliella*	57	8	9	11	10	12	2	5
5.	*Stigmela malella*	356	82	47	45	46	48	43	45
6.	*Callisto denticulella*	373	75	57	41	62	44	40	54
The total number of mined leaves by species		**1595**	**324**	**222**	**186**	**243**	**202**	**179**	**239**
%		**22,78**	**20,31**	**13,91**	**11,66**	**15,23**	**12,66**	**11,22**	**14,98**

Out of a the total of 7000 sampled leaves, mines were identified at 1595 leaves or 22,78%.

In relation to the total number of damaged leaves (1595 leaves), the highest number of mined leaves was found on the variety Idared (324 leaves or 20,31%) where *S. malella* damaged 82

leaves or 5,14%, which was the most numerous species on this variety. These results do not correspond to the findings by Tešanović (2009) where *L. clerkella* was the most numerous species on this variety. This is followed by the Melrose with 243 damaged leaves or 15,23% where *C. denticulella* was the most numerous (62 leaves), while *L. corylifoliella* (10 leaves) caused the least damage on this variety. The least damaged leaves were on the Gloster (179 or 11,22 %) where *L. malifoliella* and *S. malella* caused the most damaged leaves, while the smallest number of leaves was damaged *L. corylifoliella* (2 leaves).

In relation to the total number of damaged leaves, the most abundant species is *L. malifoliella* whose mines were registered in 402 leaves or 25,20% which is also dominant and economically the most important leafminer on apple in region Bulgaria, Croatia and Serbia (Stamenković, 2000; Andreev et al., 2001; Magud, 2002). After that, followed by *C. denticulella* (373 leaves or 23,38%) and *S. malella* with 356 damaged leaves or 22,31%, which are not considered economically important pest in according to the literature data about their presence in apple orchards (Almaši, 2004).



Graph 1. Percentage presence leafminers on apple varieties in the location Kula

The less numerous were *Lithocolletis blancardella* with 236 leaves (14,79%) and *Lyonetia clerkella* with 171 damaged leaves (10,72%). The lowest number of damaged leaves was caused by *Lithocolletis corylifoliella* (57 leaves or 3,57%), according literature data about smaller presence this species in apple orchards (Maceljski, 2002) (Graph.1).

Conclussion

Six leafminer species were found: *Lithocoletis blancardella* Fabricius, *Lithocoletis corylifoliella* Haworth, *Calisto denticulella* Thunberg, *Lyonetia clerkella* Linne, *Leucoptera malifoliella* (Costa (1836)) and *Stigmella malella* on the seven apple varieties (Idared, Jonagold, Golden Delicious, Gloster, Melrose, Elstar and Granny Smith) in the location Kula. The highest number of damaged leaves was found on the variety Idared, and the smallest number was found on the variety Gloster.

The most numerous species was *L. malifoliella*, and the least numerous was *L. corylifoliella.*

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Original scientific paper

Effect of irrigation on fruit quality and yield of 'Red Cap'apple cultivar depending on crop load

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Abstract

Effect of irrigation on fruit quality and yield of Red Cap apple variety depending on crop load were studied. The aim of the paper is to determine how many apple fruits can be left on trees in third vegetation, growth under different irrigation treatments (without irrigation, normal irrigation - control and increased irrigation - double irrigation rate). For the Red Cap variety in the third year after planting, in order to achieve good fruit quality and satisfactory yield, it is necessary to leave a maximum of 25 fruits per tree, or 3.5 fruits per $cm^2$of trunk cross-sectional area (TCSA). Different irrigation treatments have influence on the fruit size, the proportion of first class fruits, as well as the degree of ripeness of the fruits. Increased irrigation during the months of July and August affects these parameters, but to a lesser extent than the crop load. The largest irrigation effect on the increase of fruit size had trees from crop load III, who had 5,5 fruits per $cm^2$of TCSA. Increased irrigation during July and August had no statistically significant effect on tested parameters on trees that had less than 4,5 fruits per cm^2 of TCSA and trees that had more than 6,5 fruits per cm^2 of TCSA.

Key words: apple, irrigation, crop load, yield, fruit quality

Introduction

Successful cultivation of apple orchards in Serbia requires about 750 mm of precipitation per year (Mišić, 1994). Whether this quantity will actually be sufficient depends on the temperature of the air, the type of soil, the distribution of rainfall during the year, and the characteristics of the plantation itself (number of plants per ha, method of soil maintenance, crop load, etc.). The territory

of Serbia is characterized by a continental pluviometric regime (Milosavljević, 1985). According to this regime, the greatest amount of precipitation is in June and November, and the lowest in February and October. The most critical months in terms of water supply are July, August and September, since the most important varieties of apple fruits ripen in late August, September and October. There is a water deficit almost every year in our country, in this period, as these are also the warmest months (besides June). We have to make up this deficit through irrigation, if we want successful production. Flowering is shorter and the fruit set is lower, if there is a water deficit in the first part of the growing season. The lack of water leads to a massive drop of the fruits before harvesting, while remaining fruits remain smaller and ripen earlier in the second part of the growing season. Also, due to the lack of water, bud differentiation for the next year has been reduced. In addition to water, the fruit load on the tree has a great influence on the quality of the fruit, its mass, as well as the differentiation of flower buds for the next year. Excessive yield leads to a significant decrease of fruit quality and significantly reduces the yield potential for the next year. It is well known that in conditions of "dry" fruit growing, one of the ways for the fruits to reach the proper size is to reduce the yield, that is, to reduce the number of fruits left on the tree. The aim of the paper is to determine how many apple fruits can be left on trees in third vegetation, growth under different irrigation treatments (without irrigation, normal irrigation - control and with increased irrigation - double irrigation rate).

Material and Methods

The experiment was carried out in the village of Novi Slankamen, Inđija municipality. Apple orchad has been planted in the autumn of 2016. The planting distance is 3.25 meters between the rows, and the distance within a row is 0.62 meters. The drip irrigation system was installed before planting. Two-year-old nursery trees with more than five feathers were used as planting material. The variety being tested is Red Delicious (Red Cap Valtod clone) grafted on M26 rootstock. Granny Smith variety is used as a pollinator. Polinator variety was planted along each pole. Tests were conducted in the spring and summer of 2019 while the trees were in third vegetation.

The size of trees was uniform and potential yield. In May 2019, after manual thinning, a trial was set up with varying degrees of crop load: load I (25 fruits per tree), load II (30 fruits per tree), load III (40 fruits per tree) and load IV (50 fruits per tree). The average air temperature in May was 15.0 ^{o}C, in June23.4, in July 23.3 and in August 24,8 ^{o}C. The highest rainfall was in May (130 mm) and the least in August (20 mm). In June rainfal was 120.8 mm, in July was

32,7 mm. Three different irrigation treatments were established during July and August: deficit irrigation (no watering), control irrigation (one lateral in a row) and double irrigation rate (two laterals in a row). In each irrigation regime, they were trees with all four crop loads treatments. Each treatment was represented by 10 trees (120 trees in total). Each plant was irrigated with 3.2 liters of water during the day in July and 4.8 liters of water during the day in August (control irrigation).

The plant was irrigated in July with 6.4 liters of water during the day in July, and 9.6 liters of water during the day in August (double irrigation rate). Fruits from all treatments were picked at the same time. Their classification was performed according to the diameter of the fruit into three categories: I class (diameter over 70 mm), II class (diameter between 65 and 70 mm) and III class (diameter below 65 mm). Ten randomly selected fruits from every tree were immediately measured for fruit firmness, soluble solids content, titratable acidity, and starch index. Fruit firmness was determined on two positions, red blushed and unblushed portion areas, at the equator of each fruit usinga press-mounted Effegi penetrometer (model FT 327,Alfonsine, Italy) with an 11.1-mm head (HARKER et al.1996).

Total soluble solids (TSS) was determined using a hand held refractometer with automatic temperature compensation (ATC-1 Atago, Tokyo, Japan). Starch pattern index (SPI) was determined with 0.1N iodine solution using the scale 1–10 (1/early ripe, 10/fully ripe). Titratable acidity (TA) was measured by titration with 0.1N NaOH, and expressed as percent of malic acid. Data were subjected to analysis of variance (ANOVA). Mean separation was done by Duncan's multiple range test at 5 % levels of significance. Statistical procedures were performed using STATISTICA V5.5A STATSOFT.

Results and Discussion

Fruits were harvested on 31 of August. The number of fruits harvested per tree depending on load and irrigation regime was shown in table 1. The highest number of fruits was harvested from trees that were most loaded with fruits (load IV) and least from trees with load I.

The differences were statistically significant. On average, regardless of the degree of loading, the effect of different irrigation regimeon the number of harvested fruits per tree was not statistically significant. Also, the effect of different irrigation at each individual load level on the number of fruits harvested is not statistically significant.

Table 1. Number of fruits per tree

Irrigationtreatment	Crop load (number of fruits per tree)				Average
	I	II	III	IV	
Without irrigation	23.8a	29.4a	36.4a	47.0b	34.1a
Control	22.0a	27.2a	36.6a	42.0ab	31.9a
Double irrigation rate	24.0a	29.8a	36.4a	38.4a	32.1a
Average	23.3a	28.8b	36.5c	42.5d	

If we show the number of harvested fruits per cm^2 of TCSA, we obtain similar relationships as for the total number of harvested fruits per tree (table 2). The number of fruits harvested per cm^2 of trunk cross-sectional area was the lowest at load I (3.49) and the highest at load IV (6.69). The differences were statistically significant.

Table 2. Number of fruits per cm^2 of TCSA

Irrigation treatment	Crop load (number of fruits per tree)				Average
	I	II	III	IV	
Without irrigation	3.40a	4.51a	5.59a	6.85a	5.09a
Control	3.54a	4.83a	5.38a	6.55a	5.08a
Double irrigation rate	3.54a	4.66a	5.98a	6.66a	5.21a
Average	3.49a	4.67b	5.65c	6.69d	

The tress at load I had the highest fruit weight (166.3 g), while the trees at load IV had the smallest fruit weight (113.1 g). The difference in fruit weight between load III and IV is not statistically significant (table 3).

Depending on the irrigation regime, fruits on non-irrigated trees had statistically significantly lower fruit weight than fruits whose trees double irrigated.

Regarding the effect of irrigation at each crop load individually, a statistically significant difference only occurs at load III, between trees without irrigation (107.6 g) and double irrigated trees (137.8 g). The effect of irrigation on the fruit weight is only significant in trees at crop load III.

Table 3. Effect of irrigation on the fruit weight (g) depending on crop load

Irrigation treatment	Crop load (number of fruits per tree)				Average
	I	II	III	IV	
Without irrigation	164.0a	131.2a	107.6a	103.5a	126.6a
Control	162.5a	147.7a	117.4ab	117.0a	136.2ab
Double irrigation rate	172.5a	146.1a	137.8b	118.8a	143.8b
Average	166.3c	141.6b	120.9a	113.1a	

Depending on the number of fruits left on the tree, the highest yield was obtained on trees with load IV (4, 71 kg) and the lowest on trees with load I (3, 88 kg) (table 4).

Table 4. Effect of irrigation on the yield per tree (kg) depending on crop load

Irrigation treatment	Crop load (number of fruits per tree)				Average
	I	II	III	IV	
Without irrigation	3.89a	3.86a	3.89a	4.86a	4.12a
Control	3.59a	3.98a	4.28ab	4.90a	4.19a
Double irrigation rate	4.16a	4.39a	5.03b	4.37a	4.49a
Average	3.88a	4.07a	4.40ab	4.71b	

There was no statistically significant difference between load I, II and III. Yield per tree was the highest in double irrigation treatment, but this increase is not statistically significant. Many studies have shown that deficit irrigation reduces final fruit size and yield of apples (Ebel et al., 1993, Mpelasoka et al., 2001).

A statistically significant difference only occurs with load III, where the double irrigation treatment had a significantly higher yield (5.03 kg) than the non-irrigation trees (3.89 kg).

From the point of view of placing fruit on the market, only fruits over 70 mm in diameter have economic significance. The Effect of irrigation on the proportion of fruit with diameter larger than 70 mm (%) depending on crop loa is shown in table 5. The highest proportion of first class fruits (diameter over 70 mm) have trees at load I (over 84%), and the smallest trees at load IV (only 23,6%). The difference is statistically significant. Trees that are double-watered, on average, have the highest proportion of fruits over 70 mm (58.9%), and this difference is statistically significant compared to non-watered trees (40.3%).

Table 5. Effect of irrigation on the proportion of fruit with diameter larger than 70 mm (%) depending on crop load

Irrigation treatment	Crop load (number of fruits per tree)				Average
	I	II	III	IV	
Without irrigation	88.7a	32.6a	24.4a	15.4a	40.3a
Control	71.9a	67.3b	26.1a	27.1a	48.1ab
Double irrigation rate	92.7a	59.6b	54.9b	28.3a	58.9b
Average	84.4c	53.2b	35.1a	23.6a	

Regarding the effect of irrigation at each load individually, a statistically significant difference in the proportion of first-class fruits occurs at load II between normal irrigation (67.3%) and

without irrigation (32.6%), as well as at load III, between double irrigation rate (54.9%) and normal irrigation (26.1%) and no irrigation (24.4%).

The quality of the fruit, in addition to its external properties, depends on the chemical composition, and for this reason tables 6 and 7 show the content of total soluble solids (TSS)and total acids (TA), depending on the tree load and irrigation regime.

Table 6. Effect of irrigation on total soluble solids (TSS) in fruit depending on crop load

Irrigation treatment	Crop load (number of fruits per tree)				Average
	I	II	III	IV	
Without irrigation	12.4	10.8	12.1	10.2	11.38 a
Control	11.1	13.8	10.7	11.6	11.80 a
Double irrigation rate	12.5	11.7	11.3	12.3	11.95 a
Average	12.00 b	12.10 b	11.37 a	11.37 a	

Trees that were less loaded with fruits had higher content of soluble solids than trees that had a higher number of fruits (table 6).In our research, we did not find that irrigation has an effect on total soluble solids (TSS) in fruit, unlike the Djurovic at al. (2015) where the highest total soluble solids (TSS) was measured in fruits whose trees were not irrigated

The effect of irrigation intensity and different crop loadon the content of total acids in the fruit was not found (table 7). Kilili at al. (1996) also found that deficit irrigation did not affect the total acids content in fruit.

Table 7. Effect of irrigation on total acids (TA) in fruit (%) depending on crop load

Irrigation treatment	Crop load (number of fruits per tree)				Average
	I	II	III	IV	
Without irrigation	0.20	0.17	0.17	0.23	0.19 a
Control	0.19	0.21	0.22	0.19	0.20 a
Double irrigation rate	0.18	0.23	0.19	0.20	0.20 a
Average	0.19 a	0.20 a	0.19 a	0.21 a	

Trees that have more fruits had a higher firmness of the fruit (Table 8).The fruits from load IV had an average fruit firmness of 115 N, while the fruits from load I had a fruit firmness of 92 N.

An increase in fruit firmness in load IV may be an indirect effect of fruit size reduction. Smaller fruits tend to be firmer (Mpelasoka et al., 2000, Volz et al., 2003). Different irrigation had no significant effect on fruit firmness.

Table 8. Effect of irrigation on fruit firmness (N) depending on crop load

Irrigation treatment	Crop load (number of fruits per tree)				Average
	I	II	III	IV	
Without irrigation	81	102	104	116	101 a
Control	93	105	111	116	106 a
Double irrigation rate	102	121	113	114	102 a
Average	92a	109 b	109 b	115bc	

The content of starch in the fruit is a very significant indicator of the degree of ripeness of the fruit. From table 9, we can conclude that at the time of harvest, the more mature were the fruits from trees that were more loaded with fruits, compared to those that had less fruit.

The effect of irrigation on the degree of ripeness of the fruits is also evident. Increased irrigation slows down the ripening of fruits, so at the time of harvest, the least ripe fruits were from trees that were double irrigated (starch content 2.71) and the most ripe fruits from treeswithout irrigation (starch content 3.08)

Table 9. Effect of irrigation on starch pattern index (SPI) depending on crop load

Irrigation treatment	Crop load (number of fruits per tree)				Average
	I	II	III	IV	
Without irrigation	2.33	3.33	3.50	3.17	3.08 b
Control	2.17	3.00	3.50	3.00	2.92 ab
Double irrigation rate	2.83	2.17	2.83	3.00	2.71a
Average	2.44 a	2.83b	3.28 c	3.06 d	

Đurovic et al. (2015) also found that deficit irrigation increases fruit ripening. Leib et al. (2005) did not found a significant effect of treatment on the starch pattern index for ‘Fuji’.

Conclusion

Different crop load levels of trees and different irrigation treatment have a significant impact on the yield and quality of the fruit of the Red Kap apple variety in the third year after planting. Different loading of trees with fruits has a significant effect on the yield in kg per tree, on the size of the fruit, on the proportion of first class fruits, as well as on the degree of ripeness of the fruits. For the Red Kap variety in the third year after planting, in order to achieve good fruit quality and satisfactory yield, it is necessary to leave a maximum of 25 fruits per tree, or 3.5 fruits per $cm^2$of TCSA. Different irrigation treatments have influence on the size of the fruit, on the proportion of first class fruits, as well as on the degree of ripeness of the fruits. Increased irrigation during the months of July and August affects these parameters, but to a lesser extent

than the crop load. The largest irrigation effect on the fruit size increase had trees from load III, who had 5,5 fruits per $cm^2$of TCSA. On trees that had less than 4,5 fruits per $cm^2$of TCSA and those with more than 6,5 fruits per $cm^2$of TCSA increased irrigation during July and August had no statistically significant effect on tested parameters.

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Original scientific paper

The impacts of microbial fertilizer application on the health condition and quality of the tomato fruit

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Abstract

The research aims to obtain the results of the impact of an application of microbial fertilizers in combination with mineral fertilizers on the health and chemical composition of tomatoes. The study was conducted in 2013 and 2014 on the basic experimental plots in two variants (1. inorganic fertilizer and chemical crop protection + microbial fertilizer Slavol, 2. inorganic fertilizer and chemical crop protection-control). In the year 2013, there was no significant presence of diseases and pests, except for the occurrence of *Tetranychus urticae.* In early June 2014, the symptoms of *Phytophthora infestans* appeared only in the control variant. In both years of the study, the average total soluble solids, total acidity, and content of N, Mg, K, Cu were higher in the variant with applied microbial fertilizer. Content of nitrates, lycopene, P, Fe and Zn varied depending on the year and treatment. The application of microbial fertilizer has contributed to better health, and the contents of some tomato fruit quality parameters were increased.

Key words: tomato, Slavol, diseases, pests, quality

Introduction

In the region of Herzegovina, vegetable production has a long tradition, and tomatoes (*Lycopersicon esculentum L.*) are the most commonly grown vegetable species in the protected area. Tomato is an annual herbaceous plant from the *Solanaceae* family. The fruit in its technological maturity is used for nutrition.

Tomato is a rich source of lycopene, vitamin C, but also contains a specific amount of vitamin B, copper, iron, potassium, magnesium, calcium, and sodium.Tomatoes are usually grown in a protected area in the conventional way, and the application of large amounts of pesticides and fertilizers is often irrational which significantly affects the quality and fertility of agricultural land, have harmful effects on the environment, food quality and human health (Marinković et al. 2014). By applying different fertilization systems of organic, mineral, organic and mineral fertilizers together, high yields of good quality can be achieved in greenhouse vegetable production (Bogdanović et al., 2014). The application of the combination of organic and mineral fertilizers is a widespread practice in the production of vegetables in protected areas that gives satisfactory results. In addition to organic fertilizers, mineral fertilizers are used as well as commercial organic fertilizers, microbiological and phyto-eco products.

The application of microbial fertilizers in crop production contributes to reduced use of mineral fertilizers, which has economic benefits and leads to safer production. Microbiological preparations used as microbial fertilizers contain selected cultures of microorganisms that are used to inoculate seeds and seedlings or are applied to soil to intensify microbial processes that increase the content of nutrients that plant can absorb (Đurić, 2008).

The application of microbiological fertilizers increases the number of microorganisms in the soil and intensifies the microbiological processes (Govedarica et al., 2004). The optimal dose of mineral fertilizers favorably affects the microbial processes, while excessive amounts of nitrogenous fertilizers inhibit the nitrogen fixation (Milošević et al. 2003). Microbial fertilizers, such as Slavol, help the plant to collect useful substances more efficiently, using natural resources to the maximum without the soil pollution. It assists plant nutrition by converting organic and hard-to-digest components into lighter forms that the root system can absorb (Dolijanović et al., 2014). Slavol is a microbial fertilizer containing microorganisms that stimulate plant growth (*Bacillus megatherium, Bacillus licheniformis, Bacillus subtilis, Azotobacter chroococcum, Azotobacter vinelandii, Derxia sp*).

When used in vegetable cultivation, it is applied after sprouting, in the period of formation of permanent leaves, the stage of intensive growth, the flowering phase, the phase of fruit formation and the maturation phase.

The use of microbial fertilizer Slavol has shown a positive effect on plant production (Đorđević et al., 2005; Najdenovska et al., 2013) through the N and P nutrition, production of plant hormones, increasing the absorption of Fe and other microelements and indirectly acting as inhibitor of plant pathogens as increasing their resistance to disease and stress. Although microbial fertilizers are widely used in agriculture, their application still requires research,

investment and technological development to understand their impact on soil, flora and fauna as well as on human health (Carvajal-Muñoz and Carmona-Garcia, 2012).

The paper aims to contribute to the improvement of greenhouse tomato production, considering the possibility of rational use of crop protection agents and fertilizers to improve the health and quality of tomatoes.

Material and Methods

The research was carried out in the village Hodbina (43°14´N17°51´E), near Mostar (Bosnia and Herzegovina). Cultivar tomatoes Hector F1 are planted in a greenhouse of 360 m^2 (height of the object is 3,5 m) during the two growing seasons, 2013 and 2014. The planting in 2013 was done on April 3, while in 2014, the tomatoes were planted on March 11, planted in two-row strips, wherein the spacing between the rows was 80 cm, distance in the row 50 cm and 40 cm between rows. During the growing season, crop care was done by applying certain agricultural practices. The experiment was set up in a randomized block schedule in 4 repetitions with two variants on the basic experimental plots:

Variant 1. Inorganic fertilizer and chemical crop protection + microbial fertilizer Slavol

Variant 2. Inorganic fertilizer and chemical crop protection - Control

Based on the results of the soil analysis, a fertilization program was developed during tomato cultivation. In both variants during both years were used NPK fertilizers (MS Biotech, Italy) with formulations 13:40:13 (30 kg / ha); 20:20:20 (30 kg / ha); 15:30:15 (30 kg / ha); 15:15:15 (50 kg / ha); and 15:05:30 (50 kg / ha) 0-15; 15-30; 30-45; 45-60; and 60-90 days after planting. Tomato crop protection from diseases and pests was mainly applied by using preventative measures. Blue and yellow sticky boards were placed in the greenhouse to reduce and detect the pests. In the first variant in addition to the listed fertilizers and crop protection, the microbial fertilizer Slavol (Agrounik d.o.o.) was used. The first application was made during planting by immersing the roots of the seedlings. Each subsequent Slavol application (10ml / 1l water) was applied after ten days by watering the plants through the root system.

During both years of research, the harvesting was carried out successively, in 2013 the first harvesting was in mid-June, and 2014 at the beginning of June and lasted until mid-August.

Tomato fruit samples were taken for the chemical analysis from the second branch at the time of the fourth harvest. The average sample was taken from 10 plants (repetition) weighing 1 kg. The samples were packed in plastic bags and delivered to laboratory in portable refrigerator where chemical analysis of tomato fruit was evaluated and included: a total soluble solids, total

acidity (%) determined by titration with 0.1 M NaOH (OECD, 2009), vitamin C (%) determined by iodometric titration (Haan, 2015), nitrogen content (%), nitrate, lycopene (mg / 100g) using Hyman et al. (2004) method. The content of P, K, Mg, Fe, Cu and Zn were determined with the use of ICP-MS technique.

The value of all measurements are presented by means with a two-factor analysis of variance (ANOVA) for all aspects of impacts of microbial fertilizers in the years 2013 and 2014, a total soluble solids, total acidity, nitrates, vitamin C, lycopene, N, Mg, P, K, Fe, Zn and Cu. The lowest statistical significance that can exist in comparisons was determined using the Tukey Kramer test. Pearson correlation analysis was performed as a parametric analysis to analyze the correlation between the studied parameters.

Results and Discussion

Health condition of tomatoes

In June 2013, there were symptoms of an attack of the two-spotted spider mite (*Tetranychus urticae*), while there were no other pests and diseases. According to Spasov et al. (2006), organic fertilizers may reduce pest attack (*Aphididae, Helicoverpa armigera Hb., Trialeurodes vaporariorum Westwood., Thripidae* and *Leptinotarsa decemlineata Say.*). The effects of balanced crop nutrition of tomatoes on the presence of pests is shown by research by Kastori (2014), that states that excess magnesium can increase the presence of *Tatranychus sp.* in tomato cultivars.

At the beginning of June 2014, there were symptoms of the presence of *Phytophthora infestans* only in the control variant. Wang et al. (2000) point out that tomatoes grown with organic fertilizers have increased resistance to an infestation of late blight compared to plants where only mineral fertilizers have been applied and conclude that organic agriculture can effectively control this disease in tomatoes.

Tomato fruit quality parameters

According to research objectives, the numerical parameters are obtained and represented in Table 1.

Table 1. Chemical composition of tomatoes depending on treatment and year of study

	Slavol		Control	
	2013	2014	2013	2014
Total soluble solids (°Briks-a)	4.58	4.24	3.85	3.87
Fruit acids (%)	0,41	0,31	0,26	0,24
Nitrates (mg/kg)	141	209	176,3	115,6
Vitamin C (%)	39,7	27,1	18,9	28,5
Lycopene (mg/100g)	8,58	7,81	9,1	6,66
N (%)	0,18	0,44	0,17	0,26
Mg (mg/kg)	111,9	97,2	120,7	77,5
P (mg/kg)	257,2	179,5	264,5	169,8
K (mg/kg)	1756.5	1572.1	1723.0	1339.7
Fe (mg/kg)	9,1	6,9	11,7	4,3
Zn (mg/kg)	2,7	1,1	3,07	1,07
Cu (mg/kg)	1,3	0,85	0,9	0,7

According to the results of a two-factor analysis of variance (ANOVA) of the relation between the mean of total soluble solids (6.549466> 4.747225) and total acidity (8.197601> 4.747225), the treatment was statistically significant. In contrast, no statistically significant effects of years, or interaction of treatment and year were observed.

A comparison was made using the Tukey-Kramer test to determine the effect of type of treatment and the year on the parameters of total soluble solids and total acidity,

Comparing the parameter total of soluble solids in tomatoes, the impact of variant Slavol in 2013 showed statistical significance compared to the control in 2013 and 2014. The other comparisons shown in the Tukey-Kramer test for the parameter total of soluble solids were not statistically significant.

Parameter total acidity in tomatoes treated with microbial fertilizer Slavol from 2013 showed statistically significant differences compared to the control in both years and Slavol in 2014. Variant Slavol 2014 compared with variant Slavol 2013 showed statistically significant differences.

According to the results of variance analysis, neither the treatment nor the year had a statistically significant effect on the different mean of nitrate content in tomatoes, but their interaction was statistically significant. (6.203874> 4.747225). Statistically significant differences in comparisons showed the impact of variant Slavol 2013 to the control of both years.

According to the results of a two-factor analysis of variance (ANOVA), the different mean values of vitamin C in tomatoes were significantly influenced by treatment (43.7463 > 4.747225) and the interaction between treatment and year (56.66768 > 4.747225). Based on

data obtained by the Tukey - Kramer Test for Vitamin C, it was determined that no statistically significant differences were observed in the comparison of controls 2014 with variant Slavol 2014. All other correlations were statistically significant.

The results of the analysis of variance showed that there was no statistically significant effect of treatments, year, or their interaction on the reported different mean of lycopene content in tomatoes. Nevertheless, a Tukey-Kramer test showed that there were statistically significant differences in comparisons between controls in 2013 and 2014. Some research has shown that organically grown tomatoes contain lower carotenoid concentrations but also enhanced levels of flavonoids than tomatoes that are conventionally grown (Rembiałkowska et al., 2003).

According to the results of the analysis of variance, the reported different mean values of total N in tomatoes (7.643669 > 4.747225), Mg, P, K, Fe, Zn, Cu were statistically significantly influenced by year. In contrast, no statistically significant effects of treatment or the interaction of treatment and year were recorded. Statistically significant differences in comparisons for the content of N in tomatoes were shown by microbial fertilizer Slavol from 2014 compared to variant Slavol from 2013 and control in both years.

The Tukey-Kramer test for the content of Mg in tomatoes showed that there were statistically significant differences in comparisons between controls in 2013 and 2014.

For the content of P in tomatoes, the Tukey-Kramar test showed statistically significant differences between variants Slavol and control in both years of the study, while the content of K showed a statistically significant difference between controls in 2013 and 2014.

Regarding Fe and Zn, the Tukey-Kramer test showed statistically significant differences in the comparisons of the control 2013 with the control and the variant Slavol 2014. For Zn in tomatoes, the test showed statistically significant differences in the comparison of variant Slavol in 2013 and 2014.

Statistically significant differences of the Cu parameter in tomatoes were found in the comparisons of variant Slavol from 2013 with variant Slavol and control in 2014. Also, significant differences were found in the comparison of Slavol and control in 2013 for the Cu parameter.

The Pearson correlation matrix as a parameter analysis is used to determine the correlation between the studied statistical parameters.

Table 2. Correlation matrix (Pearson):

Variables	Total soluble solids	Fruit acids	Nitrates	Vitamin C	Lycopene	Total N	Mg	P	K	Fe	Zn	Cu
Total soluble solids	**1**	0.476	0.216	0.367	0.060	0.236	0.096	0.052	0.313	0.319	0.220	0.671
Fruit acids	0.476*	**1**	0.120	0.553	0.107	-0.030	-0.081	-0.029	0.210	0.124	0.046	0.289
Nitrates	0.216*	0.120*	**1**	-0.240	0.289	0.758	-0.132	-0.234	0.134	0.119	-0.106	-0.230
Vitamin C	0.367*	0.553*	-0.240*	**1**	-0.053	-0.089	-0.183	-0.057	-0.013	-0.262	-0.169	0.343
Lycopene	0.060	0.107*	0.289*	-0.053	**1**	-0.075	0.173	0.231	0.138	0.269	0.280	0.203
Total N	0.236*	-0.030	0.758*	-0.089	-0.075	**1**	-0.317	-0.467	-0.200	-0.083	-0.467	-0.257
Mg	0.096^{ns}	-0.081	-0.132*	-0.183*	0.173*	-0.317*	**1**	0.943	0.678	0.647	0.769	0.603
P	0.052^{ns}	-0.029	-0.234*	-0.057	0.231*	-0.467*	0.943*	**1**	0.692	0.609	0.809	0.574
K	0.313*	0.210*	0.134*	-0.013	0.138*	-0.200*	0.678*	0.692*	**1**	0.425	0.690	0.534
Fe	0.319*	0.124*	0.119*	-0.262*	0.269*	-0.083	0.647*	0.609*	0.425*	**1**	0.749	0.528
Zn	0.220*	0.046	-0.106*	-0.169*	0.280*	-0.467*	0.769*	0.809*	0.690*	0.749*	**1**	0.569
Cu	0.671*	0.289*	-0.230*	0.343*	0.203*	-0.257*	0.603*	0.574*	0.534*	0.528*	0.569*	**1**

Total soluble solids and total acidity relation (r = 0.476), nitrates (r = 0.216), vitamin C (r = 0.367), total nitrogen (r = 0.236), total potassium (r = 0.313), iron (r = 0.319) and zinc (r = 0.220) indicates a weak positive correlation.

Total soluble solids and copper content relation (r=0,671) indicates a strong positive correlation. Total soluble solids and Mg and P content relation indicate a negative correlation.

Total acidity in relation with vitamin C indicates moderate positive correlation, and weak positive correlation in relation with nitrates (r = 0.120), lycopene (r = 0.107), K (r= 0.210), Fe (r = 0.124) and Cu (r = 0.289).

Content of nitrates and N content relation (r=0.758) indicates a strong positive and significant correlation.

The content of Mg in relation with the content of P (r = 0.943) in tomatoes indicates a very strong positive correlation and strong positive correlation with the content of K (r = 0.678), Fe (R = 0.647) and Zn (r = 0.769).

A very strong positive correlation indicates parameter P with Zn (r= 0,809) and a strong positive correlation with the content of K (r=0.692).

Based on the Pearson's correlation coefficients presented above, it can be concluded that parameter correlations are strong, positive and statistically significant which would mean that the application of microbial fertilizer had a significant influence on analysed chemical parameters in tomatoes in both years.

Conclusion

The results of the analysis of fruit quality parameters and health conditions of tomatoes treated with microbial fertilizer Slavol in 2013 and 2014 can be used for better understanding of the complex interactions between plants and microorganisms. During the research, the health status of Slavol-treated tomatoes was better, indicating that the application of microbial fertilizer contributes to a better plant condition and increases resistance to stressful situations. The results of the research showed that the application of Slavol had an impact on some quality parameters, such as the content of total soluble solids, total acidity, N, Mg, K, Cu. It can be concluded that the application of adequate agronomic measures of nutrition and crop protection with the addition of microbial preparations can be used as the basis for the improved growth and production of high yields of high-quality tomatoes in Herzegovina.

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Original scientific paper

Influence of some enological treatments on *trans*-resveratrol and total phenolic content in wine

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Abstract

In this study, the influence of winemaking techniques and grape cultivars on *trans*-resveratrol and total phenolic content in wines was studied. The *trans*-isomer occurs in the berry skins of most grape cultivars, and its synthesis is stimulated by UV radiation, injury, and fungal infection. The *trans*-resveratrol content in wine depends of many different factors including variety, vintage, climatic conditions, UV radiation, storage conditions and winemaking process. Some wine making techniques were applied in order to investigate its impact to *trans*-resveratrol and total phenolic content. Addition of different combination of enzymes and wine yeasts, resulted in different content of these compounds. Yeast Uvaferm 299 (Lallemand, Canada) with the enzyme Lallzyme OE (Lallemand, Canada) indicated the best extraction of *trans*-resveratrol (1.56±0,04 mg/l) and total phenolic content (1774.20 mg GAE/l) in Prokupac wine samples. Also, biological deacidification, pasteurization and use of some clarifying agents (bentonite and gelatine) had no influence on *trans*-resveratrol and total phenolic content.

Keywords: *Trans*-resveratrol, Total phenolic content, Wine yeasts, enzymes

Introduction

Resveratrol (3,5,4′-trihydroxystilbene) is a phytoalexin synthesized by grapevine leaf tissue following fungal infection (*Botritys cinerea*) and UV light irradiation (Pezet et al., 2003). It is a phenolic phytoalexin that is biosynthesized in the grapevine in response to fungal infection, exposure to ozone or heavy metal ions and stress conditions, or when the environmental factors are unfavorable to their development (Téguo et al., 2001; Caruso et al., 2004; Baptista et al., 2001). *Trans*-resveratrol mostly synthesized in the skin cells, while its concentracion in flesh

and seed is low (Rodríguez-Delgado et al., 2002). The colour and other quality characteristics of wine such as astringency, bitterness and aroma are mainly responsible to these substances in a high degree. Phenolic compounds are also associated with the beneficial physiological effects deriving from moderate wine consumption due to their antioxidant and anti-inflammatory properties (Lopez et al., 2003), prevents cardiovascular diseases, Alzheimer and Parkinson′s diseases (Flamini et al., 2003).

There are a few studies about the dynamics extraction of resveratrol from solid grapes parts during fermentation and it was investigated how much resveratrol in by-product (pomace) was retained. Extractability of *trans*-resveratrol during the vinification process is also important, while its quantity in grape skin is 50-100 μg/g. Its content depends of grape variety, ripening conditions, winemaking procedures and other factors (Atanackovic et al., 2012). The aim of this study was investigated influence of using wine yeasts strain with different glicolytic activity to *trans*-resveratrol and total phenolic content in wine of Prokupac variety. Also it was obversed other factor which can contribute higher *trans*-resveratrol and total phenolic content in wine such as: biological deacidification, pasteurization and some clarifying agents (bentonite and gelatine).

Material and Methods

In this paper it was investigated content of *trans*-resveratrol and total phenolic compounds in Prokupac, Caberent Franc and Traminac variety. In 2005, grapes were harvested in state of technological maturity (phytosanitary health 100%). All of these grape varieties originating from vineyards belonging to experimental field "RADMILOVAC" of the Faculty of Agriculture in Zemun. The effect of different yeast strains and enzyme preparations on *trans*-resveratrol and total phenolic content was examined on wine samples obtained from Prokupac variety grapes. After grapes crashing and desteaming, the samples were sulfited with 10 g of $K_2S_2O_5$ per 100 kg grape pomace. Selected yeasts and enzymes preparation with different glicolytic activities "OE" and "β" (Lallemand, Canada) were used.

Alcoholic fermentation was carried out according to the microvinification method and used combination are presented in the following table (Table 1).

Table 1. Used yeast strains in combination with enzymes

Yeast (20 g/hl)	Yeast + Enzyme OE	Yeast + Enzyme β
BDX (control sample)	BDX + OE (2 mg/hl)	BDX + β (2 mg/hl)
299 (control sample)	299 + OE (2 mg/hl)	299 + β (2 mg/hl)
2056 (control sample)	2056 + OE (2 mg/hl)	2056 + β (2 mg/hl)

After fermentation (14 days at 25°C), the wine samples were separated from pomace by free-run and stored before being analysed.

Cabernet franc wine samples in which biological deacidification by use of pure culture of lactic acid bacteria *Oenococcus oeni*-Uvaferm alfa (Lallemand, Canada). Control was Cabernet franc wine sample where biological deacidification did not occured. This samples were stored at 25°C for 20 days. There was no need to add resveratrol to wine obtained from Cabernet franc grapes because its content in the control (untreated) sample was relatively high, for all experiments. Only pasteurization of half a liter of wine was performed, after which both samples (control and treated) were analyzed. Increasing amounts of gelatine were added: 0, 5 and 10 g/hl, for next experiments, respectively, in 200 ml wine samples.

The Traminac wine samples were enriched with *trans*-resveratrol (2.5 mg/l) produced by Sigma (Darmstadt, Germany). This was necessary because white wines contain lower amounts of resveratrol, and it could be more difficult to detect it during thermal treatment. Half a liter was separated as a control, while the other half was transferred to an autoclave and pasteurized at 60°C for 20 minutes, after which the treated and untreated samples were analyzed. Increasing amounts of bentonite: 0, 50 and 100 g/hl were added to wine (200 ml) obtained from the Traminac grape variety, which has been previously enriched with resveratrol. After keeping for 7 days in the fridge (5°C), the wines were separated from the precipitation and *trans*-resveratrol and total phenolic content were analyzed. After 7 days of keeping in the fridge, the wines were analyzed as in the previous case. All experiments were set up in the laboratory of the Faculty of Agriculture in Zemun.

Determination of total phenolic content in wines was measured by Folin-Ciocalteu method (Tanner and Brunner, 1979). *Trans*-resveratrol content in wine samples was determined using reversed-phase liquid chromatography (HPLC) with UV detection. Immediately prior to injection into the HPLC system, a preliminary preparation of the solid phase extraction (SPE) preparation is provided. The quantification of *trans*-resveratrol in wines were measured by the external standard method. The HPLC system for analysis is provided with the following components: Hitachi constant flow two-pump system (Model 655A-11), Gradient controller

Hitachi (Model L-5000), Hitachi Autosempler (Model 655A-40), Gilson UV detector, Bishoff Hyperchrome analytical column, ODS Hypersil shandon 25cm x 4.6 mm DB 5, CSW32 monitoring and data processing software system. The required chemicals are: methanol - HPLC purity, acetonitrile - gradient grade HPLC, acetic acid and ultra-pure water - 18 MΩ.

Results and Discussion

a) The influence of using different yeast strains and enzyme preparations

Yeast 299 used with the enzyme preparation OE showed the best extraction of *trans*-resveratrol content where a maximal recorded amount was 1.56 mg/l, while a minimal amount of *trans*-isomers was found in wine obtained from yeast 2056 in combination with enzyme β (0.48 mg/l). This confirms the results (Petrovic et al., 2019; Torre et al., 2004; Eder et al., 2000) that the activity of glucolytic enzymes increase *trans*-resveratrol content (Tab. 2).

Table 2. The effect of enzymes and yeast strains on the amount of *trans*-resveratrol and total phenolic content in Prokupac variety wine

Prokupac wine samples	*Trans*-resveratrol (mg/l)	Total phenolic content (mg GAE/l)
BDX	1.02±0.04	1645.60
BDX + β	0.75±0.04	1016.20
BDX + OE	0.70±0.04	1445.00
299	0.78±0.04	1151.92
299 + β	0.64±0.04	961.60
299 + OE	1.56±0.04	1774.20
2056	0.98±0.04	1258.90
2056 + β	0.48±0.04	903.00
2056 + OE	0.98±0.04	1609.60

The minimal amount of total phenolic content was recorded in wine obtained by yeast 2056 in combination with enzyme β (903.00 mg GAE/l), while yeast 299 in combination with the enzyme preparation OE gave the maximal amount of total phenolic content in wine (1774.20 mg GAE/l) (Table 2).

b) The influence of biological deacidification

Inoculation of Cabernet Franc wine sample with *Oenococcus oeni* bacteria had no influence on the content of *trans*-resveratrol and total phenolic content (Table 3).

Table 3. *Trans*-resveratrol and total phenolic content in treated and untreated wine

Wine sample	*Trans*-resveratrol (mg/l)	Total phenolic content (mg GAE/l)
Cabernet franc (control sample)	1.47±0.04	1620.30
Cabernet franc (treated)	1.45±0.04	1618.10

c) The influence of pasturization

There are no changes in the *trans*-resveratrol and total phenolic content were observed in pasteurized and unpasteurized samples (Table 4).

Table 4. The effect of pasteurization on the *trans*-resveratrol and total phenolic content

Wine samples	*Trans*-resveratrol (mg/l)	Total phenolic content (mg GAE/l)
Traminac (control sample)	2.61±0.04	120.10
Traminac (treated)	2.59±0.04	120.10
Cabernet franc (control sample)	1.47±0.04	1620.30
Cabernet franc (treated)	1.47±0.04	1620.30

Analysis of *trans*-resveratrol content in wine samples treated with increasing amounts of bentonite and gelatine in this range revealed no changes in *trans*-resveratrol and total phenolic content in the wine samples (Table 5). Eder et al., (2000) in their work pointed out that gelatin had no effect on reducing the amount of resveratrol in wine, which was confirmed by other authors who stated that clarification of wine with gelatin and bentonite has no effect on the concentration of different forms of resveratrol in wine (Romero-Pérez et al., 1999; Cantos et al., 2003).

Table 5. The effect of clarifying agents on the *trans*-resveratrol and total phenolic content

Wine samples	*Trans*-resveratrol (mg/l)	Total phenolic content (mg GAE/l)
Traminac (control sampe)	2.61±0.04	120.10
Traminac (bentonite 50 g/hl)	2.59±0.04	120.10
Traminac (bentonite 100 g/hl)	2.63±0.04	120.10
Cabernet franc (control sample)	1.47±0.04	1620.30
Cabernet franc (gelatine 5 g/hl)	1.45±0.04	1610.00
Cabernet franc (gelatine 10 g/hl)	1.49±0.04	1600.00

Conclusion

Analyzing Prokupac wine samples it was noticed that the combination of yeast 299 with the enzyme preparation OE showed the best extraction of *trans*-resveratrol (1.56±0.04) and total phenolic content (1774.20 mg GAE/l). Also, biological deacidification, pasteurization and use of some clarifying agents (bentonite and gelatine) had no influence on *trans*-resveratrol and total phenolic content.

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Original scientific paper

Removing astringency in persimmon with CO_2 and the effect of prolonged softening process

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Abstract

Persimmon is beloved fruit of warmer southern European areas. It is edible when it softens and astringency is naturally removed but many consumers want to eat hard fruits with astringency removed artificially. Astringency from the hard fruits can be removed by several methods of which exposure to extreme concentrations of CO_2 (>90%) seem to be the most user friendly. In this study, we investigated the effect of extreme CO_2 concentrations on astringency removal, taste, soluble solids and fruit flesh firmness. Our results show astringency removal can be sufficiently initiated by 24-hour exposure to extreme CO_2 concentrations and process is finished within the next three days. Lower CO_2 concentrations (70%) need some longer exposure but may have benefit in taste. We observed temporal decrease of soluble solids in the CO_2 exposed persimmon fruits probably due to fixation of the astringent soluble tannins. Following changes in fruits flesh firmness during and after exposure to extreme CO_2 concentrations reveal a temporal increase in exposed fruits followed by a slower softening process.

Key words: *Diospyros kaki*, temperature, soluble solids, flesh firmness, taste

Introduction

Persimmon fruit is grown in various countries in southern Europe and is increasingly appreciated for its nutritional value and rich flavour. In Asian countries where it originates, it is well known for its health benefits (Choi et al., 2019, Butt et al., 2015). Fruits of cultivars grown in Europe, as ‘Rojo Brillante’, are ordinary not edible directly from the tree due to astringency. Several non-astringent cultivars are also available, but their health benefits are less

expressed (Li et al., 2011, He et al., 2015). Astringent persimmon fruits have to be stored to ripen naturally or their astringency can be artificially removed. Several treatments for astringency removal are available among them soaking in vinegar or hot water, submerging into 500 ppm ethephon solution or warm water for 24 hours, exposing of fruits to ethanol vapour, ethylene gas or high N_2 or CO_2 concentrations (e.g. EL-Kady et al., 2008). The effectiveness of most these methods occurs through the promotion of anaerobic respiration of the fruit that accumulates acetaldehyde (Edagi et al., 2009, He et al., 2018). Accumulated acetaldehyde than reacts with soluble tannins that are responsible for the astringency and make them insoluble or polymerized (Edagi et al., 2009, Salvador et al., 2007). In our experiments, we used high CO_2 exposure of persimmon fruits in a plastic bag for astringency removal. Generalized recommendation for CO_2 treatment is 24-hour exposure to 95% concentration of CO_2 at room temperature and an additional few days of storage time prior consumption. Shorter exposure may not be sufficient for less ripe fruits (Besada et al, 2010). In this report, we investigated how lower CO_2 concentrations (70% and 80%) or temperatures (16 and 4°C) influence astringency removal, fruits flesh firmness and soluble solids during the treatment and following storage. The astringency removal method in plastic bags as used in these experiments does not need big investment and could be easily adopted by farmers.

Material and methods

Persimmon fruits grown in lower Vipava valley in West of Slovenia in session 2018 and 2019 were used for experiments. Fruits of cultivars ‘Triumph’, ‘Rojo Brillante’, ‘Tipo’, and ‘Hachiya’ were picked at the time of technological ripeness and transferred to the laboratory for three CO_2 exposure experiments. For each experiment fruits were sorted by visual ripeness status and uniform groups of six fruits for each treatment were prepared. Fruits were laid on a plate and heat-sealed into 0,2 mm polyethylene bags for individually controlled gasification treatments. For CO_2 exposure treatments bags were punctured in one corner and CO_2 was blown through a tube into the other corner form a cylinder with 100% CO_2. A target CO_2 concentration (95, 80 or 70%) was reached by ventilating bags several times. Bags were re-sealed when the defined CO_2 concentration was reached as measured by the CO_2 sensor (Geosensor-G100; Geotechnical Instruments Ltd, Coventry, UK) on the punctured corner. In the first experiment ‘Triumph’ fruits were exposed to 95, 80 and 70% CO_2 for 0, 1, 2 or 3 days while measurements were performed on the fourth day. In second experiment fruits of ‘Triumph’, ‘Tipo’ and ‘Rojo Brillante’ were exposed to 95%, 70% or room CO_2 concentration

for 0 1 or 3 days while measurements were performed immediately at the end of the treatment. In third experiments fruits of all four cultivars were exposed to 95% CO_2 concentration and stored at ordinary room temperatures (23 °C), in a cellar (16 °C) or in a cold room (4 °C) for up to 21 days. Measurements and tasting were performed on each fruit separately. After peeling of the skin, a flesh firmness was analysed with a penetrometer (Wonderful; Agrosta, Serqueux, France) on 4 corners of the fruit. Round tip with 1 cm^2 was used and measured pressure was expressed in kg. Soluble solids content measurements were done on each penetration point by digital refractometer (Ecolube, Croissy Beaubourg, France) Fruits were orally tasted for astringency (1 – no astringency to 5 – strong astringency) and taste (1 – bad taste to 5 – very good taste) by several evaluators.

Results and discussion

In the first experiment fruits of the cultivar 'Triumph' were exposed to three different CO_2 concentrations and analysis was performed on the fourth day after setup of the experiment. Exposure to high CO_2 concentrations resulted in non-astringent fruits while unexposed fruits retained their astringency (Table 1). The firmness of fruit flesh remained higher in fruits exposed to higher CO_2 concentration for a longer time. Soluble solids content decreased with prolonged CO_2 exposure. Higher concentrations and prolonged time of storage in high CO_2 had negative impact on the taste of 'Triumph' fruit in this experiment.

Table 1: Measured and tasted parameters of the 'Triumph' fruits exposed to four CO_2 concentrations (room, 70, 80 or 95%) for the annotated time while measurements and tasting was executed at the fourth day after setup of the experiment

Cultivar	CO_2 concen-tration	Days of storage	Soluble solids (Brix)	Flesh firmnes (kg/cm^2)	Taste (1-5)	Astring-ancy (1-5)
Triumph	Room	0	15.3	2.6	1.3	5.0
	70%	1	14.8	5.2	3.2	1.7
		2	15.3	4.2	4.2	1.0
		3	9.9	4.1	3.7	1.0
	80%	1	15.2	6.1	4.0	1.7
		2	15.0	7.2	3.8	1.3
		3	14.5	7.0	3.8	1.0
	95%	1	15.3	6.4	3.3	1.0
		2	15.8	6.7	3.3	1.0
		3	14.3	9.6	2.8	1.0

In the second experiment, we exposed persimmon fruits of three cultivars (‘Triumph’, ‘Tipo’ and ‘Rojo Brillante’) to different CO_2 concentration (room, 70 and 95%; Table 2) while measurements were performed directly after the defined time of treatment. In this experiment, astringency was removed in three days in all cultivars even in fruits exposed only first 24 hours (data not shown). Astringency decreased slower in fruits exposed to higher CO_2 in ‘Rojo Brillante’ and ‘Tipo’. Exposure to CO_2 kept or in case of ‘Triumph’ increased flesh firmness higher in comparison to untreated fruits. In ‘Triumph’ and ‘Tipo’, but not in ‘Rojo Brillante’, higher concentration preserved higher flesh firmness. Content of soluble solids decreased by exposure to CO_2 and higher concentration have a faster effect, while soluble solids content stayed more stable in untreated fruits. The taste was evaluated better in fruits stored at lower CO_2 concentrations especially in ‘Tipo’ while in untreated fruits astringency prevailed the taste (Table 2).

Table 2: Measured and tasted parameters of the ‘Triumph’, ‘Tipo’ and ‘Rojo Brillante’ fruits exposed to 95% CO_2 stored for the annotated time

Cultivar	CO_2 concen-tration	Days of storage	Soluble solids (°Brix)	Flesh firmnes (kg/cm^2)	Taste (1-5)	Astrin-gancy (1-5)
Triumph	room	0	19.2	6.8	1.0	5.0
		1	20,6	6.8	1.0	5.0
		3	19.6	3.4	1.3	5.0
	70%	1	18.8	7.1	2.0	3.8
		3	16.9	5.7	3.0	1.0
	95%	1	16.6	8.3	2.0	2.7
		3	16.1	8.0	2.7	1.0
Tipo	room	0	18.8	3.4	3.0	3.8
		3	18.3	2.4	3.5	1.2
	70%	1	17.5	5.2	4.3	1.1
		3	17.1	2.6	3.5	1.0
	95%	1	17.7	5.1	3.3	2.3
		3	16.3	3.5	2.7	1.0
Rojo Brillante	room	0	19.1	6.7	3.0	5.0
		1	21.0	6.8	3.0	5.0
		3	19.9	5.4	2.0	5.0
	70%	1	18.1	7.2	3.7	2.5
		3	16.2	7.1	4.5	1.0
	95%	1	17.1	7.1	2.3	5.0
		3	17.1	6.2	4.5	1.0

In the third experiment, we exposed persimmon fruits of 'Hachiya', 'Triumph', 'Tipo' and 'Rojo Brillante' to 95% CO_2 concentration at three temperatures (23 °C - room temperature, 16 °C - cellar and 4 °C - cold-room) for a different period of time. In all CO_2 treated cultivars on all three temperatures astringency was removed by 7th day (Table 3). Lower temperatures of storage in the time of CO_2 exposure caused slower astringency removal in 'Triumph'. The soluble solids content of fruits exposed to CO_2 decreased faster and reached lower levels in colder storage conditions in 'Triumph' and 'Tipo'. The drop bounced the third day at room temperature, while for colder storage conditions no rise of soluble solids content was measured. Flesh firmness is decreasing rapidly with persimmon ripening processes (e.g. Itamura et al 1997). Exposure to high CO_2 reduced speed of softening process in 'Tipo' and 'Rojo Brillante' or temporarily even increased flesh firmness in 'Triumph' and 'Hachiya'. Lower temperatures even more reduced softening process. Astringency removal does improve perceived taste, but prolonged CO_2 exposure on colder temperatures had a negative effect on taste in 'Rojo Brillante' and 'Triumph'. In some fruits of 14 days and most fruits of 21 days CO_2 exposed fruits expressed typical "fermented taste".

Table 3: Measured and tasted parameters of the 'Hachiya', 'Triumph', 'Tipo' and 'Rojo Brillante' fruits exposed to 95% CO_2 concentrations while stored on three different temperatures (23, 16 and 4 °C) for the annotated time

Cultivar	Storage temp. (°C)	Days of storage	Soluble solids (°Brix)	Flesh firmnes (kg/cm^2)	Taste (1-5)	Astring-ancy (1-5)
Hachiya	NA	0	16.6	2.8	3.2	5.0
	16	3	16.7	4.5	3.7	1.7
		7	13.9	3.2	3.7	1.0
		14	15.4	2.0	4.2	1.0
		21	15.5	2.7	3.7	1.0
Triumph	NA	0	18.6	4.5	1.0	5.0
	23	3	15.6	5.3	3.8	1.0
		7	15.6	4.5	3.7	1.0
	16	3	15.1	5.8	3.7	1.0
		7	14.3	6.1	3.2	1.0
		14	14.6	3.8	2.9	1.0
		21	14.7	3.8	3.3	1.0
	4	3	14.5	5.5	2.8	1.7
		7	14.4	5.1	3.2	1.0
		14	14.8	NA	3.0	1.8
		21	15.3	5.2	3.3	1.0

Cultivar	Storage temp. (°C)	Days of storage	Soluble solids (°Brix)	Flesh firmnes (kg/cm^2)	Taste (1-5)	Astring-ancy (1-5)
Tipo	NA	0	18.4	0.4	3.1	3.0
	23	3	16.2	0.3	4.5	1.0
		7	16.5	0.4	4.5	1.0
	16	3	17.3	0.4	4.3	1.0
		7	16.8	0.3	4.5	1.0
	4	3	16.9	0.4	4.7	1.0
Rojo Brillante	NA	0	16.6	0.0	3.2	5.0
	23	3	15.5	0.0	3.7	1.5
		7	15.9	0.0	4.8	1.0
	16	3	14.8	0.0	3.7	1.5
		7	14.4	0.0	3.3	1.0
	4	3	15.5	0.0	3.4	1.3
		7	14.7	0.0	2.8	1.0

Untreated fruits kept their astringency despite a progression of ripening and softening processes. The astringency removal was initiated by 24-hour exposure to high CO_2 concentrations and was finished in 3 to 7 days regardless of CO_2 concentration (70-95%) and temperature (4-23 °C). The process of astringency removal is initiated by hypoxic conditions what increase the ethylene production and cause acetaldehyde accumulation (He et al, 2018; Ali 2005). Acetaldehyde is believed to react with soluble tannins and make them insoluble or polymerized and thereby inactive them for taste receptors. Our measurements are in line with a drop of soluble tannins after CO_2 treatment reported by Edagi et al. (2009). Moreover, our results show that astringency removal is slower in fruits exposed to higher CO_2 and lower temperatures. But an additional lowering of CO_2 concentration may reach a level of insufficient initiation of astringency removal processes especially in less ripe fruits, similar to what Besada et al. (2010) reported for shortening of CO_2 exposure time. And when astringency is present we cannot enjoy the taste. Additionally, our results show that and higher CO_2 concentrations, longer time of exposure and lower temperatures at the time of exposure can have a negative effect on taste. In part these results may be attributed to fermentation processes detected in fruits gasified for more than two weeks, but a disturbance of secondary metabolite producing processes may also play a role.

In several treatments, we observed a rise in fruit firmness when fruits were exposed to extreme CO_2. By our manual observations, we can attribute this to change in fruit consistency since fruits felt more elastic and during measurement, more flesh bent so the pressure accumulated

before it was breakthrough. Although similar observations were reported by Salvador et al. (2005) more research and different measuring approach would be needed to reveal underlying processes. Nevertheless, reduced speed of softening when fruits were exposed to high CO_2 as observed in our experiments have been previously reported (e.g. Edagi et al., 2009). Additionally, our results show that higher CO_2 concentrations and lower temperatures at the time of CO_2 exposure decrease the softening process more. The effect of temperature on the ripening process and thereby softening of persimmon fruits is well understood while for the effect of CO_2 concentration no detailed experiments have been performed up to our knowledge. The drop of soluble solids content occurred in all CO_2 exposed fruits what can be partly attributed to the demobilization of soluble tannins. Since soluble solids content drop was more pronounced in higher CO_2 concentrations and lower temperatures where the de-astringency process was slower we can assume some other processes are involved too. When ripening continued soluble solids content increase as sugars accumulated by ordinary ripening processes.

Conclusions

Use of one fruit-carton sized bags for CO_2 treatment as in this experiment is scalable and can be introduced to the ordinary practice of non-astringent persimmon production.

Lower concentrations of CO_2 and shorter exposure times at room temperature could have a beneficial effect on fruit taste as long as the de-astringency process is sufficiently initiated.

Although exposure to CO_2 increases and/or preserves fruit firmness, prolonged storage in these conditions is not possible due to the occurrence of fermentation processes.

Acknowledgements

The author would like to thank Davor Mrzlić from Sadjarski center Bilje (KGZS -ZAVOD GO) for providing fruits for experiments and Matej Stopar (KIS) for valuable suggestions. The research presented in this study was partly financed as part Javna služba v sadjarstvu by Ministry of Agriculture, Forestry and Food, Slovenia.

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Original scientific paper

Fertilizer effects on the container production of tomato seedlings

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Abstract

In these researches the plan is to look at the effect of different fertilizers on the quality of tomato seedlings made for open field production (cv. roker). Tomato nursery were grown in containers with 104 openings and saved during the seeding period with three types of fertilizes with: *fitofert kristal*, *fitofert humisuper* and *slavol*. They were used with different percentages, and the time of use was after sowing, with the appearance of first leaf that continued during those seven days from sprouting until the end of the seedling period.

The following parameters were determined: plant height (cm), number of leaves per plant, stem thickness (mm) and the weight of the plants' above-ground parts (g). With all of the tested parameters the best results were made with continuous usage of fertilizers. In the container production of tomato seedlings, where the seedlings was being produced in small volume of substrates in short period of time, usage of mineral fertilizers gives overall better results than organic-mineral and microbiological.

Key words: tomato, seedlings, fertilisation, quality.

Introduction

Negative effects of agricultural production on the environment are increasing year after year. The increase in the level of intensification of this production results in a more negative impact on the environment. Vegetable production represents one of the most intensive branches of agriculture, while tomato is the species which is grown in the largest area in the world. It represents the most significant vegetable species worldwide and in Serbia, from the perspective of both food industry and economy. It is grown in open fields and protected spaces, by direct sowing or by means of seedlings. The seedling production is predominant, primarily due to its

pronounced earliness, higher yield and more rational land use. This is particularly evident in the tomato production in protected spaces (greenhouses/glasshouses). These production systems provide high yield, while the production is organized in the conventional manner, which involves using chemically obtained intermediate goods. In addition to using pesticides, a significant problem arises regarding the use of large quantities of mineral nutrients. The use of these nutrients begins as early as during the seedling production. Considering the complete process of tomato production, the seedling production period can be qualified as the most significant one, since during this period the plant goes through its main growth and development phases. The basic factors of the successful seedling production are temperature, light, water, relative air humidity, substrate, nutritive matter, and vegetation space. Each plant species has specific requirements regarding these factors (Jankauskiene and Brazaityte, 2008). The seedling quality, as well as the length of its production cycle, depends on these factors.

Depending on the type of production for which the seedling is intended, it can be produced in a container (for the open field) or in a pot (for the early greenhouse/glasshouse production). The seedling production also requires certain production facilities which have to provide the adequate control of climatic conditions. In the climate of our country, seedlings are predominantly produced during winter months when the consumption of energy for keeping the facility warm is the highest. Consequently, one of the main aims in the seedling production is to reduce its duration. This can be reached in numerous ways, and one of the basic ways is related to the availability of nutritive elements to plants.

Tomato seedlings which had sufficiently and continuously available nutrients during their growth and development proved to be the best. The plant nutrient regime is defined by the quality of the substrate mixture in which the plant is cultivated, but also by the type, quantity and time of applying various nutrients during the seedling production (nutrition). These nutrients have different origins. Mineral nutrients with the expressed phosphorus components are still most frequently used. Phosphorus facilitates young plants to better develop their roots and faster activate their mechanisms for nutrient uptake from the substrate. In recent times, various nutrients of an organomineral, organic, microbiological or plant origin of are used for the same purpose.

Their impact on the seedling quality is positive, but still not stable enough to increase their application to a significant degree. The application of the preparations of an organic origin (microorganisms, biostimulants) in plant production can decrease the use of mineral fertilizers, which is significant both from the point of view of economy and health safety of products. These preparations can be successfully applied to the seed, to the soil, but also foliarly.

According to their structure, they can be single- or multiple-component preparations (Miskoska-Milevska et al., 2018; Najdenovska et al., 2013). Therefore, this research aims to indicate the possibility of obtaining the high-quality tomato seedling using the preparations of the non-chemical origin.

Materials and Methods

The research was conducted in the greenhouses of the company "Superior" from Velika Plana (2019). The impact of various fertilizers on the quality of tomato seedling produced in containers was examined. 104-hole PE containers were used. The containers were filled with the sowing substrate and sown with tomato seeds (cv. Roker, Superior Seeds). The seedlings were fertilized using three types of fertilizers: Mineral - Fitofert Kristal 10:40:10+me, Organic-mineral - Fitofert Humisuper Plus 8:4:8 (Agromarket, Kragujevac) and microbiological - Slavol (Unik, Šimanovci). Each of the fertilizers was applied in three different manners: watering immediately following the sowing (A), watering following the formation of the first true leaf (B) and continuous watering each seven days during the seedling period (C).

The concentration used for watering the plants differed depending on the fertilizer type, as well as on the manner of their application. The experiment consisted of 10 treatments: **0** – control (watering by means of clear water only); **1** – 0.3% F. Kristal (A); **2** – 0.3% F. Kristal (B); **3** – 0.1% F. Kristal (C); **4** – 2% Slavol (A); **5** – 2% Slavol (B); **6** – 1% Slavol (C); **7 -** 0.3% F. Humisuper Plus (A); **8** – 0.3% F. Humisuper Plus (B); **9** – 0.1% F. Humisuper Plus (C). The sowing of the seeds was conducted manually on April 4th.

The first true leaf was recorded 11 days following the sowing. The seedling period lasted for 50 days. Each treatment included 4 containers. At the end of the seedling period, the samples for each treatment were taken – there was the total number of 40 samples (10 plants from each container). The complete experiment involved the analysis of 400 plants.

The following parameters were determined: plant height (cm), number of leaves per plant, stem thickness (mm) and the weight of the plants' above-ground parts (g). The obtained results were statistically processed using the method of the analysis of variance, while the differences between the treatments were determined using the LSD test. The results are presented in tables which include the mean values and indices.

Results and Discussion

The plant height is one of the basic indicators of the seedling quality. In our research, the average height of tomato seedlings was 16.59 cm (Tab. 1). In the complete experiment, the seedling height values were in the range from 13.31 to 18.86 cm. The lowest average value of the plant height was recorded in the treatment 4 (13.31 cm), while the highest was in the treatment 3 (18.86 cm). The plants from the control variant had the stem height values higher than the values in most of the treatments (5).

Compared to the control variant, the application of various fertilizers increased the seedling height by the maximum of 10% (treatment 3). In comparison to the control variant, this difference was statistically significantly higher. In comparison to all the variants including Slavol (treatments 4, 5, and 6), this difference was statistically much higher.

The continuous fertilization resulted in the significant increase in the plant height (Moravčević et al., 2017), except in the treatments with Slavol, which can be explained by the fact that this fertilizer leaches more easily and by the short seedling period.

The stem thickness of the seedlings is a significant quality indicator which considerably affects the early yield of a vegetable species. However, the stem thickness does not have a significant impact on the total yield (Liptay et al., 1981).

The average stem thickness in all the treatments amounted to 1.64 mm (Tab. 1). Depending on the treatment, these values ranged from 1.05 to 2.20 mm. The lowest average value of the stem thickness was recorded in the treatment 4 (1.05 mm), while the highest value was registered in the treatment 3 (2.20 mm).

Similarly, to the plant height, the thickest stem of the seedlings was obtained in the treatment with Fitofert Kristal. The measured value was statistically significantly higher than the control (33%), as well as than the values obtained in the treatments where the stem thickness index was lower than in the control (Damjanović et al., 1994).

The nutrient availability directly affects the stem thickness. Similar results have been obtained in numerous other studies (Ilin et.al., 2003; Pavlović et al., 2009).

The number of permanent leaves per plant is an important indicator of the tomato seedling quality, which has an impact on its earliness. Namely, the number of formed leaves on the main stem is directly related to the appearance of the first flowering branch. In addition to the genetic characteristics of the hybrid, this feature is also significantly affected by the plant nutrient regime and the microclimatic conditions during the seedling production (Pavlović et al., 2009).

The average number of leaves in all the treatments amounted to 4.95 (Tab. 1).

Table 1. Fertilizer effects on the container production of tomato seedlings

Fertilizers	Plant height (cm)	Index	Stem thickness (mm)	Index	Number of leaves per plant	Index	Weight of the plants (g)	Index
0	17,15	100	1,65	100	4,70	100	3,05	100
1	13,39	78	1,10	67	4,60	98	1,75	57
2	17,04	99	1,95	118	4,90	104	2,80	92
3	18,86	110	2,20	133	5,50	117	3,75	123
4	13,31	78	1,05	64	4,10	87	1,75	57
5	16,56	97	1,85	112	5,30	113	3,00	98
6	16,55	97	1,40	85	5,30	113	2,75	90
7	17,56	102	1,60	97	5,00	106	3,05	100
8	17,30	101	1,80	109	5,20	111	2,60	85
9	18,17	106	1,75	106	4,90	104	2,80	92
Average	**16,59**	**97**	**1,64**	**99**	**4,95**	**105**	**2,73**	**90**
LSD 0,05	1,56		0,47		0,60		0,75	
LSD 0,01	2,06		0,63		0,80		0,99	

This value was obtained on the basis of all the values which ranged from 4.10 to 5.50 in all the treatments. The smallest number of leaves per plant was obtained in the treatment 4, while the greatest number of leaves was registered in the treatment 3. In the treatments with all three fertilizers where the fertilization was continuously conducted, the leaf number values were not statistically different. Once again, a better nutrient regime had a positive impact on the number of leaves of the examined seedlings (Ilin et.al., 2003; Pavlović et al., 2009; Moravčević et al., 2017; Tosunov et al., 2018, Todorović et al., 2019).

The high-quality seedling includes the plants of certain weight which was reached in a particular time period owing to the rational use of all available resources (nutrients, light, and water). This parameter is directly defined by the quality of the conditions in which the plant has grown. In our study, the weight of the above-ground plant parts had the average value of 2.73 g (Tab. 1). Depending on the treatment, the average values of this parameter ranged from 1.75 to 3.75 g. The lowest value of the above-ground plant parts was obtained in the treatments 1 and 4 (1.75 g), while the highest average value was recorded in the treatment 3 (3.75 g). The difference between these two values amounted to as much as 66%. The treatment with Fitofert Kristal again provided the seedlings of the greatest weight of the above-ground parts. This value was statistically greater than all the values obtained in the treatments whose index was lower than 100. When it comes to this parameter, the use of Slavol in different variants did not offer statistically significant differences, while the same situation is seen in the relationship between the Slavol and the control variant. The quantity of the available nutrients in the seedling period, limited substrate quantity, as well as the substrate leaching in the irrigation, led to these results related to the weight of the above-ground parts (Damjanović et al., 1994;

Ilin et al., 2003; Đurovka, 2008; Pavlović et al., 2009; Moravčević et al., 2017; Tosunov et al., 2018; Todorović et al., 2019).

Conclusion

The use of fertilizers had a positive impact on the tomato seedling quality. When it comes to all the analysed parameters, the best results were provided in the variant which used the mineral fertilizer (Fitofert Kristal) in the continuous application. Similarly, the other two fertilizers, Fitofert Humisuper and Slavol, yielded the best results when they were continuously applied. It should be mentioned that these results were obtained probably due to the fact that these fertilizers leached more easily. Finally, it can be concluded that the continuous use of organic-mineral and microbiological fertilizers is justified in the production of tomato seedlings. Therefore, their use can be recommended in the production.

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Uticaj prihranjivanja na kvalitet rasada paradajza proizvedenog u kontejnerima

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Sažetak

U ovim istraživanjima je postavljen cilj da se ispita uticaj prihranjivanja na kvalitet rasada paradajza namenjenog za proizvodnju na otvorenom polju (sorta roker). Ogled je postavljen u firmi Superior iz Velike Plane. Rasad paradajza je gajen u kontejnerima sa 104 otvora i prihranjivan je tokom rasadnog perioda sa tri vrste đubriva i to sa: fitofert kristalom, fitofert humisuperom i slavolom. Korišćene su različite koncentracije preparata, a vreme njihove primene je bilo nakon setve, pri pojavi prvog pravog lista i kontinuirano na sedam dana od nicanja do kraja rasadnog perioda.

Analizirani su sledeći parametri: visina biljke (cm), broj listova po biljci, debljina stabla (mm) i masa nadzemnog dela biljke (g). Kod svih ispitivanih parametara najbolji rezultati su se ostvarivali kontinuiranom upotrebom đubriva. U tretmanu sa fitofert kristalom (kontinuirano), dobijen je rasad najboljeg kvaliteta koji je imao visinu 18,86 cm, prečnik stabla 2,2 mm, 5,5 listova i masu nadzemnog dela 3,75 g. Kod kontinuirane upotrebe mikrobiološkog preparata slavol dobijena je u proseku biljka visine 18,17 cm, debljine stabla 1,75 cm, sa 4,9 listova i masom nadzemnog dela 2,8 g. U kontejnerskoj proizvodnji rasada paradajza, gde se rasad proizvodi u maloj zapremini supstrata u kratkom vremenskom periodu, upotreba mineralnih đubriva daje bolje rezultate u odnosu na organo mineralna i mikrobiološka.

Ključne reči: paradajz, rasad, đubrivo, kvalitet.

Original scientific paper

Susceptibility of *Grapholita molesta* Busck to insecticides in peach orchards

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Abstract

In Serbia, the oriental fruit moth (*Grapholita molesta* Busck) occurs regularly in peach orchards. In this study, its susceptibility to chlorantraniliprole, cyantraniliprole, spinetoram, indoxacarb and acetamiprid based insecticides was assessed. Field trials were carried out in the northern region of the Republic of Serbia, on the slopes of Fruška Gora mountain. Trials were conducted in 2019, according to the EPPO method. Products based on chlorantraniliprole (200 g/l), cyantraniliprole (100 g/l), spinetoram (250 g/kg), indoxacarb (150 g/l) and acetamiprid (200 g/kg) were foliar applied in an amount of 0.2, 0.6, 0.2, 0.33 and 0.25 l/kg ha^{-1}, respectively. Assessment of spray liquid efficacy was based on the number of fruit damaged by caterpillars, with an overview of 100 fruits per repetition. The results were processed using one-way ANOVA and Fisher LSD test, while the efficacy was determined according to Abbott. Applied spray liquids indicate a satisfactory efficacy at both localities ranging from 92-96% (chlorantraniliprole), 91-93% (cyantraniliprole), 87-90% (indoxacarb), 84-88% (acetamiprid) and 83-85% (spinetoram), in comparison to the control.

Key words: Grapholita molesta, peach, insecticide, efficacy

Introduction

The use of insecticides is the easiest, fastest and most economical way for control harmful insects in peach, but in order to determine the insecticidal potential of a substance as precisely as possible, it is necessary to test its effectiveness by appropriate methods. In Serbia, *G. molesta* represents one of the most damaging pests of peach fruits. It is believed to originate from East Asia, from where it has spread throughout the world. Damage caused by larvae results in decay and drying of shoots and fruits. Its host plants are peach, nectarine and plum, but also apricot,

pear and apple. It can cause huge damage in orchards in a short time, which results in reduced yields (Vukasović, 1964). The indirect damage is made by making favorable conditions for the penetration of phytopathogenic bacteria and fungi.

Also, it can limit export, so the infested fruits must be discarded. Especially in importing countries where this pest belongs to a quarantine species. Chemical measures play an important role in controlling peach moth. In Serbia, *G. molesta* can be suppressed by using insecticides from several chemical groups (pyrethroids, organophosphates, juvenile analogues hormones - mimics, modulators of ryanodine receptors) (Petrović and Sekulić, 2017), among which are nervous poisons, insect growth inhibitors and those with simultaneous action on the nervous system and musculature of insects (IRAC, 2018). In this study, the susceptibility of the oriental fruit moth *Grapholita* (*Laspeyresia, Cydia*) *molesta* Busck (Lepidoptera: Tortricidae), also known as the oriental peach moth, was tested to chlorantraniliprole, cyantraniliprole, spinetoram, indoxacarb, and acetamiprid based insecticides.

Material and Methods

In 2019 field trials were conducted in Republic of Serbia at localities Čelarevo (45°18'53.2"N 19°30'05.8"E) and Šišatovac (45°07'26.5"N 19°32'45.5"E). The experiments were carried out according to the standard EPPO (2014, 2015) methods, for experimental design, data analysis, insecticide efficiency and phytotoxicity. Products based on: chlorantraniliprole (a.i. 200 g/l), cyantraniliprole (a.i. 100 g/l), spinetoram (a.i. 250 g/kg), indoxacarb (a.i. 150 g/l) and acetamiprid (a.i. 200 g/kg) were foliar applied in an amount of 0.2, 0.6, 0.2, 0.33 and 0.25 l/kg ha^{-1}, respectively, using “Solo 423 Port” back sprayer with a water consumption of 1000 l/ha. The orchards were 5-6 years old with peach cultivar Royal gem, with a planting distance of 4.5 x 2 m. The experiment was set up in four replications, with the layout of the basic plots according to the randomized block system. The basic plot consisted of four peach trees. Before setting the trial at both of localities, the oriental fruit moth was monitored and its number was determined by counting moths caught in pheromone RAG traps (Csalomon®).

Efficacy evaluations were carried out 2 weeks after the treatment and 24 days after treatment (shortly before harvest). The results of the field trials were presented as absolute and mean values for the number of damaged fruits, standard deviation from the average values (Sd), efficacy (E%) according to Abbott (Wentzel, 1963) and statistically analyzed by ANOVA (analysis of variance) and Fisher LSD test (least significant difference) for the confidence interval of 95%, in statistical program R ver. 3.6.3.

Results and Discussion

Results of the efficacy studies of insecticides based in peach orchards for control of *G. molesta* are presented in Tables 1-2. Fourteen days after the application of the insecticides for the control of the 1st generation of *G. molesta*, the number of damaged fruits at locality Čelarevo was significantly lower compared to the control. Efficacy values were ranged from 87.7-96.5%. The highest efficacy after 14 days was obtained in treatments with chlorantraniliprole (96.5%) and cyantraniliprole (92.9%). After 24 days the efficacy of chlorantraniliprole, cyantraniliprole, spinetoram, indoxacarb and acetamiprid was 91.7, 93.3, 85.0, 86.7, 88.3% respectively.

Table 1. Insecticide efficacy against *G. molesta* (Čelarevo)

Čelarevo	Dose (L,kg/ha)	14 days after the treatment				24 days after the treatment			
		x̄		Sd±	E%	x̄		Sd±	E%
chlorantraniliprole (200 g a.i./L)	0.2	0.50	b	0.50	96.5	1.25	b	0.43	91.7
cyantraniliprole (100 g a.i./L)	0.6	1.00	b	0.71	92.9	1.00	b	1.22	93.3
spinetoram (250g a.i./kg)	0.2	1.75	b	0.83	87.7	2.25	b	0.43	85.0
indoxacarb (150g a.i./L)	0.33	1.50	b	0.50	89.5	2.00	b	0.71	86.7
acetamiprid (200g a.i./kg)	0.25	1.75	b	0.43	87.7	1.75	b	1.10	88.3
Control	/	14.25	a	2.86	/	15.0	a	4.12	/
LSD (0.05%)		1.91				2.76			

x̄ -average number of damaged fruits, Sd± standard deviation, E%- efficacy

Similar results were established at locality Šišatovac, i.e. 14 days after insecticide application for control of 1st generation of *G. molesta*, the number of damaged peach fruits was on a significantly lower level compared to the control. The efficacy ranged from 83.7-95.3. The highest efficacy after 14 days was in treatments with chlorantraniliprole (95.3%), cyantraniliprole (93.0%) and indoxacarb (90.7%). During the second evaluation, 24 days after the treatment, the number of damaged fruits was also on the significantly lower level in comparison to the control, with the efficacy of 87.0% (chlorantraniliprole, indoxacarb), 91.3% (cyantraniliprole), 82.6% (spinetoram), and 84.7% (acetamiprid).

Table 2. Insecticide efficacy against *G. molesta* (Šišatovac)

Šišatovac	Dose (L,kg/ha)	14 days after the treatment				24 days after the treatment			
		x̄		Sd±	E%	x̄		Sd±	E%
chlorantraniliprole (200 g a.i./L)	0.2	0.50	b	0.50	95.3	1.50	b	0.87	87.0
cyantraniliprole (100 g a.i./L)	0.6	0.75	b	0.83	93.0	1.00	b	0.71	91.3
spinetoram (250 g a.i./kg)	0.2	1.75	b	1.48	83.7	2.00	b	1.22	82.6
indoxacarb (150g a.i./L)	0.33	1.00	b	0.71	90.7	1.50	b	0.50	87.0
acetamiprid (200g a.i./kg)	0.25	1.50	b	1.12	86.1	1.75	b	0.83	84.7
Control	/	10.75	a	1.78	/	11.50	a	1.80	/
LSD (0.05%)		1.80				1.37			

x̄ -average number of damaged fruits, Sd± standard deviation, E%- efficacy

The efficacy studies in chemical control of *G. molesta* should be permanent. A comparison of the damaged fruits number, depending on locality, showed a highly uniform presence of damaged fruits evident at both of the localities. Pesticide products that are based on chlorantraniliprol have a very low toxicity to mammals (acute and chronic), high toxicity to target pests, strong ovi-larvicidal and larvicidal properties, long-term crop protection and do not show cross-resistance with other insecticides (Bassi et al., 2009). Tamaš et al. (2015) conducted field trials according to standard EPPO method in order to evaluate the efficacy cyantraniliprole, chlorantraniliprole, in peach orchards for *G. molesta* control in Serbia. The results indicated high efficacy of cyantraniliprole (0.6 l/ha, 100 g a.i./L) ranging from 94.5 to 97.9% and 94.2-98.7% for chlorantraniliprole (0.2 l/ha, 200 g a.i./L), which is consistent with our results. Due to its high efficacy, selectivity, and favorable toxicological and eco-toxicological properties, the insecticides based on cyantraniliprole may be important in anti-resistance strategy and integrated pest management (IPM) programs (Milanese et al., 2014, Bassi et al., 2009). In Italy, Boselli and Sccannavini (2014) performed six experiments in order to assess the efficiency of spinetoram in the control of *Cydia pomonella*. The achieved results showed excellent activity of spinetoram. Also, there were no adverse effects on beneficial arthropods and other non-target organisms. The effectiveness of indoxacarb applied to larvae of the *Cydia pomonella* L., was tested by Charmillot et al. (2003). Indoxacarb based product expressed 88.6% mortality to codling moth larvae. Acetamiprid, as well, showed excellent activity against Lepidoptera. The insecticide is applicable for controlling pests of fruit trees, vegetables, and so on (Yamada et al., 1999). According to Alvial and Andres (2012), the efficacy of 84% was achieved using acetamiprid for the control of the *Cydia pomonella* larvae.

Conclusion

Applied spray liquids indicate a satisfactory efficacy at both localities ranging from 92.3-96.6% (chlorantraniliprole), 91.1-93.9% (cyantraniliprole), 87.5-90.2% (indoxacarb), 84.4-88.5% (acetamiprid) and 83.4-85.2% (spinetoram), in comparison to the control. The highest efficacy at both sites was expressed by diamide based products (chlorantraniliprole, cyantraniliprole), while spinetoram had the lowest efficacy. The population of *G. molesta* in the peach orchards on the mentioned localities, showed high susceptibility to insecticides from the diamide group, but there is also good efficacy of insecticides from neonicotinoid, spinosyn and oxadiazine groups. The high efficacy of the applied insecticides indicates the sensitivity of *G. molesta* populations to the tested insecticides.

Acknowledgement

The authors appreciate funding from the Ministry of Education and Science of the Republic of Serbia for supporting this study.

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Original scientific paper

Dissipation dynamic of indoxacarb insecticide in peach fruits

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Abstract

The aim of this study was the evaluation of the safety use of insecticide indoxacarb in peach orchards, through the analysis of residues and dissipation dynamic. The field experiment was conducted at the locality Čerević (Republic of Serbia) according to EPPO methods. An indoxacarb based insecticide (150 g a.i./l) was applied in the quantity of 0.33 l/ha, when the peaches were in the BBCH 74 phenophase. Fruit sampling was performed one hour after the treatment and after 1, 3, 5, 7, 9, 11, 13 and 15 days. For the analysis of indoxacarb residues the QuEChERS method, followed with HPLC/DAD, was applied. The maximum level of indoxacarb residues in peaches was immediately after the drying deposit (2.21 mg/kg). Residues of indoxacarb in peach fruits at the MRL (1 mg/kg) were determined between the 7^{th} and 9^{th} day after the application. Based on the obtained results, the half-life of indoxacarb in peach fruits is 4.62 days.

Key words: pesticide residues, *Cydia molesta*, indoxacarb, peach fruits, MRL

Introduction

Peach (*Prunus persica*) is a widespread fruit in the world with over 2500 varieties. It has been cultivated for a very long time, according to some authors more than 4000 years (Werner and Okie, 1998). Serbia has the fifth largest amount of peach trees in Europe, with an average yield of 50-70 t/ha, depending on the year and environmental conditions (spring frosts and low winter temperatures) (Keserović et al., 2014). Peach production is disrupted by a wide range of diseases and pests. According to the importance and occurrence frequency, large losses are caused by peach powdery mildew (*Sphaerotheca pannosa var. Persicae*), drying of flowers and twigs and brown peach fruit rot (*Monilia laxa*), peach leaf curl (*Taphrina deformans*) and

red fruit mite (*Panonychus ulmi*). The most important peach pest in our agroecological conditions is *Cydia molesta*, the peach moth. The absence of adequate protection from these harmful organisms can lead to significant loses, and sometimes complete deterioration of plantations. Peach is susceptible to the attack of *C. molesta* from fruit formation to stone formation and complete ripening (Gvozdenović et al., 1997). The contemporary fruit production and high yields are almost impossible without the use of plant protection products (PPPs) and appropriate agro-technical measures. In order to control the population of peach moth (*Cydia molesta*) in the peach orchard, PPPs based on dimethoate, chlorpyrifos, α-cypermethrin, deltamethrin, λ-cyhalothrin, pyriproxyfen, chlorantraniliprole are registered. The pre harvest intervals (PHI) of these insecticides are 14-28 days. Since the PPPs, mainly from the group of pyrethroids, have been used for many years, which causes the emergence of populations of *Cydia molesta* resistant to these compounds, the goal is to introduce active substances with different modes of action. Products based on indoxacarb for the control of *Cydia molesta* in peach orchards in our country are not registered, while in the European Union they are registered, with a PHI of 7 days.

However, intensive and/or inappropriate use of PPPs leave residues that can be accumulated at levels higher than prescribed MRLs (maximum residue levels), which makes them potentially harmful to the consumers' health. One of the most important parameters in assessing the fate of pesticide residues (Li et al., 2016) is the dissipation rate and it can be used to estimate the required time for bringing residues down below MRLs (Ambrus and Lantos, 2002). The dissipation of pesticides after application depends on different factors such as plant species, pesticide chemical structure, type of formulation, application method, climate, and photodegradation (Lazić et al., 2018a).

In this study, a field experiment was performed in order to evaluate residue levels and dissipation dynamics of indoxacarb in peach fruits, under Serbian agroecological conditions.

Materials and Methods

The field experiments were carried out in Čerević (Republic of Serbia), in peach orchards, Royal Gem variety. For the control of *Cydia molesta*, peach trees were treated with the commercial formulation of PPP (150 g/l a.i. indoxacarb, EC), in the application rate of 0.33 l/ha, according to the manufacturer’s recommendation, when fruits were in the BBCH 74 phenophase. For the residue analysis, the fruit samples were randomly picked and collected after drying deposit, one day after and every second day during 15 days.

Figure 1. Extraction and clean-up procedure

The extraction of indoxacarb was performed by using a QuEChERS based method (Anastassiades et al., 2003) (Figure 1), followed with the determination using high performance liquid chromatography (HPLC/DAD, Agilent Technologies 1100 Serie) (Lazić et al., 2018b). The mobile phase was deionized water acidified with H_3PO_4 (pH 2.8) and acetonitrile (25/75, v/v), with the flow rate 1 ml/min, column temperature 25 °C and injected volume 20 µl, while 310 nm wavelength was applied. Under these conditions, the retention time of indoxacarb was 1.501 min.

The method was developed and validated in accordance with SANTE/11813/2017 and applied to the real samples.

Results and Discussion

Method validation

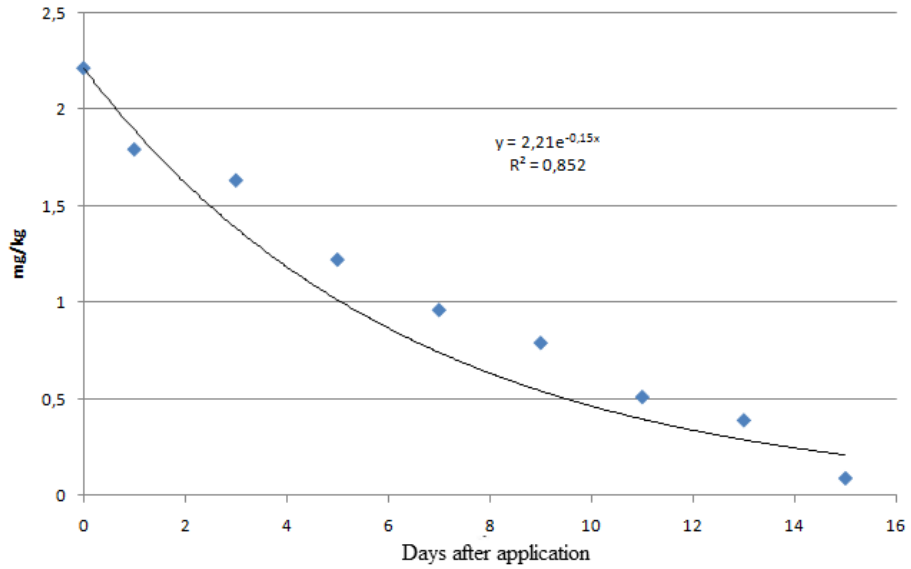
Validation of the method was evaluated through the linearity, precision, recovery, limit of detection (LOD) and quantification (LOQ) and the matrix effect. The results obtained for the parameters (Table 1) completely fulfilled SANTE/11813/2017 (Lazić et al., 2018b).

Table 1. Validation parameters

Insecticide	LOD (mg/kg)	LOQ (mg/kg)	R^2	Precision (%)	Recovery (%)	Matrix effect (%)
Indoxacarb	0.006	0.02	0.996	0.27	83.3-91.6	102.39

Dissipation dynamic

Validated method was applied for the analysis of indoxacarb residues in peach samples (Graph. 1). Residues contents were expressed as the sum of indoxacarb and its R-enantiomer. The highest content of indoxacarb residues in peach fruits (2.21 mg/kg) was determined directly after drying the deposit. After three days it decreased to 1.63 mg/kg and after 5 days it had fallen to 52.59% of the initial quantity. During the following days, the content of indoxacarb continuously decreased.



Graph. 1. Dissipation of indoxacarb in peach fruits

The maximum residue level (MRL) of indoxacarb in peach fruits in the European Union is 1 mg/kg (European Commission). The residues of indoxacarb in peach fruits, at the MRL level, were reached between 7th and 9th day after the treatment. Fifteen days after the application, the content of indoxacarb (0.09 mg/kg) in peach fruits was far below MRL.

Using the obtained results, the half-life (DT_{50}) of the analyzed insecticide was determined. The dissipation kinetics of the indoxacarb in peach fruits was determined by plotting residue content against time. The half-life of insecticide was calculated using the equation $C_t=C_0e^{-kt}$ where C_t represents the concentration of the pesticide residue at time t, C_0 represents the initial

concentration and k is the rate constant per day. DT_{50} was determined from the k value (DT_{50}=ln2/k) (Gupta and Shanker, 2008).

Table 2. Half-life (DT_{50}) of indoxacarb in peach fruits

Insecticide	Regression equation	Constant	R^2	DT_{50} (day)
Indoxacarb	$y = 2.21e^{-0,15x}$	0.15	0.852	4.62

The regression coefficient of indoxacarb (0.852) indicates a continuous and gradual decrease. Indoxacarb half-life in peach fruits obtained in this experiment, after application in the recommended quantity, was 4.62 days. According to the results of Yoon (2013), after the application of PPP based on indoxacarb in cauliflower in the amount of 100 g a.s./ha, the half-life was 6.33 days. In the experiment conducted in Greece, residues of indoxacarb + R enantiomer in peach samples were 0.12, 0.13 and 0.18 mg/kg (FAO, 2005). Also, according to research in Italy, after the application of indoxacarb in peach orchards in the quantity of 0.075 kg/ha with PHI of 7 days, indoxacarb residues were 0.16 – 0.06 mg/kg, while residues in peaches from experiments in Spain and France performed according to the same GAP as in Italy, were 0.05 mg/kg and 0.10 mg/kg, respectively (FAO, 2005). In our trial, indoxacarb residues 7 and 15 days after the application of plant protection product Avaunt 15 EC (0.33 l/ha), were 0.96 mg/kg and 0.09 mg/kg, respectively.

Conclusion

This study indicates that PPPs based on insecticide indoxacarb could be safety used in peach orchards in the recommended dose in our agroecological conditions, as well. The results would be helpful for involving indoxacarb in the control of *C. molesta* population and for reducing the residues and the potential risk of its presence.

Acknowledgement

This study was funded by the Ministry of Education and Science of the Republic of Serbia.

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Original scientific paper

Analysis of vitamin C content of pomegranate (*Punica granatum*) fruits in different localities of Herzegovina

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Abstract

The area of Herzegovina is known for the intensive cultivation of pomegranate, which is consumed fresh or processed into juice throughout the year. This study aimed to determine, through physico-chemical analysis, basic parameters on the quality of existing plantations of the investigated fruit species with special reference to the vitamin C content.

The sampling localities are approximately 40 km away from each other, and the following locations have been selected: Dubrave, Blagaj, Mostar, Zalik, Vrapčići, and Potoci. The analysis included pH% dry matter, acidity, % total sugars, and vitamin C content. The results of the analysis showed that pomegranate samples were sour fruits, with a small percentage of total sugars. The pH was quite low, while the vitamin C content is quite high. The content of vitamin C is slightly higher in four localities in mesocarp samples in comparison to pure grain samples, while in the other two localities its content was higher in pure grain samples. The highest amount of the vitamin C were determined in samples from the Mostar site, namely in a grain sample with mesocarp, as much as 11 mg / 100g.

Key words: pomegranate, physio-chemical properties, vitamin C

Introduction

Pomegranate (*Punica granatum*) is native to Persia, and in Latin, it is called the apple full of pits. It is a deciduous plant that belongs botanically to the Punicaceae family, and today about 1000 species of cultivated pomegranate are known (Levin, 1994). For optimum yield, it requires a long, warm summer, but withstands quite cold days as well as drought. It grows mainly in the southern parts of Bosnia and Herzegovina, with the fruits ripening in late autumn. In terms of resistance to low temperatures, it can withstand temperatures of -17^{o}C and even up

to -30^{o}C. The fruit is a berry and its taste and size depend on whether it is a cultivated or a wild species. The fruit of the tame rod is slightly larger and heavier than the fruit of the wild rod and it has larger light red seeds of 8-12 mm polygonal shape. All parts of the tree (leaves, flowers, and root) have been used in medicine for centuries (Gowda et al., 2009). The fruits are consumed fresh or processed into juice. The edible part of the fruit is grains rich in sugar, vitamins, polyphenols and minerals. The basic chemical composition of the fruit expressed per 100g of an edible portion is shown in Table 1.

Table 1. Chemical composition of the edible portion of pomegranate (USDA National Nutrient Database for Standard Reference, 2011).

NUTRIENT	SHARE IN THE EDIBLE PART OF THE FRUIT
Water (g)	77,93
Energy (kcal)	83,00
Proteins (g)	1,67
Fat (g)	1,17
Carbohydates (g)	18,70
Total sugar (g)	13,67
Fibers (g)	4,00
Ash (g)	0,53
Vitamin C (mg)	10,20
Folate (µg)	38,00
Pantothenic acid (mg)	0,38
Niacin (mg)	0,30
Vitamin E (mg)	0,60
Vitamin K (µg)	16,40
Choline (mg)	7,60
Iron (mg)	0,30
Calcium (mg)	10,00
Magnesium (mg)	12,00
Potassium (mg)	236,00
Sodium (mg)	3,00
Phosphorus (mg)	36,00
Zink (mg)	0,35
Selenium (µg)	0,50

Vitamin C in pomegranate

Ascorbic acid, as a reduced form of vitamin C, readily transfers two hydrogen atoms with electrons, thus becoming a dehydroascorbic acid (oxidized form) that does not have the antioxidant property of the vitamin. Due to its antioxidant properties, food manufacturers often add it as a preservative, as it prevents the food from browning and contributes to the vitamin C

content of the food. Vitamin C can be added as an antioxidant in the form of lemon juice as a natural substitute for the synthetic form (E300) (Marti et al., 2001). Vitamin C is a hydro-solubilizing compound that cannot be synthesized by the human body so that it is completely dependent on food intake. Ascorbic acid is an important antioxidant and is present in neurons in high concentrations. (Pavlović et al., 2015). Interest in the effects of vitamin C on the common cold arose shortly after purified vitamin C became available. The first controlled examinations of vitamin C were conducted in the 1940s. For example, in the 1950s, a British study examined the clinical effects of vitamin C deprivation and reported that "the geometric mean duration of colds was 6.4 days in subjects deprived of vitamin C and 3.3 days in non-deprived subjects, and the authors concluded that the absence of vitamin C tends to cause colds to last longer (Hemila H., 2017). The anticancer effects of vitamin C (ascorbic acid) have been known since 1969 when Benade et al. published a paper showing that the sodium salt of this nutrient (sodium ascorbate) is highly toxic or deadly to Ehrlich ascites cancer cells *in vitro* (Mastrangelo et al. 2017). Of the total fruit mass, about 52% are grains, and their composition is 80% juice and 20% seeds, which have been shown to have significant antioxidant activity (Kulkarni et al., 2004). Various studies have been conducted indicating the anti-inflammatory (Lansky & Newman, 2007), anticancer (Adhami & Mukhtar, 2006) antimicrobial (Reddy et al., 2007) and antiviral (Kotwall, 2007) effects of pomegranate juice.

Materials and Methods

The choice of locations was in different zones of the urban and suburban area of Mostar. Six sites were selected in total: the Dubrava Plateau area in the southern part of Herzegovina, the Blagaj suburban settlement, the Mostar locality, the Zalik suburban settlement, the Vrapčići suburban settlement and the Potoci area at the northern entrance to the Mostar town. The fruit sampling took place during the period when they were ripe and ready for consumption. Two types of pomegranates were sampled: sweetcorn, which is consumed in the largest quantities in the fresh state for about one month and chickpeas, which during this period in large quantities is squeezed into juice consumed throughout the year. Pomegranates were harvested from several trees randomly selected at each site for a total of about 30 ripe intact fruits, of which an average sample was subsequently prepared. Samples were stored in plastic containers at –20 ° C until laboratory determinations were made. Laboratory analyses of pomegranate and mesocarp grains included the following parameters: pH, total dry matter,

acidity, total sugars, and vitamin C. All laboratory analyses were performed at a laboratory certified by the Federal Agricultural Institute in Sarajevo.

Determination of total dry matter

Total dry matter is the total amount of substance from a sample that does not evaporate under defined conditions. Depending on the composition of the sample, three drying procedures are applied to determine the total dry matter: drying at 105°C, vacuum drying and distillation (Medar A. 2013). In this work, the drying method at 105ºC was used. This procedure determines the residue of the sample after drying at 105°C to a constant weight.

$$Dry\ matter\ (\%) = \frac{m_2 - m_0}{m_1 - m_0} \times 100$$

in which:

m_0 (g) - a mass of the container and auxiliary material

m_1 (g) - a mass of the test sample and container before drying

m_2 (g) - the mass of the vessel with the residue after drying

Determination of total acidity

The method of determining total acidity is based on potentiometric titration with sodium hydroxide solution. The method applies to the determination of total acidity in fruits and vegetables, as well as in fruit and vegetable products (Medar A. 2013).

$$\text{Total acidity}\ (\%) = \frac{\mathrm{V} \cdot \mathrm{F} \cdot \mathrm{G}}{\mathrm{D}} \times 100$$

where:

V (ml) - volume of NaOH used for titration

F - NaOH normality factor

G (g / ml) - gram equivalent of the most represented acid in the sample

D (g) - a mass of the sample in the titrated liquid

Determination of sugar content

The sugars were determined by the "Luff - Schroorl" volumetric method (Medar A. 2013). This method is based on the principle that, under certain conditions, reduced sugar (natural invert) translates Cu^{2+} ions into Cu^{+} ions. The unused amount of Cu^{2+} ions is retitrated with a thiosulphate solution. The difference between the consumption for the blank and the values in

the table gives the result of the amount of sugar. The untreated disaccharide must be pre-inverted, that is, hydrolyzed to the unreacted monosaccharides by acid, and then determined using a Luff reagent. In this way, the total amount of sugar in the test sample is obtained.
The content of the total invert is calculated according to the following formula:

$$\text{content of total invert (\%)} = \frac{V \cdot V_2 \cdot a}{Ok \cdot V_1 \cdot V_3 \cdot 1000} x100$$

where:
V - cm^3 of stock solution,
V1 - cm^3 of filtrate I,
V2 - cm^3 of diluted filtrate I after inversion,
V3 - cm^3 of the diluted filtrate I in which the sugars are determined,
a - amount of invert sugar,
Ok - weighed sample volume.

Determination of L-ascorbic acid (Medar A. 2013)
Determination principle:
2,6-p-dichlorophenolindophenol oxidizes L-ascorbic acid to dehydroascorbic acid until the color of the reagent turns into a colorless leuco base and serves as an indicator of this redox reaction. This method applies to the determination of ascorbic acid in vegetable and fruit products.

$$\text{Vitamin C } (\frac{mg}{100g}) = \frac{VxF}{D} x100$$

V (ml) - volume of 2,6-dichlorophenolindophenol consumed during titration,
F - normality factor of 2,6-dichlorophenolindophenol,
D (g) - a mass of the sample in the titrated liquid

Results and Discussion

The high biological value of the fruit is due to the content of dry matter, acids, invert sugars, vitamins and other substances. Table 2 shows the results of the physicochemical analyses of the pomegranate samples.

Table 2. Physicochemical analysis of pomegranate samples

Locality	sample	pHof the fruit (±0,01)	% Dry matter	Degree of acidity g/100ml	% Total sugar	Vitamin content C mg/100g of sample
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
Mostar	Grain	4,14	23,52	1,10	12,40	9,50
	With mesocarp	4,04	22,11	1,55	7,20	11,00
Zalik	Grain	3,18	26,29	2,42	10,60	10,50
	With mesocarp	3,06	26,33	2,64	8,20	5,60
Vrapčići	Grain	3,33	22,51	1,44	12,00	6,80
	With mesocarp	3,37	21,45	1,68	10,00	7,70
Potoci	Grain	3,48	19,83	1,20	7,40	8,50
	With mesocarp	3,44	17,85	1,66	5,20	7,15

According to the pH value of the fruit, which ranged from 3.04 to 4.14, it can be concluded that it is an acidic fruit species. The percentage of dry matter ranged from 17.85 in the Potoci site in the mesocarp sample, to 27.47 in the pure grain sample from the Blagaj site. The acidity level is quite low, the lowest values are at the Dubrave site of the pure grain sample, while the highest value is at the Zalik site of the sample with mesocarp. Acidity in all sites is higher in mesocarp samples. The percentage of total sugars is ranging from 5.20 to 12.40. According to the content of total sugars, it can be concluded that higher values are in the samples of pure grain. At four localities, cultivated pomegranate samples were taken, while the other two localities were wild pomegranate, i.e. peppercorn samples. The results of vitamin C content at the sites where the cultivated pomegranate sample was taken show a higher presence of this vitamin in mesocarp samples, which is not the case in wild pomegranate samples, where higher values are in pure grain samples. The highest values are in the sample from the Mostar site, the sample with mesocarp, and amount to 11.00 mg / 100g. From the results, it can be concluded that vitamin C is present in large quantities in samples with mesocarp. The results of the study by Dumlu and Gurkan (2007) confirmed a high content of vitamin C in different types of pomegranates, varying from 1,050 - 312 mg / 100g of edible portion. Interestingly, in addition to the pulp, vitamin C was also found in the pomegranate bark, and this content varies depending on the species and the area from which it originated. For example, the results of some studies show that pomegranates from India have a significantly higher proportion of vitamin C compared to those from Oman and Egypt (Opara et al., 2009). Amela M. (2013) states in her comparative study of the chemical composition of some pomegranate cultivars in

Herzegovina that the highest average vitamin C content ranged from 4.7 to 8.45 mg / 100g for the Glavas variety and from 3, 36 to 9.2 mg / 100g for "bar sweet" variety. The pH value was recorded for the bar sweet variety 4.09 and the Glavas cultivar 3.45. Ammararante et al. in 2012, in a study of phenolic substances and vitamin C content in pomegranate juice in Sri Lanka, reported that the content of vitamin C ranged from 6.3 - 8.4 mg / L.

Conclusion

A study conducted on pomegranate (*Punica granatum*) grown in the wider area of Mostar, of pure grain and mesocarp grains, showed the following:

Vitamin C content in all samples showed values at the upper literary range ranging from 5.60 mg/kg at the Zalik site in the mesocarp grain sample to 11.00 mg/kg at the Mostar site in the sample with mesocarp.

In four localities of Dubrava, Blagaj, Mostar, and Vrapčići, the content of vitamin C was slightly higher in samples with mesocarp grains in comparation with pure grains, while at two localities Potoci and Zalik, the higher content of vitamin C was in samples of pure grain in comparation with samples with mesocarp.

The obtained results of the pH value of a 3.04 sample of pure grain from the Blagaj locality to 4.14 samples of pure grain from the Mostar locality shows that the pomegranate is a highly acidic fruit. Acidity in all sites is higher in mesocarp samples.

The percentage of total sugars is quite low, ranging from 5.20 at the Potoci site in the mesocarp grain sample to 12.40 in the pure grain sample from the Mostar site. According to the content of total sugars, it can be stated that higher values are in the samples of pure grain.

The results of the study show that, according to the content of vitamin C, consuming pomegranates in the fresh state or as a juice can have a positive effect on the human body. In climatic conditions, Herzegovina is favorable for the cultivation of this plant, so it would be advisable to plant as many plantations of this crop as possible.

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Analiza sadržaja vitamina C u plodovima šipka na različitim lokalitetima Hercegovine

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Sažetak

Područje Hercegovine poznato je po intenzivnom uzgoju šipka, koji se konzumira u svježem stanju ili prerađen u sok, tokom cijele godine. Cilj ovog rada bio je, putem fizičko-hemijske analize, odrediti osnovne parametre o kvaliteti već postojećih nasada istraživane voćne vrste sa posebnim osvrtom na sadržaj vitamina C.

Lokaliteti su međusobno udaljeni oko 40 km vazdušne linije, te su odabrane sljedeće lokacije: Dubrave, Blagaj, Mostar, Zalik, Vrapčići i Potoci. Analiza je obuhvatila pH-vrijednost ploda, % suhe materije, stepen kiselosti, % ukupnih šećera, te sadržaj vitamina C.

Rezultati analize su pokazali da se radi o kiselom voću, sa malim postotkom ukupnih šećera. pH-vrijednost je dosta niska, dok je sadržaj vitamina C dosta visok. Nešto veći sadržaj ovog vitamina je na četiri lokaliteta u uzorcima sa mezokarpom, dok je na ostala dva lokaliteta njegov sadržaj veći u uzorcima čistog zrna. Najveće količine ovog vitamina su određene u uzorku sa lokaliteta Mostar i to u uzorku zrna sa mezokarpom, čak 11,00 mg/100g.

*Ključne riječi***:** šipak, fizičko-hemijske analize, vitamin C

Original scientific paper

Determination of the presence of aflatoxin b1 in feed in Republic of Srpska (BiH) in the period of 2017-2019

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Abstract

Aflatoxins are a mixture of related chemical compounds of bisfokumarine type. They are synthesized in a variety of agricultural and food products such as oilseeds, spices, cereals and other products.

In the period of year 2017– year 2019, 418 samples were analysed for the presence of aflatoxin B1, using the ELISA method. The analysis included samples of maize (370 samples), concentrated feed (284 samples), and other types of feed (silage, cereals, soy bean, etc.) (240 samples). The presence of aflatoxin B1 was determined in all of the analysed samples. The highest detected concentration of aflatoxin B1 was 30 μg/kg, in the maize samples, 25 μg/kg in the concentrated feed samples, and 15 μg/kg in the other analysed samples. Higher concentration of aflatoxin B1 from maximum residual level specified in valid document was detected in samples of concentrated feed.

Key words: Aflatoxin B1, ELISA, food, feed

Introduction

Mycotoxicosis problems burden agriculture and livestock production worldwide. Mycotoxins in food and feed are recognized as a public health problem. Fungal toxins dealt with many researchers which established their carcinogenicity. Aflatoxins (AFB1, AFB2, AFG1, AFG2, AFM1, AFM2) are a potential teratogenic and carcinogenic metabolic products of Aspergillus flavus, *Aspergillus parasiticus* and *Aspergillus nomius* (Kurtzman at al., 1987). Fungi are commonly found on the grains, almonds, walnuts, and peanuts. According to FAO data (FAO, 2018) cereal contamination with mycotoxins at the world level is 25-30% of the harvest.

Aflatoxins are a mixture of related chemical compounds. Series of aflatoxin B has the structure of molecules, which a cyclopentane ring is replaced by a G in the series of the -lactone. Three

structural variations give 18 molecules of aflatoxin, of which 8 is toxic and so far known aflatoxin B1 is the most toxic (Sinovec et al., 2006). Clinical signs of an acute aflatoxicosis include loss of appetite, lethargy, weight loss, neurological disorders, jaundice mucous membranes and cramps. High doses of aflatoxins are acutely toxic, causing substantial damage to the liver and intestinal and peritoneal bleeding, which can be lethal (Marriott and Gravani 2006).

Material and Methods

As the material in our testing we used a feed (silage, maize, concentrate feed). Analyses were performed by ELISA method, using test kit produce by manufacturer Bioscinetific, Max signal, (Austin USA).

Test protocol

5.0 g of representative and ground samples were extracted with 25 mL of 70% methanol. Extraction was performed by combination of shaking, vortex and centrifuge of the samples. The obtained supernatants were diluted with solution C, and vortexed. 50 µL of the diluted samples was used for the analysis.

Results and Discussion

The obtained results for feed samples are shown in Table 1.

Table 1. Feed samples

	2019 year				2018 year				2017 year			
	µg/kg											
	<0,5	0,5 - 5,0	5,0 - 20,0	>20,0	<0,5	0,5-5,0	5,0-20,0	>20,0	<0,5	0,5-5,0	5,0-20,0	>20,0
Maize	49	14	-	1	129	20	10	-	106	11	29	1
Concentrate feed	27	27	1	-	31	59	8	1	57	50	22	1
Other feed	30	26	-	-	29	19	3	-	22	92	19	-

Table 1 shows review for period 2017-2019 where total of 894 samples were analysed. 46,30% (414 samples) were contaminated by aflatoxin B1. 2,46% (22 samples) at concentrations higher than allowed by valid regulation. The greatest number of samples (480) had a concentration of aflatoxin B1 less than 0,5 µg/kg, and 318 samples in the concentration range from 0,5µg/kg - 5,0 µg/kg, 92 samples in the concentration range from 5,0 µg/kg -20,0 µg/kg. The highest

detected concentrations of aflatoxin B1 in maize was 30,0 μg/kg, and at concentrate feed was 25 μg/kg, and other feed 15 μg/kg.

Results of this studies are compared with results of available literature. Kos et al. (2013) in their studies found that of the 78 analysed samples, in 44 was detected presence of aflatoxin B1. The most contaminated samples had a concentration (17.9%) of 1-10 ppb. Škrinjar et al. (2013) reported that in 12 of the tested samples the concentration aflatoxin B1 in feed material was from 6 μg/kg to 145.8 μg/kg, while in this study a highest concentration of 30 μg /kg were recorded. Nedic et al. (2014) in their work detected 7.21% of positive samples in concentrate feed analysed in year 2013, which was slightly higher than the results of this examination. Increased values of concentration of aflatoxin B1 (51%) was found by Škrinjar et al. (2013) in their studies. Almeida et al. (2013) in their research reported that the largest number of analysed samples has a concentration of aflatoxin less than 5 μg/kg, which would correspond to this study test results. Results of analysis of Nedic et al. (2014) and Škrinjar et al (2013), indicates on increased concentration of aflatoxin B1 in feed in 2012 which is direct consequence of drought that happened that year in this region. Similar results were also obtained by Dojčinović et al (2017).

According to official data (FAO, 1995), the average content of aflatoxin B1 varied between 4 and 8 μg/kg with a maximum value of 30 μg/kg. In world-wide scale in the period 1986-1997 from 2,460 tested samples of grain 1273 of the sample contained B1 at concentrations of 7-44 μg/kg. Furthermore, in Brazil, from 2 546 samples of corn, 51% were contaminated with aflatoxin B1, with a maximum quantity amounted to 2440 μg/kg (Sinovec et al, 2006), which was more than determined in this research. Higher concentrations of aflatoxin B1 (251 μg/kg) were also recorded in China (Villers,2017).

Researchers in Spain (Bonnet et.al.,2013) by analysing the rice from the different areas have found that the rice is contaminated by aflatoxin B1 in the range from 0.8 μg/kg to 91.7 μg/kg. The same researchers examined rice in Mexico where they have found concentration aflatoxin B1 in range from 4.5 μg/kg to 8,1μg/kg (Bonnet et al., 2013). The mean concentration of aflatoxin B1 in peanut samples, in season 2014/2015 amounted to 38.24 μg /kg (Villers, 2017), which was higher than the value that obtained in this study.

Conclusion

Based on the results obtained in this experiment it is possible to conclude that is necessary to do monitoring of concentration aflatoxin B1. Concentration aflatoxin B1 depends on climate

changes. Therefore, it is necessary to apply adequate agro technical measures and select the hybrids of corn and other food and feed, which are more resistant to contamination of aflatoksin B1.

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Одређивање присуства Афлатоксина Б1 у храни за животиње у Републици Српској (БиХ) у периоду 2017-2019

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Апстракт

Афлатоксини су смеша сродних хемијских једињења типа бисфокумарин. Синтетишу се у различитим пољопривредним и прехрамбеним производима као што су уљане семенке, зачини, житарице и други производи.

У периоду 2017-2019. године, 418 узорака је анализирано на присуство афлатоксина Б1, коришћењем ЕЛИСА методе. Анализа је обухватила узорке кукуруза (370 узорака), концентроване хране (284 узорка) и друге врсте крме (силажа, житарице, соја итд.) (240 узорака). У свим анализираним узорцима утврђено је присуство афлатоксина Б1. Највећа откривена концентрација афлатоксина Б1 била је 30 µg/kg, у узорцима кукуруза, 25 µg/kg у концентрованим узорцима хране и 15 µg/kg у осталим анализираним узорцима. У узорцима концентроване хране откривена је већа концентрација афлатоксина Б1 од максималне заостале вредности наведене у важећем документу.

Кључне речи: *афлатоксин Б1, ЕЛИСА тест, храна, храна*

Original scientific paper

Microbiological criteria in the production of sterilised milk

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Abstract

Ultra-high temperature milk processing is generally defined as heating milk between 135 and 145ºC for 1-10 s. The product defined in this way indicates that it does not contain vegetative forms of microorganisms and it has been packaged in a hermetically sealed container to be stable at room temperature. This is difficult to achieve 100% of the time and so some ultra-high temperature milks contain microorganisms. Many of these are thermophilic spore-forming bacteria, whose spores survive the heat treatment.

Samples of sterilized milk come from a dairy from the Republic of Srpska (Bosnia and Herzegovina), and were sampled over a six-month period. Within self-control, 302 samples from production and 21 samples from the distribution centre were tested.

The aim of the research was to determine the safety of sterilized milk and hygiene conditions in the production process based on the test results of sterilized milk in the production process, as well as to consider a proposal for recommended microorganisms to be tested in the sterilized milk production process.

For microbiological testing of sterilized milk were used methods BAS EN ISO 4833-1, BAS ISO 15213 and BAS EN ISO 11290-1.

In the self-control of sterilized milk, 12.30% of the samples were unsatisfactory according to the recommended criteria. An increased colony count was the cause in 97.30% of unsatisfactory samples, while in 2.70% of the samples the cause was sulphite-reducing anaerobic bacteria. *Listeria monocytogenes* was not detected in the tested samples. The main cause of unsatisfactory samples of sterilized milk are aerobic bacteria, with or without the possibility of spore formation. In the self-control of sterilized milk, it is justified to test the colony count and sulphite-reducing anaerobic bacteria according to the recommended criteria.

Keywords: sterilised milk, microbiological criteria, hygiene process

Introduction

Ultra high temperature (UHT) milk processing is generally defined as heating milk between 135 and 145ºC for 1-10 s. In practice, such a range of temperatures and times accounts for most commercial plants (Tran et al., 2008), although some commercial treatments are more severe, operating as high as 152ºC with holding times up to 13 s (Cattaneo, 2008).

Raw material for the production of UHT milk is raw milk, which is suitable medium for the growth of microorganisms due to high aw values, moderate pH (6.4-6.6) and sufficient nutrient content. This requires high standards of hygiene in the production of milk and milk products. Milk obtained from healthy animals, aseptically taken, is generally sterile or contains very few microorganisms, usually less than 10^2-10^3 CFU/ml. Microorganisms mature in milk in the case of mastitis when their number may be about 10^5 CFU/ml or by subsequent contamination from the outside environment. In fresh milk, heat resistant microorganisms that survive the pasteurization process can be present (Adams and Moss, 2008). These are mainly Gram positive bacteria that form spores, as well as members of the genus *Micobacterium*, *Micrococcus, Enterococcus* and *Lactobacillus*. Minimum combinations of time and temperature for pasteurized milk are 63°C for 30 minutes or 72°C for 15 seconds (Burton, 1986; Rulebook, 2011). The application of heat treatment during which pasteurization takes place is sufficient to reduce 99.99% of microorganisms from raw milk (Juneja, 2003). Some bacterial species are capable of forming heat-resistant spores that survive high temperature short time pasteurization (Collins, 1981).

Two major factors influenced the introduction of UHT processing of milk. The first was the desire to produce a shelf-stable, safe product with better sensory characteristics than in-container sterilised milk; this milk has a very distinct heated flavour and a brownish colour. The second factor was the development of aseptic packaging. High-temperature heat processing had been available for some time but it was not until aseptic packaging was commercialised that the treated milk could be effectively stored at room temperature without spoilage (Robertson, 2003). UHT milk has a better microbial quality as compared to other types of milk available (Agarwal et al., 2012). Since the introduction of UHT processing combined with continuous aseptic packaging, the technology has been reasonably stable and many of the challenges facing UHT processors have remained. These include minimising heat induced flavours, destroying bacterial spores and enzymes, particularly proteinases, while avoiding

unacceptable chemical change, and minimizing fouling or burn-on on heat exchangers. However, new challenges have also arisen. A major one was the identification in UHT milk of *Bacillus sporothermodurans*, a mesophilic sporeformer, which produces extremely heat resistant spores which are difficult to remove from UHT plants (Hammer et al., 1996). Several instances of spore-forming bacteria in UHT milk believed to have survived the heat treatment have been reported (Meier et al., 1995; Forschino et al., 1990). The spores capable of surviving the UHT process are mainly *Geobacillus stearothermophilus* (previously *Bacillus stearothermophilus* and before that *Bacillus calidolactis*), *B. subtilis* (Muir, 1990), *B. megaterium* (Hassan et al., 1993), *B. sporothermodurans* (Pettersson et al., 1996; Scheldeman et al., 2004; Scheldeman et al., 2006) and *Paenibacillus lactis* (Scheldeman et al., 2004). *G. stearothermophilus* has a high survival potential with some strains showing extreme heat stability. Decimal reduction times (the time taken for a 1-log reduction) at 121ºC of up to 6.2 minutes have been reported (Burton, 1988). Intaraphan (2001) found that the spores of one strain of *G. stearothermophilus* isolated from a farm hot water supply survived a laboratory heat treatment of 154ºC for up to 9 s. While the bacterial contaminants in UHT milk can be either heat-resistant spore-formers or post-sterilisation contaminants, most failures are due to post-sterilisation contamination (Tran et al., 2008; Lewis and Heppell, 2000; Cattaneo, 2008). Rulebook on microbiological criteria for food (2012; 2013) prescribes general and special conditions of food hygiene at any stage of production, processing and trade (microbiological criteria for food) as well as rules which food business operators have to respect when applying general and special hygienic measure based on risk analysis of critical control points. Food safety criteria define the acceptability of a product and is applied to products in the market. Food safety criteria and their limit values apply to food from the dispatch phase until the expiration date. In cases where food is in the dispatch phase and transport documents have already been drawn up, food safety criteria are considered to be applied (Guide, 2013). The process hygiene criterion is a criterion that applies to food production and processing and indicates the proper functioning of the production process by representing the value of the contamination above which corrective measures are taken to maintain the process hygiene (Rulebook, 2012; Rulebook, 2013). Recommended microorganisms, with the category of food they refer to, are applied primarily at the end of the production process, but their limit values are recommended throughout the shelf life of the product, and can be used in the definition of microbiological criteria in the preparation of the manufacturer's raw material specifications (Guide, 2013).

Food business operators decide on the frequency of sampling, except in cases for which the sampling frequency is specified in the Rulebook on microbiological criteria for food (2012; 2013). Sterilized milk may be regarded as ready-to-eat foods. In this context, a parallel can be drawn with microbiological criteria for ready-to-eat foods. The food safety criterion for ready-to-eat foods provides for testing for *Listeria monocytogenes* (Rulebook 2012; Rulebook 2013). The test method is BAS EN ISO 11290-1 (Microbiology of food chain, 2018), and the limit value is absence in 25ml (M=m, n=5, c=0). However, regular testing does not apply to the criterion under normal circumstances for heat-treated or otherwise processed food that effectively eliminates *Listeria monocytogenes* when recontamination is no longer possible after such treatment. Sterilized milk is produced at temperatures that destroy *Listeria monocytogenes* (Tran et al., 2008; Rulebook, 2011; Juneja, 2003).

The recommended microbiological criteria for sterilized milk (Guide, 2013) are:

- colony count M<1CFU (colony-forming unit)/ml (n=5, c=0),
- number of sulphite-reducing anaerobic bacteria M<1CFU/ml (n=5, c=0).

The aim of the research was to determine the safety of sterilized milk and hygiene conditions in the production process based on the test results of sterilized milk in the production process, as well as to consider a proposal for recommended microorganisms to be tested in the sterilized milk production process.

Materials and Methods

Samples of sterilized milk come from a dairy from the Republic of Srpska (Bosnia and Herzegovina), and were sampled over a six-month period (January-June). Within self-control, 302 samples from production and 21 samples from the distribution centre were tested. Each sample consisted of 5 units. Testing of sterilized milk was performed according to the plan given in Table 1.

Table 1. Sampling plan for sterilized milk

Month	Production/ Distribution centre	Number of samples	Parameter
January	Production	50	Colony count sulphite-reducing anaerobic bacteria
	Distribution centre	2	*Listeria monocytogenes*
February	Production	37	Colony count sulphite-reducing anaerobic bacteria
	Distribution centre	4	*Listeria monocytogenes*
March	Production	52	Colony count sulphite-reducing anaerobic bacteria
	Distribution centre	4	*Listeria monocytogenes*
April	Production	71	Colony count sulphite-reducing anaerobic bacteria
	Distribution centre	4	*Listeria monocytogenes*

Month	Production/ Distribution centre	Number of samples	Parameter
May	Production	43	Colony count sulphite-reducing anaerobic bacteria
	Distribution centre	4	*Listeria monocytogenes*
June	Production	49	Colony count sulphite-reducing anaerobic bacteria
	Distribution centre	3	*Listeria monocytogenes*

Laboratory testing of samples was performed at the Public Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka.

The following standard test methods were used for microbiological testing of sterilized milk:

- BAS EN ISO 4833-1 (Microbiology of food chain, 2014.) for enumeration of colony count,
- BAS ISO 15213 (Microbiology of food and animal feeding stuffs, 2008.) for enumeration of sulphite-reducing anaerobic bacteria,
- BAS EN ISO 11290-1 (Microbiology of food chain, 2018.) for detection of *Listeria monocytogenes*.

In our research and in the statistical analysis of the obtained results, we used, as basic statistical methods, descriptive statistical parameters. The research results are presented in tables.

Results and Discussion

According to the Rulebook on dairy products and starter crops (2011), sterilized milk is defined as a product that is subjected to a treatment that "involves a continuous flow of the process at high temperature for a short time (at least 135ºC in combination with an adequate maintenance time), so that there are no surviving microorganisms or spores that could develop in the processed product if kept in an aseptically sealed container at room temperature." The product defined in this way indicates that it does not contain vegetative forms of microorganisms and that there are no criteria for sterilized milk within the microbiological criteria for food safety. The same regulation requires that the sterility of this product be checked before it is placed on the market by incubating the product for 15 days at 30ºC or 7 days at 55ºC, to determine that there is no spore that can germinate during storage and consequent growth and metabolic activity cause the product to spoilage within a given shelf life.

Aseptic processing means that the product has been subjected to sufficient heat processing to render it commercially sterile and that it has been packaged in a hermetically sealed container. These dairy foods are stable at room temperature (Sperber and Doyle, 2009).

Microbiology is central to UHT processing and products. The UHT process arose out of the need to destroy or exclude microorganisms which are likely to cause milk to spoil during ambient storage. This means that the process has to be severe enough to destroy any microorganisms in the raw product which might grow at room temperature and also has to ensure that in the post-sterilisation section of the plant, i.e. the cooling sections, the aseptic storage tank and the packaging stage, no such microorganisms gain access to the product. This is difficult to achieve 100% of the time and so some UHT milks contain microorganisms. Many of these are thermophilic spore-forming bacteria, whose spores survive the heat treatment and will not grow below ~50ºC, but other types of microorganisms can be present and some can cause spoilage (Mansel, 2010)

Self-control performed by food business operators in the process of producing sterilized milk was performed according to the Guide for microbiological criteria for food (2013). The results of self-control in the process of production of sterilized milk according to the recommended criteria are shown in Table 2.

Table 2. Results of self-control in the process of production of sterilized milk according to the recommended criteria

Month of sampling/ Number of samples	Parameter	Sampling plan		Interpretation of results
		n	c	
January / 50	Colony count	5	0	44 satisfactory (88%) 6 unsatisfactory (12%)
	Sulphite-reducing anaerobic bacteria	5	0	50 satisfactory (100%)
February / 37	Colony count	5	0	32 satisfactory (86,50%) 5 unsatisfactory (13,50%)
	Sulphite-reducing anaerobic bacteria	5	0	37 satisfactory (100%)
March / 52	Colony count	5	0	49 satisfactory (94,20%) 3 unsatisfactory (5,80%)
	Sulphite-reducing anaerobic bacteria	5	0	52 satisfactory (100%)
April / 71	Colony count	5	0	52 satisfactory (73,20%) 19 unsatisfactory (26,80%)
	Sulphite-reducing anaerobic bacteria	5	0	70 satisfactory (98,60) 1 unsatisfactory (1,40%)
May / 43	Colony count	5	0	40 satisfactory (93%) 3 unsatisfactory (7%)
	Sulphite-reducing anaerobic bacteria	5	0	43 satisfactory (100%)
June / 49	Colony count	5	0	49 satisfactory (100%)
	Sulphite-reducing anaerobic bacteria	5	0	49 satisfactory (100%)

In the period January-June, the food business operators conducted self-control on 302 samples of sterilized milk according to the recommendations given in the Guide (2013). Of the total number of sterilized milk samples, 12.30% (37) of samples were unsatisfactory. Of these, 97.30% is due to an increased colony count, while 2.70% is due to sulphite-reducing anaerobic bacteria. Similar results were obtained Jeppu et al. (2015), where is only seven out of the 60 (11.67%) UHT milk samples failed the colony count test. Non-spore-forming bacteria, both Gram positive and Gram negative, have also been isolated from UHT milk in several studies. In a South African study, 12% of the non-sterile UHT milks contained non-spore-forming bacteria (Lück et al., 1978) and in a Brazilian study 7% of the microorganisms isolated from UHT milks were non-spore-formers (Coelho et al., 2001). The bacteria isolated include *Micrococcus* and *Corynebacterium*-like Gram-positive bacilli (Coelho et al., 2001), *Streptococcus lactis* and unspecified Gram-negatives (Von Bockelmann and Von Bockelmann, 1998) and *Staphylococcus aureus*, *Streptococcus faecalis* and *Enterobacter sakazakii* (Skladal et al., 1993). The presence of these organisms in a product that is supposed to be commercially sterile emphasises the constant battle that UHT processors have to maintain sterility in the whole processing and packaging line.

Observed on the total number of tested samples, it is noticeable that a negligible number of unsatisfactory samples is due to sulphite-reducing anaerobic bacteria (0.35%). This indicates that the main cause of unsatisfactory samples are aerobic bacteria, with or without the possibility of spore formation (11.95%).

A significant percentage of samples labelled unsatisfactory indicate that good manufacturing and good hygiene practices are not applied during the sterilized milk production process. The cause of microbial contamination of sterilized milk should be sought in the adequacy of the sterilization process, the packaging process and the sterility of the packaging.

Any microorganism in UHT milk which is not a heat-resistant spore-former must enter the product after the sterilisation step, assuming the appropriate UHT heating conditions are used. In addition, some spore-formers may also be post sterilisation contaminants. Since *Bacillus* species make up the majority of the contaminants (Lück et al., 1978; Forschino et al., 1990; Skladal et al., 1993; Von Bockelmann and Von Bockelmann, 1998; Coelho et al., 2001), it is possible that many of these are post-sterilisation contaminants.

However, Cerf and Davey (2001) suggested that non-sterility rates in UHT milk could be explained statistically on the basis of residence time distribution in the UHT plant, whereby a very small percentage of spores pass through the holding tube too fast to be destroyed.

The subject introduced a test for the presence of *Listeria monocytogenes* as part of the self-control of the sterilized milk production process. The results obtained are shown in Table 3.

Table 3. Test results of sterilized milk for the presence of *Listeria monocytogenes*

Month of sampling/ Number of samples	Parameter	Sampling plan		Interpretation of results
		n	c	
January / 2	*Listeria monocytogenes*	5	0	2 satisfactory (100%)
February / 4	*Listeria monocytogenes*	5	0	4 satisfactory (100%)
March / 4	*Listeria monocytogenes*	5	0	4 satisfactory (100%)
April / 4	*Listeria monocytogenes*	5	0	4 satisfactory (100%)
May / 4	*Listeria monocytogenes*	5	0	4 satisfactory (100%)
June / 3	*Listeria monocytogenes*	5	0	3 satisfactory (100%)

As part of self-control, 21 samples of sterilized milk were tested for the presence of *Listeria monocytogenes*. *Listeria monocytogenes* was not detected in any of the samples tested. The results obtained are in line with those of Agarwal et al. (2012). *Listeria monocytogenes* is a microorganism from the group of mesophilic microorganisms and grows at an interval of 1-45°C (Walker et al., 1990).

The thermoresistance of *Listeria monocytogenes* is determined on the basis of the D-value. The D value for Listeria monocytogenes at 71,1°C was 0,17 minutes (Juneja, 2003). In sterilized milk, where temperatures of 136°C are applied for a few seconds, the listeria is certainly destroyed, so it is not justified to prove it in sterilized milk. For this reason, *Listeria monocytogenes* is not even listed as the recommended microbiological criterion for sterilized milk (Guide, 2013).

Conclusion

In the self-control of sterilized milk, 12.30% of the samples were unsatisfactory according to the recommended criteria. An increased colony count was the cause in 97.30% of unsatisfactory samples, while in 2.70% of the samples the cause was sulphite-reducing anaerobic bacteria. *Listeria monocytogenes* was not detected in any of the samples tested. The main cause of unsatisfactory samples of sterilized milk are aerobic bacteria, with or without the possibility of spore formation. In the self-control of sterilized milk, it is justified to test the colony count and sulphite-reducing anaerobic bacteria according to the recommended criteria. There is a reasonable risk of post-contamination in the production of sterilized milk, and for this reason special attention should be paid to controlling the sterility of packaging and the packaging process of sterilized milk.

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Original scientific paper

Yolk sac conversion efficiency of brown trout (*Salmo trutta m. fario*) and rainbow trout (*Oncorhynchus mykiss*) during endogenous nutrition

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Abstract

The subject of this paper's research is endogenous nutrition for brown trout (*Salmo trutta* m. *fario*) and rainbow trout (*Oncorhynchus mykiss*). The goal of the paper was to determine the efficiency of yolk sac conversion in brown and rainbow trout during endogenous nutrition. The experiment was conducted in the Aquaculture Laboratory of the Faculty of Agriculture, University of Banja Luka. The eggs was placed in trays with three repetitions for brown trout, and three for rainbow trout. Standard body length, length and height of yolk sac were measured after hatching, 27th day of incubation (357 dd) of the rainbow trout (n = 20) and 30th day of incubation (492 dd) for the brown trout (n = 20) and before swimming, 36th day of incubation (492 dd) of rainbow trout (n = 20) and 43rd day of incubation (571 dd) for the brown trout (n = 20). Obtained data was used for determining volume and yolk sac conversion efficiency for the brown and rainbow trout. Standard body length of brown trout after hatching was not in corellation with the yolk sac volume (Pearson's r = 0.25; P = 0.141), and the rainbow trout standard body length was in corellation with the yolk sac volume after hatching (Pearson r = 0.47; P = 0.019; α=0,05). Standard body length, and the yolk sac length and height for brown trout from hatching to swimming were higher, but the yolks sac conversion efficiency for brown trout was lower. Yolk sac conversion efficiency for brown trout from hatching to swimming was 0.121, and for rainbow trout it was 0.178.

Key words: yolk sac conversion efficiency, brown and rainbow trout

Introduction

Brown trout (*Salmo trutta* m. *fario)* and rainbow trout (*Oncorhynchus mykiss)* represent important salmonid fish species in aquaculture and sport fishing. These two species were the subject of many studies, rainbow trout especially, which represents one of the most researched fish species in aquaculture. Fish growth and development are generally highly dependent on the water temperature. Desirable water temperatures for spawning and incubation fertilized eggs range from 8 to 10^{o}C, for fry from 10 to 12^{o}C and for juvenile fish from 12 to 16^{o}C (Savić et al. 2018). Kamler and Kato (1983) state that, during incubation of fertilized rainbow trout eggs, to complete yolks sac resorption at water temperatures 9, 10, 12 and 14^{o}C, with increasing the water temperatures larvae mass was increasing as well, and From and Rasmussen (1991) state that at water temperatures 5, 10 and 15^{o}C, larvae has the highest mass at the temperature of 10^{o}C. Salmonid fish species have bigger eggs in comparison to many other fish species, and big embryos, that contain a large yolk sac. Embryo size is determined by the area and volume which shrinks with the increase of embryo size (Cabrita et al. 2009.). Bonislawska et al. (2001) releases data that the duration of embryonic development measured in thermal units points to a significant level of interconnectedness between egg size and embryogenesis duration. Many researches about eggs size and properties were conducted without relation to other characteristics (area and volume, yolk sphere and perivitelline space) and because of that, they lack a common denominator, that is, a unifying theory of phylogenetic correlation between species that would properly describe the rate of change during embryogenesis (Imanpoor et al., 2009). Rainbow trout growth and development from hatching to first feeding depends on the nutritional substances content in the yolk sac (Hodson and Blunt, 1986). Reiser et al. (2019) state that the length and mass growth of the rainbow trout larvae with yolk sac in degree days function. Khan (2018) states that obtained results of yolk sac conversion efficiency for brook trout (*Salvelinus fontinalis)* in the realized research they can facilitate the management of the hatchery in order to determine the optimal method of rearing the larvae immediately after the use of the yolk sac. The subject of this paper's research is endogenous nutrition for brown trout (*Salmo trutta m. fario*) and rainbow trout (*Oncorhynchus mykiss*). The goal of the paper was to determine the efficiency of yolk sac conversion in brown and rainbow trout during endogenous nutrition.

Material and Methods

The experiment on the yolk sac conversion efficiency for brown trout (*Salmo trutta m. fario)* and rainbow trout (*Oncorhynchus mykiss*) during endogenous nutrition (from hatching to

swimming) was conducted in Aquaculture Laboratory of the Faculty of Agriculture, University of Banja Luka. The eggs was placed in three repetition trays for brown and rainbow trout. Water analysis included water temperature (°C) and dissolved oxygen content in water (mg/l). Water temperature and dissolved oxygen content in water were measured with a digital oximeter – Oxi 330i / SET 2B20-0011 (WTW - Germany).

The diameter (d) of brown trout eggs (n = 20), and rainbow trout eggs (n = 20) was measured at the embryonic stage of eyes, after which the average diameter of the eggs was determined.

$$d = \frac{(d_1 + d_2)}{2}$$

d_1 - diameter, width egg (mm); d_2 - diameter, height egg (mm)

Standard body length, yolk sac length and height were measured after the hatching, on the 27th day of incubation (357 dd) of rainbow trout (n = 20) and the 30th day of incubation (404 dd) of brown trout (n = 20) and just before the swimming, 36th day of incubation (492 dd) of rainbow trout (n = 20) and 43rd day of incubation (571 dd) of brown trout (n = 20). The data obtained was used to determine the volume and yolk sac conversion efficiency for brown and rainbow trout.

$$V_{ys} = l_{ys} \cdot h_{ys}^2 \cdot \frac{\pi}{6}$$

V_{ys} – yolk sac volume (mm^3); l_{ys} – yolk sac length (mm); h_{ys} – yolk sac height (mm); π – 3,14

Yolk sac conversion efficiency (CE_y) was calculated according to Fraser et al. (2010):

$$CE_{ys} = \frac{L_{t2} - L_{t1}}{V_{ys,\,t1}}$$

CE_{ys} – yolk sac conversion efficiency; L_{t2} – body length after yolk absorption; L_{t1} – body length after hatching; $V_{ys,\,t1}$ – yolk volume during hatching

Digital images inserted into a computer and analyzed using ImageJ 1.49v (Wayne Rasband, National Institutes of Health, USA) were used to measure the diameter of eggs, standard body length, length and height of the yolk sac. The SPSS17 statistical program was used to determine the parameters of the descriptive statistics and the Pearson's correlation coefficient.

Results and Discussion

The results obtained from the analysis of water temperature and dissolved oxygen content in water (Table 1) do not indicate a significant difference between treatments during the observation period.

Table 1. Water temperature (°C) and dissolved oxygen (mg/l) (average±SD) during the observation period

Parameter		*Brown trout (Salmo trutta m. fario)*		*Rainbow trout (Oncorhynchus mykiss)*	
		The number of days		*The number of days*	
		from fertilization to hatching	*from fertilization to swimming*	*from fertilization to hatching*	*from fertilization to swimming*
		30	*43*	*27*	*36*
Water temperature	*°C±SD*	*13,45±0,69*	*13,28±0,87*	*13,23±1,43*	*13,67±1,00*
	CV	*5,11*	*6,55*	*10,78*	*7,31*
Dissolved oxygen	*mg/l±SD*	*8,48±0,81*	*8,81±0,84*	*8,68±0,13*	*8,54±0,22*
	CV	*9,60*	*9,56*	*1,52*	*2,62*

SD – Standard deviation. CV – Coefficient of variation.

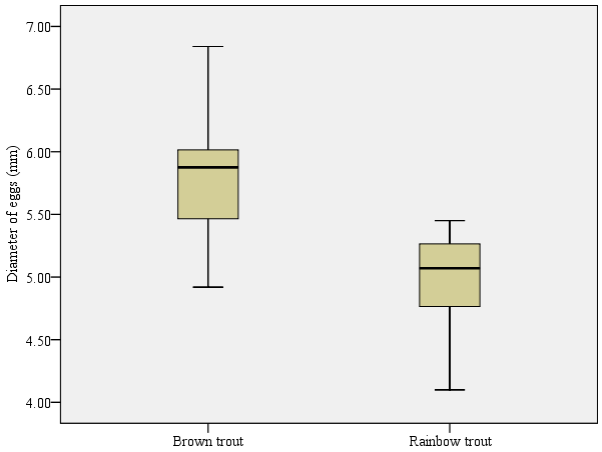
The dissolved oxygen content of water was within the allowed limits, but still lower, while the water temperature was higher compared to the study by Reiser et al. (2019) on rainbow trout, which was realized at a water temperature of 10.22-10.26°C and dissolved oxygen content of 11.05-11.09 mg/l. Kocaman et al. (2009) state that incubation of fertilized rainbow trout eggs at a water temperature of 9.5°C takes 28-30 days, and brown trout 37-43 days, and the yolk sac phase lasts 19-20 days for rainbow trout and 34-38 days for brown trout. The incubation duration in this study for rainbow trout and brown trout is shorter considering higher water temperatures during incubation.

The diameter of brown trout eggs in the eyes stage is larger, with a more pronounced coefficient of variation, than rainbow trout (Table 2).

Table 2. The diameter of the eggs (average±SD) brown and rainbow trout in the eyes stage

	Diameter of eggs (mm)			
	prosjek±SD	*Min*	*Max*	*CV (%)*
Brown trout (Salmo trutta m. fario)	*5,82±0,53*	*4,92*	*6,84*	*9,07*
Rainbow trout (Oncorhynchus mykiss)	*4,99±0,35*	*4,10*	*5,45*	*7,09*

SD – Standard deviation. CV – Coefficient of variation.



Graph 1. The diameter of eggs of brown and rainbow trout in the eyes stage with variation range and median (horizontal line in box)

The diameter of the analyzed brown trout eggs (Table 2; Graph 1) is larger than that of Bonislawska et al. (2001), who report the average diameter of hydrated brown trout eggs of 5.01mm, while rainbow trout eggs diameter in this paper is slightly higher than the results (4.9 mm) of Bonislawske et al. (2001). The diameter of the eggs has wider limits of variation depending on the age of the females, nutrition, etc.

Table 3 shows the standard body lengths, lengths and heights of the yolk sac (average±SD) of brown trout and rainbow trout during the observation period.

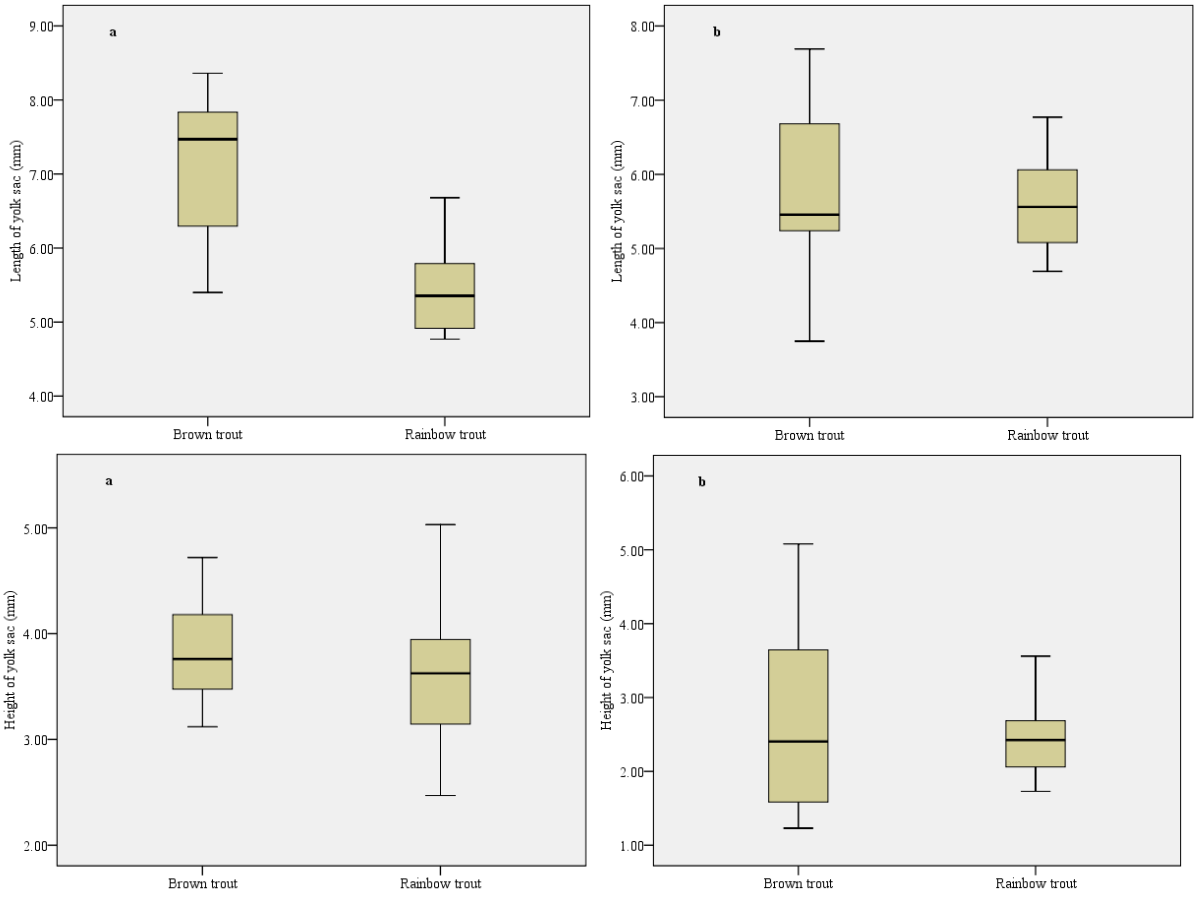
Table 3. Standard body length (SL), length and height of yolk sac (average ±SD) of brown trout 30th and 43rd days after fertilization and rainbow trout 27th and 36th days after fertilization, the efficiency of the conversion yolk sac and the cumulative degree days (dd)

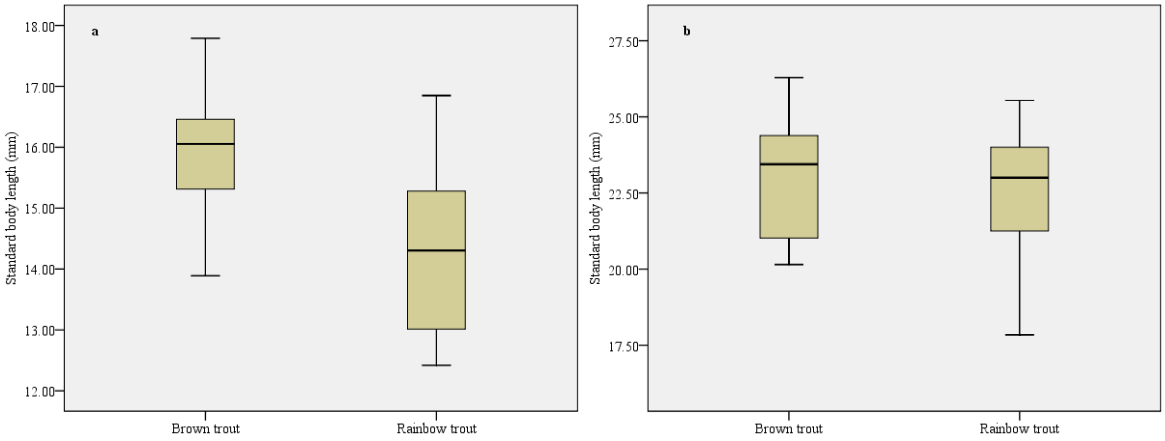
Parameter		*Brown trout (Salmo trutta m. fario)*		*Rainbow trout (Oncorhynchus mykiss)*	
		The number of days		*The number of days*	
		from fertilization to hatching	*from fertilization to swimming*	*from fertilization to hatching*	*from fertilization to swimming*
		30	*43*	*27*	*36*
Standard body length	*mm±SD*	*15,99±1,03*	*22,94±1,89*	*14,38±1,36*	*21,23±1,88*
	CV	*6,46*	*8,25*	*9,44*	*8,84*
Yolk sac length	*mm±SD*	*7,16±0,90*	*5,84±0,95*	*5,42±0,54*	*5,15±0,33*
	CV	*12,51*	*16,28*	*9,93*	*6,35*
Yolk sac height	*mm±SD*	*3,86±0,46*	*2,56±1,02*	*3,59±0,69*	*2,43±0,45*
	CV	*11,94*	*39,74*	*19,07*	*18,71*
Yolk sac conversion efficiency		*0,121*		*0,178*	
Cumulative degree days from fertilization	*dd*	*404*	*571*	*357*	*492*

SD – Standard deviation. CV – Coefficient of variation. dd – Degree days.

The embryonic development of rainbow trout lasts shorter than that of brown trout and some other salmonid fish species, as confirmed by Kocabaş et al. (2011) stating that the embryonic development of *Salmo abanticus* lasts 589 degrees days. Savić et al. (2012) state that the period from fertilization to swimming of the rainbow trout lasts from 44 to 47 days at an average water temperature of 10.54ºC.

The standard trout body length after hatching was not correlated to the volume of the yolk sac (Pearson's r = 0.25; P = 0.141), while the standard body length for rainbow trout was correlated with the volume of the yolk sac after hatching (Pearson's r = 0.47; P = 0.019; α = 0.05). The standard length after swimming had similar trends as after hatching, higher in brown trout than in the rainbow trout. Frasier et al (2010) state that the body length of salmon after hatching did not correlate with the volume of the yolk sac, while the ratio of length between hybrids at first feeding had similar trends as after hatching.





Graph 2. Length and height of yolk sac, standard body length of brown trout and rainbow trout from hatching (a) to swimming (b) with variation range and median (horizontal line in box)

The conversion efficiency of the brown trout yolk sac from hatching to swimming was 0.121, while for rainbow trout it was 0.178. Reiser et al. (2019) state that the efficiency of rainbow trout yolk sac, at lower water temperature, after 53 days of incubation in different treatments is 0.13-0.15. Although the volume of the brown trout yolk sac was higher after hatching, compared to rainbow trout, the yolk sac conversion efficiency was determined to be poorer, which matches the statements of Frasier et al. (2010) that larvae of Atlantic salmon with a larger volume of yolk sac have poorer conversion of yolk sac.

Conclusion

Standard body length, length and height of brown trout yolk sac from hatching to swimming were higher in comparison to rainbow trout, but the yolk sac conversion efficiency of the brown trout was poorer.

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Original scientific paper

Impact of the training process on the level of stress in horses

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Abstract

The aim of this study is to examine stress level of horses impacted by the training process. Sample included 82 horses from five countries (RS=36.6%, MNE=11.0%, CRO=8.5%, BiH=29.3%, NM=14.6%), age (4-7y=20.7%, from 8 to 15y=45.1%, above 16y=34.1%) and various breeds (Axx=6.1%, Exx=4.9%, Ex=89%). Out of the total sample, 37.8% are involved in police service and 62.2% in sports. T-test of paired samples examined the impact of intervention on stress test results. Significant increase of stress was detected between the first (M=37.00, SD=4.84) and the second measurement (M=43.71, SD=6.96), t= -10.61, p=0.00. Average increase of stress value was 2.52, while the interval of 95-percent trust stretches from -7.96 to -5.45. Value of eta square (0.58) shows that the impact of training process on increase of horses stress level is very high. Actions to eliminate potential distraction factors were implemented during measurements. In explored case, research would be enhanced by introducing control sample, that would not be impacted by training process, but would resemble this sample in all other aspects.

Key words: Stress, horse, training, pulse, space.

Introduction

Horse, as a grazing animal that has been hunted, with the exceptional sensibility, with the instinct for leading and following in the function of survival, is motivated by fear to behave in different ways, and which can make handling a horse easier or harder, more or less safe (Grandin, 1997; Grandin, 1999; Sighieri, C., Tedeschi, D., De Andreis, C., Petri, L., & Baragli, P. (2003). A confused sense of threat in horses, induced by the unexpected situation, is a predicament for development of the “fear memory”, which represents a very high stress level

which can be additionally increased or decreased by the intervention of the human (de Jonge, & van den Bos, (Eds.). 2005; Grandin, & Johnson, 2009;). Various methods of horse training induce different psychological and physiological stress responses (Zebisch, May, Reese, & Gehlen, 2014). Stable phycological condition and high level of the physical competency represent a base for achieving individual optimal performances of the each engaged horse. Complex pattern of behavior and individual reaction of a horse is indicated by the previous experience and genetical predispositions produced by the complex complementary interactions between the horse and the rider (Wipper, 2000; Chapman, & Thompson, 2016).

Besides the body temperature, cortisol concentration, eye temperature, behavior model, stress level can be detected through the change of the pulse of the horse as the most sensitive physical reaction. Quantification of hearth rhythm variable is a domain that indicates the level of activity of the sympathetic and parasympathetic nervous system. Monitoring of the hearth frequency, as the momentary indicator of the stress level, can indicate the level of preparedness of the horse for the activity. A number of authors stipulates the impact of the training process as the stress factor on the phycological state of the horse, implying their correlation (Rietmann, Stuart, Bernasconi, Stauffacher, Auer, & Weishaupt, 2004; Cayado, Muñoz-Escassi, Dominguez, Manley, Olabarri, De La Muela, ... & Vara, 2006; McBride, & Mills, 2012; Ille, Aurich, Erber, Wulf, Palme, Aurich, & von Lewinski, 2014; Borstel, Visser, & Hall, 2017).

Acute changes of the autonomous nervous system may be caused also in the idle state, caused by the sensitivity and the memory of different ways of handling of the horse in certain places (Birke, Hockenhull, Creighton, Pinno, Mee, & Mills, 2011; Dorey, Conover, & Udell, 2014). Cognitive capabilities of the horse enable discriminative model of learning, memory and development of concepts (Murphy, Waldmann, & Arkins, 2004; rphy, & Arkins, 2007; Murphy, 2009). Regardless of the wide use of horses in sports and recreation, in police and military service, in therapy and recreation, a number of authors stipulates the lack of exploration of horses' cognitive capabilities and the ability of various learning (Goodwin, 2007; Cooper, 2007).

The aim of this study was to examine the situational stress level in horses impacted by the training process, based on the internal mental state caused by the memory of the training stressors, indicated through the heart frequency.

Material and Methods

The study included 82 clinically healthy horses from the five countries, out of which 36.6% from Serbia (RS), 11.0% from Montenegro (MNE), 8.5% from Croatia (CRO), 29.3% from Bosnia and Herzegovina (BiH) and 14.6% from North Macedonia (NM), the age structure 4-7y=20.7%, from 8 to 15y=45.1%, over 16y=34.1% and the breeds structure Arabic thoroughbred (Axx) 6.1%, English thoroughbred (Exx) 4.9%, thoroughbred * (Ex) 89%. The term "a half-blooded breed" is being used for all the other breeds that may be thoroughbred or in that type, but are, compared to the English thoroughbred, considered for a half-blooded breed. Out of the total sample 37.8% of the horses are included in the police service and 62.2% in sports. Measurement of the frequency of the hearth rhythm was conducted using the device Polar RS800CX N G3 watch, Polar Electro Oy, Finland. The first measurement was conducted in the morning hours in boxes where horses are being fed, where they are resting and live. Second measurement was conducted in the training area in the same order, 10 minutes after the first measurement. Measurement of the pulse in the training area was conducted in the idle state without any physical activity. All training areas were located next to the club stables and after the entering in the training area, horses were in the idle state for 3 minutes in order to avoid the potential effect of the walking on the decrease of the pulse. Pulse measurement was conducted in the presence of the rider that is handling the horse, that has been handling the horse in the box and in the training area. Pulse measurement was conducted in the duration of one minute, and the number of the heart beats was noted by the assistant of the spot. During the pulse measurement, horses were wearing only the head collar. All the measurements were conducted in the period between 24th April and 9th November 2019. During the measurement, necessary precautions were taken to eliminate the potential impact of the disruptive factors. In the moment of the measurement all horses were in the training process.

Data analysis was conducted using the statistical program "SPSS 19.". For obtaining data for this study, descriptive statistics and frequency methods were implemented. Normal distribution of the data for this research was examined using the Kolmogorov-Smirnov test, while T-test of paired samples was used to estimate the impact of the intervention on the results of the examination of the stress level in horses. Value of the eta square was used to determine the impact of the training on the decrease of the stress level in horses.

Results and Discussion

According to the Table 1., prevalence of the stallions and geldings is clear. Horses aged from 8 to 15 are also prevailing, along with the thoroughbred breed of horses.

Table 1. Descriptive statistical indicators of the category variables

Sex			**Breed**		**Age**		**Country**	
	Males	Females	Axx	6.1%	do 7.g	20.7%	RS	36.6%
Stallions	8.5%		Exx	4.9%	8-15.g	45.1%	MNE	11.0%
Geldings	58.5%	32.9%	Ex	89%	16 i viš	34.1%	CRO	8.5%
Purpose	Police	Sport					BiH	29.3%
	37.8%	62.2%					NM	14.6%

Legend: Axx- Arabic thoroughbred, Exx English thoroughbred, Ex- half-blooded, RS-Serbia MNE-Montenegro, CRO-Croatia, BiH-Bosnia and Hercegovina, NM-North Macedonia.

Results of the pulse measurement at the first measurement (Table. 2), indicate that the most of the horses have normal pulse distribution, but that certain individual specimens show significant deviation from the arithmetic middle. It is noticeable that there is an asymmetry of the distribution of the positive direction in the first measurement, which indicates that the pulse in the most of the horses is in the lower values. Tendency of grouping pulse results towards the zone of the better results group indicates that horses are in the good shape and that they are not under the stress in the box. Middle values of the pulse in the first measurement (Mean=37) are within expected limits and values for the horses who are resting in the box. Kurtosis values variables after the first measurement indicate the leptokurtic distribution and a couple of the extreme results that significantly deviate from the average. Values of the standard deviations and the range indicate large heterogeneity (dispersion) of the pulse results in the examined horses. Based on the insight in the results of the second measurement presented in the Table 2. the normal distribution of the middle values is evident, even though there is a significant deviation in the distribution from the arithmetic middle in the positive direction. The pulse values have a slightly worse distribution, which indicates that these results move towards the worse group of the results. Presence of a couple of the extreme results contributed to the rounding coefficients movement above average values which makes the distribution leptokurtic. Based on the value of the standard deviation and the range, large heterogeneity of the results is evident, which indicates larger variations in the pulse manifestations, namely the

stress level. The increase of the middle value of the pulse in the second measurement indicates the increased excitement in horses in the stress situation when entering the training area.

Table 2. Descriptive statistics, asymmetry and flattening of the results of the first and the second measurement

PULSE								
	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
First m.	29	56	37.00	4.841	1.526	.266	3.586	.526
Second m.	30	67	43.71	6.958	.813	.266	1.535	.526
Valid N (listwise)								

Legend: N-number of examined horses, Mean-middle value, Std. Deviation-average standard deviation of the middle value, Skewness-asymmetry, Kurtosis-flattening.

Results Sig in the Table 3. for the first and the second measurement indicate that the premise of the normal distribution of the pulse results was not confirmed and would have to be rejected.

Table 3. Kolmogorov-Smirnov[a]

	Kolmogorov-Smirnov[a]		
	Statistic	df	Sig.
First m.	.192	82	.000
Second m.	.134	82	.001

Results of the T-test of the paired samples (Table 4.) suggest that there is a statistically significant difference in the pulse values between the first and the second measurement (p=0.00). Based on the insight in the results on the Table 2. it is visible that there is a significant increase of the pulse in the second measurement (M=43.71, SD=6.96) in regard to the first measurement (M=37.00, SD=4.84) t= -10.61, p=0.00 which indicates the increase of the stress level when horses are in the training area compared to when they are in the box. Average increase on the value of the stress was 2.52, while the interval of the 95-percent trust stretches from -7.96 to -5.45. Value of the Eta square (0.58) according to the Cohen's guidelines indicate that the impact of the training on the stress level of the horses is high.

Table 4. T-test

Paired Samples Test								
	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
First m. – Second m.	-6.707	5.725	.632	-7.965	-5.449	-10.609	81	.000

Results of the descriptive statistics of this study showed that the highest number of participating horses are males (67.1%) half-blooded (89.0%) aged between 8 and 15 (45.1%), out of which geldings represent 58.5% of the total sample. Results of the descriptive statistics of the pulse are within normal distribution. Standard deviation and the range of pulse results are heterogenic and detect larger pulse related individual differences in horses, which is to be expected taking into consideration variations in breeds, genetics, phenotype, age and purpose. Using the method of the repetitive measurement and the T-test, significant difference in the pulse variability was detected, which indicates different levels of excitement, while the eta square detected that the training has large impact on the stress level of the horses. Detected changes of the autonomous nervous system, indicate that the level of anxiety in horses in the training area is caused by the stress subdued during the training process that did not have to be of the high intensity (Rietmann, Stuart, Bernasconi, Stauffacher, Auer, & Weishaupt, 2004). Gathered findings in this study suggest the acute sensibility of the horse in regard to the training treatment, which is also indicated by other authors (Goodwin, 2007; Birke, et. al., 2011; Dorey, et. al., 2014). It is necessary to bear in mind that the implementation of the training techniques may suggest the conflict with the biological urges that are the predictors of survival throughout the evolution, which can lead to the confusion of the horse (McGreevy, & McLean, 2007; McLean, & McGreevy, 2010). Synchronization of the training with the biological base of the horse would significantly contribute to the reduction of the stress level and to the improvement of the wellbeing of the horse. Taking into consideration that the horse has the ability to detect positive and negative emotions of the rider, it is reasonable to assume that positive emotions will promote the stimulus of award and satisfaction, while the opposite will promote the stimulus of the threat and anxious mental state. Behavioral and physiological response to the negative human expressions may lead to the anxiety in the horse, which is important for the riding population (Smith, Proops, Grounds, Wathan, & McComb, 2016). Assumed close cooperation full of understanding between the horse and the rider, especially comes forth in sports and in the random context in the function of the good actors (Thompson, McGreevy, & McManus,

2015). Positive context of the human relation towards the horse, with the award outcome, may be a significant contribution to the reduction of the stress level during the training period of the horse (Keeling, Jonare, & Lanneborn, 2009). The state of stress indicates the conflict behavior, which was not manifested by any of the horses in this study. Decrease of the frequency of the heart rate indicates the stress, which suggests that the lack of the conflict behavior does not necessarily indicate the lack of stress. Methodologically well based training which includes a good partnership between a horse and a rider, may produce positive emotions and may not necessarily lead to the stress. It is necessary to stipulate that the repetition of the certain sources of the stress through the adequate quantified training exercises can improve the ability of the organism of the horse to cope with the stress (Borstel, et. al., 2017).

Conclusion

Heart rate and changes in its frequency are frequent parameters used for the identification of the stress. Taking into consideration the stress, it can be concluded that the significant component may be the interaction between the horse and the rider. It may be suggested that the analysis of the heart frequency and its variability represent a useful tool for the research of the interaction between horses and humans. Training program does not necessarily have to produce the negative stress, which strongly stipulates the role of the competency of the rider in order to decrease the exposure of the horse to the negative stress. Established riding praxis may be insufficiently acceptable towards the research achievements in the domain of the cognitive abilities and mental states of the horse. It may be concluded that the partnership is a multilayered interaction and that it represents an important predictor in the reduction of the negative stress level in horses.

Difficulties in isolating the disruptive factors are known to decrease the visibility of the results and to make their interpretation harder. Inability of the insight in the psycho-social aspects of the specimen may be a disruptive factor in the process of making premises. Limitations of the research in this area may be the lack of consent of the horse owner to conduct experimental work with the horse. Limited number of specimens within the population leads towards the conclusion that the wider population of horses should be included in the research on the horse behavior and well-being.

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Original scientific paper

Antibacterial activity of different types of honey on pathogenic bacteria

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Abstract

Honey has been used as a food and natural sweetener since ancient times. Thus, its application is mentioned in Sumerian records, and this not only as food but also as a therapeutic agent. Between in many crops and civilizations during the history of use in medicine and for the improvement of the general physiological state. However, only in recent times have medically recognized and its positive drug activity. The composition of honey is variable and depends primarily on the botanical origin, but also the same can affect multiple environmental factors that are holders of such activities that constitute the main obstacle to its possible clinical application. It is necessary to investigate the antibacterial properties of different types of honey due to the botanical and geographical origin for several consecutive years. The aim of this study was to determine the antibacterial activity of five samples of honey from different geographic origin, from the Republic of Srpska (Bosnia and Herzegovina), Slovenia and New Zealand and also to deterimine the type of antibacterial activity. The disc diffusion method on agar was used to test the honey samples with incubation at 37ºC for 24 hours.

The results showed that all tested types of honey (acacia honey, acacia comb honey, floral honey, chestnut honey and manuka honey) demonstrated good inhibitory properties on *Streptococcus* group D, *Escherichia coli, Staphylococcus aureus, Salmonella* Enteritidis and *Salmonella* Typhymurium (clinical isolate) and *Staphylococcus aureus* WDCM 00034 (certified reference material), except acacia honey and acacia honey comb which are not acting inhibitory at clinical isolate of *Staphylococcus aureus.* The best antimicrobial effect was manifested by manuka and flower honey, with the strongest bacteriostatic effect, while chestnut honey showed the strongest bactericidal effect.

Key words: honey, antibacterial activity, pathogens

Introduction

Honey is a sweet substance produced by honey bees (*Apis mellifera*) from the nectars of different plants or honeydew (Anklam, 1998). This is a bee product that has always been used as a natural sweetener and for medical purposes in apitherapy (Bogdanov et al., 2008). The existence of some anthropological findings indicate the possibility that during the nutrition honey began to used as early as 30,000 years ago in tribal communities in today's Ukraine. However, solid evidence is of a more recent date - around 7,000 BC used it as food. Honey was extracted from bees in the wild, as evidenced by the drawings in the Cueva de la Arna cave near the Spanish city of Valencia (Crane, 1984).

It is considered that the first beginnings of bee breeding are in the Far East, while organized, true beekeeping can be talked about as early as around 2,400. BC on the territory of ancient Egypt (Adewumi and Ogunjinmi, 2011; Molan, 2001; Mutsaers et al., 2005). Scientific beekeeping was developed in Greece in the time of Hippocrates and Aristotle. In his numerous writings, watching the life of bees, they have presented a number of theories about beekeeping and medicinal properties of honey.

Beekeeping in ancient Rome represented an important part of the economy and the production of honey has brought considerable profits, so the bee was so widespread that there was no property that did not have at least one apiary. As in Greece, and in Rome was consider as a universal medium for treatment and is recommended as an aphrodisiac and used against depression. Honey contains about 200 different compounds, about 80-85% carbohydrates, 15-21% water, up to 0.3% protein and 0.02-1.03% minerals (Lugomer et al., 2017; Ball, 2007; Ullah Khan et al., 2018; White et al., 1963). In the small quantities honey contains organic acids, vitamins, minerals and trace elements, pigments, phenolic compounds, volatile compounds and solid particles derived from honey during its formation (Missio da Silva et al., 2016; Anklam, 1998).

The chemical composition of honey is variable because bee grazing includes different plant sources. Variability in composition is also evident in honeys derived from the same vegetable source, and the result of seasonal changes of climate or of different geographical origin (Anklam, 1998; Lugomer et al., 2017). Uniflorne honeys that originate entirely from one plant species are extremely rare.

Also important is the use of honey in the food industry, where it is used as an additive in many beverages and food products (Pasias et al., 2017).

Acacia honey is, due to its characteristics, a highly respected among consumers and is one of the most valued types of honey that can be foundon on the European market (Persano Oddo and Piro, 2004; Belčić and Sulimanović, 1982). It is extremely light-colored (almost colorless), low odor and a pleasant mild taste. Due to its higher content of fructose compared to glucose, it crystallizes slowly. The presence of even small amounts of foreign nectar can impair the properties of acacia honey and make it less acceptable to consumers (Persano Oddo and Piro, 2004; Plantea, 2015a). This type of honey fits most people because its aroma is practically not felt in drinks. It is an excellent choice for people recovering from an illness. It is also used in problems of the cardiovascular system and constipation, helps with insomnia, nervousness and tension.

Chestnut honey is dark brown in color, with a very strong and pungent odor on the plant (Plantea, 2015b). It is characterized by a bland and bitter taste in the mouth and long lasting aftertaste. Due to its high water content and higher content of fructose than glucose, chestnut honey is long-held in the liquid state (slow crystallizes) (Persano Oddo and Piro, 2004; Belčić and Sulimanović, 1982). It contains a large amount of minerals and pollen. It helps with digestive problems (stomach, liver, duodenum), blood circulation, strengthens the heart system, balances blood pressure, and is also recommended for anemia. Due to its strong antibacterial activity it is one of the most healing types of honey. It works at a stomach ulcer, hepatitis, bacteria in the urinary system, poor circulation and weakened heart muscle. Helps the digestive system. Floral (meadow) honey is derived from meadow flowers and contains different types of pollen. Depending on the location where the bees collected the nectar, it can be light in color to a deep dark, with a mild to aromatic aroma and taste. It has a beneficial effect on digestion, helps in recovery after an illness. It is suitable for people with heart disease, and recommended for people suffering from low blood pressure.

Manuka honey is a monofloral honey obtained from the manuka plant, *Leptospermum scoparium*, *Myrtaceae* family, which grows as a tree or bush (2 to 4 m), in New Zealand and eastern Australia. Manuka honey color varies from light to deep brown color, of the liquid to a viscous consistence. Manuka honey is less sweet, aromatic, pungent to spicy (Stephens, 2006). In traditional medicine, the herb has been used as a sedative and for wound healing, and manuka honey for various skin conditions (abscesses, surgical wounds, burns and ulcers of different etiologies). The problem of bacterial resistance to antimicrobial drugs has increased the interest of the scientific community to manuka honey as an active ingredient, in particular due to its antibacterial properties in the treatment of wounds (Anyanechi and Saheeb, 2015; Alvarez-Suarez et al., 2014).

Antibacterial activity is an important property of honey in the fight against infections caused by pathogenic bacteria. The first scientific evidence of the antibacterial activity of honey dates from Van Ketela, as early as 1892 (Jerković-Mujkić and Memić, 2005).

Honey has antibacterial properties mainly against Gram-positive bacteria and has a bacteriostatic and bacteriocidal activity in many pathogens. Antibacterial activity is conditioned by the heterogeneity of the composition (Dustmann, 1979; Gobin et al., 2014) and depends on the botanical origin of the honey (Molan, 1992). The high concentrations of honey sugar and low pH value are responsible for antibacterial activity. Many studies have confirmed that honey has anti-inflammatory (Kouchesfahani et al., 2010; Al-Waili and Boni, 2003; Al-Waili, 2003; Al-Waili, 2004; Bilsel et al., 2002), immunomodulatory and antitumor activity (Abuharfeil et al., 1999; Al-Waili, 2003; Chepulis, 2007; Othman 2012; Orsolic and Basic, 2004; Wen et al., 2012). It was found that a diet enriched with honey can reduce the level of malignant processes of breast, prostate, endometrium (Tsiapara et al., 2009).

Honey also inhibits foodborne pathogens and, and in synergistic combination with other food ingredients, reduces the risk of poisoning caused by bacterial toxins (Taormina et al., 2001). Vegetative forms of pathogenic bacteria survive in honey for only a few hours (Snowdon and Cliver, 1996). Laboratory studies have shown that honey can slowdown the growth of bacteria such as *E. coli* and *Salmonella*, and successfully fights against bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (*S. aureus*) which are common pathogens in hospitals (Almasaudi et al., 2017).

Consumption of honey stimulates the growth of healthy specific *Bifidus* and *Lactobacillus* spp. in digestive tract. It is known that honey from pickled wood, alfalfa, sage and clover have a very probiotic effect (Shin and Ustunol, 2005). Probiotic effect of chestnut honey is greater than the probiotic effects of acacia honey (Lucan et al., 2009).

There are significant differences in the antimicrobial activity of certain types of honey. It was found that honey of the same species but from different geographical origin also showed significant differences in antibacterial activity.

Insufficient knowledge of the composition of the honey, and variability of the results of individual studies, determination of antibacterial activity of honey, from different geographical and botanical origin is extremely important in order to systematically monitor antibacterial activity of honey and for the purpose of using honey for therapeutic purposes.

What is interesting is that there is no published data on any antibacterial activity of honey obtained from flora from Republic of Srpska (Bosnia and Herzegovina), despite the great

tradition of production and sale of raw honey and obvious interest in the development of local, biological and therapeutically useful curative for wound care.

The aim of this study was to examine the antibacterial activity of five different types of honey originating from the Republic of Srpska, Slovenia and New Zealand against pathogenic bacteria isolated from clinical materials and the reference bacterial strain and to determine the type of antibacterial activity.

Material and methods

In this study we used five honey samples: acacia honey, acacia honey comb, chestnut honey, flower (meadow) honey and manuka honey. Acacia honey, acacia honey comb and flower honey is raw honey from the Republic of Srpska, raw chestnut honey is from the area of Slovenia, and Manuka Honey UMF 15+ is originally from New Zealand (purchased at a commercial sale at Žužemberk Pharmacy, Grajski trg 41, Žužemberk, Slovenia).

Raw honey sold on markets, households or local stores are not heated in the production process. This is mainly due to the fact that raw honey comes from local beekeepers who immediately after extracting honey poured in glasses ready for sale, as opposed to commercial honey, which is heated to dissolve after storage. This direct processing without heat preserves natural enzymatic properties of honey, but there is a small risk of bacterial contamination. In order not to jeopardize the antibacterial effect of the honey and to eradicate micro-organisms, such as spores of *Clostridium botulinum* which sometimes honey contains, medical honey should be sterilized by gamma radiation which kills spores, and doesn't affect biologically active ingredients and a therapeutic effect of honey (Molan and Allen, 1996; GHC, 2010; Lisby et al., 2002).

For testing the antimicrobial activity of five different types of honey we used the following bacterial culture: *Streptococcus* group D (*S.* group D), *Escherichia coli* (*E. coli*), *S. aureus*, *Salmonella* Enteritidis (S. Enteritidis) and *Salmonella* Typhymurium (*S.* Typhimurium) from the collection of isolates laboratory for microbiology (Public Institution Veterinary Institute Republic of Srpska "Dr Vaso Butozan", Banja Luka) and certified reference material *S. aureus* WDCM 00034. The cultures were seeded in nutritive broth and incubated at 37°C for 18 hours. Petri plates with an appropriate substrate (Müeller Hinton Agar) were seeded with 100 µl of the bacterial suspension at concentrations of 10^5cfu/ml.

The disks diffusion method was used by placing 9 mm diameter paper disks on a solid sterile medium (Müeller-Hinton agar). The micropipette was piped with 100 µl of honey on paper

disks. Sugar syrup was used as a negative control and it was prepared by dissolving 1.5 g sucrose, 7.5 g maltose, 40.5 g fructose and 33.5 g glucose in 17 ml sterile deionized water (Cooper et al., 2002). This solution represents the proportions of the four predominant sugars in natural honey samples. After filling, the plates were left in the refrigerator for 30 minutes to diffuse the sample into the medium and then incubated at 37°C for 24 hours.

The ability of the growth and reproduction of the bacterial strain depends upon its sensitivity to the tested honey. If there is an antibacterial effect around the cylinder will form clear, transparent zone in which there is no growth of micro-organisms.

For each honey sample, three replicates were made and, after an incubation of 18-24 hours, the results were read by determining the inhibition zone diameter and the mean value.

In order to determine whether honey acted bactericidal or bacteriostatically, the type of action was determined. A small slice of agar was added from the inhibition zones in nutrient broth. The incubation was performed at 37ºC for 24 hours. If after incubation occurred blur of broth is considered to be a bacteriostatic effect of honey, but, if the broth remained clear its bactericidal effect of honey.

Results and Discussion

Testing the antibacterial or inhibitory activity of honey on the growth of microorganisms is important to explain the healing effects of honey, as well as the discovery of new potential application of honey in modern medicine. The research results of the antibacterial activity of honey from different botanical and geographical origin are shown in Table 1.

Table 1. Antibacterial activity of different types of honey on selected strains of pathogenic bacteria

Pathogenic bacteria	Inhibition zone in mm				
	Acacia honey	Acacia honey comb	Flowerhoney	Chestnut honey	Manuka honey
S. group D	37.00	39.00	33.33	24.66	34.00
S. aureus (clinical isolate)	0.00	0.00	30.00	23.66	31.00
S. aureus WDCM 00034	39.33	30.33	28.33	22.00	30.00
E. coli	29.00	25.33	31.33	26.66	27.00
S. Enteritidis	28.00	26.00	22.00	33.33	27.66
S. Typhimurium	28.66	24.66	27.66	30.00	28.33

Sugar syrup (negative control) did not inhibit growth of any of the tested pathogens.

All types of honey, except chestnut, proved greater inhibitory activity against Gram positive bacteria. Chestnut honey had greater inhibitory activity against Gram-negative bacteria,

particularly *S.* Enteritidis and *S.* Typhimurium, which is in accordance with the observations of other authors (Kwakman et al, 2011; Mandal and Mandal, 2011; Estevinho et al., 2008).

The best antimicrobial effect expressed a flower and manuka honey. The results of this study are different from the results of other authors (Kunčić et al., 2012) which are shown that chestnut honey had the best antimicrobial activity as a number of other honey species, while the weak antibacterial activity of acacia honey was shown against *E. coli* (Molan, 1992).

The results of our study showed that tested honey's had the highest antibacterial effect on *S.* group D. Other studies have shown that bacteria *E. coli* O157:H7 are most sensitive of action of almost all types of honey in very low concentrations, which is not surprising, because bacteria is relatively sensitive to avarious chemical compounds and is thus known, its sensitivity to different types of honey (Taormina et al., 2001; Kunčić et al., 2012).

The difference between the sensitivity on honey and other antibacterial agents between Gram positive and Gram-negative bacteria may be because of structure of cell wall. Gram-positive bacteria don't have an external membrane which protects the layer of peptidoglycan, compared to gram negative bacteria, which simplify the penetration of the antibacterial agents, and cause damage (Madigan et al., 2015).

Antibacterial activity of honey may significantly differ in various bacterial species, and even among related bacteria, such as in this case with Gram-positive species, respectively, reference strain of *S. aureus* from clinical isolates of same bacteria. This is in agreement with a study that investigated the antibacterial effect of acacia honey on *S. aureus* and it was concluded that the acacia honey has less antimicrobial activity against bacterial strain of *S. aureus* from some other type of honey from different parts of the world (Dugalić-Vrndić et al., 2005).

The inhibitory activity of honey is attributed to various factors such as osmotic pressure, low pH, high sugar content, hydrogen peroxide formation (White et al., 1963; Weston, 2000), then the presence of phenolic acids (Alvarez-Suarez et al., 2010), lysosomes (Israili, 2013) and flavonoids (Taormina et al., 2001; Kwakman and Zaat, 2011), and more recently have been identified methylglyoxal and defensin-1 (anti-bacterial peptide bee) such important anti-bacterial compound in the honey (Israili, 2013).

It is believed that the antibacterial activity of the honey is very complex because of the various types of compounds which are present in different concentrations in different types of honey.

Sugar syrup for its sugar composition and pH values is similar to the natural honey (Cooper et al., 2002) so that the osmolarity as well as the single most important factor of antibacterial activity can be turned off. Sugar syrup has about the same osmolarity as natural honey, and how he did not inhibit the growth of any pathogens tested, it can be argued with great certainty

that osmolarity was not a factor contributing to the antibacterial activity of honey. Also, since the pH of artificial honey is close to the pH of natural honey, we can assume that acidity is not the deciding factor alone responsible for the inhibitory antibacterial potential of honey, but only in combination with many other factors that are an integral part of a honey-making mixture. These findings are consistent with the research conducted by Molan (1992) and Oliveira et al. (2017), who showed that antibacterial activity was higher in dilute honey because osmolarity decreased with honey dilution.

Various studies have shown that the main antibacterial factors in honey are hydrogen peroxide, catalase and glucose oxidases, while Gil et al. (1995) in their research showed among the first that non-peroxide factors can contribute to the antibacterial properties of honey, and that flavonoids and other phenolic components prevent the growth of numerous Gram-negative and Gram-positive bacteria. For the samples of acacia honey and acacia honey-comb, which are not acting inhibitory at clinical isolates and certified reference material of *S. aureus*, it can be assumed that they have the necessary ingredients which would act on the bacteria, whether it be on phenols, flavonoids, proteins, hidroksimetilfurfurolu, methylglyoxal or their combination. The antibacterial activity of honey is very complex due to the large number of compounds, because of a large difference in the concentrations of the various substances and compounds between different types of honey it is not possible completely to define which is the most effective type of honey.

In a number of further studies, honey has been shown to be bactericidal to many types of Gram-positive and Gram-negative bacteria, as well as fungicidal to different fungi (Almasaudi et al., 2017; Jerković-Mujkić and Memić, 2005; Mutsaers et al., 2005). Honey is a food that in many ways prevents the development of microorganisms.

Today it is also known that the antibacterial activity of the honey is expressed due to the presence of various bacteriostatic and bactericidal compounds and substances, and also, under the influence of certain constituents of honey in the body leads to increased the release of cytokines (Tonks et al., 2001) and immunomodulatory (Weston, 2000; Kwakman and Zaat, 2012). Results of antibacterial effects are shown in Chart 1.

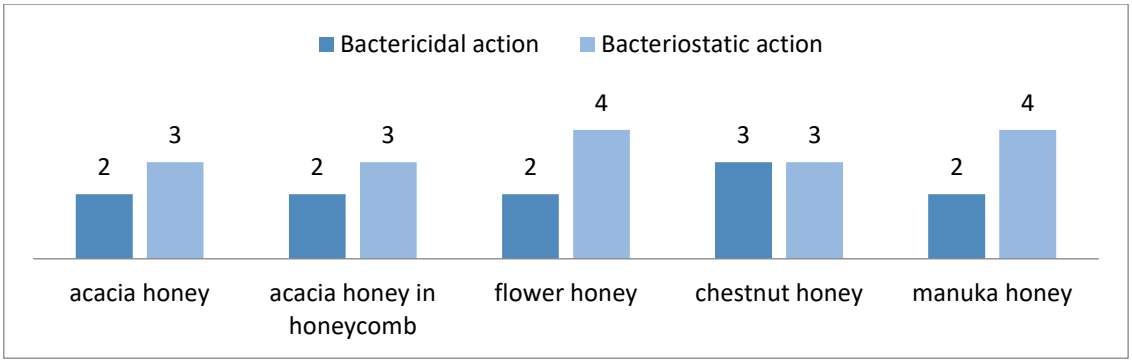

Chart 1. Bactericidal and bacteriostatic activity of different types of honey

All tested honey samples showed good bactericidal and bacteriostatic properties. It is also observe that manuka and flower honey is almost same in terms of its properties, which supports the fact that honey from Republic of Srpska have also very good antibacterial properties. These results indicate that honey from different plant species may have good medicinal potential, that is, may have advantages or similarities with manuka honey due to enhanced antibacterial activity, local production, and greater selectivity for bacteria significant in medicine.

A more detailed determination of antibacterial compounds and their activity, or involvement in the complex antibacterial activity of honey, can enable standardization and help eliminate major barriers to the use of honey in medicine and also in therapy.

Conclusion

Acacia honey, acacia comb honey, floral and manuka honey expressed higher antibacterial activity against Gram-positive bacteria. Chestnut honey had a higher antibacterial activity against Gram-negative bacteria, especially against *S.* Enteritidis and *S.* Typhimurium. All tested honey's showed the strongest antibacterial effect on *S.* group D. Acacia honey and acacia honey comb did not show antibacterial activity against the clinical isolate of *S. aureus*. The best antimicrobial effect was manifested by manuka and flower honey, with the strongest bacteriostatic effect, while chestnut honey showed the strongest bactericidal effect. Although some researchers have concluded that honey from certain plants has better antibacterial properties than others, there is little evidence to support this claim. Some claims are based on the data of a very small number of samples, while other studies show that there are different antimicrobial activities of honey originating from the same floral pattern.

The osmolarity and acidity of honey is not the decisive factors which are responsible for the inhibitory and antibacterial potential of honey, but only in combination with many other factors that are integral to the honey-forming mixture. The antimicrobial properties of honey are

generally little explored, a lack of knowledge on the composition and antimicrobial factors holders is a major problem possible clinical application of honey in the treatment of certain infections. It is necessary to carry out research of this type over a number of consecutive years and on a large number of samples of honey of different botanical composition, in order to determine more precisely the effectiveness of certain types of honey and finally determine honey for use in medicine within the honey varieties in Republic of Srpska (Bosnia and Herzegovina), as is done in Australia (manuka honey).

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Original scientific paper

Production results of broiler chicken farms of different capacities in region of Banja Luka

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Abstract

The aim of this study was to investigate influence of broiler chicken farm capacity on main production results. A total of six farms were categorized according to capacity (number of chickens per round) in three equal groups as small (6.000), medium (10.000) and large (20.000). Production records of total 60 fattening rounds achieved during 2013 and 2014 were analyzed using descriptive statistics and ANOVA. There were no statistical differences among farms of different capacity regarding fattening duration, initial and final chicken weight, feed conversion ratio, total mortality and European production efficiency factor, whereas first-week mortality was significantly higher on large compared to small and medium capacity farms ($p<0.05$). Similar production conditions and cooperation model that seeks to ensure uniform input quality and farm management probably contributed to comparable production results, but further researches are advised.

Key words: Broiler, chicken, farm capacity, productive results, Banja Luka

Introduction

Broiler chicken production in Republic of Srpska generally characterizes compliance of fattening farms with modern standards in terms of housing, nutrition and health care of broiler hybrids (Mitrović et al., 2010; Salihbašić et al., 2015). The average estimated consumption of cca 17 kg poultry meat *per capita* is ahead of the consumption of pork and beef (RSIS, 2018) and production is increasing, despite some financial and organizational constraints (Salihbašić et al., 2015). Growth rate, final weight, feed conversion efficiency, mortality and fattening duration are considered as important production indicators in fattening of broiler chickens (Gussem et al., 2013; Horne & Bondt, 2014), while mortality could be also used as indicator

of welfare and health (Yerpes et al., 2020). The profitability of broiler fattening is directly influenced by these parameters, so the provision of high standards in terms of chick quality, housing and farm management is crucial (Szőllősi & Szűcs, 2014; Sasaki et al., 2014; Tandoğan & Çiçek, 2016; Györke et al., 2016; Mesa et al., 2017; Jong et al., 2020). Farm capacity may be considered as one of the important factors of production performance. Impact of farm capacity on mortality was reported by Simsek and Ozhan (2015), impact on final weight, feed conversion ratio and mortality by El-Tahawy et al. (2017), while Tandoğan and Çiçek (2016) found similar final weight, mortality, feed conversion ratio and European production efficiency factor in commercial farms of different capacity. Also, monitoring and analysis of production results can be one of the ways to examine the current state of technology and management of broiler farms in domestic conditions, which can be useful in improving production and establishing closer cooperation among all participants in this sector (Sasaki et al., 2014; Jong et al., 2020). Therefore, the aim of this study was to determine and compare the selected production results depending on the broiler farm capacity, and to present its average values in order to evaluate the state of this production in the region of Banja Luka.

Material and Methods

This study was based on data obtained from production reports of a total of 60 fattening rounds of Cobb 500 chicken archived during 2013 and 2014 on six broiler farms in the region of Banja Luka. Farms were categorized into three equal groups according to capacity (chicken per round): small (6.000), medium (10.000) and large (20.000). Following paremeters were examined: fattening duration (days), initial (g) and final (kg) chicken weight, feed conversion ratio, first-week and total mortality (%), as well as European production efficiency factor (EPEF) calculated according to the formula given by Tandoğan and Çiçek (2016) as follows:

$$EPEF = \frac{average\ final\ body\ weight\ \times\ livability}{feed\ conversion\ ratio\ \times\ fattening\ duration} \times 100$$

Methods of descriptive analysis (mean, standard deviation and interval of variation) and one-way analysis of variance were used in statistical processing of data, while the significance of differences was determined at $p<0.05$.

Results and Discussion

According to data shown in Table 1, no differences in fattening duration, initial and final chicken weight, as well as feed conversion ratio depending on farm capacity were found. These results are consistent with the results from Tandoğan and Çiçek (2016), obtained from a commercial farms with different capacity (≤10.000; 10.000-30.000 and ≥30.000 chickens per round). Also, Simsek and Ozhan (2015) did not confirm the impact of farm capacity (15.000; 25.000 and 35.000 chickens per round) on the final weight and feed conversion ratio in Ross 308 chickens for 37 days of fattening. El-Tahawy et al. (2017) found differences in production results after fattening of Cobb 500 chickens for 35 days, according to farm capacity (<10.000; 11.000-30.000 and 31.000-50.000 chickens per round), because the feed conversion ratio was better on farms with medium than on farms with small and large capacity, while there was no difference in the final chicken weight.

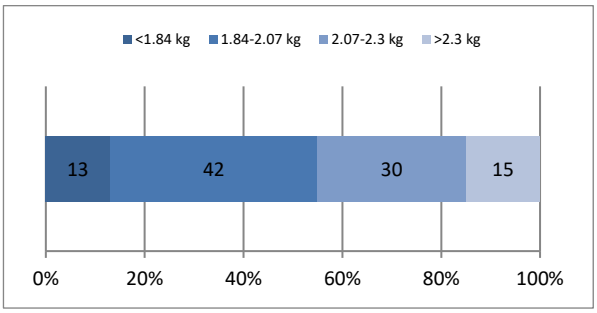
Table 1. Fattening duration, initial and final chicken weight and feed conversion ratio (M – mean, SD – stand. deviation, IV – variation interval)

Parameters		Farm capacity			Total (mean±SD)
		Small	Medium	Large	
Fattening duration (days)	M±SD	38.86±2.14	37.81±2.49	38.01±1.86	38.23±2.19
	IV	36.00-45.00	35.00-43.00	35.00-42.00	
Initial weight (g)	M±SD	43.34±3.18	42.70±2.34	42.87±2.34	42.97±2.62
	IV	35.00-48.00	38.00-46.20	38.00-46.20	
Final weight (kg)	M±SD	2.08±0.24	2.05±0.24	2.07±0.16	2.07±0.21
	IV	1.67-2.53	1.61-2.52	1.93-2.61	
Feed conversion ratio	M±SD	1.80±0.07	1.78±0.08	1.78±0.04	1.79±0.06
	IV	1.71-1.98	1.68-1.94	1.69-1.86	

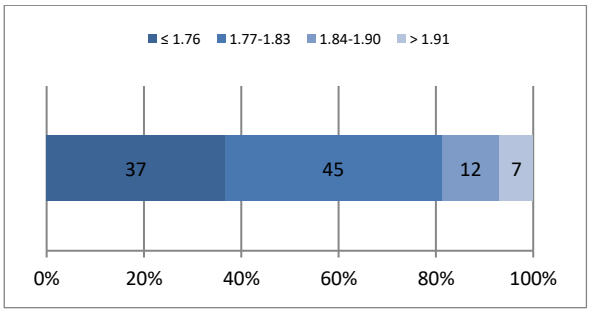
Farm capacity (chicken/round): Small – 6.000, Medium – 10.000, Large – 20.000
[ab] – Values with different letters in the same row indicate statistically significant differences ($p<0.05$)

Mitrović et al. (2010) analysed production records of one broiler farm (cca 15.000 chickens per round) in the region of Semberija from period 2004-2009, and found average fattening duration of 38.49 days (34.5-44.0), average final weight of 2.02 kg (1.80-2.38 kg) and average feed conversion ratio of 1.72 (1.61-1.89), which are similar to the average values obtained in this study (38.23 days, 2.07 kg and 1.79, respectively). Also, the average fattening duration found in this study is close to its estimated optimal duration of 39.34 days for Cobb 500 chickens calculated on basis of the average target performance of that hybrid, and prices of feed, chicks and broiler meat (Cicek & Tandogan, 2016). The fattening duration is usually a function of acheivement of the target weight, but feed conversion ratio is also highly

influenced. Feed conversion ratio, as an indicator of feeding efficiency, has a special role in improvement of productivity and reduction of production costs (Tandoğan & Çiçek, 2016). Importance of this is confirmed by report that the price of day-old chicks and feed account for 80-85% of fattening costs (Horne & Bondt, 2014). Body weight is a quantitative indicator of day-old chick quality, so weights over 40 g ensure a good start and potential to achieve satisfactory results of fattening, especially in terms of mortality (Tumer, 2013). When observing the share of the rounds depeding on final weight in the total number of analyzed rounds showed in Graph 1, it can be seen that in 42% rounds the final weight in the range of 1.84-2.07 kg, while 28% of rounds had values out of range 1.84-2.3 kg. Distribution of rounds according to feed conversion ratio, presented in Graph 2, showed that almost half of all rounds (45%) was in the range of 1.77-1.83, and then followed by rounds with values lower than 1.76 (37%).



Graph 1. Share of rounds according to final weight



Graph 2. Share of rounds according to feed conversion ratio

The average final weight of chickens in EU countries is between 2 and 2.5 kg, and the feed conversion ratio between 1.65 and 1.85, as reported by Horne and Bondt (2014) for year 2013, although conversion ratio should be observed in correlation with the final weight. Beside that, the influence of chick origine and health, feed quality, as well as housing and weather conditions should be taken into account during the assessment of these results (Schmidt, 2008; Campe et al., 2013; Liang et al., 2013; Sasaki et al., 2014; Györke et al., 2016; Jong et al., 2020).

Parameters of mortality, shown in Table 2, indicate a significant difference in first-week mortality depending on farm capacity ($p<0.05$), because it was higher on large (0.98%) compared to small (0.53%), and medium capacity farms (0.59%), while no significant difference in total mortality was found.

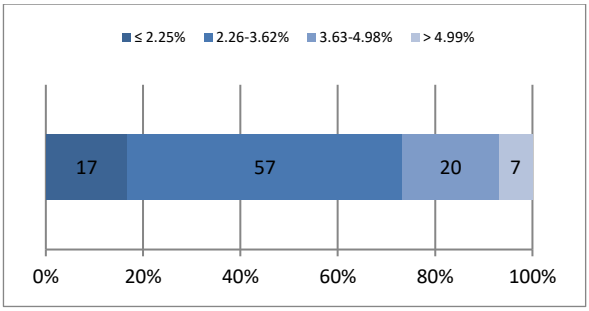
Table 2. Mortality parameters and European production efficiency factor (EPEF) according to farm capacity (M – mean, SD – stand. deviation; IV – interval variation)

Parameters		Farm capacity			Total (m±SD)
		Small	Medium	Large	
First-week mortality (%)	M±SD	0.53±0.29[b]	0.59-0.49[b]	0.98±0.27[a]	0.71±0.41
	IV	0.10-1.17	0.12-1.61	0.50-1.50	
Total mortality (%)	M±SD	3.32±0.84	2.84±1.14	3.57±0.98	3.24±1.02
	IV	1.72-5.50	1.19-5.85	2.42-6.34	
EPEF	M±SD	287.35-19.95	296.01±18.76	294.46±18.16	292.61±19.03
	IV	241.78-321.69	263.32-338.64	259.30-333.31	

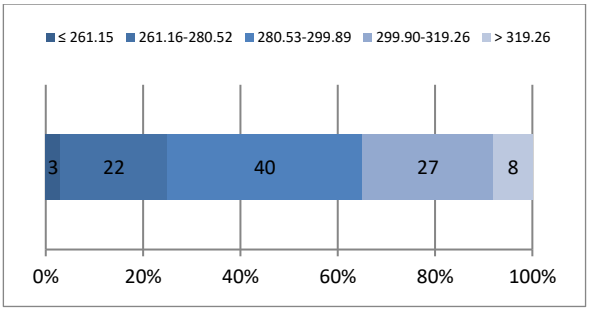
Farm capacity (chicken/round): Small – 6.000, Medium – 10.000, Large – 20.000

[ab] – Values with different letters in the same row indicate statistically significant differences ($p<0.05$)

Mortality usually peaks on day 3 or 4, and then gradually declines to about day 9 or 10 when it maintains a relatively constant value until day 30, when it gradually increases again to end of fattening (Tabler, 2004). Heier et al. (2002) found that first-week mortality was lower on larger farms, in contrast to Yerpes et al. (2020) who found a lack of impact of farm capacity. The first-week mortality in several European countries reported in field studies varies, so it had values such as 1.5% (Yassin et al., 2009), or 1.82% (Yerpes et al., 2020), while a maximum limit of 1.0% can be set as a target (Gussem et al., 2013). Year and season, chick origin and quality, brooding conditions, age of fattening farms and type and functionality of farm equipment are also factors of the first-week mortality identified in commercial production (Heier et al., 2002; Yassin et al., 2009; Yerpes et al., 2020). Relatively unvarying total mortality on farms with different capacities observed in this study was also reported by Tandoğan and Çiçek (2016). However, according to Simsek and Ozhan (2015), total mortality is significantly lower on small compared to medium and large capacity farms (5.94, 6.99 and 7.05%, respectively), which contradicts the findings of El-Tahawy et al. (2017) that mortality is higher on small than on medium and large farms (6.15, 1.89 and 2.60%, respectively) or Tumer (2013) that mortality decreases with increasing of flock size. Mitrović et al. (2010) reported that total mortality was 3.24% with a range of 2.13-4.99%. Average value of this parameter for all rounds in this study (3.24%) is in line with recommendation given by hybrid breeder, i.e. it is lower than 5.0% (tolerable for fattening duration of 42 days) or to the recommendation that allows a maximum of 3.5% (Gussem et al., 2013). Moreover, according to the data given in Graph 3, almost two-thirds of the analyzed rounds (57%) had total mortality in the range of 2.26-3.62%.



Graph 3. Share of rounds according to total mortality



Graph 4. Share of rounds according to value of EPEF

Housing, quality and transport duration of day-old chicks, health care, duration of inter-round period, and season could also influence a total mortality in field conditions (Tabler, 2004; Schmidt, 2008; Campe et al., 2013; Liang et al., 2013; Sasaki et al., 2014; Györke et al., 2016; Mesa et al., 2017).

EPEF, as performance indicator, in this study did not differ statistically between farms with different capacity and its averages 292.61. This concurs with findings of Tandoğan and Çiçek (2016). Mitrović et al. (2010) reported that EPEF averaged 296.32 with the range of 260.11-322.83, and the highest values were registered for rounds lasted from 39 to 41 day, indicating that the best production effects were achieved with that fattening duration. Similarly, a decrease of EPEF with an extension of the fattening duration was found by Schmidt (2008), while Sasaki et al. (2014) identified age of breeder flock, duration of inter-round period, season and stocking density as significant factors in commercial production. Shane (s.a.) states that 200-225 is acceptable minimum for EPEF in Europe, because lower values than this indicate a low level of technological equipment, poor quality of feed and day-old chick. When the share of rounds according to the value of EPEF presented in Graph 4 were observed, the largest number was in the range of 280.53-299.89 (40%), and then in the range 299.90-319.26 points (27%).

Production results in broiler fattening are influenced by day-old chick quality, farm housing and management, so maintaining conditions aligned with the requirements of the hybrid used regardless of farm capacity allows the expression of their genetic potential in terms of growth, feed conversion and vitality (Liang et al., 2013; Tandoğan & Çiçek, 2016; Mesa et al., 2017; Jong et al., 2020). Realization of this targets will also financially reward the farmer due more favorable ratio between cost and profit of fattening, which makes the farm more competitive and profitable (Schmidt, 2008; Szőllősi & Szűcs, 2014; Tandoğan & Çiçek, 2016). Also, the analysis of production records in all production segments allows corrective actions to be taken to improve broiler production (Yassin et al. 2009; Sasaki et al., 2014; Jong et al., 2020).

Conclusion

Based on the obtained results, it can be concluded that there is no difference between key production parameters between broiler farms of different capacity, which can be interpreted by similarities in used technologies and a model of cooperation that seeks to ensure uniform input quality and continuous production control. It is necessary to continue this type of research on larger number of farms with more identified production factors in order to comprehensive evaluation of this production in the region of Banja Luka.

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Original scientific paper

Assessment of wheat self-sufficiency in Bosnia and Herzegovina

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Abstract

Wheat and corn are the dominant cereals in Bosnia and Herzegovina (BiH), and the additional importance of wheat is that it is the most important bread grain. In Bosnia and Herzegovina, there is no official calculations on production and consumption comparison of agricultural products. Therefore, the aim of the paper is to determine the degree of self-sufficiency of Bosnia and Herzegovina and wheat in the period 2014-2018. A descriptive method is used to describe production, foreign trade, and consumption. Wheat self-sufficiency balance is calculated using formulas recommended by FAO. The research was conducted based on a secondary data. The Republic of Srpska (RS) has a larger role in production, than in wheat consumption within Bosnia and Herzegovina. Foreign trade in wheat as well as wheat products has great economic importance in BiH. Consequently, this analysis is essential from the aspect of reconciling wheat production and consumption. Bosnia and Herzegovina had a deficit in foreign trade of wheat grain and flour in the analyzed period. In the analyzed period BiH had a low degree of self-sufficiency in wheat (29-48%). Since wheat consumption is fairly stable, if the balance of production and consumption wants to be improved, BiH would have to produce more wheat by increasing sown areas and/or yields.

Key words: wheat, production, consumption, self-sufficiency, BiH.

Introduction

Wheat is a main bread grain and is considered one of the strategic food products in BiH. Wheat is the second crop in sown areas, as well as in production in Bosnia and Herzegovina. In the analyzed period, wheat production in BiH tended to grow until 2017. More detailed data on production and consumption of wheat in BiH are given in chapter Discussion. Wheat is considered as the important crop and in EU as well as in the world. The largest producers of wheat in the European Union are France, Germany, the United Kingdom and Poland. In 2017/2018, common wheat output recovered

compared to the previous low harvest and was slightly above the 5-year average, with a total of 141.8 million t. Production in France and Romania rose by 33% and 17%, respectively. In contrast, wheat harvest dropped by almost 45% in Spain and slightly less in the Czech Republic, Slovakia and Hungary (EC, 2018).

The effort to provide sufficient quantity and high technological quality of wheat grain and other cereals is a prerequisite for satisfaying basic living needs for human consumption, animal nutrition and industrial processing (Paunović et al, 2016). More than two-thirds of global wheat is used for food, 20% is used for livestock feed and another 3% to 5% each for seed, industrial use and other uses (Vogel, 2017). As a result, it is the country's endeavor to provide sufficient quantities of this product, not only in quantity but also in quality, to meet the needs of the local population with regard to this product.

Stevanović (2009) states that Serbia in wheat production from the once-surplus country has been approaching to the border of self-sufficiency, and that in the long run it cannot count on safe country contingents for export of this product, which significantly reduces the competitiveness of the Republic of Serbia in export of wheat to foreign markets.

Internationally, wheat is heavily traded. Starting from year 2000, Russia became a major grain exporter, along with Ukraine and Kazakhstan. Russia exported 35 million metric tons (mmt) of grain a year during 2014–2016 (average annual), while Russia, Ukraine, and Kazakhstan collectively exported 80 mmt (average annual, excluding any sales to each other. Over this period, Russia supplied 10% and 15% of world exports of total grain and wheat, respectively (Liefert et al, 2019).

A rate of self-sufficiency measures the ability of a given country to satisfy all needs of consumers from the country's domestic production. The term self-sufficiency is often confused with the term food security (Slaboch et al, 2018).

Most self-sufficiency ratio (SSR) analyses focus on key staple crops, such as cereals and starchy roots, in order to give an approximation of food self-sufficiency of a country (Clapp, 2017). According to him, food self-sufficiency is more issue of a country's domestic capacity for food production than issue of limitation of food foreign trade.

The key point is that if a country is food self-sufficient, it produces an amount of food that is equal to or greater than the amount of food that it consumes. The self - sufficiency index (SSI), expresses food production as a ratio of consumption (SEA, 2019).

Production - consumption balances provide information on wheat supply and demand as well as degree of self-sufficiency as well as per capita consumption. Wheat consumption is conditioned by both rational and irrational demand factors.

Atar (2018) considers that "wheat, with its direct and indirect use, is one of the most important food sources of our country and the world". According to him, Turkey is one of the countries with the highest per capita consumption of wheat (213 kg), with the expected tendency to reduce that consumption. Despite being a self-sufficient country in wheat production, this balance has changed in recent years and the sufficiency rate has dropped to 90% (2014 -2015). Kuzman et al. (2007) state that the level of consumption is conditioned, both by the movement in production, or the conditions of cultivation, and by habits of consumption in certain countries. The population of Bosnia and Herzegovina uses considerable amounts of wheat in its diet, which is a consequence of consumer habits.

Material and Methods

The aim of the research is to analyze the production and consumption of wheat and wheat products and to calculate the degree and trends of wheat self-sufficiency in Bosnia and Herzegovina in years 2014-2018.

Combination of desk research from literature sources and own data calculation from secondary sources were the method of the research. Wheat production and consumption data were taken from statistical publications and self-sufficiency was calculated on the basis of available data and own estimation of authors.

Foreign trade is the result of the data obtained on imports and exports from the Indirect Taxation Authority database. Total production, imports and exports are converted to the equivalent of the basic product, which in this case is wheat grain.

Methodologically, self-sufficiency in wheat was calculated as Ostojić and Vaško (2019) and Ostojić et. al (2019) did in the case of meat and fruit for BiH. In the calculation of self-sufficiency balance, total production is reduced by losses and recalculated, because the grain wheat is not consumed directly, then processed for human and industrial consumption. Certain ratios and coefficients have been taken over as technological norms or estimated by the authors and used to calculate the quantities of flour and other processed wheat from wheat to grain.

The number of wheat consumers is equal to the population and a constant population number was used for all years. Population data were obtained from the last census (3,531,159) (ASBiH, 2013).

Calculation of self-sufficiency level starts from the relations which is based on the balance between sum of production and import and sum of consumption and export:

$$Production + Import = Consumption + Export$$

The assessment of the self-sufficiency level is calculated according to the formula recommended and used by FAO (2012):

$$Import\ Dependency\ Ratio = Import \times 100 / (Production + Import - Export)$$

neglecting the impact of initial and closing stocks. Initial and final stocks are neglected in calculating the degree of self-sufficiency, among other things due to the lack of any baseline for their quantification. However, in the long run, their impact on individual coefficients is minor.

Estimation of the level of dependence on imports is calculated according to the formula (FAO, 2012):

$$Self\ Sufficiency\ Ratio = Production \times 100 / (Production + Import - Export)$$

The indicative per capita consumption of wheat is calculated according to the following formula:

$$Per\ capita\ consumption = (Production + Import - Export) / Number\ of\ population$$

The accuracy of the calculated coefficients is conditioned by the accuracy of the non-computing data. Without prejudice to the accuracy of import and export data, the same cannot be said for wheat production data, since they are largely based on subjective estimates of harvested areas and harvested yields that are needed for statistical research. If this data is undervalued or overestimated, it indirectly reflects the data on consumption of wheat, total and per capita.

Results and Discussion

Wheat production in BiH

Wheat production in Bosnia and Herzegovina (tab. 3) during the analyzed period averaged 253,986 tons, with an average of 68,334 ha. The analysis of the data shows that wheat production has grown in the first three years. The decrease in production in 2017 compared to 2016 was almost 6%. The analogous condition is also with the sown surfaces and corresponds to the picture of the movement of production (-2.7%). In 2016 and 2017, the highest yield per unit area was achieved and the average yield in the analyzed period was 3.7 t / ha, which is below the European average.

Wheat production in the Republika Srpska during the analyzed period averaged 161,148 tons, with an average of 44,800 hectares. During all years, there has been an evident increase in areas and production of wheat, especially until 2016, and production in 2017 is almost identical to the previous year. Wheat sown areas in Republika Srpska are increasing, inter alia, due to the incentives per unit of sown area that has been paid in the RS in recent years, as well as the premium per kg of purchased wheat. In five years the area under wheat has increased by 11 thousand ha or by 23%. In the RS, around 15% of the arable land is sown wheat, or 22% of the arable land are sown cereals.

Wheat areas in RS represent about 2/3 of the total wheat sown area in BiH, and production of 60-76% of total BiH wheat production.

Table 1. Area hrvested, production and yield in BiH and RS (2014-2018)

	The Republika Srpska (RS)			Bosnina and Herzegovina (BiH)		
Year	ha	Yield t/ha	Production tons	ha	Yield t/ha	Production tonnes
2014.	38,219	2.8	106,777	59,110	2.9	170.055
2015.	38,897	3.3	127,140	60,407	3.5	213,015
2016.	45,305	4.3	194,310	71,394	4.3	306,605
2017.	45,596	4.3	195,315	69,435	4.1	288,738
2018.	49,716	4.1	202,197	72,677	4.0	291,515

Source: For the Republika Srpska Statistical Yearbook of the RS for 2019, issued by Republika Srpska Institute of Statistics, and for BiH annual announcements on harvested areas, total production of yields of important crops for the corresponding year (2014, 2015, 2016, 2017 and 2018), issued by Agency for Statistics of BiH.

Republika Srpska is a significant producer of wheat within BiH. The share of RS in total areas was on average 65%, while in terms of production it was about 64%. These data confirm that the main wheat producer in BiH is Republika Srpska. Republika Srpska's maximum share in wheat production in 2018 was 69%.

The average wheat yield in RS is almost identical to the average yield achieved throughout BiH. Compared to neighboring countries, the average yield in BiH is lower than in Serbia and Croatia, but higher than in Northern Macedonia. If the average yield of wheat is compared with the lowest and highest yield in EU-28, the average yield in BiH is slightly higher than the minimum (mainly recorded by Portugal) and 2-3 times lower than the highest yield (which is mainly achieved in Ireland). The relatively low wheat yield in BiH is partly due to the unfavorable ownership structure.

Table 2. Average yield wheat (t/ha) (2014-2018)

Year	RS	BiH	Serbia	Croatia	North Macedonia	EU-28 min.	EU-28 max.
2014.	2.8	4.3	4.8	5.7	3.8	2.4	9.5
2015.	3.3	3.5	4.1	5.4	2.8	2.0	10.7
2016.	4.3	4.3	4.8	5.7	3.8	2.4	9.5
2017	4.3	4.2	4.1	5.9	2.7	2.1	10.1
2018.	4.1	4.1	4.6[1]	5.44	3.4	2.6	8.6

Source:http://www.fao.org/faostat/en/#data/QC, for 2014-2017
https://appsso.eurostat.ec.europa.eu/nui/submitViewTableAction.do, for 2018.

Wheat foreign trade

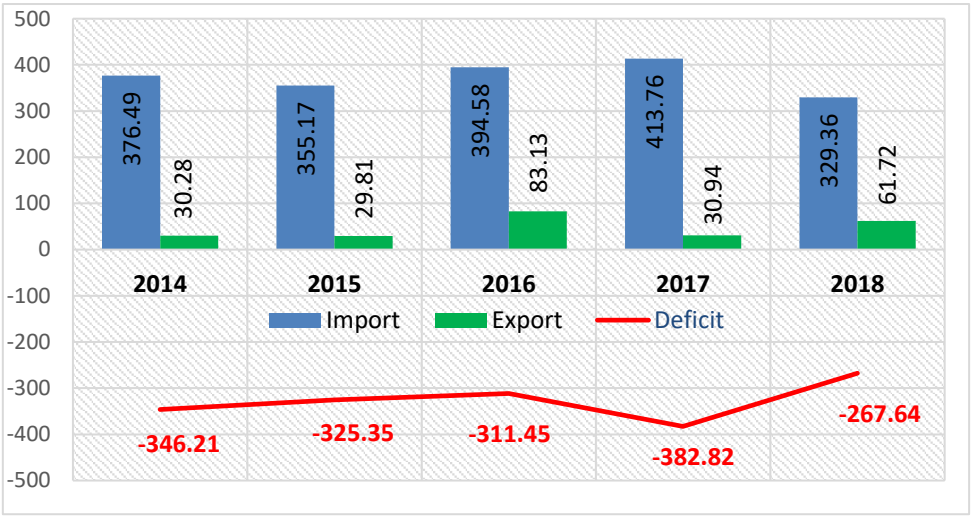
Bosnia and Herzegovina has traditionally been a net importer of wheat, in the former SFRY it imported wheat from neighboring republics of the former SFRY. In 1990, BiH accounted for 18.9% of the population of the SFRY and produced 7.1% of the wheat in the SFRY (Miljković and Nikolić, 1996). Ten years ago (2009), wheat imports to BiH amounted to 316,776 tonnes of wheat, and 60% of that amount came from Hungary (Muratović and Crnica, 2010). Even today, wheat is imported from neighboring countries, Serbia and Croatia and from Hungary. Between 250 and 400 thousand tonnes of wheat are imported annually. It serves human consumption, and since 2015, significant exports of flour have started, so that part of the wheat is imported for re-export through flour. Wheat grain exports were minor (Graph 1).

In world, almost a quarter of global wheat production is traded. Four regions supply the world with wheat: the EU, the Black Sea region, North America, and Oceania. (EU, 2019). Although the analysis of exports and imports by calendar year may seem illogical, since wheat is harvested in the middle of the calendar year, approximately the same wheat stocks are transferred from one production year to another, so the analysis is again reduced to a 12-month period, from one harvest to another. Data on imports and foreign trade deficits in wheat grain confirm that BiH does not produce enough of this grain to meet its own needs. In the period 2014-18. the deficit ranged between 311 and 382 thousand tonnes (Graph 1). The deficit has a decreasing trend, with the exception of 2017, when justification for its increase was the largest flour export in the observed five years. The lowest deficit was in 2018, the year in which BiH achieved the largest domestic wheat production. The average of wheat grain imports is 68% of domestic production, which indicates the dependence of BiH on its imports and the chance to replace part of the deficit by increasing domestic production. In that regard, Republika Srpska is in a more favorable position because it produces 2/3 of the total wheat produced in BiH and

[1]https://publikacije.stat.gov.rs/G2019/Pdf/G20192052.pdf, accessed:24.01.2020.

1/3 of the BiH population lives there. The uneven distribution of wheat production is certainly influenced by agro-ecological conditions (RS has more arable land than FBiH), but also by the fact that RS has been encouraging wheat production for years.

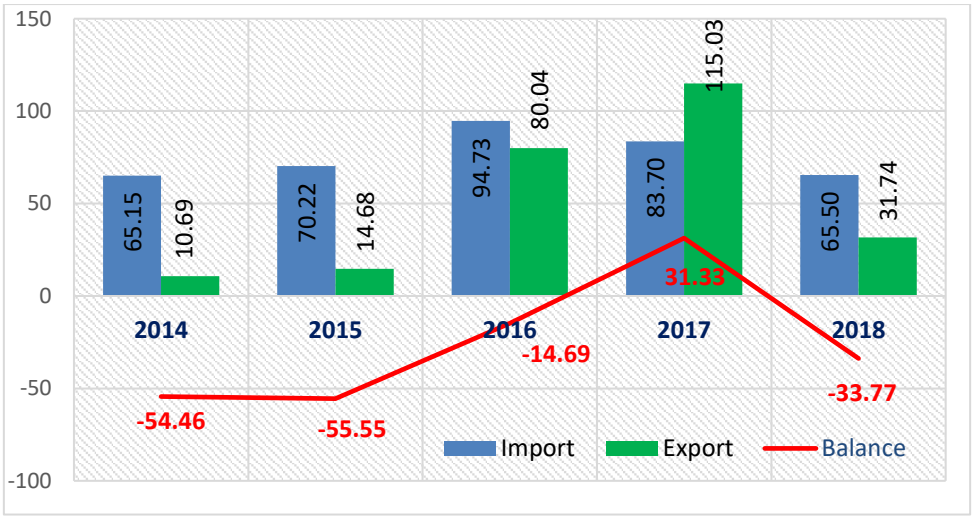
In addition to the increase in areas sown with wheat, the deficit in foreign trade could also be reduced by higher yields. So e.g. Analyzing the situation in Romania, Kruzslicka (2018) states that "total EU cereal production in the period 2007-2017 had an increase of 18%, while in Romania the increase was higher by 255%, mainly due to the average yield increase by 246 %", which is certainly a roadmap for other countries in transition.



Source: *Data processing by authors based on BiH Indirect Taxation Authority data.*

Graph 1. Foreign trade balance wheat in the grain (000 tons) (BiH, 2014-2017)

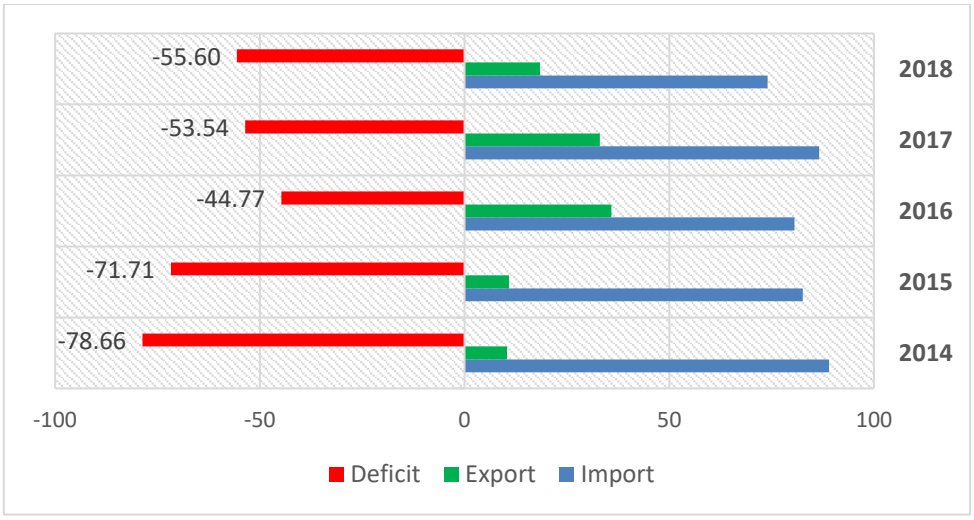
Due to insufficient quantities of wheat from domestic production, BiH imports flour and other wheat products in the range of 65-95 thousand tons per year (Graph 2). At the same time, flour is also exported from BiH, so that in 2017 BiH had a surplus in foreign trade in wheat products, and their imports and exports were approximately the same in 2016. This means that BiH, on the basis of wheat grain imports, was able to achieve significant exports of wheat products in the two mentioned years, and this was achieved mainly thanks to a special arrangement for exporting flour to Turkey in those years. Export prices of flour were 20-30% higher than import prices of flour. Ministry of Foreign Trade and Economic Relations (2019), states that one of the four most exported agricultural products (2018) to Turkey was wheat or husk flour.



Source: *Data processing by authors based on BiH Indirect Taxation Authority data.*

Graph 2. Foreign trade balance products of processed wheat (000 tons) (BiH, 2014-2017)

In terms of value, foreign trade in wheat and grain products can be viewed in a coherent manner. The data processed indicate a constant deficit of between 45 and 79 million EUR per year (Graph 3). The deficit was smallest in 2016 due to significant exports of wheat and wheat products, as well as the lowest import price of wheat.



Source: *Data processing by authors based on BiH Indirect Taxation Authority data.*

Graph 3. Foreign trade of grain and processed wheat (million EUR) (BiH, 2014-2018)

In general, due to the lack of domestic production relative to consumption, in the period 2014-2018, BiH had a deficit in foreign trade in wheat and grain products. The size of this deficit depends on domestic wheat production, which varies from year to year and import and export prices. Depending

on the quality of domestic wheat, the ratio between the quantities used for human consumption and for livestock feed varies.

Wheat self-sufficiency in BiH

Wheat is primarily used for human consumption (milled into flour and processed into bakery products), but a certain amount is also used to feed livestock, primarily because of its favorable wheat price relative to the price of corn. According to statistics, BiH produced an average of 71.93 kg per capita of wheat in the observed period. Bosnia and Herzegovina imported an average of 105.88 kg per capita of wheat per grain during the analyzed period alone. The data in tab. 3 show a high import dependency (IDR) of BiH with respect to this product. IDR averaged 77.79% over the observed period. The highest import dependency was in 2016 and 2017, when imports and exports were the largest. In 2018, there was a significant decline in IDR.

Table 3. The level of wheat self-sufficiency in BiH (2014-2018)

Year	2014	2015	2016	2017	2018
Production (t)	170,055	213.015	306,605	288,738	291,515
Import (t)	466,477	452,818	526,382	530,112	420,610
Export (t)	45,224	50,335	195,179	191,972	106,148
Import Dependency Ratio (IDR) (%)	78.89	73.57	82.53	84.56	69.41
Self Sufficiency Ratio (SSR) (%)	28.8	34.6	48.1	46.1	48.1
Production + Import – Export (t)	591,309	615,498	637,808	626,878	605,978
Per capita consumption (kg)	167.45	174.30	180.62	177.53	171.61

Source: *Author's own calculation.*

The estimated quantity available for wheat consumption averaged 615,494 tonnes, while per capita consumption averaged 174 kg, equivalent to flour of 131 kg. Consumption has varied over the observed period, but is also quite high. According to MOFTER (2010), wheat consumption for human consumption in 2009 was estimated at 400,000 tonnes, while per capita consumption was approximately 138 kg per year, equivalent to about 103.7 kg per capita of flour. However, in BiH, part of the wheat from domestic production is consumed for livestock, so wheat consumption for human consumption is slightly lower.

According to the survey, the level of self-sufficiency in BiH ranged between 29% and 48%. Therefore, Bosnia and Herzegovina has a deficit of production because it does not cover the total needs with wheat and self-sufficiency averaged around 41%. According to MOFTER (2019) and ten years earlier (2009) the satisfaction rate for wheat was 45.7%. The degree of self-sufficiency depends largely on the volume of domestic production, which varies from year

to year. According to the Short-term outlook for the EU agricultural market (EC, 2018), EU-28 had an excess of wheat during 2017-2019 and Self Sufficiency Ratio was 116% (2016/17); 122% (2017/18) and 117% (2018/19) and BiH is one of the countries to which this surplus is placed.

In many countries, the share of cereals in the diet is very high, especially in those with a low standard of living (over fifty percent of the nutritional requirements are settled by the grain), while in countries with a high standard of living, the direct share of cereals in the diet is small, but indirectly over fodder, ie animal products such as meat and milk very high (Kuzman et al, 2007). This is confirmed by the data in the table below.

Table 4. Human consumption of wheat per capita, (kg per capita)

Area	Year				
	2014	2015	2016	2017	2018
World	67.03	66.83	66,.90	67.08	67.16
Africa	50.36	50.65	50.63	50.43	50.14
Asia	64.93	65.20	65.33	65.50	65.55
Europe	107.80	108.14	109.06	109.01	109.08
Latin America	55.93	51.78	52.52	54.27	56.30
North America	81.37	81.21	79.60	80.27	80.23
Oceania	67.00	67.05	66.95	70.07	70.02
European Union	111.02	111.88	112.88	112.64	113.00
Developed countries	95.01	95.17	95.22	95.47	95.47
Developing countries	60.36	60.14	60.28	60.50	60.66

Source: *https://stats.oecd.org/viewhtml.aspx?datasetcode=HIGH_AGLINK_2019&lang=en#*

Consumption of wheat per capita is highest in the European Union and Europe. However, although developing countries have lower wheat consumption than developed countries per capita, they use significantly higher amounts of human consumption, while developed countries use significantly higher amounts for livestock consumption. In developing countries, according to OECD-FAO data, one tone for livestock consumption is consumed by almost 8 tones for human consumption. The closest 1:1 ratio is in Europe and EU-28, respectively. At a global level, this ratio is about 1:3. Romania also has high per capita consumption of wheat, as confirmed by the Kruzslicka survey (2018), which states that the average net annual consumption of wheat per capita has decreased since the year 2000 by 14%, to 122 kg / capita / year in 2016. Morgounov et al (2016) states that annual consumption of bread and other wheat products in Turkey exceeds 200 kg per capita and is one of the highest in the world.

Conclusion

Wheat is one of the strategic products in Bosnia and Herzegovina and is second crop in importance. Balance sheet calculations provide information on supply and demand in the market, foreign trade, per capita consumption and the extent to which domestic production is met. In 2014-18, Bosnia and Herzegovina achieved maximum wheat production in 2016-18. Foreign trade in wheat in BiH has a constant quantitative deficit. It is positive that it is decreasing, although in 2017 it increased, and the target deficit was in the last year of analysis. In terms of flour, the deficit in terms of quantity was the largest in 2015. Only in 2017 was the foreign trade surplus for flour. In terms of value, wheat and flour together accounted for a negative balance in all years (2014-2018), between 45 and 79 millions EUR per year. Overall, wheat production in BiH varies and is not sufficient to meet the needs of the indigenous population. According to the degree of self-sufficiency, BiH is deficient in this product. The average five-year self-sufficiency rate was 41%. This data indicates that BiH depends on wheat imports, even though wheat has a significant share in the crop production structure. By using additional currently cultivated areas, wheat production could be increased, as agotechnical procedures for its production are realistically simple and well known to domestic producers, of course, if domestic producers find their financial interest in it. As good example is Republika Srpska, where, thanks to monetary incentives, wheat production has increased by 10,000 ha in five years.

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Original scientific paper

Drought analysis for the region of North Backa, Serbia

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Abstract

This study aims to investigate the characteristics of meteorological and agricultural drought conditions in North Backa. The meteorological data for weather station Palic over the period 1966-2018 were used to calculate several drought indices. According to the Aridity Index, semi-arid growing seasons are more frequent in the last two decades of the studied period. Drought was present in 14% of growing seasons (according to both SPI and RDI) and 16% cases (according to SPI) and 17% (according RDI) during July and August. De Martonne Index revealed that irrigation was required every second year from July through September, except for crops resistant to drought. The Climatic Precipitation Deficiency Index indicated that water shortage was present during the whole growing season in the studied period. The largest water deficit was recorded in July (83mm) and August (76mm) when crops have the largest water requirements.

Key words: Drought, Drought indices, Lowland, Palic, Serbia.

Introduction

North Backa, a subregion of Vojvodina, is located in the northern part of Serbia, in the Pannonian Plain. Vojvodina is predominantly a lowland region situated in the south-eastern part of the Pannonian Plain. The studied area is characterized by the temperate continental climate with strongly pronounced continental features (Lalić et al., 2011). Agriculture represents an important economic sector in this region, since approximately 90% of the land area is covered by arable land, mostly fertile plough-fields. Agricultural production is largely conducted without the application of irrigation, so the impact of climate is of crucial significance for plant production. In Vojvodina, precipitation varies according to the amount and order (Pejić et al., 2011), while drought represents an occasional or common phenomenon

which limits high production (Bošnjak, 2001). Droughts occur frequently, affect large territories, damage crops (Dragović, 2012), and decrease the yield of the cultivated crops (Stričević et al., 2011). In the area of Vojvodina, droughts have been increasingly present in the last few decades (Dragančić et al., 2017; Matović et al., 2013).

The aim of this paper is to determine the humidity conditions in the North Backa District by analyzing years-long climate data (1961-2018) and to study the meteorological and agricultural aspects of drought by means of several various indicators.

Materials and Methods

Meteorological data (from 1961/62 to 2017/18) for weather station Palic, representing the region of North Backa, were provided by the National Hydrometeorological Service of Serbia (NHSS). Drought analysis was based on the following climatic parameters: annual, growing season (April-September) and monthly mean air temperature; number of tropical days (Tmax>30^{o}C); annual, growing seasonal monthly precipitation. The annual data refer to the hydrological year, from October of one year to September of the next year.

Meteorological and agricultural aspects of drought was assessed using following groups of drought indices: 1. Indices based on precipitation data only - The Standardized Precipitation Index: SPI-6 for September 30th , SPI-3 and SPI-2 for August 31th (Zarch et al., 2015; WMO, 2016); 2. Indices based on precipitation and temperature data - De Martonne Index (De Martonne, 1926; Stričević, 2007a); 3. Indices that also include potential evapotranspiration (PET) - Reconnaissance Drought Index (RDI): RDI-6, RDI-3 and RDI-2 (Tsakiris &Vangelis, 2005), Aridity Index (AI) (UNEP, 1992) and Climatic Precipitation Deficiency (CPD) (Spasov, 2003).

DrinC software (http://drinc.ewra.net) was used to calculate SPI and RDI. Other indices were calculated using Microsoft Excel. Drought indices are determined on an annual basis and/or for growing season and summer months. PET for determining CPD was calculated using the FAO method Penman-Monteith (Allen et al., 1998), while Thornthwaite method (Thornthwaite, 1948) was used to calculate PET for AI and RDI.

Results and Discussion

The North Backa District is an area of temperate continental climate with warm summers and cold winters. The analysis of the data from 57 hydrological years (1961/62-2017/18) showed that the annual mean air temperature amounted to 11.1^{o}C, while the mean air temperature

during the growing season of spring crops (April - September) was 18.1°C. The hottest months in the year are July (21.9°C) and August (21.3°C). On average, the highest number of tropical days occur in August (10) and July (9). The average annual precipitation in Serbia ranges from approximately 550mm in Kikinda to around 950mm in Zlatibor (Tošić et al., 2017). In Palic precipitation amounts to 570mm, so it is close to the minimum values. Gocić & Trajković (2014) concluded that in the total area of Serbia the annual amount of precipitation had a rising tendency in the period from 1946 to 2012. Its average value is 0.851mm/year. However, in the northern and northeastern parts of Serbia, where Palic is situated, it is 0.656mm/year. During the growing season, the average rainfall in Palic amounts to 337mm, which is lower than the average precipitation in the agricultural regions in Serbia (365mm) (Matović et al., 2013). The lowest amount of precipitation (128mm) was recorded during the growing season of the 1999/00 hydrological year, when the precipitation was somewhat higher than the third of the years-long average precipitation. Palic has a continental precipitation regime with June being the month with the largest amount of rain (74mm). The most arid period during the growing season is April-May, with the average amount of precipitation of 100mm. During the summer, the lowest recorded amount of precipitation was in July 2013 (4mm) and August 2012 (3mm). In addition to the growing season, the July-August period has been selected for a detailed analysis regarding the occurrence of drought. This period was chosen due to its high air temperatures and low precipitation.

The calculated annual value of the Aridity Index (AI) is 0.6, which indicates that the climate in this area is semi-humid (AI=0.50-0.65). This is confirmed by the De Martonne's Index amounting to 27.1. The results of the analysis of the summer season and the complete growing season in terms of aridity are presented in the following text.

The mean air temperature during the growing season, as well as during the summer months (July, August), shows mainly the negative deviation from from the reference period in the first thirty years of the studied period (Fig. 1). In the following period, the temperatures have risen and had an increasingly positive deviation, which was also confirmed in other studies conducted in Serbia (Ruml et al., 2017; Matović et al., 2013; Gregoric et al., 2019; Idrizović et al., 2018). Analyzing the SPI-6 for September 30th on the basis of the criterion SPI<-0,935 (NHSS), the occurrence of mild or severe drought was noticed 8 times (Fig. 2). This means that there were 14% of arid growing seasons. SPI-3 (June, July, and August) shows the occurrence of droughts during 7 years, i.e. in 12% of the cases, while SPI-2 (July-August) shows droughts in 17% of the cases (10 years). Exceptional drought (SPI $\leq$ -2.326) was registered during the year of 1999/00 (SPI-6 and SPI-3), as well as in 2011/12 (SPI-3). Extreme

drought appeared during four years in the July-August period (SPI-2), while in the period June-August (SPI-3) it occurred during two years. Also, extreme drought (-2.326 < SPI ≤ -1.645) in the growing season occurred during two years (Fig. 2).

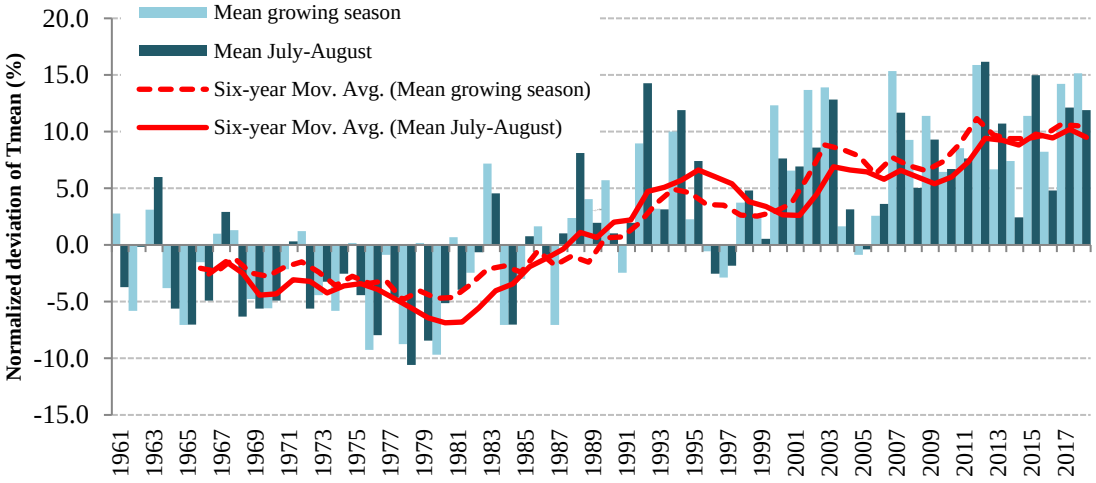

Figure 1. Mean temperature deviation from the reference period 1971–2000 during the summer period (July-August) and growing season. The red lines represent a six-year moving average for the July-August period (solid line) and growing season (dashed line).

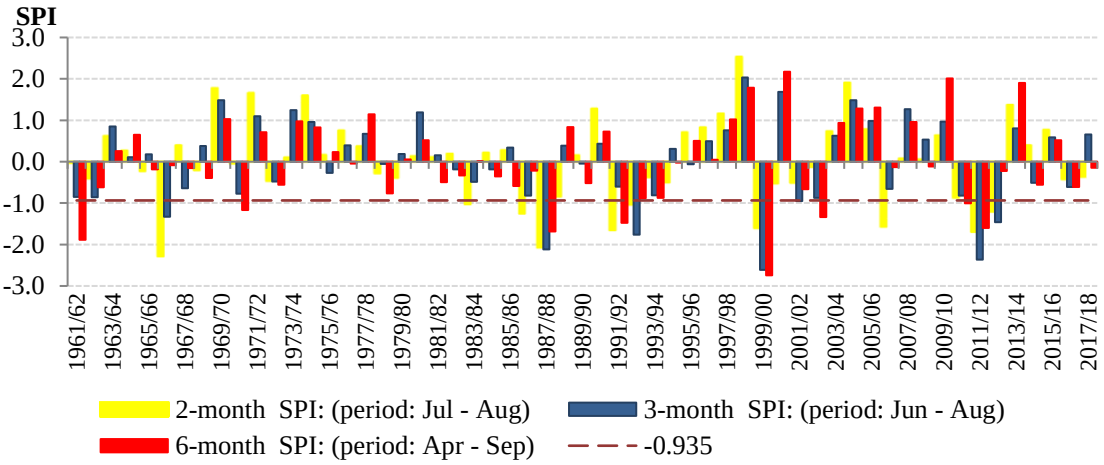

Figure 2. SPI-6 values for September 30th, SPI-2 and SPI-3 values for August 31th

The average values of the De Martonne's Index (1961-2018) were in the range from 20 to 30 during the complete growing season (Tab. 1), which indicates that irrigation is required as an additional measure (Stričević, 2007a). A more exhaustive analysis for the period July-September for each of the 57 years (Tab. 2) shows that irrigation is necessary (except for some

drought-resistant crops) in 48% of the situations in July, 56% of the cases in August and 52% of the cases in September.

Table 1. Monthly values of the drought index according to De Martonne (Im).

1961-2018	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Im	44.7	36.3	25.7	23.5	25.7	29.1	23.2	20.4	20.7	21.8	34.5	50.1

Table 2. The analysis results of the De Martonne's Index monthly values for July-September

	Im<5	Im=5-10	Im=10-20	Im=20-30	Im>30
Jul	3%	9%	36%	24%	28%
Aug	10%	10%	36%	26%	17%
Sep	10%	14%	28%	31%	17%

The RDI shows similar results as the SPI. The analysis of the RDI-6 showed that some of the forms of drought occurred during 9 years (16% of arid growing seasons). RDI-3 (June, July, and August) detected droughts during 8 years, i.e. 14% of the cases, while RDI-2 (July-August) showed droughts in 16% of the cases (9 years). The comparison of the results of the RDI and SPI calculations showed a very good statistical compatibility of the values both for the growing season and for the summer period.

For the growing season, the coefficient of determination amounts to 0.977, for the period June-August it is 0.981, while for the period July-August it is 0.984 (Fig. 3). When it comes to the compatibility of SPI and RDI indicators on a yearly scale, similar results were obtained by the studies conducted in Srem (Stričević et al., 2007b), Greece (Tsakiris et al., 2007) and southern Serbia (Idrizović et al., 2018).

The Aridity Index for the period of the growing season (the average for 1961-2018) is 0.6, which indicates semi-humid conditions. A higher incidence of the semi-arid growing season (AI=0.2-0.5) was observed in the last two decades of the research (50%) than in the previous period of 38 years (32%).

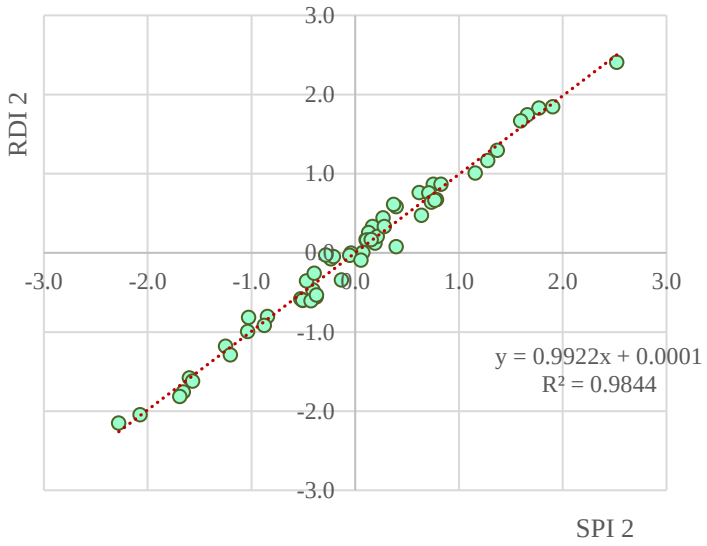

Figure 3. Relation between RDI-2 and SPI-2 for August 31th for the meteorological station Palic (1961-2018).

The values of the Climatic Precipitation Deficiency (CPD) were calculated for each month during the growing season on the basis of the average values for the 1961-2018 period. The CPD shows the lack of water during the complete growing season (-352mm). The largest precipitation deficit is in July (-83mm) and August (-76mm), when the water requirements are the highest (Tab. 3). Similar CPD values were presented by Spasov (2003) for the Banat, Timocka Krajina and the far south of Serbia.

Table 3. Monthly values of CPD, calculated on averaged values for the period 1961–2018. P – precipitation, PET – potential evapotranspiration.

Month	Apr	May	Jun	Jul	Aug	Sep
P – PET (mm)	-39.2	-52.9	-55.4	-83.2	-76.3	-45.0

The distribution functions (Fig. 4) created for the interval of the growing season and the July-August period show that once in ten years the CPD was 253mm in the July-August period, and 529mm in the complete growing season. Once in 57 years, the CPD was 671mm for the growing season, while it amounted to 308mm for the period of July-August.

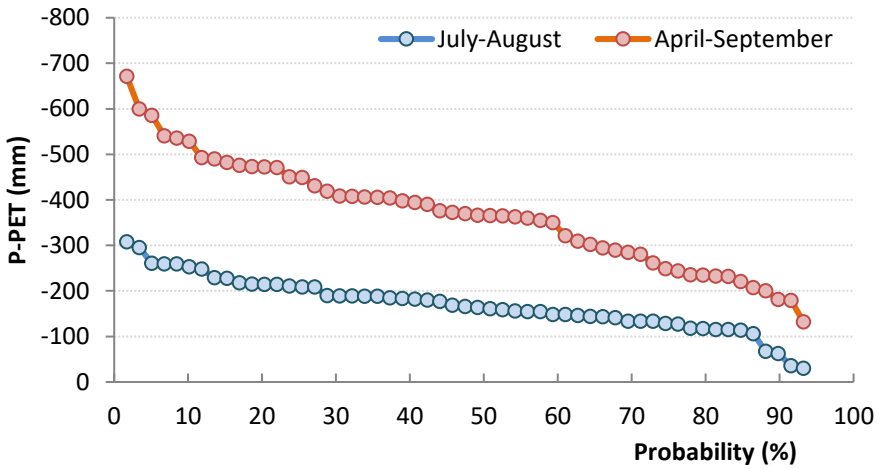

Figure 4. Distribution function of CPD (mm) for the growing season and the period July-August for meteorological station Palic (1961–2018).

Conclusions

Study results suggest that climatic conditions for agricultural production in North Backa are very variable, deteriorating in the last few decades. Temperature and number of tropical days showed positive trends in the last three decades of the studied period. According to the Aridity Index, the occurance of semi-arid growing season was more frequent in last two decades (every second year) than in the previos period (every third year). Drought was present in 14% of growing seasons (according to both SPI and RDI) and 16% cases (according to SPI) and 17% (according RDI) during July and August. De Martonne Index revealed that irrigation was needed as a supplementary measure during the growing season, while every second year was necessary from July through September for all crops except for those very resistant to drought. The CPD index indicated that water shortage was present during the whole growing season (352mm), with the largest water deficit in July (83mm) and August (76mm), which is the period when crops have the largest water requirements.

Acknowledgements

This paper is part of research project #31005 (A Modern Biotechnological Approach to Resolving Drought Issues of Serbia's Agriculture), #37005 (Assessment of Climate Change Impact on Serbia's Water Resources), and #43007(Studying climate change and its influence on the environment: impacts, adaptation and mitigation), all funded by the Ministry of Education and Science of the Republic of Serbia.

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Original scientific paper

Wine production in Serbia and competitiveness on international market

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Abstract

The market environment in Serbia is characterized by integration with the international market, and this brings a numerous changes in the level of competitiveness of individual segment of food sector. Namely, as Serbia has been in the process of market liberalization for a long period of time, there have been numerous changes in the food market resulting from the integration (free trade agreements with European Union, CEFTA, Eurasian Union,...), which has not bypassed the wine sector as well. Serbia is a significant wine producer in the world, and according to FAO, Serbia is the 20th largest wine producer in the world by production quantity. However, wines from Serbia face stiff competition on the international market, where wine producers from other countries are more competitive that Serbian producers, both in quality and price. The main aim of this paper is to determine the potential for exporting Serbian wine to the international market, and to consider the competitiveness of this sector. The results of this research indicate unfavourable tendencies in the export of wine from Serbia, especially to EU countries. In that context, the potentials for further exchange, as well as recommendations for improving competitiveness in the international market, have been considered.

Key words: wine, Serbia, competitiveness.

Introduction

The market environment in Serbia is characterized by integration with the international market, which implies numerous changes in the agribusiness sector. Namely, as Serbia has been in the process of adapting its economic system to European Union (EU) and World Trade Organization (WTO) for a long period of time, there have been numerous changes in the market of agri-food products resulting from the integration and necessity to respect the rules and principles in the international trade of these products. In addition, changes in foreign trade were

caused by the liberalization of the Serbian market, which resulted from the signing of trade agreements with the countries of the EU, as well as the signing of the regional CEFTA agreement and agreements with other foreign trade partners (Eurasian Union, EFTA, and Turkey). The current liberalization of the market has caused changes in the foreign trade flows of agri-food products, in terms of intensification of the exchange, but also changes in the level of competitiveness on the international market under the influence of free trade agreements. Specifically, studies that analyse the effects of liberalization on the agri-food sector of Serbia indicate a significant contribution of the Stabilization and Association Agreement with the EU, as well as the CEFTA agreement on the exchange of agri-food products. It is emphasized that the Stabilization and Association Agreement with EU has achieved stronger effect, both in exports and imports of agri-food products (Matkovski et al., 2018).

Numerous papers show that Serbia is at a lower level of competitiveness than most EU countries in the agribusiness sector, and one of the main causes is the low level of productivity. In terms of Serbia's competitiveness against the World Economic Forum, Serbia has progressed, especially in the last three years. In 2018 it was ranked on 65th out of 140 countries analysed. Serbia lags behind all EU countries except Croatia, which was ranked on 68th place in 2018, but Serbia is better ranked than all Western Balkan countries (WEF, 2020).

Grape and wine production in Serbia has a long tradition. However, Serbia is not recognized on the world map of wine producers. Wines from Serbia face strong competition in the international market, where foreign wine producers are more competitive both in quality and price. According to Food and Agricultural Organization (FAO), Serbia represented the 20th wine producer in the world in 2014 (FAO, 2020). In addition to the relatively large volume of production, there are numerous problems in the export of wine and the achievement of appropriate positions in the international market. Namely, there is very strong competition in the EU wine market. Recent reforms in the EU wine production sector are promoting a market-oriented approach, although quantitative production quotes are still present, under certain conditions (Rizzo, 2019). The most pronounced comparative advantages in the export of wine have: France, Italy, Spain and Portugal. In addition to these countries, Bulgaria, Cyprus and Greece have comparative advantages in exporting wine from EU countries (Balogh and Jambor, 2017). Within the CEFTA countries there is significant competition in wine production. Countries such as Moldova, Montenegro and North Macedonia have a positive foreign trade balance of wine, while in Serbia, Bosnia and Herzegovina and Albania there are negative foreign trade balance of wine (Vlahović et al., 2017).

The objective of this paper is to examine the position of wine from Serbia on the international market, i.e. to determine the potential of foreign trade in wine. Additionally, the paper covers a regional analysis of exports and imports, as well as an overview of the foreign trade balance. Also, the potentials of further trade and recommendations for improving competitiveness in the international market were examined.

The structure of the paper consists of five sections. The introduction elaborates the subject of the research and sets the main objective. The second section is the methodological framework. The third section presents the results of the research and it is divided into two parts. The first part deals with the production tendencies of grapes and wine in Serbia. The second part of the research results is related to the analysis of foreign trade in wine. The fourth section is discussing the potentials for further development of the wine sector in Serbia. The last section represents the main conclusions, as well as recommendations for future research.

Materials and Methods

For the purposes of the analysis, Statistical Office of Republic of Serbia (SORS) data were used, namely the data from the Census of Agriculture in 2012, as well as data available in the database relating to foreign trade. For the purposes of export and import analysis, data were used for the wine product according to the Standard International Trade Classification - Revision 4. The period of the analysis is the 2012-2018. This analysis of production includes analysis of areas under the grapes, the total production of grapes, grape yields, as well as wine production tendencies. The same period of analysis was used in the analysis of foreign trade of wine (export, import and foreign trade balance of wine in Serbia).

Results and Discussion

Production of grapes and wine in Serbia

In Serbia, the production of grapes is around 21,000 ha, which is less than one percent of the total land used. The areas under vineyards make up about 10% in the structure of permanent crops, and grape production has a similar share in the total production of permanent crops. In terms of production, the grapes are behind the apples and plums, which are dominant in Serbian fruit production. Most of the vineyards are located in the territory of central Serbia, just over 17,000 ha, which makes up about three quarters of the total area under grapes. In Vojvodina the vineyards grows on about 5,000 ha, with an average annual production of about 37 thousand tons, which represents about a quarter of the total Serbian grape production. Wine varieties are

dominant and make up about ¾ of the total area under grapes in Serbia, and in Vojvodina about 84%. Central Serbia has a significant share of varieties intended for fresh consumption - about 30%. As the red wine varieties are concerned, the largest areas have: Merlo, Cabernet, Frankovka, Prokupac and others, while at white varieties: Graševina, Chardonnay, Rhine Riesling, Sovignon and others (Jakšić et al., 2019).

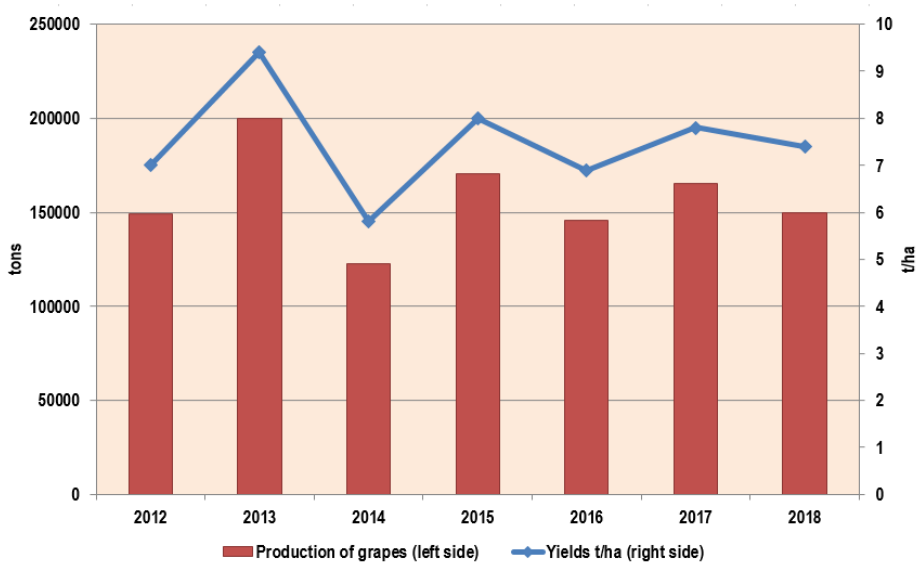
Central Serbia and Vojvodina along with Kosovo and Metohija comprise three wine regions of Serbia, so there are a total of 22 wine regions with 77 vineyards and more wine-growing oases. The districts with the largest areas under the vineyards are: Rasinski District, South Banat District and Sremski District. This is not surprising, since the first two districts contain two of the largest grape and wine producers in Serbia - Rubin AD Krusevac and Vršački vinogradi AD (Swisslion) from Vršac. In terms of vineyards, the largest areas are in the Vršac Ninogorje - about 1,300 ha, followed by Fruška Gora vineyards - 924 ha and Trstenik vineyards with about 756 ha (Jakšić et al., 2019).

The characteristic of wine production in Serbia is that it takes place on a relatively large number of agricultural holdings, which indicates that the production units are small. Namely, according to the Census of Agriculture in 2012, about 80,000 holdings owned vineyards, which make up over 10% of the total holdings. Over one third of these holdings produce grapes at less than 0.1 ha, which indicates that it is mainly production for own needs, without market potential. The importance of these agricultural holdings is reflected in the preservation of autochthonous and regional varieties, as well as possibly in organic production (Jakšić et al., 2019). Most agricultural holdings have vineyards on the area of 0.1-0.5 ha, which is not enough to achieve more serious economic results and further development of production. Such agricultural holdings cultivate over 8 thousand hectares. Agricultural holdings with vineyards up to 0.5-2.0 ha cultivate about 4,430 ha and do not have significant development opportunities. Agricultural holdings with more than 2 ha under vineyards are potentially serious producers, while agricultural holdings with more than 10 ha may be serious wine producers, whose wines can also be recognized on national market. Farms of 2-10 ha cultivate 2,830 ha, while those of 10-100 ha cultivate 2,310 ha. Only six wineries in Serbia own more than 100 ha, and cultivate a total of 2,800 ha (Census of Agriculture, 2012).

The structure of wine producers is similar to grape producers, i.e. wineries with less capacity predominate. Namely, about half of the total 312 wineries have limited capacities (below 20,000 litres), while 93 wineries have slightly larger capacities - from 20,000 to 40,000 litres, but still insufficient for competitive production. The largest capacities are found in 48 wineries

with capacities of over 100,000 litres per year, while 13 wineries have capacities over one million litres per year (Jakšić et al., 2019).

The areas under vineyards in Serbia show stagnant tendencies despite the policy of encouraging this production. The Census of Agriculture in 2012 showed significantly less acreage under vineyards than the statistical estimates before it. Namely, until the Census of Agriculture in 2012, it was estimated that there were about 50,000 ha under vineyards in Serbia, while the results of the Census showed only slightly over 21,150 ha under vineyards in Serbia (Strategy of agriculture and rural development of Republic of Serbia in period 2014-2024). The volume of grape production varies mainly due to changes in the yield level, which ranges from 5.8 to 9.4 tonnes per hectare and tends to decline (Graph 1).

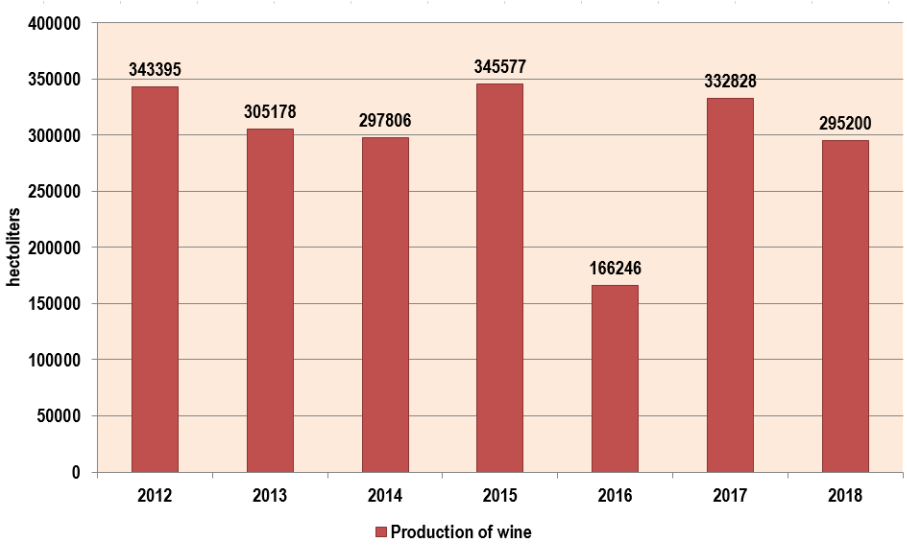


Graph 1. Production tendencies and yields of grapes in Serbia

Source: Authors' calculations based on SORS (2020)

According to SORS data, wine production in Serbia is around 300 thousand hectolitres per year (Graph 2). However, this data should be taken conditionally, since the SORS since 2014 does not record the production of wines on agricultural holdings that make wines from their own grapes. The structure of production is dominated by table wines with about 65% share in wine production; wines with geographical origin make up 20% of production, while the rest of 15% of production consists of wines with controlled and guaranteed geographical origin and quality. (Vlahović, 2015). Increasing production of wines with a geographical origin is one of the main priorities in the wine industry; as such wines are increasingly in demand on the market and will have a better competitive position, both domestically and internationally. So far, seven

geographical indication of origin have been established in Serbia: Knjazevac, Negotin Krajina, Sumadija, Subotica-Horgoski sandstone, Toplica, Srem and Three Moravas (MAFW, 2020). Also, one indication for a flavoured Bermet wine has been registered.



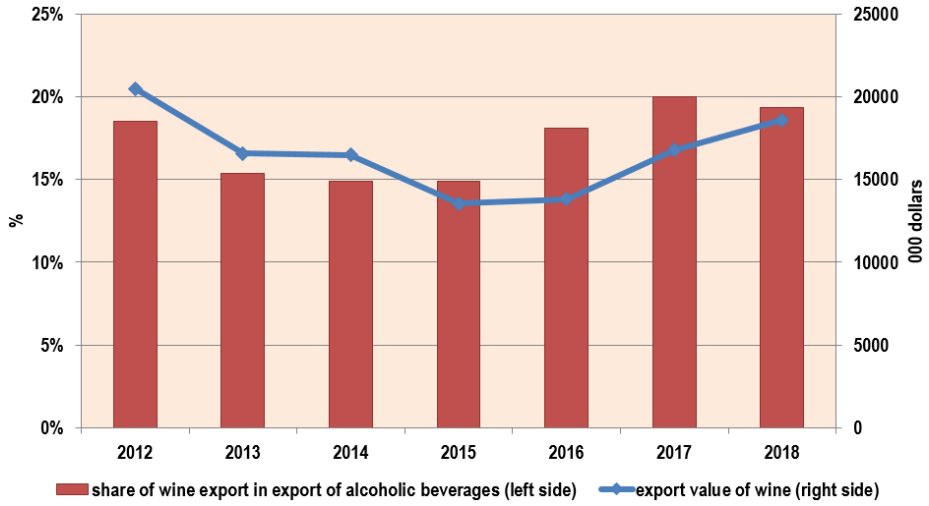
Graph 2. Production of wine in Serbia

Source: Authors' calculations based on SORS (2020)

Most wineries sell wine through large market chains and/or specialty stores, as well as HoReCa channels. A smaller part of the wine is sold directly in the wineries, although this type of sale is becoming more interesting, especially for smaller wineries. Namely, agricultural holdings through direct sales of wines can increase their total income, both through higher wine prices and through additional gastronomic and agro-tourism activities that they can practice on their holdings.

Foreign trade exchange and competitive positions of wine from Serbia

Exports of wine from Serbia are very low, and on average for the analysed period, exports from Serbia amounted to 16.5 million dollars (Graph 3). The highest value of exports was achieved in 2012, when the value of wine exports reached 20.5 million dollars, after which the value of exports was decreasing until 2015. In recent years there was an increase in the value of exports, so in 2018 wine exports reached a value of about 18.5 million dollars. Wine exports represent a significant part of total alcoholic beverage exports, and in the analysed period wine participated for almost one fifth of total alcoholic beverage exports from Serbia.

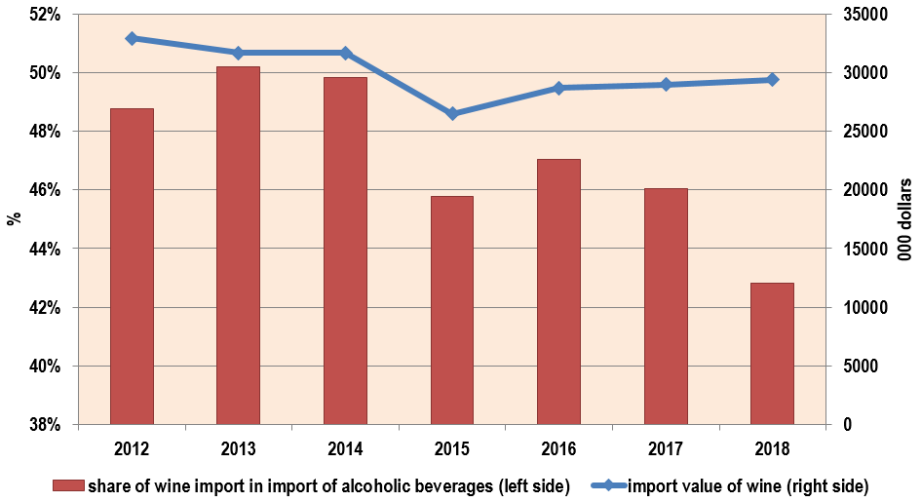


Graph 3. Exports of wine from Serbia

Source: Authors' calculations based on SORS (2020)

Regarding the regional structure of wine exports, CEFTA countries dominate, while exports to EU countries, despite the export quotas established by the Stabilization and Association Agreement, were very low. The reason for this should be found in the lower quality of wine from Serbia, i.e. the lower level of competitiveness, both in quality and in price, in comparison with the European countries who are large wine producers. Individually, the highest value in the analysed period was achieved in the export of wine to Russia, followed by Bosnia and Herzegovina and Montenegro. Of the EU countries, the most significant exports was to the Czech Republic and Croatia. Also, looking at the regional structure, it is noticeable that the significant value of wine was exported to China. (SORS, 2020)

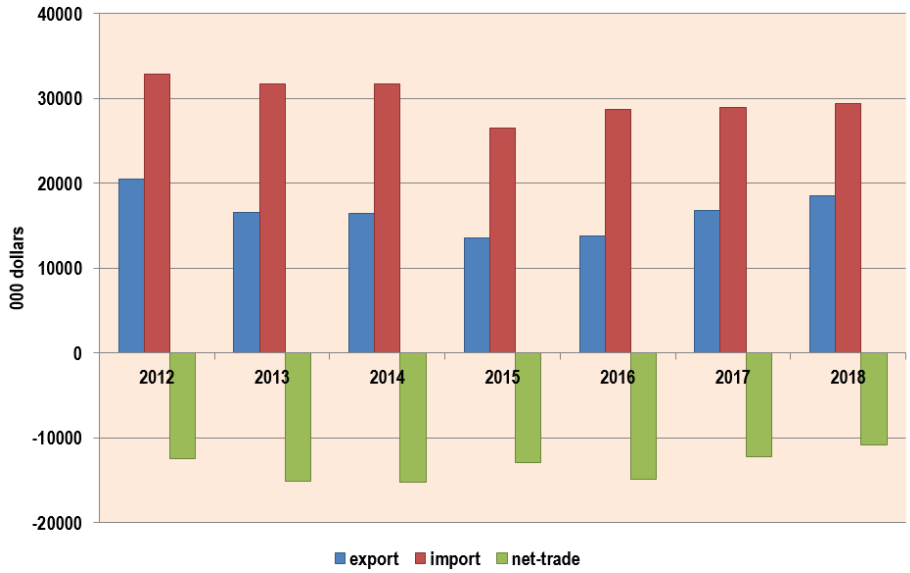
As imports of wine to Serbia is concerned, it is analysed period 2012-2018 on Graph 4. On average, it was imported wines to Serbia in value of about 30 million dollars annually, with the highest value being recorded in 2012. Graph 4 also shows that wines represent a significant part of total alcoholic beverage imports, for example in 2013 wine participated for over 50% of the value of total alcoholic beverage imports.



Graph 4. Imports of wine in Serbia

Source: Authors' calculations based on SORS (2020)

In the regional structure of wine imports into Serbia dominated CEFTA countries with over 80% of the total value of wine imports. The most significant was the import of wine from North Macedonia, with over 50% of total wine import in the analysed period, followed by Montenegro. Less than 20% of wine was imported from the EU, while imports from other countries were very low. Most of the wines in the analysed period from the EU countries were imported from Italy and France. (SORS, 2020)



Graph 5. Foreign trade balance of wine in Serbia

Source: Authors' calculations based on SORS (2020)

The balance of foreign trade of wine in Serbia was negative throughout the analysed period (Graph 5), i.e. an average trade deficit was about 13 million dollars. The reason for the deficit in trade is still the high value of wine imports from the surrounding countries, primarily North Macedonia, where the biggest trade deficit was found. The reason for the high import from North Macedonia is the relatively low price of wine, but also the fact that some Serbian companies have bought wineries with vineyards in this country (Vlahović et al., 2017).

Discussion

Due to numerous limiting factors, such as small vineyard areas, fragmented production, lack of quality and top quality wines, the opportunity to export wine should be found in adding value through investment in top quality. Namely, taking into account the quotas for export to the EU, and the focus on exports to Russia and CEFTA countries, these are markets with high absorption power, and it is necessary to adjust the structure of production to the requirements of these markets. In that context, Serbian wine producers should differentiate their production according to consumer preferences in terms of quality, packaging and prices, but also they should make additional efforts to build specific brands.

However, building the recognition of a country as a destination from which "good" wines come is a lengthy process with an uncertain outcome. Serbia, as a country with an increase in the quality of the wine produced, cannot yet be imposed on the foreign market, even when the countries of the region are concerned. In addition to this, another reason for lower level of competitiveness is the mismatch of price and quality. Namely, wines of the same or similar quality are imported to Serbia at much lower prices and that also reduces the competitiveness of Serbian wines on the domestic market. One of the way to increase the attractiveness of Serbian wines may be targeting autochthon and local varieties, which may be of interest on the international market, due to the rarity and geographical recognition. Also, such wines should be part of the rural tourist offer on the domestic market, which is especially interesting for smaller wineries that cannot be competitive into foreign markets.

A particular problem for Serbian grapes and wine producers is the lack of comprehensive institutional support, as well as the lack of producer associations. Anticipated subsidies for the raising of vineyards are certainly a good measure, but they can only represent an initial stimulus in wine production. Institutional support must cover the entire product chain - from vineyards to wine market, whether in the domestic or foreign markets. Also, the association of agricultural producers should have important role, as they are highly represented in countries with

developed agriculture. The contribution of these associations can be both in marketing activities, as well as in the procurement of raw materials and in the provision of producer's knowledge and information services.

Conclusion

Grape production in Serbia is concentrated in seven wine regions within which 22 vineyards are located. The dominant part of production is concentrated in central Serbia, where the Rasinski District, i.e, the Tri Morava Region, is emphasized. In Vojvodina, grapes are mostly grown in Vrsac vineyards, in Fruska Gora and around Subotica. Wine varieties dominate, which is more pronounced in the Vojvodina area. Production units are small, but the emergence of commercially oriented producers is noticeable, where the quality of wine is receiving increasing attention. The production and sale of wines of controlled geographical origin on agricultural holdings, especially in the case of autochthon and local varieties, can become an attractive part of the rural tourist offer.

Market liberalization, which has taken place in recent years, has led to significant changes in the foreign trade of agri-food products. Foreign trade was intensified and various changes in the level of competitiveness on the international market under the influence of concluded preferential agreements were occurred. As foreign trade in wine is concerned, it is noticeable that Serbia is not particularly competitive in the international market, both in terms of price and quality. Nevertheless, in recent years there have been positive changes in the structure of the varieties, especially when it comes to the introduction of new clones of vine. In addition to this, there is a low representation of high quality varieties in certain regions. For this reason, it is necessary to direct the attention of producers towards quality improvement, but also to make additional efforts to build brands with geographical origin. The subject of future research will be an examination of the competitiveness of Serbian wines in key market segments.

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Preliminary communication

Intermediation to increase agricultural land use by ICT support: explanation of its application in the case of the City of Trebinje

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Abstract

Competitiveness is one of the main sources of profitability and sustainability in open economies. In agriculture, competitiveness is based on specialization and the effect of the economy of scale. In a situation of dominance of small and fragmented land holdings, market opening and creating chances to generate opportunity income have resulted in, among other things, reduced use of agricultural land. The subject of the research presented in the paper is non-utilized agricultural land in the conditions of its imperfect mobility. The aim of the research was to determine the area of non-utilized agricultural land in the world and in Bosnia and Herzegovina and to find ways to increase its use. The research was conducted combining literature review and modelling of the increase of the utilization of agriculture land by its virtual consolidation, i.e. temporary renting using intermediaries, also known as land bank. Business Process Modelling Notification (BPMN) was used to graphically interpret information flows. The City of Trebinje was taken as an example for the case study. The result is developed and a schematically elaborated model of communication, information and cash flows between landowners and renters with the mediation of the Agrarian Fund and the involvement of several other institutions. The financial effects of applying that model were also evaluated by the simulation method is presented. The conclusion is that applying proposed solution of intermediation in the temporary renting of agricultural land could have multiple effects for land owners, renters, the local community and the state.

Key words: agriculture land, land abandonment, intermediation, ICT.

Introduction

Paul Krugman called competitiveness as a "dangerous obsession" (Psofogiorgos and Metaxas, 2016) and today, in the conditions of open market economy, to be better than others, i.e. be competitive, is a precondition of survival in the business. The term competitiveness has different definition and different sources, depending of the approach, author or sector, without a final consensus on the answer to the question of what competitiveness really is. Porter (1990) is an advocate of productivity-based competitiveness theory. According to him the productivity is the key determinant of the level of competitiveness that producer on some location can achieve over time thanks to the favourable unit cost. At this point, in addition to the input factor conditions, no other factors of competitiveness from Porter diamond will be elaborated. Ketkes (2006) draws attention to the difference between inherited and crated productivity of location, because some of the unheralded natural and other advantages of the location can be offset by increased productivity based on better utilization of other factors. According to Schumpeter's theory of economic development, it is exactly the role of entrepreneurs to create certain advantages over competitors by "changing in existing production systems and introducing some innovative conditions for making profits and reducing costs" (Langroodi, 2017).

In agriculture, the quality of agricultural land is mainly cited as a source of relative competitiveness, especially in crop production, because better land quality provides higher yield. This neglects another aspect of the same production factor, namely the size of agricultural land. According to the theory of economies of scale, increase of production volume is a source of reduction of fixed and average costs, and therefore the cost price of such products also become more price competitive (Vaško, 2019).

This leads to another theory of competitiveness, starting from the premise of perfect competition and based on a new trade theory at the company level, according to which both parties involved in the exchange have mutual benefits, advocated by Paul Krugman (1996). According to him, the source of competitiveness is specialization, which causes cost reduction. A company that has failed to sell its products at a reasonable price is bankrupt, but its place is taken by another company that is doing the same more successfully. This comes to agricultural land, a limited resource that must be used to meet the needs for food. If it is not profitable to one producer, it may be profitable to others based on the use of the same resource (agricultural land). According Krugman (1996), anyone who takes part in the market trade has a chance to achieve "market share" advantage.

It is not easy to obtain data about utilized and unutilized agricultural land at the world level. As Merlet (2013) noted "there are different databases on the real and potential agricultural use of land at the global level". He mentions three possible sources of such data, FAOSTAT, GAEZ, and SAGE/GATP[2], and outlines the advantages and disadvantages of each of these approaches. A similar problem is encountered when these areas are identified at the national level. The question is how much is actually arable land, due to the constant deforestation or expansion of forest vegetation and change in use of certain areas of agricultural land? There are also different definitions and classifications of particular categories of agricultural land, especially pastures and meadows (Le Mouël et al., 2018, 47-54). EUROSTAT and FAOSTAT monitor and publish data on cultivated but not non-utilized land. Therefore, such data are obtained through sporadic and partial surveys. In some parts of Europe, two processes are taking place at the same time, abandoning land cultivation and expanding arable land. Kuemmerle et al. (2016) found that "in the EU between 1990 and 2006 were cropland decline (~136,660 km^2), followed by expansion of grazing land (~75,670 km^2), and expansion of forest areas (~70,630 km^2). The least common conversion among broad land-use categories was urban expansion (~16,820 km^2). Agricultural abandonment (i.e., of cropland and grazing land) amounted to 20,500 km^2 between 2000 and 2012, whereas recultivation after 2006 affected 16,430 km^2." The EU is exposed to the loss of agricultural land, mainly in Central, Eastern and Southern Europe. The farms in Central and Eastern Europe are facing problems of small and fragmented landholdings. These are countries that have undergone several agrarian reforms that have consolidated and subdivided land tenure, from "liberation" of the peasants, through confiscation of privately owned land and its collectivization, to limiting maximum tenure. Primarily, these are countries now called 'transitional countries' in which adequate solutions are seek for restitution, privatization, replacement and exchange of agricultural land e.g. only in Romania the extent of uncultivated land has been estimated at around 3,000,000 ha or 20% of the agricultural area (Cartwright, 2010, 6).

Hatna and Bakker (2011) emphasize concerns about the effects of abandonment on biodiversity and ecosystem functioning. They highlight increased awareness of the importance of agricultural practices in a particular agro-ecosystem. By them, several land-use studies suggested that abandonment of agricultural land is more likely to occur in marginal areas where biophysical conditions are relatively poor and economic activity is low. In developing

[2] FAOSTAT - Food and Agriculture Organization Corporate Statistical Database, GAEZ system managed by International Institute for Applied Systems Analysis and FAO and SAGE/GATP - Center for Sustainability and the Global Environment / Global Trade Analysis Project database.

countries, abandoning cultivation and use of agricultural land is generally seen as a missed income, and issues of the consequences of such practices on biodiversity are not taken seriously and systematically by either the farmer or the institutions.

Various instruments are available for the consolidation of agricultural land, including land funds and land banking (Van Dajk, 2003). According to Hartvigsen (2015) the term land banking is used with different understandings in different European countries and is often synonymous with the term "land fund". The interpretations range from the mediation of the temporary lease of the land to the purchase of the land and its sale in order to consolidate the property. In addition to land consolidation, the term land mobility is used, which Hartvigsen (2014) defines as "the coordinated extent of re-structuring of land rights through sale, purchase, exchange or lease from one owner to another as it proves possible during the re-allotment process". In some countries there are also programs for the replacement of abandoned farmland for retirement, mainly in the case of the elderly (e.g. Hungary and Romania), and further care of the land is taken over by local mayors (Thelen et al., 2008).

According to the World Bank (2010), land fragmentation is the cause of its lower productivity. According to the same source, the average farm in Bosnia and Herzegovina (BiH) has 2 hectares, which is divided into 6-8 plots. Such small farms face difficulties in entering into profitable markets and participating in supply chains because of the limited quantities if the products they are able to produce. According to the data of the Republic of Srpska Institute of Statistics (RS IS, 2019a), the Republic of Srpska (RS) has 971 thousand ha of agricultural land, of which 525 thousand ha is arable. Even if the area of arable land is overestimated, previous data indicate the severity of the problem of uncultivated land for which solutions should be sought.

Material and Methods

The aim of the research is to determine how cultivation and the use of agricultural land in the Republic of Srpska and Bosnia and Herzegovina could be incrassated.

In accordance with the set goal, literature review and collection of data from secondary sources were summarized using analysis and synthesis methods. The modelling method was used to show the results of the research. A model of the impact of land, as one of the production factors, on increasing the competitiveness of agriculture has been created. Through the case study, the application of this model was elaborated on the example of the City of Trebinje. Land leasing is modelled on an e-commerce platform whose "main components are: communication systems, data management systems and security" (Nanehkaran, 2013). It is based on the identified product, which in

this case is the service of leasing and renting agricultural land, and the ITC infrastructure that enables that type of service. Methodologically, it is a modified C2C (consumer-to-consumer) model because contact between the landlord and the tenant is not direct, but it takes place thanks to an intermediary, so that the model is closer to Zwass (2013) 5-C model (commerce, collaboration, communication, connection and computation). Given the level of IT literacy of the rural population, alternative non-digital channels of communication between the central database and end-users are provided for the exchange of certain data. Due to the asymmetry of information of the owner and tenant of agricultural land, reintermediation model is developed in which the role of electronic market maker is elaborated (Wigand, 2007). Business Process Modelling Notification (BPMN) was used to graphically interpret information flows.

Results and Discussion

In BiH and the countries of South-eastern Europe, a significant part of arable land is not cultivated. In this way, the volume and value of agricultural production outputs are reduced and ultimately the gross value added (GVA) of agriculture at the national level. One of the reasons for abandoning the arable land is their small average size and fragmentation. According to statistics, 45% (180 thousand ha) of potentially arable land is not cultivated in the Federation of BiH (FBiH), 44% (about 250 thousand ha) in RS, and 83% (4 thousand ha) in the City of Trebinje, which represents a significant unutilized potential for food production (IS RS, 2019b).

Table 1. Uncultivated land in FBiH, RS and the City of Trebinje (2014-2020) (ha)

	2014	2015	2016	2017	2018
FBiH	199,000	189,000	181,000	183,000	179,000
RS	278,839	270,530	262,089	253,137	254,940
City of Trebinje	4,870	4,744	4,662	4,605	4,370

Source: The Republic of Srpska Institute of Statistics (2019a), Statistical Yearbook 2019, p. 272; The Republic of Srpska Institute of Statistics (2019b), Cities and municipalities of the Republic of Srpska, p. 153; and Institute for Statistics of FBiH (2019), Statistical Yearbook 2019, p. 199.

The agricultural land market is underdeveloped, on the one side there is inadequately formulated offer for sale or rent of mostly small plots of agricultural land by old-age and deagrarized farms, and on the other side there is the insufficiently articulated demand for additional agricultural land by commercial farms. The practice of temporary leasing of agricultural land is common in many countries, and there is often a problem of communication in connecting landlords and tenants and the presence of certain transaction costs. The solution

for both parties is a kind of institutionalized mediation in the approaching of the supply and demand of agricultural land, and thus at least a temporary increase in the cultivation and use of agricultural land. This paper is based on the elaboration of the idea of institutionalizing the mediation in the exchange of data on the offer of free agricultural land and the need for it and providing support in the conclusion of lease agreements and the substantiation of the right to incentives. In that way, instead of permanent consolidation of land tenure through the purchase and sale of agricultural land, it is virtually consolidated by one-year or more-years lease.

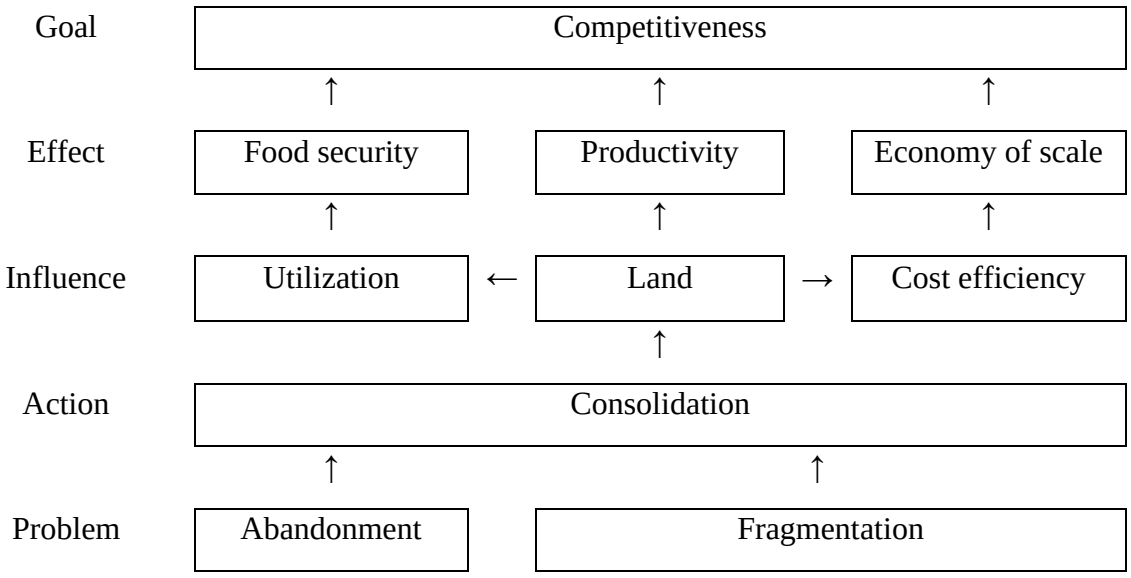

Figure 1. Effects of virtual land consolidation

The problems of the present abandonment of agricultural land and its fragmentation can, at least temporarily, be solved by its virtual consolidation. This would lead to a better utilization of agricultural land and increase the quantity of food produced, thereby raising the level of food self-sufficiency of BiH, which is still a net food importer. To a certain extent can be also increased the competitiveness of the country as it would provide the opportunity to increase exports and reduce imports of food. Aggregation of fragmented plots would also result in increased productivity due to mechanized land cultivation and saving working time. The third effect of land consolidation is increased profitability in agricultural production due to the effect of economies of scale, i.e. lower average cost. Since the experience and scope of permanent land consolidation are modest (last serious land consolidation is carried out in Bosnia and Herzegovina 30 years ago), in the short term it is realistically possible to achieve the above-mentioned effects only through virtual land consolidation by the establishment of a sort of bank

of agricultural land. The model of functioning of the land bank is elaborated on the example of the area of the City of Trebinje (Figure 2).

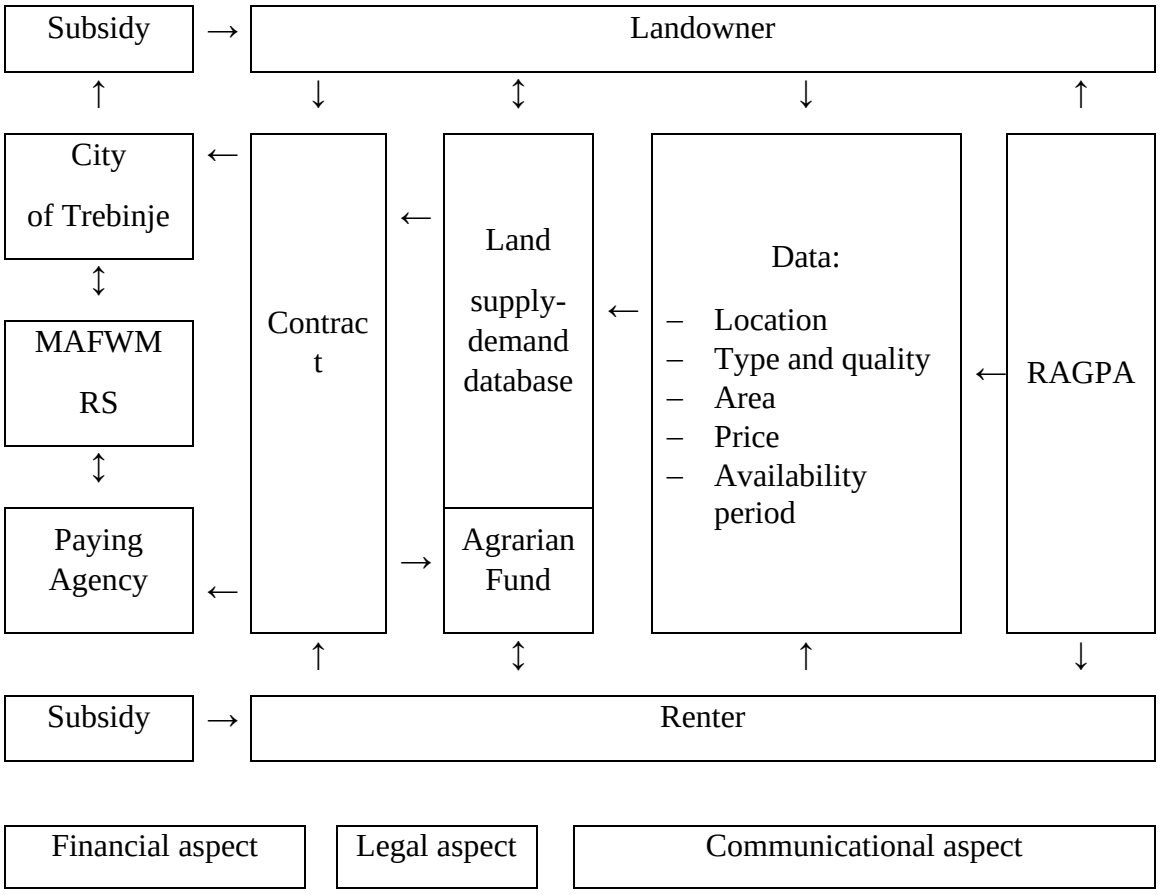

Figure 2. Model of intermediation in the lease of agricultural land

Legend: MAFWM – Ministry of Agriculture, Forestry and Water Management; RAGPA – Republic Administration for Geodetic and Property Affairs.

Nowadays, when information and communication technologies have entered almost all spheres of human life, including agriculture, it is logical that hardware and software solutions are used for the purposes of data collection, processing and storage. From the IT point of view, the previously presented model is described in more detail in BPMN format in Appendix 1.

Similar solutions exist in the case of purchase and sale of real estate, and therefore agricultural land, but they are of a commercial nature and are mainly focused on making profit from advertising and providing mediation services. The proposed model is more complex and provides multiple individual and social benefits. The City of Trebinje, selected for the case study, has an initial advantage because it has the Agrarian Fund, an institution that already has the human and technical capacity to implement such a concept. Its application in that local

community could be a pilot project which, if confirmed by his success, could be multiplied to other municipalities.

The model is based on the assumption that there is unused agricultural land and that there is an interest of the owner to rent it, which could be stimulated by the granting of certain, even symbolic, incentives by the local community to those land owners who decide to conclude a lease arrangement. Another assumption is that there are farmers interested in the use of more agricultural land, which cannot be only arable land but also pastures. The conditions for accomplishing the right to certain incentives are proof of ownership or lease of land. The cultivation of leased land must be documented and recorded in the farm registry, and this has been one of the constraints on the hitherto getting incentives. If an institution, in the analysed case, the Agrarian Fund of the City of Trebinje, mediates the conclusion of contracts, the contracting would be easier for both parties, and the fact that it is done by an official institution reduces the level of distrust and reduces transaction costs, since this service would be free of charge.

Creating a land bank model is crucial to establishing a database, based on a specific IT platform. This database would be maintained in the case of Trebinje by the Agrarian Fund of the City of Trebinje, and it would be supplied with data from landowners and potential tenants. Key data are the location of land, surface, type and quality, period of its availability, rental price and other conditions. It should not be neglected that in practice there is a dispensation of arable land or pasture for use at no charge, just to prevent it from overgrowing, in which intermediation can also help. Thanks to modern ICT technologies, search and data exchange might be via smartphone or personal computer, leaving the possibility to the less computer literate farmers to do it by the phone, filling out paper forms, or visiting the office where the data is stored. The Agrarian Fund in Trebinje has field officers who could also collect data in the field by visiting villages and farms. One of the links in this model is to the Republic Geodetic and Property Administration (local acronym RUGIP), which keeps records of ownership of real estate, including agricultural land. Temporary linking of agricultural land owners and tenants could also result in permanent sale of rented land, without forgetting the need to link the land market and the capital market (appropriate credit for the purchase of land), whereby the land seller get the opportunity to invest the money gained from the sale of land in other non-agricultural businesses.

The summation model is based on five possible uses of rented land, for the production of cereals, industrial crops and forages (X_1), vegetables (X_2), fruits and grapes (X_3), natural meadows (X_4) or pastures (X_4). Starting from the area of at least 500,000 ha (A) in different

ways of unused agricultural land in BiH, it was assumed that with the additional intermediation, at least 3% of that area (A) will be "returned" in productive use, divided into five possible uses (B). Each ha is associated with an average annual production value of 1 ha (C), which is determined by the expert estimations of the authors. The utility function (Y) to restore the use of agricultural land has the following form:

$$f(Y) = b_1 * X_1 + b_2 * X_2 + b_3 * X_3 + b_4 * X_5 + b_5 * X_5$$

500,000	ha						A
↓							
3%		0.45	0.15	0.10	0.20	0.10	B
↓		cereals	vegetable	fruit	meadows	pastures	
X =	→	X_1	X_2	X_3	X_4	X_5	
12,500	ha	5,625	1,875	1,250	2,500	1,250	C
	KM/ha	2,400	25,000	20,000	1,500	500	
	KM	13,500,000	46,875,000	25,000,000	3,750,000	625,000	Y
	KM			89,750,000			

If from the currently unused 500,000 ha of agricultural land in BiH, an additional 12,500 ha were put back into use, it would increase the value of agricultural production by about 90 million convertible marks (KM) per year (1 KM = 0.51 EUR). Subtracting the value of intermediate consumption, the real benefit would be an increase in GVA of agriculture by about 5%. In the case of the Trebinje, 3% that would be returned into use through the Agrarian Fund, with a slightly different land use structure, would increase the value of agricultural production by KM 1.2 million per year.

From municipality to municipality, the effect would vary depending on the percentage of cultivated and otherwise utilized agricultural land, which depends on the supply/demand ratio, as well as on the success of the mediation presented in the described intermediation model. It is crucial that one of the institutions takes on the role of intermediary and assume the transaction costs of keeping records, dissemination and contracting. In the case of the City of Trebinje, such institution already exists, and in the case of other municipalities, it could be a municipal administration or, at the regional level, offices of agricultural advisory services.

Conclusions

The cessation of arable land cultivation is a phenomenon seen in a number of transition countries which, after opening their market, have become net food importers from net exporters. In most post-socialist countries acceding to the EU, the agricultural land market has become functional and, in combination with agrarian policy measures, uncultivated land has largely disappeared. Unfortunately, in Bosnia and Herzegovina, which is also in the process of transition of its economic system, the problem of uncultivated land has become even more pronounced due to the war happenings and post-war migration of the rural population. In Bosnia and Herzegovina, one of the serious problems that diminishes the value of agricultural production and causes many other problems is the large percentage of uncultivated land (according to statistics about 45%). In the absence of some other solutions (land consolidation and redistribution, crediting and subsidizing the purchase of agricultural land, taxation of uncultivated land, etc.), certain advances could be achieved through the application of provisional measures such as virtual consolidation of arable land by the land bank model.

This process can be accelerated and driven by institutional mediation in renting and leasing of agricultural land and the establishment of the digital databases that enable and facilitate that. The concept and manner of functioning of such a solution is demonstrated by the example of the City of Trebinje where the role of an intermediary could be successfully carried out by the Agrarian Fund, which has most of the necessary capacities. The application of this model in Trebinje may be a pilot project to show whether such solutions are acceptable and can be applied in other local communities. This model is particularly suitable for a country such as BiH and because of the tradition of retaining family legacy, even if the land has no useful value to the successors, and at the same time represents a loss of added value and problem of environmental protection.

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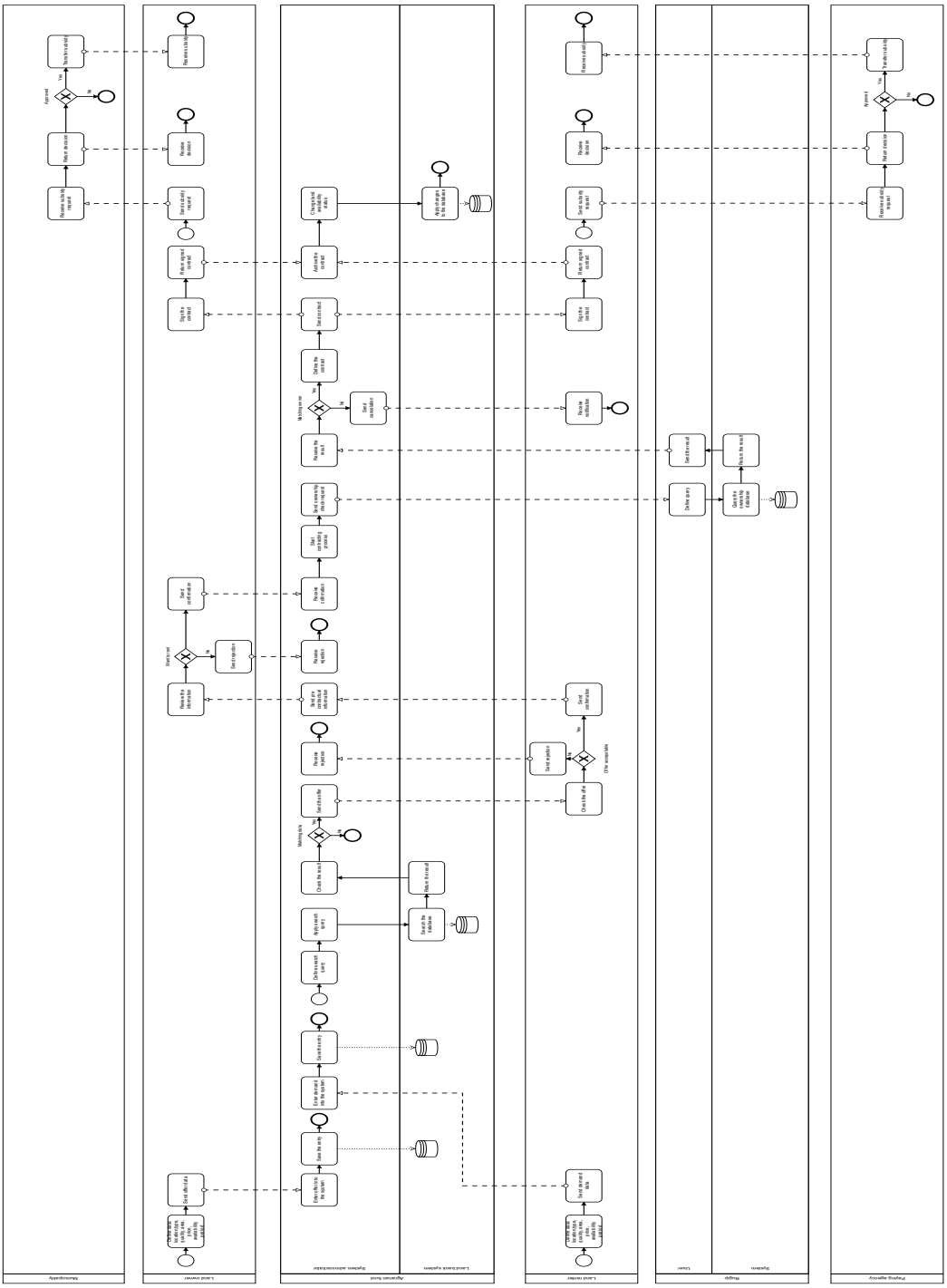
Appendix 1. Information flow recorded in BPMN format



Original scientific paper

Treatment of other gainful activities and marketing costs according to various FADN regulations

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Abstract

FADN accounting system is specific because it covers not only regular activities in agricultural production but also other gainful activities (OGA) and some technical indicators. The goal of this paper is to present recording of OGA throughout different FADN regulations. Besides, the goal is to present recording of marketing (sales) costs in FADN methodology (related not only to agricultural products but also to products gained through processing activities). To clarify the issue, authors compared methodological approaches presented in the following EU regulations – 868/2008, 385/2012 and 2015/220. It has been determined that there are not significant differences between given regulations in terms of recording marketing costs, whereas in newer regulations much more attention is paid to OGA. New research should be directed to a more detailed analysis of costs related to OGA. Comparing to an average farm in the EU, farms in Serbia should increase participation of OGA in total output.

Key words: Other gainful activities, Processing, Marketing, Costs, FADN

Introduction

Farm Accountancy Data Network (FADN) has been established in the European Union in 1965 and it is obligatory in all EU member states. The FADN system has been formed with a goal to unify methodology regulating data collection and accountancy on agricultural holdings in all countries which are members of the EU (Njegovan and Nastić, 2011). Introduction of FADN system in Serbia has started in 2011 as a technical assistance project supported by the EU. The goal of FADN introduction is to enable gathering of data (from all types of holdings – family farms and agricultural enterprises in Serbia) applying the same methodological principles that

are applied in the EU. Having in mind significance of these data, introduction of FADN system is one of preconditions for Serbian accession to the EU (Ivkov et. al., 2013). In the EU, Farm Accountancy Data Network represents the primary and the only source of microeconomic data from agricultural holdings which are consistent and comparable on the level of the entire EU (European Commission, 2010).

Before the introduction of FADN system there was an absence of permanent and systematic monitoring of accountancy data on family farms in Serbia (Vasiljević et. al., 2012). Because of the importance of FADN system, many institutions in the Republic of Serbia are involved in its development, such as Ministry of Agriculture, Forestry and Water Management, academic institutions, Agricultural Extension Service, Statistical office of the Republic of Serbia among others (https://www.fadn.rs/fadn-u-srbiji/organizaciona-struktura/). Having in mind significance of FADN system, there are number of research conducted in Serbia related to the use of FADN and comparison of Serbia and the EU (Vasiljević, 2011; Figurek and Vukoje, 2011; Cvijanović et al., 2014; Bojcevski et al., 2015; Bojčevski et al., 2016; Nastić et al., 2017; Nastić et al., 2017; Ivanović, 2018; Ivanović et al., 2018).

Introduction of FADN system enabled monitoring of large number of parameters regarding commercial family holdings operation and, among others, data regarding other gainful activities. Besides that, determining and monitoring of data regarding different marketing costs is also enabled, regardless of whether they are costs of agricultural products or products of processing activity. Being familiar with these costs is of special importance, because selling of agricultural products (as well as products of processing activity) is becoming an increasing challenge for small agricultural producers which dominate in agricultural structure of the Republic of Serbia, especially when the process of accession to the EU is taken into consideration.

During the last decade, three important regulations defining FADN methodology have been adopted in the EU (Commission Regulation No 868/2008 of 3 September 2008, Commission Implementing Regulation No 385/2012 of 30 April 2012 and Commission Implementing Regulation 2015/220 of 3 February 2015). The goal of this paper is to describe how FADN system (according to various regulations) records data on other gainful activities and marketing costs (related not only to the most important crop and livestock products, but also to products of processing activity).

Discussion

Other gainful activities directly related to the holding are not agricultural activities but other activities performed on the holding. Such activities (regulation 2015/220) use products of the holding or resources of the holding (buildings, machinery etc.). In other words, according to regulation 385/2012 "Other gainful activities (OGA) directly related to the holding refer to the non-agricultural activities having an economic impact on the holding and which are using the agricultural resources (means of production or products) of the holding".

Other gainful activities assume active work (use of labour) while activities which do not require work are not considered to be other gainful activities. Therefore, pure financial investments or renting out resources of the farm (without involvement of farmer in further activities) is assumed to be regular activity of the holding. Other gainful activities are usually related to processing of agricultural products, "regardless of whether the raw material is produced on the holding or bought from outside" (regulation 385/2012). It should be taken into consideration that there are certain exceptions which are clearly defined in appropriate regulations. For example, wine production is considered as regular agricultural activity, not other gainful activity.

Term 'other gainful activity' is closely related to terms 'diversification' and 'pluriactivity'. Similarities and differences among these terms according to number of researches were discussed by Blad (2010). According to EU standards (Augère-Granier, 2016), creation of gainful activities on the farm is considered to be diversification, while pluriactivity is discussed at the level of the farmer. Schuh et al. (2019) stated that pluriactivity and farm diversification "has been on the rise in many of the case study regions (including Campania, Aragon, South-west Oltenia, and Southern and Eastern Ireland), and is seen as an important development targeted by various local institutional actors". Presence of other gainful activities is a useful tool for the description of type of rural areas so that Vidal et al. (2001) used indicators such as percent of farmers with other gainful activities, percent of young farmers and percent of old farmers with other gainful activities. Besides, Longhitano et al. (2012) used other gainful activities as one of the indicators for evaluation of farm sustainability.

Analysing presence of other gainful activities across EU – 27 countries Krakowiak-Bal (2009) determined that five clusters comprised of countries with similar level of farm diversification could be formed. Authors also confirmed "big differences of types and scale of other gainful activity across the EU", while "the highest percentage of households with another gainful activity" is present in Germany, Luxemburg and Austria. Research conducted by Buchta and

Federičova (2009) in Slovak Republic revealed that diversification of activities on agricultural holdings depends on level of development of region where the farm is situated. In more productive regions the most common diversification activities are processing of farm products, contractual work and sale of agricultural products for energy production. On the other hand, the less productive regions are characterized by agro tourism and rural tourism, as well as wood processing. Authors also concluded that enterprises dominated in all diversification activities over family farms, except for handicraft.

Turtoi et al (2013) discussed orientation of smallholders in Romania regarding other gainful activities as a way to reduce dependence on agriculture as a sole income source. It was determined that small farms are more oriented towards processing of agricultural products, while bigger farms are more directed towards contractual work. Similar analysis conducted by Thomson (2019) revealed that in 2013 only 5.2% of the EU farms had some type of other gainful activities, while the same farms produced 18.9% of the EU agricultural output. It was also determined that bigger farms, farms specialized for livestock production as well as farms situated near cities were more oriented towards other gainful activities. Busck et al. (2006) also found out that peri-urban area (around Copenhagen) is characterised by the decline of number of farms, but at the same time by increasing farm size as well as increasing involvement of farmers in other gainful activities.

Treatment of other gainful activities in FADN regulations

In the oldest analyzed regulation (868/2008) other gainful activities are not mentioned directly (that term was not used), but there are appropriate elements within category – production. Apart from crops and animal products, forestry is also mentioned (sales of felled timber and standing timber, other forestry products) as well as other products. In latter directives forestry and other products are referred together as other gainful activities. At the same time, costs related to other gainful activities are not separately recorded according to this regulation.

The next important regulation, which regulates FADN system, paid special attention to this kind of output (regulation 385/2012). In this regulation, holdings are classified in three classes, depending on share of other gainful activities in the total output of the holding. According to this classification (and regulation 2015/220), the division could be:

- Minor (share of other gainful activities is from 0% to 10%),
- Moderate (share of other gainful activities is higher than 10%, but below 50%),
- Significant (share of other gainful activities is higher than 50%).

Other gainful activities directly related to the holding are presented in these regulations via large number of elements, such as:

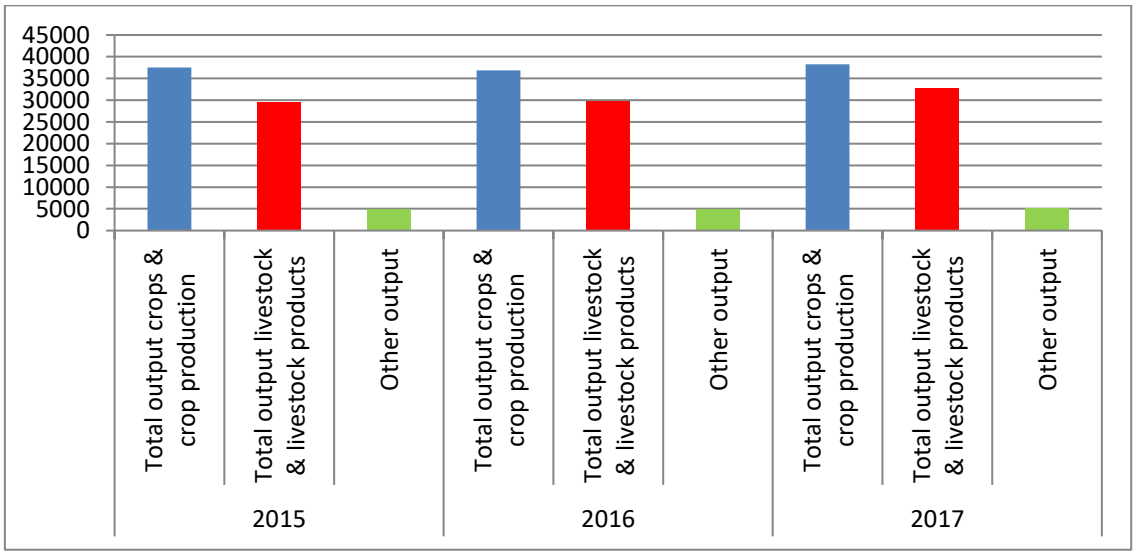
- Analysis of labour - share of work for OGA directly related to the holding, which means – percent of annual time spent working, and percent of Annual Work Unit. The issue of labour analysis by FADN methodology was not dealt with by many authors, and the introduction of other gainful activities broadens the possibilities of these analyses (Ivanović et. al., 2013).
- Analysis of costs related to other gainful activities and
- Analysis of output from other gainful activities.

Other gainful activities usually assume processing of agricultural products, such as processing of milk (cow's milk, sheep's milk, goat's milk), processing of meat and other animal products, processing of crops (it includes production of alcohol, but excludes wine). Nevertheless, other gainful activities also include:

- Forestry and wood processing, Contractual work (using production means of the holding), Tourism, accommodation, catering and other leisure activities,
- Production of renewable energy (includes production of renewable energy for the market such as biogas, bio-fuels or electricity produced by wind turbines; it excludes production of renewable energy only for the holding's own use,)
- Other 'other gainful activities' directly related to the farm, such as production of fur animals, handicraft, aquaculture and similar activities.

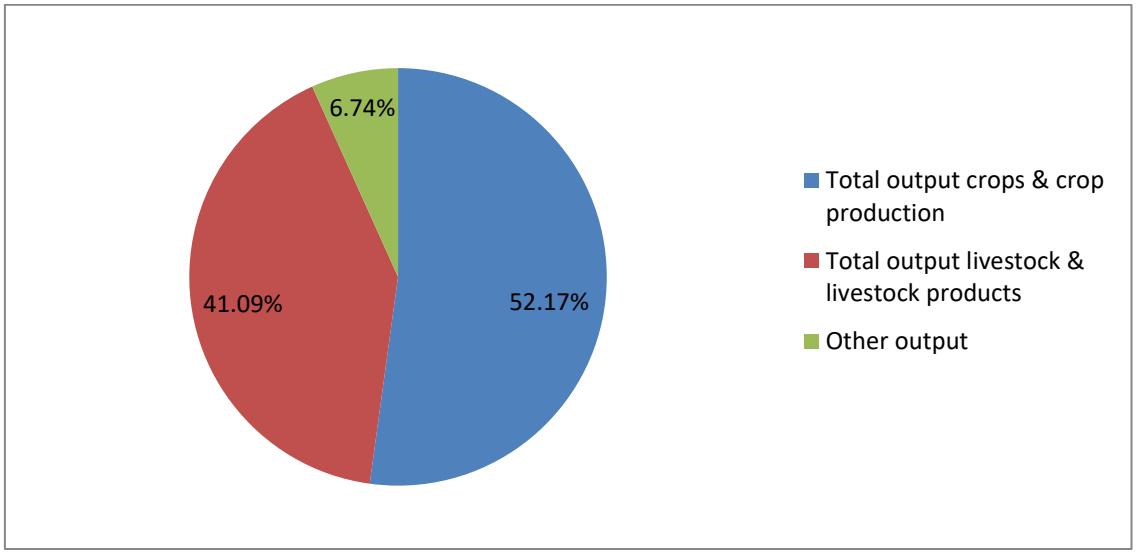
If available data for the period of last three years (the period of 2015-2017) are considered, it is possible to analyze the relation of the total output related to crop production, total output related to livestock and livestock products as well as other output – output related to other gainful activities for all EU countries (graph 1). In all the years observed, the biggest significance was that of output from crop production, while the least important is output related to other gainful activities (in all observed years it is somewhere around 5,000 EUR per farm). On the other hand, in 2015 an average output from other gainful activities in Serbia was 530 EUR. To make comparison between Serbia and the EU authors used data from year 2015 because data for this year are the newest publicly available data for Serbia. Participation of other output in total output in the EU in 2015 (graph 2) was rather low (6.74%), while in Serbia it was only 1.18% (graph 3). This indicator remained at the approximately same level in the EU in the following years, so that it is, by the latest data available for the year 2017, 6.68%. This is an indicator of the fact that (in Serbia) possibilities of agricultural holdings providing additional output through other gainful activities are not used sufficiently. If certain types of

production are taken into account in Serbia, farms specialized in poultry production do not perform other gainful activities at all, while farms specialized in crop production have the highest participation of other gainful activities (other output) in total output (2.22%). In other words, output from other gainful activities in 2015 for Serbian farms specialized in crop production was 1,560 EUR.



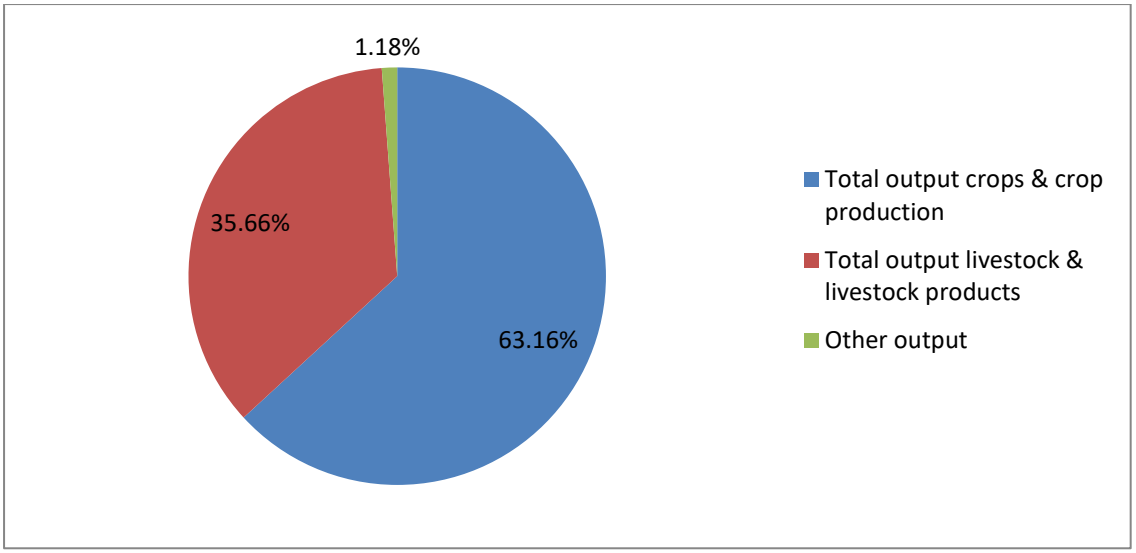
Graph 1. Amount of various types of outputs on the EU level (EUR)

Source: https://ec.europa.eu/agriculture/rica/database/database_en.cfm



Graph 2. Structure of total output on the EU level in 2015 (based on FADN data)

Source: https://ec.europa.eu/agriculture/rica/database/database_en.cfm and authors' calculation



Graph 3. Structure of total output in Serbia for 2015

Source: Farm Return 2015 and authors' calculation

Treatment of costs and inputs in FADN regulations

When comparing previously mentioned regulations, it can be seen that, in its structure, table Costs (regulation 868/2008) and its corresponding table Inputs (regulation 385/2012) have the following elements in common: Labour and machinery costs, Specific livestock costs, Specific crop costs and Farming overheads. On the other hand, there are certain differences in the structure of these tables. Therefore, table Costs by regulation 868/2008 has the following elements Land charges and Interest paid. These two elements are excluded from the regulation 385/2012 and Specific costs for other gainful activities are added.

Since the costs of other gainful activities were not recorded separately in the previous regulation (868/2008), it is clear that Land charges and Interest paid are now reassigned to other groups of costs, so that there is a different reassignment of costs in regulation 385/2012, which is much more complex than it could seem at the first sight. Detailed analysis of these complex changes was dealt with by Marković et. al. (2014).

As one of the advantages of regulation 385/2012, we can name the precision of differentiating output and costs from other gainful activities (in our practice, these are most commonly processing of agricultural products and contractual work). On the other hand, changes that were made when presenting the costs will make comparing the costs of agricultural holdings by 2014 and after 2014 more difficult. Inclusion of other gainful activities in the regulation 385/2012 clearly shows an increased interest of Common agricultural policy for this segment of agricultural holdings operation. Another, completely new, segment of activities included in the

regulation 385/2012 refers to the environmental protection, and it is conducted by monitoring of quantity (but not the value) of N, P_2O and K_2O used in mineral fertilizers. Having in mind that agricultural policy in Serbia is not tightly related to Common agricultural policy yet, it is hard to estimate whether these changes in FADN methodology are going to influence agricultural policy in Serbia. The newest regulation 2015/220 important for FADN system (as well as its latest update from 01/01/2020) mostly corresponds to the regulation 385/2012 regarding all aspects dealing with other gainful activities. Nevertheless, regulation 2015/220 contains rules for determining the size and type of agricultural holdings, which did not exist in the regulation 385/2012.

Marketing (sales) costs

Marketing costs are present in various places within FADN regulations (in different tables and under different codes or serial numbers), depending on the product which is sold (table 1).

Table 1. Recording of marketing costs

Type of cost	Position in regulation 868/2008	Position in regulations 385/2012 and 2015/220
Marketing of livestock and livestock products: Packing materials for livestock products and supplies for processing of livestock products, costs of storage and market preparation of livestock products of the farm performed outside the farm, cost of marketing the livestock products of the farm, short-term rent of buildings used to house animals or store products in connection therewith.	Table Costs within point 71. Other specific livestock costs (serial number 271)	Table Inputs under code 2090 – Other specific livestock costs
Marketing of crops and crop production: Packing and binding materials, string and rope, supplies for the preservation of crops, storage and market preparation of crops done outside the farm, cost of marketing the crop products of the farm.	Table Costs within point 76. Other specific crop costs (serial number 276)	Table Inputs under code 3090 – Other specific crop costs
Specific costs for other gainful activities: Packaging and marketing costs	-	Table Inputs under codes from 4010 to 4090
Costs related to the use of private cars for farm purposes:	Table Costs within point 62. Motor fuel and lubricants (serial number 262) or point 63. Car expenses (serial number 263)	Table Inputs under code 1040 – Motor fuel and lubricants or code 1050 – Car expenses

Source: Regulations 868/2008, 385/2012, 2015/220 and authors' presentation

If the holding possesses its own fixed assets which are used with the aim of selling agricultural products, the costs of their depreciation are calculated the same way as with other fixed assets at the holding. It should be born in mind that introduction of regulations 385/2012 and 2015/220 significantly changed the way depreciation is calculated (comparing to regulation 868/2008), as well as that these regulations introduced new category named accumulated depreciation.

Conclusion

In order to improve economic effects of agricultural operations, family holdings are increasingly inclining towards other gainful activities (primarily contractual work and agricultural products processing), as well as towards the improvement of sales methods (of primary agricultural products and products of processing activity, as well). It was determined that there was a significant change in newer regulations (number 385/2012 and number 2015/220) comparing to the old one (number 868/2008) regarding recording of output and costs of agricultural products processing, that is to say, additional attention was paid to this segment of holdings' operation. FADN methodology in all the regulations observed (number 868/2008, number 385/2012 and number 2015/220) records the costs of sales (marketing) and the analysis showed that there were not any significant changes of methodology in the regulations observed.

Acknowledgement

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia based on the agreement between the Ministry and the Faculty of Agriculture, University of Belgrade (Contract No. 451-03-68/2020-14/200116) as well as between the Ministry and the Institute of Agricultural Economics, Belgrade (Contract No. 451-03-68/2020-14/200009) on the realisation and financing of scientific research in 2020.

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