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PREFACE

The Proceedings contains 38 papers presented at XI International Symposium on Agricultural Sciences "AgroReS 2022" in Trebinje, Bosnia and Herzegovina, from 26 to 28 May, 2022. In the Proceedings are published only papers for which their authors choose that way of publishing

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Original scientific paper

Analysis of soybean varietal trials in 2015 and 2016 / Danube Soya project

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Abstract

The varietal trials with soybean were performed at the experimental site of the Agricultural Institute of the Republic of Srpska (abrr. the Institute) at Banja Luka in 2015 and 2016. The Danube Soya Association coordinated these activities through the research network in six European countries. The main objective of the research was to assess the suitability of the selected varieties for the production in the Banja Luka region. The material consisted of 12 varieties from three breeding institutions (Banja Luka, Novi Sad, SELSEM). The trial was designed in four replications. In addition to the standard measurements and observations, climate data were analysed on a daily basis. Due to the extremely unfavorable weather conditions in 2015, grain yield and quality failed on all varieties. The complex of fungi *Diaporthe/Phomopsis* was strongly activated in the rainy October causing significant soybean seed decay. Unlike 2015, optimal weather conditions in 2016 resulted in record grain yields, while the protein content was relatively low. More varieties in sowing structure is a measure for mitigating and preventing the risks of drought and heat stress. It can be concluded that all tested varieties can be recommended for commercial production in the region Banja Luka.

Key words: soybean, variety, extreme weather conditions, grain yield, protein

Introduction

The previous decennium will be remembered as the warmest one in written meteorological history as well as for the largest number of extreme weather events that had a significant impact on the entire agricultural production. Some plant species, such as small grains, have benefited from warmer winters, while spring crops such as soybean have been increasingly exposed to various forms of environmental stress, most commonly heat stress (Nožinić et al., 2016). Varietal trials on soybeans are always relevant because they indicate the advantages of new genetics, which are gradually replacing the old ones. Kondić (1991) cites the results of a varietal trial at the location Banja Luka, which included 42 soybean genotypes, none of which are in production anymore. Numerous soybean varieties created in Serbia and Croatia are also suitable for cultivation in our country, where selection and breeding work on this plant species has been disabled due to technical, personnel and financial limitations. Climatic conditions till the end of 20^{th} century had been temperate and more favorable for soybean cultivation (Kondić, 1987; Kondić, 1988; Kondić, 1993; Todorović et al., 2007). Today, the situation is completely different. Global warming and climate instability become the main challenges to successful soybean production. The behavior of varieties in extreme weather conditions is of great importance for scientific knowledge and defining directions for further research. Nožinić et al., (2015) analysed varietal trials in extreme weather conditions in 2013 and 2014 (Danube Soya Project). This paper deals with the varietal experiments at the Banja Luka field in 2015 and 2016. Weather conditions in the first year of research were extremely unfavorable for soybean cultivation, which affected grain yield and quality.

The second year of research was a school example of favorable weather conditions for soybean cultivation. The highest soybean yields in research experiments were achieved in that year.It is important to point out that the field experiments at the location Banja Luka in the period from 2014 to 2016 were included in the network of European soybean field experiments organized in six countries by the Danube Soya Association. In addition to the varietal experiments, adjacent trials with sowing dates, sowing densities as well as herbicide trials on the variety Sonja (Babić et al., 2017) were monitored in the mentioned period. The main objective of the research was to assess the suitability of the selected varieties for commercial production in the region of Banja Luka. The notion of variety suitability is primarily identified with grain yield, because local buyers still do not value soybean according to protein and oil content.Since the Department of Industrial Plants is committed to soybean processing, the determination of the protein content was also the goal of this research.

Materials and Methods

In 2015, the research material in the varietal trial consisted of ten soybean varieties from three seed companies (Tab. 3). Category, certified seed of the first generation was used for sowing. The choice of varieties was guided by practical reasons, with special emphasis on the availability of seeds for local farmers. Due to the extreme amount of precipitation during 2014, it was not possible to perform autumn plowing on the plot planned for the trial with soybean in 2015. January and February 2015 were extremely rainy, so plowing of the plot for varietal trial was done in March, which adversely affected the next tillage operations. Diagonal disking showed limited effect due to large "baked" soil lumps. NPK mineral fertilizer (formulation 6:26:16) in the quantity of 300 kg/ha^{-1} was applied after disking, and mixed into the surface soil layer (about 15 cm) with rotary harrow. The seeds were treated with the inoculant Nitragin in quantity of 100 g per 100 kg of seed. Sowing was done on May 3, while the harvest was very late (October 28). Varietal trial was set up in four replications with a random arrangement of varieties. The length of the elementary plot was 16 m, and the width 5 m. The width of the trails between the plots (tested varieties) was 1 m, and the width of the trails between replications was 2,5 m. The trails were somewhat wider than usual for practical reasons (Field day with a lot of participants). Pre - emergence herbicide treatment was performed with the herbicide Dual Gold 960 EC in the quantity of 1,2 l/ha^{-1} and the herbicide Sencor WG 70 in the quantity of 0,8 kg/ha. Post - emergence herbicide treatment against broad-leaved weeds was done with the herbicide Corum by split application (0,9 + 0,8 l/ha^{-1}) while the herbicide Fokus Ultra was used against grass weeds (2 l/ha^{-1}).

In 2016, the research material consisted of nine soybean varieties from three seed companies (Tab. 5). Unfortunately, the companies did not provide three varieties (Sava, Victoria and Dana) from the first year of research for the field experiments in 2016. On the other hand, they sent two additional varieties (Pelican and Apollo) which had not been sown in the first year (Tab. 5). Plowing was done in the fall of the previous year. Due to the high winter precipitation, the soil was dried only in the surface layer, so deep harowing was not feasible. Therefore, the pre-sowing tillage was only possible in the surface soil layer. NPK mineral fertilizer (formulation 6:26:16) in quantity of 300 kg/ha^{-1} was applied pre-sowing. The seeds were treated with the inoculant Nitragin. Due to favorable weather conditions, the sowing of trials was done on April 12. The methodology of setting up the trials and weed control were identical to the methodology from the previous year. Although more measurements and observations were made during the vegetation period (date of emergence, plant height, number of plants per m^2, lodging), the disscussion has been focused on grain yield and protein content. The protein content was determined according to the method BAS EN ISO 5983-2 in the Institute's laboratory. Coefficient of variation (Kv) is calculated for the yield and protein content. Analysis of variance has been done in the SPSS Statistics program for each year separately. The results are obtained in a confidence interval of 95%. The comparison of differences among the varieties is done by LSD (adjusted according to Sidak). The data of the Hydrometeorological Service of the Republic of Srpska are quite representative for the experimental field, which is two km away from the meteorological station Banja Luka.

Results and Discussion

Global warming is related to the increasing occurrence of extreme weather events. That is why every farmer's day starts by looking at the sky. In 2015, the "sky" conspired against spring crops. How the weather conditions can play with soybean can be seen from the devastating results from the varietal trial in that year (Tab. 3). The trial was set up on relatively fertile alluvial soil on May 3, 2015. The problems started a few days after sowing. Occasional poor rainfalls provoked the germination of grains close to the surface of soil. The first roots could not find enough moisture for further development. For that reason, 10-20% of seeds perished by the third decade of May. Although May 2015 offered higher rainfall of many - year average, the most of rain fell in the third decade (90 l/m^2). Therefore, soybean sprouting started a month later (early June). The summer months were significantly drier and warmer than the many - year average (Tab. 2).

July 2015 has been the warmest and the driest since 1951. Such weather conditions did not suit soybean, but it favored early appearance of mites (*Tetranychus urticae*). Although acaricides have been applied twice, the mites have not been completely defeated. In addition, temperatures above 32˚C lead to declining flowers and pods in the most of soybean varieties (Vratarić and Sudarić, 2008). During summer months, Banja Luka valley has more "tropic days" than the regions Potkozarje and Posavina. In July 2015, Banja Luka experienced 18 days with temperatures above 32˚C. Heat wave continued in August when temperatures over 32˚C appeared in 17 days. The heat led to a significant decline of flowers and pods, the first on the lower floors of the stem, then higher ones. It was a reason for prolonged flowering. As a rule, the negative effect of drought in the flowering phase is smaller than in the phase of pods formation (Đorđević and Nožinić, 2013).

September 2015 was the warmest in the period of measurements. It is interesting to point out that Banja Luka registered the absolute maximum temperature for this month (40,2˚C) on September 17. The extreme heat was caused by the strong circulation of the southern wind. Other lowland meteorological stations did not record September's temperatures over 40˚C. After the heat wave, earlier varieties began ripening, while the varieties of longer vegetation (Sonja, Marina, Galeb, Apolo) needed an additional period to be harvested. For technical and practical reasons, the harvest of all varieties was planned in the first decade of October, which was a fatal mistake.

October brought additional problems related to high rainfall, 14 rainy days, the highest number of foggy days since 1961, the highest relative humidity since 1961 and the lowest number of sunny hours with the exception of 1977 (Tab. 2). Such weather conditions caused rapid development of the soybean seed decay on all varieties. Dominant fungi belonged to the genus *Diaporthe/Phomopsis*, while the most common and harmful species was *P. longicolla.* The biology and damage by this genus have been described in detail by Serbian and Croatian authors (Vidić et al., 2006, Santos et al., 2011). The fast development of disease caused significant reduction of soybean yield in 2015 (Tab. 3). Though the yields were low, NS varieties Sava and Victoria had significantly higher yields than other ones.

Unlike the previous year, the varietal trial in 2016 was sown much earlier (April 12). The Selsem varieties (Biser, Galeb, Dukat, Pelikan) emerged earlier (April 22-24) then the other ones. BL varieties emerged two or three days after Selsem ones while NS varieties emerged at the end of April. Warm and periodically hot weather had lasted until April 23. Then the winter returned. Fortunately, a frosty night on April 26 with minimum temperature -2˚C at 2 m (about -4˚C at 5 cm over the ground) did not cause damage to the soybean in the field trial. At this stage, cotyledons contain "herbal antifreeze" which protects plant from short - term frosts. Research on soybeans in the middle of the last century indicates that young soybean plants tolerate frosts from -3˚C to -4˚C (Gutschy, 1950). Vratarić and Sudarić (2008) state that soybeans can withstand temperatures up to -3,8˚C in the simple leaves phase. Some authors state that young soybean plants tolerate short - term cold up to -7˚C without serious damage (Kurnik, 1976; Todorović et al., 2007). The first signs that the year could be "fruitful" for soybean were visible at the end of May 2016. The roots of all cultivars were covered with large bacterial nodules, blood - colored in cross - section, what indicated tothe productive work of nitrate reductase. All varieties were in the phase of the first trefoil on June 19/20. At the end of May, NS varieties had more intensive leaves colour then the other ones as well as smaller stems.The amount of rain in June was slightly higher than the average.

The mean temperature in June was 1,5˚C higher than the average (1961-2019). Shifts of rainy and sunny periods caused the appearance of downy mildew, caused by *Peronospora manshurica* on all varieties. The application of fungicides stopped the progression of the disease.The July heat was mitigated with highrainfall in the middle of the month (110 l/m^2). Slightly longer flowering with minimum flower decline was observed in the complete trial.

After a few years, August in 2016 was to be desired. One hundred liters of well - distributed rain, moderate heat and abundance of sunny days have been ideal preconditions for the formation of a large number of pods and grains (phases R3-R6) as well asexcellent grain filling. Unlike August in the previous year, the temperature over 32˚C was registered only once in August 2016. The abundance of summer rain, especially in August, stimulated the growth of soybean. In the beginning of September the average trial height reached 118 cm. Like in the previous years, the varieties Sonja and Milica were 10 - 20 cm higher than other varieties. At the end of the vegetation, the varieties Sonja Milica and Galeb slightly lodged in different directions. The maturing of BL varieties lasted ten days longer then other varieties from 0 maturing group. The harvest was done on October 25. In 2016, all varieties achieved very high grain yields, especially NS variety Apollo (5.011 kg/ha^{-1}), (Tab. 6).

In contrast to the record grain yields in 2016, the protein content was very low (Tab. 5). Grain tissue in the first year of research was partially damaged by soybean seed decay, which also affected the chemical composition of the grain. Traceable tests of protein content in the region Banja Luka have been performed only on BL variety Sonja. The lowest protein content (31,7%) was determined at the experimental Institute's site in 2021, while maximum content of 35,7% was measured in 2016 (Tab. 4). In 2009, the protein content of BL variety Milica on very fertile soil along the river Vrbas was 38,2%, and for the cultivar Sonja 33,5% (Nožinić et al., 2010). The application of two component inoculant "Preside Ultra" (Verdesian company) to the variety Sonja in 2017 resulted in high protein content (39,4%). Inoculant Biofor provided just 33,3% of protein. The stem and leaves treated with the inoculant "Preside Ultra" had greener color than the plants treated with the standard inoculant Biofor, but also slightly longer vegetation. More complex research of the chemical composition has been done through the network of experiments in Serbia. Đordević et al. (2010) state that NS varieties with longer vegetation have a lower protein content than cultivars of shorter vegetation, and that the protein content is negatively correlated with the oil content. The results of recent research indicate that high protein content can be achieved in new NS varieties with longer vegetation too, so grain yield and protein yield need not be negatively correlated (Miladinov et al., 2019). These authors point out that the variety Apolo showed the highest protein variation and that the variety Galina had the lowest protein content. Varieties Dukat and Galeb obtained significantly higher protein content in three-year trials in Serbia compared to the trials in Banja Luka (Nenadić et al. 2008). The "golden" grain variety Dukat holds the absolute record in protein content (49,5% of dry matter), achieved at the location Vajska in Vojvodina in 2005 (Plazinić et al., 2007). Due to the chronic shortage of domestic soybean as well as the lack of a sophisticated processing industry (tofu cheese, soy milk, lecithin and many other products), domestic buyers have not yet defined standards regarding the chemical composition of soybeans.

Table 1. Results of soil fertility control on the trial with soybean

pH in H_2O	pH in KCl	Humus (%)	P_2O_5 (mg 100 g^{-1} soil)	K_2O (mg 100 g^{-1} soil)
6,3	5,1	3,0	12,6	15,8
Slightly acid	Slightly acid	Average	Medium supplied	Medium supplied

Table 2. Climatological elements for the meteorological station Banja Luka in 2015

2015	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual
Mean month (˚C)	3,3	2,4	7,3	11,8	17,4	20,9	25,2	24,0	18,3	11,5	7,1	3,2	12,7
Maximum temp.	19,7	14,6	24,0	27,6	33,1	34,4	39,3	38,0	40,2	26,4	27,1	18,0	40,2
Minimum temp.	-16,0	-8,2	-2,6	-1,4	4,0	8,0	11,5	10,8	6,1	1,0	-2,4	-4,9	-4,9
Precipitation l/m^2	111	91	79	54	118	61	21	23	75	143	86	8	863
Rainy days	19	13	11	15	15	12	5	7	11	14	8	7	137
Days, snow cover	13	9	1	-	-	-	-	-	-	-	4	-	27
Rel. humidity (%)	82	83	69	60	69	65	59	64	70	86	80	87	73
Insolation	74	60	126	207	229	300	346	271	172	66	145	116	2.112
Days with fog	9	10	3	1	1	-	-	-	3	23	12	16	78

Table 3. Results of varietal trial on the location Banja Luka in 2015

No.	Variety	Seed company	Maturity group	Grain yield (kg/ha^{-1})	Proteins (%)
1.	Sonja	Institut Banja Luka	II	1.624	31,9
2.	Milica	Institut Banja Luka	II	1.538	25,3
3.	Valjevka	Institut Novi Sad	0	1.600	29,2
4.	Galina	Institut Novi Sad	0	1.599	28,8
5.	Sava	Institut Novi Sad	I	1.834	26.9
6.	Victoria	Institut Novi Sad	I	1.838	27,7
7.	Galeb	Selsem Beograd	I	1.376	27,3
8.	Dukat	Selsem Beograd	0	1.638	27,5
9.	Biser	Selsem Beograd	0	1.357	26,8
10.	Dana	Selsem Beograd	0	1.659	27,0
Mean value				1.606	27,8
Coefficient of variation				9,9%	1,3%
Least significante difference (adjusted by Sidak)				161	No replications

Table 4. Analyses of variance in 2015

The source of the variation in the data	Sum of Squares	df	Mean Square	*F* – statistic.	Sig.
Corrected Model	920775,100	9	102308,344	32,957	0,000
Intercept	103182288,4	1	103182288,4	33238,683	0,000
Variety	920775,100	9	102308,344	32,957	0,000
Error	93128,500	30	3104,283		
Total	104196192,0	40			
Corrected Total	1013903,600	39			

Table 5. Climatological elements for the meteorological station Banja Luka in 2016

2016	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual
Mean month ˚C	2,3	7,6	8,0	13,5	16,2	21,5	23,3	20,5	17,8	10,6	7,4	0,4	12,4
Maximum temp.	19,0	20,9	26,4	29,6	31,9	34,5	37,3	34,3	31,7	27,3	23	14,1	37.3
Minimum temp.	-11,0	-3,9	-2,2	-1,0	3,0	9,9	13	7,4	5,1	0,5	-6,0	-8,9	-11
Precipitation l/m^2	110	109	112	71	101	118	126	100	63	76	69	5	1.058
Rainy days	12	17	20	13	16	13	10	9	8	15	13	5	151
Days, snow cover	12	-	-	-	-	-	-	-	-	-	1	-	13
Rel. humidity (%)	79	76	74	66	71	69	70	74	75	81	79	82	75
Insolation	83	63	98	164	227	250	291	248	220	132	84	99	1.959
Days with fog	8	4	5	4	3	1	2	1	3	11	5	12	59

Table 6. Results of varietal trial on the location Banja Luka in 2016

No.	Variety	Seed company	Maturity group	Grain yield (kg/ha^{-1})	Proteins (%)
1.	Sonja	Institut Banja Luka	II	4.856	35,7
2.	Milica	Institut Banja Luka	II	4.521	34,4
3.	Valjevka	Institut Novi Sad	0	4.699	35,6
4.	Galina	Institut Novi Sad	0	4.795	35,3
5.	Apolo	Institut Novi Sad	I	5.011	33,8
6.	Pelikan	Selsem, Beograd	0	4.878	32,3
7.	Galeb	Selsem, Beograd	I	4.686	33,3
8.	Dukat	Selsem, Beograd	0	4.559	33,7
9.	Biser	Selsem, Beograd	0	4.551	34,0
Mean value				4.728	34,2
Coefficient of variation				3,6 %	3,3 %
Least significant difference (adjusted by Sidak)				274	No replication

Table 7. Analyses of variance in 2016

The source of the variation in the data	Sum of Squares	df	Mean Square	*F* – statistic.	Sig.
Corrected Model	913125,000[a]	8	114140,625	10,948	0,000
Intercept	805708225,0	1	805708225,0	77279,297	0,000
Variety	913125,000	8	114140,625	10,948	0,000
Error	281500,000	27	10425,926		
Total	806902850,0	36			
Corrected Total	1194625,000	35			

Conclusion

No variety showed resistance to soybean seed decay caused by complex of fungi *Diaporthe/Phomopsis* thatwas strongly activated with the humid conditions in October

2015.Desication can be considered as a preventive measure in order to avoid such a situation.

All tested varieties obtainedvery high grain yield under favorable climatic conditions in 2016, so all can be recommended for cultivation in the region Banja Luka. By sowing more varieties from different maturing groups, one can reduce the risk of the influence of extreme weather conditions to soybean.

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Final reports of the soybean trials in Banja Luka in 2015 and 2016/Danube Soya project

Analiza sortnih ogleda na soji u 2015. i 2016. godini / Dunav soja projekat

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Sažetak

Sortni ogledina soji praćeni su na oglednom polju Poljoprivrednog instituta Republike Srpske (skraćeno Institut) na lokaciji Banja Luka u 2015. i 2016. godini. Udruženje "Dunav soja" koordiniralo je navedene aktivnosti u šest zemalja. Osnovni cilj istraživanja odnosio se na procijenu pogodnosti odabranih sorti za komercijalnu proizvodnju u banjalučkoj regiji. Materijal rada činilo je 12 sorti iz tri oplemenjivačke institucije (Banja Luka, Novi Sad, SELSEM). Ogled je postavljen u četiri ponavljanja. Pored standardnih mjerenja i opažanja, klimatski podaci praćeni su na dnevnoj bazi. Zbog izuzetno nepovoljnih vremenskih uslova u 2015. godini, prinos i kvalitet zrna podbacili su kod svih sorti. Kompleks gljiva Diaporthe/Phomopsis snažno se aktivirao u kišnom oktobru uzrokujući značajno propadanje sjemena soje. Za razliku od 2015. godine, optimalni vremenski uslovi u 2016. godini rezultirali su rekordnim prinosima zrna, dok je sadržaj proteina bio relativno nizak. Više sorti u strukturi sjetve predstavlja mjeru za ublažavanje posljedica i prevenciju rizika od suše i toplotnog stresa. Sve sorte iz ogleda mogu se preporučiti za komercijalnu proizvodnju u banjalučkoj regiji.

Ključne riječi: soja, sorta, ekstremni vremenski uslovi, prinos zrna, protein

Original scientific paper

Response of *Xanthium orientale* L. to glyphosate

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Abstract

Xanthium orientale L. with three subspecies is annual weed species wide distribution, in many countries characterized as invasive species. This species is also wide spread as a weed on agricultural land, coast areas as on the ruderal area on the territory of Bosnia and Herzegovina. Mechanical, agrotechnical and chemical measures can be applied to control these species. The objective of this study was to conduct a biological study of the phytotoxic symptoms and effectivness of glyphosate on control *X. orientale*. The efficacy of glyphosate was expressed through biometric parameters (fresh shoot mass, dry shoot mass, fresh root mass, dry root mass) of plants treated in different growth phase. The regression relationship between the biometric parameters, as a dependent quantity, and the glyphosate rate, as an independent quantity, is represented by a sigmoid curve according to the logistic model based on the "dose-response" relationship. Although visible effects were observed on all treated plants the highest percentage of inhibition was achieved on the largest treated plants. *X. orientale* showed high sensitivity to the glyphosate. On all treated plants, regardless of the growth stage, the percentage of mass inhibition ranged 60-85%.

Key words: *X. orientale*, control, glyphosate, biometric parameters, growth phase

Introduction

Xanthium orientale L. (common cocklebur, lampourde glouteron; family Asteraceae) with three subspecies and a great number of synonyms (e.g: *X. strumarium, X. chinense, X. cavanillesii, X. maculatum, X. echinatum*) is annual weed species wide distribution, in many countries characterized as invasive species (Sudhakar Reddy et al., 2007; Boršić et al., 2008; Vrbničanin, 2015). This species is present on agricultural land, coast areas as on the many disturbed habitats on the territory of Bosnia and Herzegovina (Topalić-Trivunović and Pavlović-Muratspahić, 2008; Kovačević et al., 2008; Vrbničanin et al., 2020; Kelečević et al., 2020). In general, seed production is strongly correlated with above-ground biomass at the time of floral initiation. The harmfulness of this species is increased thanks to high seed production 7000 per plant (Senseman and Oliver, 1993), the seed can tolerate water submersion for ten months (Wang et al., 2014) and high biological plasticity (cross-pollination occurs to 12%) (Love and Dansereau, 1959). Seedling as well as fruit of *Xanthium* species are toxic containing high amount of carboxyatractyloside (Karadas et al., 1997; Xue et al., 2014).

X. orientale has an economic impact in many crop and non-crop fields. *X. strumarium* has an economic impact in reduced maize grain number per ear and grain weight. At the 16 plants per m^2 of this species caused 45-54% reduction in maize grain number per ear, whereas grain weight was reduced by 33-40% (Karimmojeni et al., 2010, 2021). Also, *X. strumarium* had for 20-50% finer root system and was more competitive belowground than soybean during early growth period (Bozsa, and Oliver, 1990). Further, Hussain et al. (2013) indicate that increasing density of *X. strumarium* from 0-6 plants per m^2, maize plant height progressively increased, however the plant heights declined at 8-12 *X. strumarium* plants per m^2. Therefore, it is concluded that both the crop and weed density do affect the phonological parameters of maize. However, crop density alone is not enough to cope effectively with the higher populations of *X. strumarium* weed. Hence, control of this weed should be focused to improve the crop phenology when in competition with this species.

Due to the high distribution of *Xanthium* species on arable land, significant attention has been paid to research and practical solutions for the control of these species. This group of weeds can be controlled by agro-technical and chemical measures. Within chemical control measures, it is possible to use pre- and post-em herbicides. However, due to the specific germination and seedling, low efficacy is achieved by pre-em herbicides (Baldwin and Frans, 1972; Soltani et al., 2010) as a mechanical control measures (Gunsolus, 1990). The reason for the low efficacy of pre-em herbicides is very short and late germination period (Dorado et al., 2009) as well as the specific structure of the fruit that allows it to germinate from depths >8 cm (Saeed et al., 2020) to which pre-em herbicides it can't reach. Post-em aplication of dicamba, dicamba+atrazine, dicamba+diflufenzopyr, mesotrione + atrazine, 2,4-D+atrazine, bromoxynil+atrazine, prosulfuron+dicamba, primisulfuron+dicamba, mesotrione+atrazine, and topramezone+atrazine provided from 88% to 98% control of common cocklebur (Soltani et al., 2010).

Post-em application of 2,4-D, dicamba, clopyralid, bentazon, bromoxynil, foramsulfuron, imazamox, thifensulfuron-methyl, metsulfuron-methyl and desmedipham provide efficiency more then 75% in control *X. italicum* and *X. strumarium* (Lešnik, 2017).

With the invention of acetolactate synthase inhibitors (ALS), this group of active substances was also included in the list of herbicide for control of *X. strumarium* (Griffin et al., 1992). Active substance prosulfuron and primisulfuron achieved an efficacy from 90% (Soltani et al., 2010) to 95% (Obermeier and Kapusta, 1996). ALS inhibitors pose a great risk for the development of resistant forms of weed, because they act only on one reaction site within the ALS gene, but at different sites on the protein, ie. α, β and γ region (Pang et al., 2004). This claim is supported by the fact that by 2019, 162 weed species were registered that have developed resistant forms to herbicides of this site of action. One of these, which has developed resistant forms, is the *X. strumarium* (Heap, 2022). Glyphosate as a highly effective broad-spectrum herbicide has wide application. *X. strumarium* is very sensitive to the glyphosate. Glyphosate is very poorly mobile outside the parts of plants that have not been treated, which indicates that the translocation of herbicides through the plant is not the most important factor determining the sensitivity or resistance of treated plants. The difference in sensitivity is not caused by differences in the metabolism of this compound, but in the intensity of absorption (Skora Neto et al., 2000).

The aim of this study was to conduct a biological investigation of the phytotoxic symptoms and effectiveness of glyphosate on control of *X. orientale* as problematic weed species of agricultural and ruderal areas. By defining regression between herbicide rate and the growth phase, as independent quantities and the efficiency of glyphosate, as a dependent quantity, the effective herbicide rate in relation to the plant development phase can be defined. By knowing the relationship between efficacy for each growth phase and glyphosate rate, an effective dose of glyphosate can be recommended.

Material and Methods

As experitmental plant it was used seed of *Xanthium orientale* L. subsp. *italicum* (Moretti) Greuter. Glyphosate was applied as a Clinic 480 SL (480 g ai/L) at rate 180, 360, 720, 1440 and 2880 g ai/ha and control. Herbicide was applied by using a spray chamber ("boom sprayer") calibrated to deliver of 400 L/ha spray volumes.

Glyphosate was applied with distilled water in order to avoid the influence of ions from water on herbicide molecules at three growth stage: F1 - 2 true leaves were developed (average plant height was about 15 cm); F2 - 3-4 true leaves (average plant height was about 20 cm) and F3 - 5-7 true leaves (average plant height was about 30 cm). Seeds were sown in pots with a diameter of 10 cm, for plants treated in the I and II phase, and plants treated in the III phase were sown in pots with a diameter of 19 cm. Commercial humus was used as a substrate. The pots were thinned after emergence to five plants per pot. Each treatment was done in four replication. Twenty days after treatment the herbicide efficacy was expressed through biometric parameters of treated plants, by measuring: fresh shoot mass (FSM), dry shoot mass (DSM), fresh root mass (FRM), dry root mass (DRM) and visual assessment of phytotoxicity symptoms. The weed weight data were exspresed as a percentage of the untreated control. The linear regression between biometric indicators, as a dependent quantity, and the herbicide dose, as an independent quantity, is represented by a sigmoid curve according to the logistic model based on the dose-response. To determine the regression dependence between plant mass and herbicide dose, a four-parameter logistic function was used, represented by the formula:

$$f[x,(b,c,d,e)] = \mathrm{C} + \frac{\mathrm{D} - \mathrm{C}}{1 + exp\{b \times [log(x) - log(e)]\}} \qquad [\,1\,]$$

where b, C, D, e are four parameters. The parameter e is also denoted ED_{50} and it is the dose producing a response half-way between the upper limit, D, and lower limit, C. The parameter b denotes the relative slope around e (Ritz and Streibig, 2005). This functional dependency was done using the statistical program OriginPro 8.

Due to differences in plant susceptibility at different stages of development, the reaction of plants is expressed through an effective dose (ED) that reduces growth by 50% in regard to control, ie. ED_{50}. The value of effective doses (inhibition of growth, %) is calculated according to the formula:

$$\mathrm{ED}_y = \mathrm{ED}_{50} \times \left(\frac{\mathrm{In}[\%]}{100 - \mathrm{In}[\%]}\right)^{1/\mathrm{b}} \qquad [\,2\,]$$

ED_y – effective dose for the required inhibition,

ED_{50} – mean effective dose,

In – inhibition (%),

b – regression coefficient.

Results and Discussion

The first symptoms of phytotoxicity were observed five days after the treatment in the form of turgor loss and wilting of plants. Seven days after treatment, necrotic areas appeared on the leaf margin (Picture 1), and after the tenth day, necrosis of whole plants occurred.

Picture 1. Symptoms of glyphosate phytotoxicity, 7 days after treatment

X. orientale showed great sensitivity to glyphosate, as a *X. strumarium* (Skora Neto et al., 2000). Although visible effects were observed on all treated plants, the highest percentage of inhibition was achieved on plants treated in F3 (on root mass) which confirmed the study by Skora Neto et al. (2000) that the highest sensitivity is due to the intensity of glyphosate absorption. The least phytotoxic effect was observed on the plants treated in F2 (Table 1).

Table 1. Reactions of *X. orientale* to different rate of glyphosate depending on growth stage

Growth stage	BP (biometric parameter)	Glyphosate rate (g ai/ha)					
		Control	180	360	720	1440	2880 g
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$
F1	FSM	14.10±0.39	13.92±0.58	13.32±1.08	8.40±0.55	7.16±0.54	4.51±0.97
	DSM	3.30±0.12	3.11±0.14	2.94±0.08	1.89±0.19	1.22±0.11	0.83±0.22
	FRM	9.01±1.47	7.88±1.05	6.37±1.01	3.44±0.34	3.24±039	1.46±0.44
	DRM	1.08±0.09	1.05±0.07	0.77±0.08	0.54±0.08	0.44±0.02	0.19±0.07
F2	FSM	21.93±2.03	18.76±0.51	18.52±0.61	13.06±1.09	11.00±1.90	8.10±0.67
	DSM	5.59±0.50	4.72±0.25	4.39±0.21	3.53±0.36	2.67±0.56	2.12±0.06
	FRM	13.35±2.21	8.87±0.89	8.08±0.77	6.61±0.74	5.41±2.09	3.07±0.69
	DRM	1.65±0.15	1.11±0.04	0.96±0.08	0.84±0.05	0.56±0.22	0.34±0.08
F3	FSM	75.77±5.02	62.63±9.74	53.07±3.20	46.60±6.54	28.91±3.53	22.71±5.32
	DSM	24.45±0.95	19.73±2.49	17.09±1.35	13.78±1.71	8.14±0.87	7.77±1.56
	FRM	33.12±4.03	22.19±4.40	20.71±4.97	16.09±2.47	11.41±2.56	6.84±2.50
	DRM	5.71±0.40	4.39±0.63	3.23±0.29	2.90±0.52	1.44±0.30	0.97±0.32

On plants treated in F2, at application rates of 720, 1440 and 2880 g ai/ha, yellowing and necrosis of leaves occurred, while at lower rates (180 and 360 g ai/ha) there was slight yellowing of leaves and plants did not lagged in compared to untreated plants. In the observed period of 20 days, the greatest effect in reducing the mass of plants was achieved in plants treated in F3, because larger plants absorbed a larger amount of glyphosate. At higher rates 1440 and 2880 g ai/ha, yellowing, necrosis and drying of whole plants occurred.

Regression dependence of biometric indicators development of treated plants, as dependent variable, in relation to the glyphosate rate, as independent quantities are shown in Table 2.

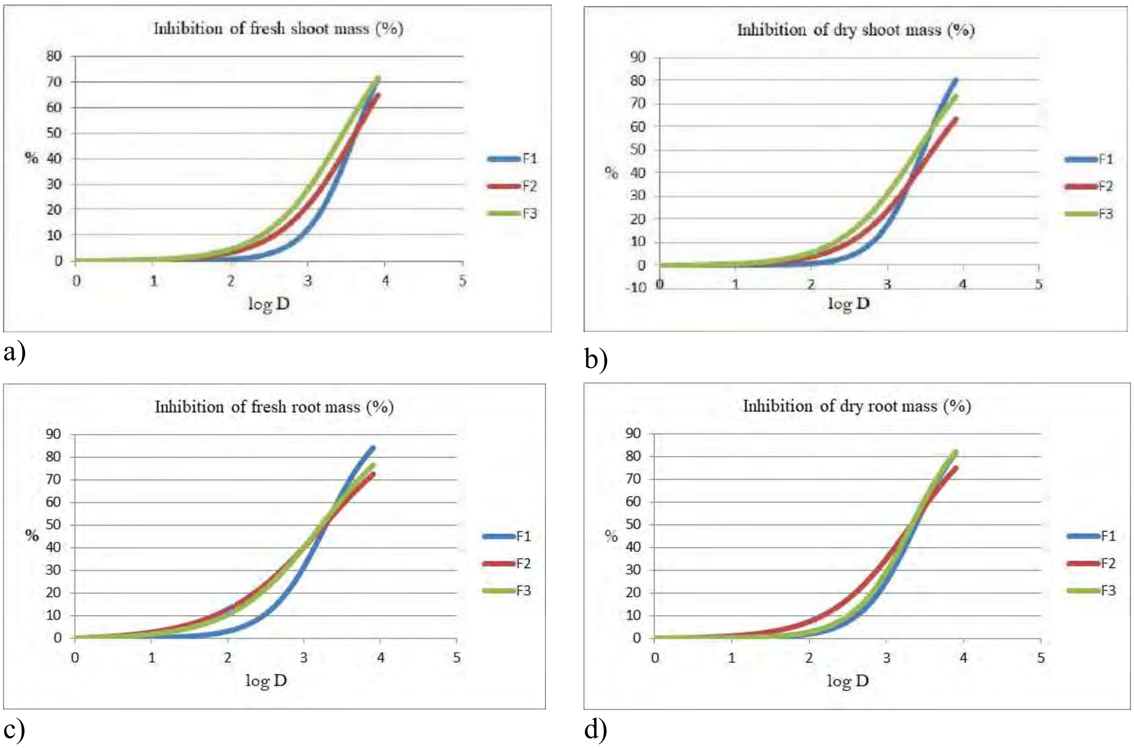
Table 2. Regression parameters (ED_{50}, b, R^2, χ^2/DF) for all measured parameters of glyphosate effect on plant treated in growth stage F1, F2 and F3 *X. oreintale* (corresponds to Graph 1)

Growth stage	Regression parameters	Biometrical parameters			
		FSM	DSM	FRM	DRM
F1	ED_{50} (g/ha)	1480.68	1060.92	686.16	870.12
	b	1.367	1.419	1.171	1.250
	R^2	0.895	0.969	0.939	0.959
	χ^2/DF	1.760	0.034	0.553	0.005
F2	ED_{50} (g/ha)	1621.08	1141.2	637.92	760.32
	b	0.904	0.903	0.649	0.825
	R^2	0.966	0.994	0.984	0.961
	χ^2/DF	0.979	0.009	0.198	0.008
F3	ED_{50} (g/ha)	1014.84	903.6	597.6	773.64
	b	0.896	0.867	0.751	1.159
	R^2	0.987	0.974	0.987	0.939
	χ^2/DF	5.369	1.099	1.079	0.189

These results showed that using the sigmoid curve according to the logistic model based on the “plant-dose” relationship, it can be used as a biological parameter in expressing the effect of glyphosate in relation to biologically equivalent doses (ED values) (Kudsk and Mathiassen, 2007). The very high R^2 value (89.5- 98.7%) for all mesured biometrical parameters suggesting that basipetal translocation of the glyphosate towards undergraund organ was significant.

The glyphosate rate that reduced plant weight by 50%, depending on the growth stage, was 597.6-1621.08 g ai/ha (Table 2). Unlike *X. orientale*, the species *Conyza canadensis* showed significantly higher sensitivity to glyphosate and GR_{50} (the amount of application that reduced the fresh weight of plants by 50%) is 170 g ai/ha (de Oliveira Latore, 2017). Higher sensitivity to glyphosate also shown species of the genus Lolium (*L. rigidum* and *L. multiflorum*), mean value ED_{50}=206 g ai/ha (Panozzo et al., 2020), as well as *Kochia scoparia* ED_{50} =187 g ai/ha (Ou et al., 2016). In this study, a slower effect of glyphosate was observed on plants treated in F1, which can be explained by the lower shoot height at the time of treatment, so that the plants absorbed a smaller amount of glyphosate, and therefore the effect was lower. The glyphosate at rate 2880 g ai/ha reduced the dry weight of plants 80-82% compared to untreated plants, similar results were obtained in studies by other authors where the efficacy ranged between 80% (Sikkema et al., 2008) and 90% (Ferrell and Witt, 2002). The glyphosate efficacy at the largest applied amount 2880 g ai/ha was high and renged 72-100%, which indicates that the control of *X. orientale* with glyphosate gave good results. Given the fact that resistance to glyphosate species of this genus has not been reported yet (Heap, 2022), glyphosate can be recommended for the control of *Xanthium* species. However, glyphosate should be used with caution (in rotation with other measures), as well as in combination with non-chemical control measures, in order to delay the resistance.

Regression dependence, observed as an absolute biometric parameters expressed in grams, was transformed into percentages, with the value of inhibition of control plants expressed as 0%, which is presented in the graphs (Graph 1). On all treated plants, regardless of the time of treatment, the % of mass inhibition ranged 60-85%, with the same effect being achieved on both the root and the shoot of the treated plants.



Graph 1. Regression dependence of biometric parameters on the glyphosate rate: a) fresh shoot mass (FSM), b) dry shoot mass (DSM), c) fresh root mass (FRM), d) dry root mass (DRM)

Predicting the beginning and duration of germination can contribute to making correct and timely decisions related to weed control. On agricultural fields, a significant agrotechnical and indirect measure that affects on the reduction of *X. strumarium* population is earlier sowing and minimal soil tillage (Norsworthy and Oliveria, 2007). *Xanthium* species, as a thermophilic species that has higher temperature requirements, germinate from May to August. So, timely and proper sowing of cultivated plants provides more favorable conditions for growth and development, which would cover the space between the rows, reduce soil temperature, and thus reduce the germination of this weed species.

Conclusion

X. orientale showed high sensitivity to glyphosate. On all treated plants, regardless of the growth phase, the percentage of inhibition ranged from 60-85%. Thus, in order to achieve satisfactory efficacy, a minimum amount of preparation Clinic 480 SL is 3-6 l/ha is recommended. Also, in order to show high efficacy, it is necessary that plant reach a certain height, in order to absorbe a sufficient amount of herbicides. The best efficacy is achieved with the plant treated in the stage 5-7 true leafe (plant height about 30 cm). The very high R^2 value for all biometrical parameters suggesting that basipetal translocation of the glyphosate towards underground organ was significant so researched biometrical parameters can be used as indicator of glyphosat efficacy. Glyphosate may be recommended to control *X. orientale* on agricultural and ruderal areas, where this species can pose a significant problem as a weed.

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Preliminary report

Interdependence of seed yield components of pumpkin (*Cucurbita pepo* L.) genotypes

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Abstract

The aim of this study was to analyze the interdependence of seed yield components of the pumpkin (*Cucurbita pepo* L.) genotypes, which are grown in Western and Central Serbia. The experiment was performed in the period 2021-2022. In the initial phase, the fruits of genotypes of pumpkin grown in the villages from Priboj in the south to Šabac in the north, Smederevska Palanka and Krusevac in the east were collected. A total of 81 pumpkin fruits of 27 different genotypes were gathered. The following yield components were analyzed on selected fruits: fruit weight, number of seeds per fruit, dry seed weight per fruit, thousand seed weight, kernel weight per fruit and kernel percentage. Fruit weight was significantly positively correlated with number of seeds per fruit, dry seed weight per fruit, kernel weight per fruit and thousand seed weight, and significantly negatively correlated with kernel percentage (r = -0.44). Fruit weight was not significantly correlated with number of seeds per fruit, while number of seeds per fruit was significantly positively correlated with seeds weight per fruit and kernel weight per fruit. Seeds weight per fruit was in a significant positive correlation with kernel weight per fruit and thousand seed weight. Kernel percentage was significantly positively correlated with the number of seeds per fruit, seeds weight per fruit, kernel weight per fruit and the thousand seed weight. The analyzed genotypes represent an important source of diversity that could be used in plant breeding studies in the future.

Key words: correlation, pumpkin genotypes, seed

Introduction

The variety of uses and the possibility of growing in different agroecological conditions classifies pumpkins as very important crops (Rabrenović et al., 2011). Lately, pumpkins have become increasingly important, especially as a raw material for obtaining cold-pressed oil. *Cucurbita pepo* L. is the most widespread species of pumpkin, which is characterized by numerous varieties and forms (Berenji, 2010).

Distinct polymorphism complicates the botanical, and especially the practical division within this species. According to Rabrenović et al. (2012) ordinary forage pumpkin, in a narrow sense, can be considered among pumpkins for oil, considering that its seeds can be used to obtain the oil. For the production of cold-pressed oil, the seeds of pumpkin genotypes with the shell or its inner part, which we call the kernels, can also be used, as well as the seeds of hull-less genotypes (genotypes with naked seed) (Rabrenović et al., 2012). Today in Serbia, pumpkins for oil are grown exclusively for the production of seeds from which oil is obtained, and the area under hull-less seed pumpkins is 2-3,000 ha with a tendency to grow (Berenji, 2010). In addition, a large number of smaller farms that are otherwise engaged in mixed agricultural production, among other things, have gardens in their backyards where various indigenous genotypes of the pumpkin are grown. They have their value, which is still not recognized on the market. The obtained fruits of domestic pumpkin are used for human and farm animal nutrition. A by-product in this production is pumpkin seeds, which are discarded due to poor digestibility in the digestive tract of animals and remain unused. The best seeds are sown in the next year, whereby uncontrolled selection is performed.

During the planned breeding of pumpkins, one of the most important characteristics on which the selection is made is the seed yield. Since seed yield is a quantitative trait of low heritability, this can be achieved by indirect selection on one or more traits that are correlated with yield. However, selection for one trait may result in a response of other genetically related traits. Therefore, knowledge of the relationship between yield components is very important in the breeding program (Espitia-Chamacho et al., 2006). The aim of this study was to through the analyze the interdependence of seed yield components of pumpkin, to analyze the traits of genotypes grown in Western and Central Serbia and assess their potential importance. The aim of this study was to analyze the interdependence of the seed yield components of the pumpkin (*Cucurbita pepo* L.) genotypes (which have seeds with shell), grown in central and western Serbia.

Material and Methods

The experiment was conducted in the period 2021-2022. In the initial phase, the fruits of the pumpkin genotype grown on the territory of Central and Western Serbia were collected. Fruit collection was done during the autumn and winter of 2021 in the villages in the area from Priboj in the south to Šabac in the north, Smederevska Palanka and Krusevac in the east. A total of 81 pumpkin fruits of 27 different genotypes were collected. All genotypes of pumpkin had mature fruits of yellow to the orange colour of lower or higher intensity. After collection, the fruits were stored in the Laboratory for Plant Production at the Faculty of Agronomy in Čačak, at a temperature of 15 °C until the moment of seed separation. In the next phase, fruit weight measurement, fruit cutting and seed separation were performed.

The seeds were dried to storage humidity, and then stored in closed paper bags at a temperature of 4 °C in the dark. Considering the components of seed yield, the following were determined: the number of seeds per fruit, dry seeds weight per fruit and the thousand seed weight. Then, the kernel was separated from the shell by hand, and then the total kernel weight per fruit of the pumpkin was measured. Kernel percentage (%) was calculated based on fruit weight and dry seeds weight per fruit.

Meteorological conditions in 2021 in Central and Western Serbia were out of average, with cold springs and hot and dry summers with a large number of days with temperatures above 35 °C, which was not conducive to the growth and development of pumpkins. The interdependence of yield and seed yield components was assessed by calculating simple correlation coefficients using STATISTICA 8 software (StatSoft Inc., 2007).

Results and Discussion

Fruit weight in this experiment was significantly positively correlated ($p \leq 0.05$) with seed number per fruit, dry seed weight per fruit, and kernel weight per fruit (Table 1). Since according to Espitia-Chamacho et al. (2006) fruit weight has the greatest direct impact on the yield of pumpkin fruit, which means that higher mass of individual fruits indicates higher yield of fruits, seeds and seed kernels per unit area. This is confirmed by the results of other research.

Fruit weight of the pumpkin was significantly negatively correlated with number of fruits per plant ($r = -0.65$) (Espitia-Chamacho et al. 2006) and ($r = -0.01$) (Badr et al., 2021), and in a significant positive correlation with fruit yield per plant ($r = 0.67$) (Espitia-Chamacho et al., 2006) and ($r = 0.24$) (Badr et al., 2021). In 21 genotypes of pumpkin grown in Bangladesh, fruit weight was significantly positively correlated with pumpkin leaf size ($r = 0.30$) and fruit yield per unit area ($r = 0.43$) (Sultana et al., 2015). Similar results were obtained with *Cucurbita moschata* Duch. Fruit weight of *Cucurbita moschata* was significantly positively correlated with fruit yield per unit area in 20 genotypes grown in Bangladesh (r = 0.58) (Akter et al. 2013) and 23 genotypes grown in India (r = 0.57) (Srikanth et al., 2015). According to Kumar et al. (2018) in 28 genotypes of *Cucurbita moschata* in India, a significant positive correlation (r = 0.61) was observed between fruit weight and fruit yield per plant. According to Chaudhari et al. (2017) in *Cucurbita moschata*, a significant positive correlation (r = 0.94) was observed between fruit weight, fruit yield per unit area and number of fruits per plant (r = 0.72).Fruit weight in these studies was significantly positively correlated with the thousand seed weight. In contrast, Sekerci et al. (2017) indicate that in 38 genotypes of pumpkin from different areas of Turkey, no significant correlation was observed between fruit weight and the thousand seed weight. A significant negative correlation was observed in this experiment between fruit weight and kernel percentage. The average fruit weight was 4.41 kg, and it ranged from 1.33 to 12.52 kg.

The average number of seeds per fruit was 355, and it ranged from 240-444. According to Berenji (2010), there are about 400-500 seeds in one fruit of an pumpkin. Aruah et al. (2012) state that the average number of seeds per fruit in 10 pumpkin genotypes was in the range 315-335 depending on agroecological conditions. The number of seeds per fruit in these studies was significantly positively correlated with dry seed weight per fruit, kernel weight per fruit, kernel percentage and fruit weight.

Aruah et al. (2012) state that number of seeds per fruit of the pumpkin was in a significant positive correlation with the diameter of the fruit (r = 0.62). In 23 *Cucurbita moschata* genotypes, number of seeds per fruit was also significantly positively correlated with the average fruit weight (r = 0.45) (Srikanth et al., 2015). In contrast, according to Sekerci et al. (2017) in 38 pumpkin genotypes from different areas of Turkey, no significant correlation was observed between number of seeds per fruit and the weight and diameter of the fruit. Similar results are reported by Chaudhari et al (2017) according to which number of seeds per fruit in 40 genotypes of *Cucurbita moschata* grown in India was not significantly correlated with fruit weight and the thousand seed weight.

The number of seeds per fruit in these studies was not significantly correlated with thousand seed weight. Similar results are indicated by Sekerci et al. (2017), according to which in 38 genotypes of pumpkin from different areas of Turkey, no significant correlation was observed between seeds number per fruit and seeds weight per fruit. In contrast, in 28 genotypes of *Cucurbita moschata* in India, seeds number per fruit was significantly negatively correlated (r = -0.60) with thousand seeds weight (Kumar et al., 2018).

Table 1. Correlation coefficients between seed yield components of pumpkin; n=81

	Number of seeds per fruit	Seed weight per fruit	Kernel weight per fruit	Thousand seed weight	Seed yield
Fruit weight	0.39*	0.57*	0.52*	0.39*	-0.44*
Number of seeds per fruit		0.71*	0.74*	0.18	0.31*
Seed weight per fruit			0.94*	0.71*	0.33*
Kernel weight per fruit				0.76*	0.32*
Thousand seed weight					0.25*

*Statistically significant according to p≤0.05

Dry seeds weight per fruit of pumpkin in these studies ranged from 27.2 to 111.0 g. The average of dry seeds weight per fruit was 65.1 g. According to Berenji (2010), the mass of air-dried seeds per fruit is about 80-120 g. The lower average seed weight per fruit in this experiment is a consequence of the pronounced drought in the period of fruit and seed formation. Seed weight per fruit was significantly positively correlated with kernel weight per fruit, thousand seed weight and kernel percentage. Similar results are indicated by Kumar et al. (2018) in 28 genotypes of *Cucurbita moschata* in India, in which the weight of the seeds per fruit was in a significant positive correlation (r = 0.46) with the thousand seed weight.

Kernel weight per fruit pumpkin fruit in this experiment was significantly positively correlated with the thousand seed weight and kernel percentage. The average kernel weight per fruit was 49.2 g, and it ranged from 27.2 to 105.5 g, which indicates that about one quarter of the seed weight is shell. Similar results are indicated by Rabrenović et al. (2012), in which the share of shells in the seed of pumpkin for oil ranged from 22.45-27.02%, with the mean value of share of shells being 24.68%.

The average thousand seed weight in this experiment was 194.7 g, and it ranged from 100.8-338.0 g. According to Meru et al. (2018) the average thousand seed weight for 35 genotypes of pumpkin in SAD ranged from 61-287 g. Rabrenović et al. (2012) found that the thousand seed weight in a pumpkin with a shell ranged from 236 to 254 g. The large variations in the thousand seed weight in our experiment are a consequence of different growing conditions, since the genotypes were collected from a wider geographical area. This is confirmed by the research of Seymen et al. (2016), according to which the average thousand seed weight in 20 genotypes originating from Turkey varied significantly depending on the water supply of plants. In the irrigated variant it was 246.6 g, with a variation interval of 149.0-323.3 g, while in the non-irrigated variant the average a thousand seeds weight was 188.8 g, and ranged from 127.3-239.9 g.

Thousand seeds weight of pumpkin in this experiment was significantly positively correlated with kernel percentage. In contrast, Sekerci et al. (2017) state that in 38 genotypes of the pumpkin from different areas of Turkey, no significant correlation was observed between the thousand seed weight on the one hand and the weight and size of the fruit on the other hand. Seymen et al. (2016) indicate that thousand seeds weight of pumpkin was significantly positively correlated with stem height, fruit size, seed size and seed yield. According to Nagar et al. (2017) the thousand seed weight in *Cucurbita moschata* genotypes was significantly positively correlated with seed size (r = 0.31) as well as with seed yield per plant (r = 0.41) and fruit yield per plant (r = 0.37). Meru et al. (2018) indicate that thousand seeds weight of pumpkin was significantly positively correlated with seed size (r = 0.80) and seed oil content (r = 0.70), and significantly negatively correlated with seed protein content (r = -0.39). Oil content and protein content in seeds were also significantly negatively correlated (r = -0.51). The authors state that the positive correlation between seed size and seed oil content (r = 0.50-0.70) suggest that during breeding work it is possible to indirectly choose in which direction further selection will continue. By selecting genotypes with larger seeds, selection takes place in the direction of obtaining ~~a~~ higher oil content, and by selecting genotypes with smaller seeds in the direction of increasing the protein content. Similar results have been observed for soybeans (Filho et al., 2001) and sunflowers (Tang et al., 2006).

The average kernel percentage in this experiment for all genotypes was 1.89%, and ranged from 0.61 to 3.55%. Kernel percentage of pumpkin was significantly positively correlated with number of seeds per fruit, seeds weight per fruit, kernels weight per fruit and thousand seed weight, and in a significant negative correlation with fruit weight.

Conclusion

Fruit weight was significantly positively correlated with the number of seeds per fruit, the dry seeds weight ~~of~~ per fruit, kernel weight per fruit and thousand seed weight. A significant negative correlation was observed between fruit weight and kernel percentage (r = -0.44). Fruit weight was not significantly correlated with the number of seeds per fruit, while the number of seeds per fruit was significantly positively correlated with dry seed weight per fruit, kernel weight per fruit and kernel percentage. Number of seeds in the fruit was not significantly correlated with the thousand seed weight. Seed weight per fruit was significantly positively correlated with kernel weight per fruit, thousand seed weight and kernel percentage. Kernel weight per fruit was in a significant positive correlation with thousand seed weight and kernel percentage. Thousand seed weight was significantly positively correlated with kernel percentage. Kernel percentage was significantly positively correlated with number of seeds per fruit, seed weight per fruit, kernel weight per fruit and thousand seed weight, and significantly negatively correlated with fruit weight. Correlation coefficients and their significance in these studies largely agree with the studies of other authors, but in some studies conducted with other genotypes in different conditions there are discrepancies. Accordingly, the analyzed genotypes represent an important source of diversity that could be used in the future in new plant breeding studies.

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Original scientific paper

Effect of glyphosate on wheat seedlings depending on the characteristics of the water

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Abstract

The aim of the research is to examine the dependence of the impact of glyphosate on wheat seedling depending on the characteristics of water it is dissolved in. The impact of water on effective concentration of glyphosate has been researched by bioassay methods. Wheat seeds were germinated on the filter paper in the Petri dishes on the temperature of 22˚C for six days whereupon the measurements of the mass of the shoot and root were taken. Wheat variety BELIJA was used in the experiment. Glyphosate was used in the following mass concentrations: 720, 1080, 1620, 2430 and 3645 µg a.s./L, as well as three types of water: spring water, tap water and distilled water. EC_{50} of glyphosate for the shoot weight in the natural water varied from 2571.9 to 3114.83 µg/L, as in the distilled water it was from 2251.99 µg/L. EC_{50} of glyphosate for the root weight in the natural water varied from 1571.49 to 2793.56 µg/L, as in the distilled water it was 1240.24 µg/L. By simple linear correlation it is determined that value $EC_{50Shoot}$ for the shoot weight statistically significantly depends on carbonate hardness and total water hardness while the linear correlation coefficients are r= 0.922* (p=0.026) and r=0.897* (p=0.039), respectively. It is determined that effective concentrations of glyphosate for the shoot weight $EC_{10Shoot}$ and root weight EC_{10Root}, statistically significantly depends on the concentration of magnesium in water and the linear correlation coefficients are 0.923* and 0.892*, respectively.

Key words: Glyphosate, effective concentration (EC), water characteristic , wheat, bioassay

Introduction

Glyphosate [IUPAC: N-(fosfon-metil)-glicin] is nonselective herbicide from the class of phosphonate. Glyphosate is strong and specific inhibitor of enzyme 5-enolpyruvylshikimate

3-phosphate (EPSP) synthase. Glyphosate is weak acid and has four acid dissociation constant: two for the phosphate groups (pKa1. pKa3), one for amino group (pKa4) and one for carboxyl group (pKa2), where pKa2. pKa3 and pKa4 are values determined by various analytical methods such as potentiometric titration (Madsen, et al., 1978. Barja and Dos Santos Afonso, 1998), nuclear magnetic resonance NMR (Appleton, et al., 1998) and infrared spectroscopy FTIR. The acidic constants of dissolution are pKa1=2.22; pKa2=5.44 and pKa3=10.13 (Borggaard and Gimsing, 2008).

Molecule of glyphosate at higher pH of water is mostly negatively charged. On the other side, in most of the natural water there are ions with positive (cations) as well as with negative electrification (anions), and the most important ones are: calcium (Ca^{++}), magnesium (Mg^{++}), sodium (Na^{+}), sulfate (SO_4^{-}), chloride ($C1^{-}$) and bicarbonate (HCO_3^{-}). Small amounts of potassium (K^{+}), iron (Fe^{++}, Fe^{+++}), nitrate (NO_3^{-}) or the other ions can be present (Petroff, 2003). Hard water which contains high level of cations of Ca, Mg, Na or Fe can reduce the efficacy of some herbicides of weak acids such as glyphosate, dicamba, 2.4-D (Hull et al.,1982). Ions Mg^{2+} whose concentration can be particularly high in the river water can have negative effect on the performance of glyphosate (Thelen et al., 1995). Water that contains 200 mg/L $CaCO_3$ can cause the problems if it is used to dissolve the pesticides. The higher level of hardness of water that is used as herbicide solvent is 300 mg/L (McDougall, 2012). Water containing a lot of calcium and magnesium can decrease the efficacy of glyphosate. For such hard water it is recommended to add ammonium sulphate (AMS). Schortgen and Patton (2020) determined that antagonism of herbicides of weak acids (specifically 2.4-D) towards the hard water is surpassed by adding 20 $g \cdot L^{-1}$ ammonium sulfate (AMS) into the mixture. When AMS was included in spray mixtures, no differences were observed at 600 mg $CaCO_3$ $L^{-1.}$ compared with distilled water. Ammonium nitrate can increase the efficacy of the glyphosate at many weed plants. Nitrate ion (NO_3) forms conjugated salts with hard water while in the presence of the ionized form of ammonium ion (NH_4) glyphosate is easily absorbed (Petroff, 2000).

Material and Methods

The effect of water on the effective concentration of glyphosate was examined by the bioassay method . The herbicide glyphosate was used as commercial formulation CLINIC, containing 480 g/L of the isopropylamine salt of glyphosate and was formulated as soluble concentrate (SL). Wheat variety BELIJA was used for the experiment as well as three types of water for making solution: spring water, tap water and distilled water (Table 1). The effect of the herbicide glyphosate on the mass of the wheat seedlings was investigated.

Table 1. Physical and chemical characteristics of water

Parameter	Unit	Measured values				
		SW1	SW2	SW3	TW	DW
pH	pH unit	7.15	7.15	7.12	7.19	7.12
Water electrical conductivity (20 °C)	µS/cm	370	371	370	371	371
Calcium, Ca	mg/L	109.3	148.5	81.6	78.3	1.6
Magnesium, Mg	mg/L	15.5	5.8	34.8	14.5	<0.1
Carbonate hardness ($CaCO_3$)	mg/L	309.0	378.8	368.8	229.3	24.9
Total water hardness	mg/L	334.6	393.4	341.8	252.8	3.6
Legend: SW1. SW2. SW3= spring water; TW= tap water; DW= distilled water						

Glyphosate was used in the following volume concentrations of the formulation Clinic: 0.00015; 0.00023; 0.00034; 0.00051; 0.00076% that is in the mass concentration of glyphosate from 720, 1080, 1620, 2430 and 3645 µg/L. The weight of the seedlings was taken as the biometric indicator, separated on the weight of root and weight of shoot. Wheat seeds were germinated on the filter paper in the Petri dish in the dishes with the diameter of 95 mm. In each of the dishes there were 30 seeds and 5 mL of the working herbicide solution. Germination was preceded in the thermal chamber on 22˚C for six days. On the sixth day, it was done the separation of the over ground part and root and the remaining grains (seed coat and endosperm). After the final separation, the shoot weight and the root weight were measured. The measurements are done on the technical balance scale with the precision of 0.001 g.Regressive dependence among the indicators of the growth of the test plant, as the dependent value and the volume concentration of the glyphosate as the independent value was done by the sigmoid curve according to logistic model based on "dose-response" relationship.

The four-parameter logistic function was specifically used given by the formula

$$f(x,(b,c,d,e)=c+\frac{d-c}{1+exp\{b[log(x)-log(e)]\}}$$

where *b, c, d, e* are four parameters. The parameter e marks the effective dose ED_{50} and it is the concentration which produces a response half-way between the upper limit, *d*, and lower limit, *c*. The parameter *b* denotes the relative slope around e (Ritz and Streibig, 2005). This functional dependence is performed by the statistical programme Origin®Pro-6.1. The statistical programme determines besides these four basic parameters of the functional dependence the interval of trust for all the parameters they can be found at, so the effective dose is presented as EC_{50} (±SE).

Results and Discussion

During the short bioassay in five water types , test plant is exposed to the doses of glyphosate from 720 µg/L to 3645 µg/L of water. The respond of test plant grown in five different types water to the effect of glyphosate was emphasized depending on both the glyphosate concentration and the characteristics of water where glyphosate is dissolved in. The impact of glyphosate to the wheat seedlings weight in five different water types is shown in table 2.

Table 2. Influence of glyphosate on the wheat seedlings weight (g) grown in 5 different water types

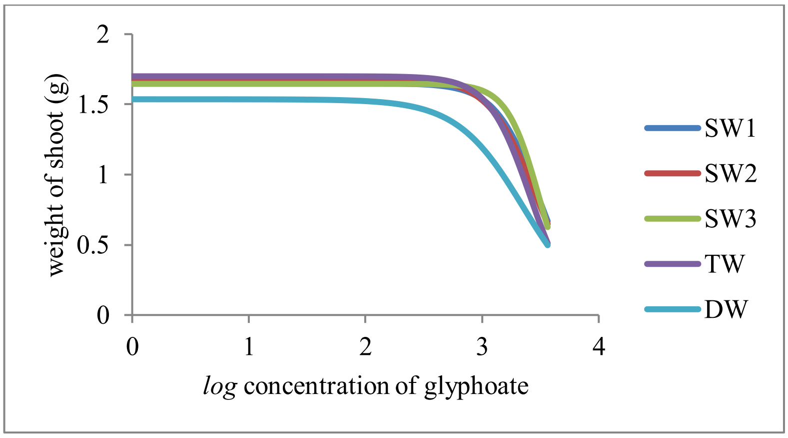
Water types	Part of plants		Mass concentration of glyphosate (µg a.s./L water)					
			Control	720	1080	1620	2430	3645
			K0	K1	K2	K3	K4	K5
			Average weight of 30 seedlings of wheat (g)					
SW1	Shoot	$\bar{X}$ (g)	1.570	1.632	1.668	1.216	1.049	0.696
		±SE (g)	±0.015	±0.053	±0.033	±0.084	±0.054	±0.038
		$F_{0.05(5\ i\ 18)}$=2.77; $F_{0.01(5\ i\ 18)}$=4.25; $S_{\bar{d}}$=0.071; $t_{(0.05;18)}$=2.101; $t_{(0.01;18)}$=2.878; $lsd_{0.05}$= 0.149 g; $lsd_{0.01}$=0.204g; F_{exp}= 59.114**						
	Root	$\bar{X}$ (g)	0.590	0.570	0.543	0.408	0.387	0.191
		±SE (g)	±0.063	±0.076	±0.027	±0.020	±0.021	±0.010
		$F_{0.05(5\ i\ 18)}$=2.77; $F_{0.01(5\ i\ 18)}$=4.25; $S_{\bar{d}}$=0.063; $t_{(0.05;18)}$= 2.101; $t_{(0.01;18)}$= 2.878; $lsd_{0.05}$=0.133 g; $lsd_{0.01}$= 0.182 g; F_{exp}= 12.110**						
SW2	Shoot	$\bar{X}$ (g)	1.696	1.568	1.564	1.261	1.034	0.637
		±SE (g)	±0.039	±0.053	±0.110	±0.064	±0.102	±0.036
		$F_{0.05(5\ i\ 18)}$=2.77; $F_{0.01(5\ i\ 18)}$=4.25; $S_{\bar{d}}$=0.105; $t_{(0.05;18)}$= 2.101; $t_{(0.01;18)}$= 2.878; $lsd_{0.05}$=0.221g; $lsd_{0.01}$=0.302g; F_{exp}= 30.186**						
	Root	$\bar{X}$ (g)	0.651	0.365	0.512	0.274	0.229	0.075
		±SE (g)	±0.021	±0.026	±0.046	±0.011	±0.027	±0.006
		$F_{0.05(5\ i\ 18)}$=2.77; $F_{0.01(5\ i\ 18)}$=4.25; $S_{\bar{d}}$=0.045; $t_{(0.05;18)}$= 2.101; $t_{(0.01;18)}$= 2.878; $lsd_{0.05}$=0.095g; $lsd_{0.01}$=0.130 g; F_{exp}= 43.865**						
SW3	Shoot	$\bar{X}$ (g)	1.489	1.660	1.781	1.409	1.064	0.669
		±SE (g)	±0.058	±0.046	±0.056	±0.092	±0.092	±0.085
		$F_{0.05(5\ i\ 18)}$=2.77; $F_{0.01(5\ i\ 18)}$=4.25; $S_{\bar{d}}$=0.105; $t_{(0.05;18)}$= 2.101; $t_{(0.01;18)}$= 2.878;$lsd_{0.05}$=0.221; $lsd_{0.01}$=0.302g; F_{exp}= 31.105**						
	Root	$\bar{X}$ (g)	0.430	0.532	0.535	0.300	0.312	0.136
		±SE (g)	±0.018	±0.037	±0.024	±0.022	±0.038	±0.011
		$F_{0.05(5\ i\ 18)}$=2.77; $F_{0.01(5\ i\ 18)}$=4.25; $S_{\bar{d}}$=0.039; $t_{(0.05;18)}$= 2.101; $t_{(0.01;18)}$= 2.878; $lsd_{0.05}$=0.082 g; $lsd_{0.01}$= 0.112g; ; F_{exp}= 29.835**						
TV	Shoot	$\bar{X}$ (g)	1.660	1.694	1.497	1.260	0.911	0.519
		±SE (g)	±0.047	±0.069	±0.061	±0.065	±0.065	±0.057
		$F_{0.05(5\ i\ 18)}$=2.77; $F_{0.01(5\ i\ 18)}$=4.25; $S_{\bar{d}}$=0.087; $t_{(0.05;18)}$= 2.101; $t_{(0.01;18)}$= 2.878; $lsd_{0.05}$=0.183g; $lsd_{0.01}$=0.250g; F_{exp}= 57.697**						
	Root	$\bar{X}$ (g)	0.640	0.595	0.452	0.440	0.337	0.076
		±SE (g)	±0.046	±0.076	±0.042	±0.037	±0.029	±0.002
		$F_{0.05(5\ i\ 18)}$=2.77; $F_{0.01(5\ i\ 18)}$=4.25; $S_{\bar{d}}$=0.067; $t_{(0.05;18)}$= 2.101; $t_{(0.01;18)}$= 2.878; $lsd_{0.05}$=0.141g; $lsd_{0.01}$=0.193g; F_{exp}= 17.033**						
DW	Shoot	$\bar{X}$ (g)	1.512	1.420	1.046	0.983	0.700	0.527
		±SE (g)	±0.022	±0.018	±0.106	±0.060	±0.044	±0.065
		$F_{0.05(5\ i\ 18)}$=2.77; $F_{0.01(5\ i\ 18)}$=4.25; $S_{\bar{d}}$=0.087; $t_{(0.05;18)}$= 2.101; $t_{(0.01;18)}$= 2.878; $lsd_{0.05}$=0.183g; $lsd_{0.01}$=0.250g; F_{exp}= 41.104**						
	Root	$\bar{X}$ (g)	0.448	0.386	0.210	0.183	0.102	0.023
		±SE (g)	±0.047	±0.022	±0.023	±0.021	±0.012	±0.014
		$F_{0.05(5\ i\ 18)}$=2.77; $F_{0.01(5\ i\ 18)}$=4.25; $S_{\bar{d}}$=0.039; $t_{(0.05;18)}$= 2.101; $t_{(0.01;18)}$= 2.878; $lsd_{0.05}$=0.082g; $lsd_{0.01}$=0.112g; 35.548**						
Legend: SW1. SW2. SW3= spring water; TW= tap water; DW= distilled water; SE= standard error								

Observing the weight of shoot grown in five different water types depending on the glyphosate concentration, it is seen that in water SW2 and distilled water (DW) as the mass concentration of glyphosate increases, the shoot weight decreases. At SW1, SW3 and tap water (TW) there are variations so that small glyphosate concentrations stimulated the growth of the shoot.

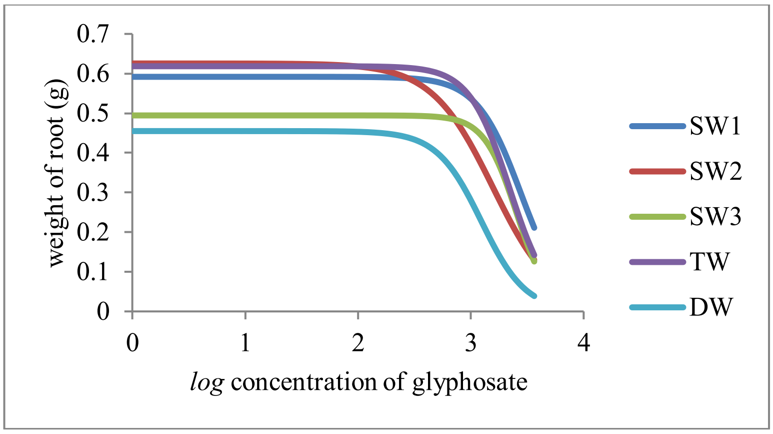
Perceiving the results for the mass of the root of the wheat seedlings depending on the glyphosate concentration in five water types , it can be concluded the roots weight was as expected in three water types, water SW1, TW and DW, as the as the mass concentration of glyphosate increased, the shoot weight decreased. At water SW2 and SW3 the small concentrations of glyphosate caused the stimulation of the growth of the root. Perceiving the dependence of the wheat seedlings weight on the glyphosate concentration it can be noticed that there are no significant differences between the smallest concentration of glyphosate and the control.

The research has shown that small doses of glyphosate stimulate the growth of wheat seedlings but it is not statistically larger than the control. Cedergreen (2008) also determined that glyphosate can stimulate the plant growth if it is applied in the doses from 5-60 g/ha a.i. Results that Velin et al. (2008) and Brito et al. (2018) as well achieved show that subtoxic doses of glyphosate stimulate the growth of some weeds and grown plants, which means that they cause effects of hermetic.

Regression dependence of wheat shoot weight on logarithm of glyphosate mass concentration is shown in graph 1. Regression dependence of wheat seedlings weight on logarithm of glyphosate mass concentration is shown in graph 2.



Graph. 1. Regression dependence of weight of wheat shoot on logarithm of glyphosate concentration



Graph. 2. Regression dependence of wheat weight root on the logarithm of glyphosate concentration

Indicators of the regression function and effective concentrations (EC) of glyphosate for the the shoot weight (SW) are shown in table 3.

Table 3. Effective concentration of glyphosate for shoot of wheat (EC_{Shoot}) and regression parameters

Water types	Effective concentration of glyphosate $EC_{50Shoot}$ (µg/L) (±SE)			
SW1	$EC50_{Shoot}$(SW1)=3085.99 µg/L (±350.24 µg/L)			
	d=1.650 g	c=0 g	b=2.309	R^2=0.935
SW2	$EC50_{Shoot}$(SW2)=2916.73 µg/L (±143.89 µg/L)			
	d=1.688 g	c= 0 g	b=2.120	R^2=0.990
SW3	$EC50_{Shoot}$(SW3)=3114.83 µg/L (±328.44 µg/L)			
	d=1.646 g	c=0 g	b=3.092	R^2=0.917
TW	$EC50_{Shoot}$(TW)=2571.97 µg/L (±102.08 µg/L)			
	d=1.700 g	c=0 g	b=2.417	R^2=0.993
DW	$EC50_{Shoot}$(DW)=2251.99 µg/L (±294.42 µg/L)			
	d=1.536 g	c=0 g	b=1.530	R^2=0.962
$EC50_{Shoot}$= Effective concentration of glyphosate on which the weight of the shoot is reduced by 50%; SE= Standard error; *c*, *d*, *b*= parameters of logistic function; R^2= coefficient of determination				

Indicators of the regression function and effective concentrations (EC) of glyphosate for the roots weight (RW) are shown in table 4.

Table 4. Effective concentration of glyphosate for root of wheat (EC_{Root}) and regression parameters

Water	Effective concentration of glyphosate EC_{50Root} (µg/L) (±SE)			
SW1	$EC50_{Root}$(SW1)=2793.56 µg/L (±289.10 µg/L)			
	d=0.592 g	c=0 g	b=2.212	R^2=0.957
SW2	$EC50_{Root}$(SW2)=1571.49 µg/L (±508.29 µg/L)			
	d=0.625 g	c=0 g	b=1.589	R^2=0.853
SW3	$EC50_{Root}$(SW3)=2551.47 µg/L (±520.54 µg/L)			
	d=0.494 g	c=0 g	b=3.006	R^2=0.823
TW	$EC50_{Root}$(TW)=2209.73 µg/L (±353.41 µg/L)			
	d=0.618 g	c=0 g	b=2.416	R^2=0.928
DW	$EC50_{Root}$(DW)=1240.24 µg/L (±173.77 µg/L)			
	d=0.455 g	c=0 g	b=2.200	R^2=0.962
EC_{50Root}= Effective concentration of glyphosate on which the weight of the root is reduced by 50%; SE= Standard error; *c*, *d*, *b*= parameters of logistic function; R^2= coefficient of determination				

Percieving the influence of water on the effective concentration of glyphosate is done by linear correlation which is shown in table 5.

Table 5. Correlation matrix between effective concentration of glyphosate and chemical characteristics of water

Effective concentration (µg/L)		Ca	Mg	Carbonate hardness ($CaCO_3$)	Total water hardness
$EC_{50Shoot}$	*r*	**0.759**	**0.694**	**0.922***	**0.897***
	p	0.137	0.193	0.026	0.039
$EC_{10Shott}$	*r*	**0.568**	**0.923***	**0.856**	**0.808**
	p	0.318	0.025	0.067	0.098
EC_{50Root}	*r*	**0.373**	**0.777**	**0.577**	**0.296**
	p	0.537	0.123	0.309	0.296
EC_{10Root}	*r*	**0.095**	**0.892***	**0.417**	**0.387**
	p	0.879	0.041	0.486	0.527
r= The linear correlation coefficient; *=*statistical significance* (*p*<0.05)					

By simple linear correlation it is determined that value EC_{50} for the shoot weight statistically significantly depends on carbonate and total hardness. Coefficient of the linear correlation for the relation of EC50 for the shoot weight and carbonate hardness of 0.922* (*p*=0.026) while the coefficient of the linear correlation between EC50 for the shoot weight and total hardness is 0.897* (*p*=0.039). Regression dependence of the effective concentration ($EC50_{Shoot}$) on carbonate hardness (CH) actually on total water hardness (TWH) is:

– $EC_{50Shoot}$ =2175.37+2.339×CH (R^2=0.849; p=0.026; $F_{exp.}$=16.972*, $F_{0.05;1.3}$=10.13)

– $EC_{50Shoot}$ =2219.85+2.143×TWH (R^2=0.800; p=0.039; $F_{exp.}$=12.376*; $F_{0.05;1.3}$=10.13).

By insight into the relations between the measured variables over the linear correlation, it is observed that between EC10 and the concentration of magnesium in water is strong positive connection for the shoot weight as well as for the the roots weight. That correlation between $EC10_{Shott}$ actually $EC10_{Root}$ and concentration of magnesium in water is 0.923 meaning 0.892, respectively.

Regression dependence of the effective concentrations of glyphosate on the content of magnesium in water is:

- $EC_{10Shoot}$=710.762+25.107×Mg (R^2=0.854; p=0.0253; $F_{eksp.}$=17.282*, $F_{0.05;1.3}$=10.13)

- EC_{10Root}=454.732+25.101×Mg (R^2=0.7986;p=0.0419;$F_{eksp.}$=11.679*; $F_{0.05;1.3}$=10.13).

Results of linear correlation between the effective concentrations of glyphosate and concentration of magnesium (Mg), carbonate hardness ($CaCO_3$) and total water hardness in water refer to a general conclusion that as concentration of cations in water and water hardness increase, the activity of glyphosate on the wheat seedlings in water decrease.

Conclusion

Results of the research confirmed that biological activity of glyphosate significantly depends on the water characteristics. It is found that by increasing the concentration of magnesium (Mg), carbonate hardness ($CaCO_3$) and total water hardness it would be necessary to increase the concentration for glyphosate necessary to achieve the particular effect at the test plant. It all points out that in practice the chemical characteristics of water which is used for solution of the herbicides of weak acids must be considered.

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Review paper

Analysis of climate changes in peri - Pannonian Basin and Dinaric region: basis for future agricultural strategies

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Abstract

The aim of this study was to describe the process of climate changes on the selected meteorological stations in the Pannonian Basin and Dinaric region. The material has been collected from different sources for complete periods of measurement. The main method was based on comparing data among more stations for different periods. Extreme weather events are described too. The trends of temperature regime (temperature increase) in the period of measurements show similarity on all stations. Past decennium was the warmest one with sudden deviation from previous ones. Global warming has been followed with frequent occurrences of extreme weather conditions. This analysis can be used as a basis for more detailed studies of climate changes as well as for the development of agricultural strategies of adaptation to global warming in future.

Key words: climate changes, extreme events, the warmest decennium

Introduction

The climate of the Earth has been constantly changing. "Not so long ago", from 5000 – 2400 BC, the Sahara and the Middle East use to have steppe vegetation (Šegota & Filipčić, 1996). Climate and its importance were highlighted in the oldest records of the Bible. So seven skinny and seven fat cows in Pharaoh's metaphorical dreams symbolize seven dry and seven fertile years. Records from the early medieval church and old books indicate periods when the winyards thrived in England, Prussia and southern Norway. In the period from 1000 to 1300, numerous English farmers produced wines of similar quality as French ones. Thanks to warmer climate in Europe in the period from 400 to 1200 AD, Vikings lived and traveled easier, spreading their influence over a large area from Paris to Greenland. Occasionally visits of extremely cold winters in the period of warmer climate brought unusual appearances as ice on the River Nile (1010/11) or partially frozen Northern Adriatic Sea (859/60). The period from 1200 – 1400 was characterized with extremely warm or harsh winters, wild floods and strong storms.

Than the climate got colder. The period from the mid-fifteenth to the mid-nineteenth century is known as the Little Ice Age. Old Dutch masters (*Pieter Brueghel the Elder and Pieter Brueghel the Younger)* often painted skaters on frozen lakes. These pictures look unreal because today's Netherlands has mild, rainy winters. They lived in the 16th and 17th century when Europe faced harsh winters. Poland experienced 26 exceptionally cold winters in the 16th century (Twardosz and Kossowska - Cezak, 2016).

Before the period of global warming, Balkan region had been of unique importance in Europe due to its great biodiversity and respectable potential for various branches of agricultural production. Unfortunately, global warming has diminished the agricultural importance of this region. For that reason, in the recent two decades, local scientists have been paying more attention to the effects of the climate changes to the field crops production (Nožinić et al., 2004; Nožinić, 2008; Nožinić et al., 2009; Nožinić et al., 2012, Nožinić et al., 2015; Nožinić et al., 2016; Nožinić et al., 2022). In the past decennium, agricultural production was affected with extreme drought in six years and the worst flood (2014) in the 20th century.

Since the climate of continental part of Balkan and Central Europe has certain similarities, this paper deals with the available climate data and trends of changes at several meteorological stations in Bosnia and Herzegovina, Croatia, Slovenia and Austria. These stations are selected in order to represent different environmental conditions. Having in mind negative effects of global warming to the environmental balance, the focus was on the temperature regime and changes. As the measurements in Vienna began eighty five or more years earlier than in the Balkan region, the method of interpolation can offer a fairly realistic picture of the climate on the other stations (regions) before the period of measurement. The aim of this study was to describe the process of climate changes on the selected meteorological stations in some peri-Pannonian, Pannonian and Dinaric regions.

Material and Methods

As meteorological stations in Bosnia and Herzegovina and Croatia have operated in five countries, climate data have been taken from several sources (details in References). Some data are still not available, such as the temperature series for Banja Luka in the period 1914-1948. Šarić (1977) presented the average temperatures for this station, as well as other stations in Bosnia and Herzegovina for the period 1931-1960. However, the data per years in that period are not available. During the war period (1941-1945) many stations in Bosnia in Herzegovina did not work (or worked with breaks), so the data ought to be interpolated with neighboring Croatian stations. The most of data for the stations out of Bosnia and Herzegovina have been taken from HISTALP data basis. The periods of availability of data series are presented in the Tab. 1.

Table 1. Basic data of the selected meteorological stations

Meteorological station	Country	Longitude	Latitude	Altitude (m)	Period
Vienna - Hohe Warte	Austria	16.3560	48.2490	209	1765 - 2020
Munich	Germany	11.5500	48.1700	525	1781 - 2015
Banja Luka	Bosnia and Herzegovina	17.2200	44.7800	153	1881 - 2020
Tuzla	Bosnia and Herzegovina	18.6700	44.5400	305	1880 - 2020
Sarajevo	Bosnia and Herzegovina	18.4300	43.8700	630	1880 - 2020
Zagreb - Grič	Croatia	15.9800	45.8200	162	1862 - 2014
Gospić	Croatia	15.3800	44.5300	573	1862 - 2014
Osijek	Croatia	18.6700	45.5500	91	1899 - 2014
Slavonski Brod	Croatia	18.0188	45.1618	88	1963 - 2020
Ljubljana	Slovenia	14.5125	46.0656	299	1851 - 2015

The location of the meteorological station in Banja Luka has been changed four times in the circle of two kilometers. It should not have a significant influence to precipitation amounts which have been available in a homogeneous series since 1881. However, the comparison of temperature series among three locations is not reliable, especially for minimum temperatures. Valley climate is characterised with frequent temperature inversions, when the minimum temperature can significantly vary depending on position in the valley (valley edge and sunny slopes are wormer), vegetation cover, temperature of the river water, relative moisture and wind characteristics. The methodology has been based on comparing data and trends of climate changes among more meteorological stations at different heights, longitudes and latitudes (Tab. 1). These stations represent the climate of the wider space of the peri - Pannonian, Pannonian as well as the Dinaric and Alpine regions. The original data for rainfall and sunny hours are rounded to whole numbers. In this way the data are clearer with negligible mathematical error. Lang's rain factor is calculated as a simplified indicator of aridity (or humidity) oscillations over a long period.

Results and Disscussion

The comparison of the mean annual temperature of thirty-year periods shows that the last period (1990-2020) was the warmest one with the most significant temperature increase on all stations (Tab. 2). On the basis of the longest data series (Vienna), it can be estimated (method of interpolation) the temperature regime before the periods of measurements on the other stations (Tab. 2). A bit higher mean temperature in the first period of the records (1781-1810) is still under the mean temperature for the period 1961-1990. As precipitation amounts among the analysed periods do not show significant differences or clear trends of increasing or decreasing, the significant increase of temperatures lead to increase of aridity which is proved by Lang's rain factor (Tab. 3). Analyses of extreme temperatures as well as extreme values of other climatic factors have great importance for strategic planning in agriculture as well as in the mathematical calculation for rough seasonal weather forecasts (Nožinić et al., 2009). The coldest months and years have been related to the earlier period of measurements, while the warmest ones mainly appear in the recent decades (Tab. 4-6).

The year 1940 deserves to be mentioned as the coldest one in the 20th century. It was mainly a consequence of a very cold winter. However, even colder years and winters occured in the 19th century. After severe winter frosts in 1829/30, peasants had to pull out all plum trees in some Dinaric regions. Balkan's winter (1941/42) will be remembered with extreme coldness and tragic events in the beginning of the Second World War. After Partisans' march over the mountain Igman during the frosty night (January 28, 1942; estimated minimum temperature -40˚C), 172 partisans suffered severe hypothermic injury, six of whom died. This is just one case of human suffering caused by combined evils; war, cold and hunger. Thousands of soldiers and prisoners in war camps had lost body parts from freezing during these winters. Exceptionally cold winters have covered larger area in Europe then exceptionaly mild winters (Twardosz and Kossowska - Cezak, 2016). Some absolute minimum temperatures in January in Dinaric and Pannonian regions can be listed as follows; Igman -42.5˚C (1963), Sjenica -38˚C (1954), Gračac -34.6˚C (2003), Negotin -33.2˚C (1947), Vinkovci -30.5˚C (1963), Zrenjanin -30.4˚C (1963), Otočac -30.1˚C (2003), Prijedor -30˚C (1963), Kruševac -30˚C (1947). The average temperature of February does not show its real nature. The temperature oscillations from year to year can be fascinating (Tab. 4-6). February can bring Siberian coldness or the first flowers and buds. For its very changeable nature, in the Croatian language this month is called "Veljača, premetača". The apposition noun "premetača" can be translated as changeable or fickle month. Most of the winter minimums and maximums are related to this month. During the coldest February in 1929, Lika (the area divided from the Adriatic Sea by the mountain Velebit) was the most affected by winter troubles. Unlike Pannonia, this area has a winter maximum of rainfall (snowfall). Gospić experienced this through the highest snow cover (285 cm) in February 1929. In addition wild winds on mountain peaks and saddles created snow hills. In 1929, Balkan and Middle Europe faced lower mean February temperatures than those typical for Moscow region (Gospić -12.2˚C, Brno -12.2˚C, Tuzla -10.5˚C, Sarajevo -9.4˚C, Osijek -9.6˚C, Wien -10.2˚C). One strange winter deserves to be mentioned too. January 1956 was 2-4˚C warmer than the average, then winter showed its teeth in February, what can be seen from the mean temperatures on more locations (Gospić -11.6˚C, Osijek -9.6˚C, Banja Luka -9.4˚C, Zagreb -7.1˚C). Official minimum for Gospić (-33.5˚C) was registered on February 17, 1956. The minimum temperature -36˚C in February 1929 was not accepted as official one for some doubts due to the measurement method. Extreme heat is another face of February. The best example comes from 1966. The warmest February (Tab. 4-6) came after a very cold January (Tuzla -3.4˚C, Zagreb -3.5˚C, Banja Luka -3.9˚C). March 1966 was colder than that year's February. The differences between the temperatures in the lowland and mountain regions are more emphasized in March than in winter months. Agricultural activities in the peri - Pannonian regions usually begin in March. However, winters did not withdraw in the first Spring month in each year. March with a negative mean temperature in Tuzla occurred in 1907 (-2.0˚C), 1932 (-0.6˚C) and 1987 (-0.1˚C), while Sarajevo experienced negative mean temperatures in this month five times. Osijek had the same mean temperature of March as Tuzla in 1932, but it happened just in that year. Banja Luka had lower mean temperature (-1.5˚C) than Tuzla and Osijek in March 1932. Zagreb - Grič is the only continental station without a negative mean temperature of March. As a rule, cold March almost always comes after cold winter months. An example is the winter in Tuzla in 1906/07 with the following mean temperatures; December 1906 (-0.5˚C), January 1907 (-4.4˚C), February 1907 (-4.3˚C) and March 1907 (-2°C). Four months with a negative mean temperature also occurred in 1931/32 (December - March).

The data for "endless" winter 1874/5 are presented in the tab. 7. In that prolonged winter Gospić and Ljubljana faced five months with a negative mean temperatures while Zagreb - Grič recorded just one month with a negative mean temperature. The significant difference in the mean temperatures between Zagreb - Grič and other two stations can not be explained with latitude effects or strong influence of urban climate. In that period Zagreb was a relatively small town. By part this difference appeared because of the altitude effect and by part for protected location on the slope under the mountain Medvednica. Unlike open fields round town, location Zagreb - Grič has no emphasized temperature inversions, which results in about 1˚C higher mean annual temperature compared with lowlands round town (Šegota, 1986). Banja Luka valley also has about 1˚C lower annual temperature than Zagreb - Grič though both locations share similar altitude. Some protected locations on the slopes round Banja Luka valley have similar temperature regimes like Zagreb - Grič. Sunny slopes and small hills around Banja Luka (up to 400 m of altitude) exposed to warm southern wind (germ. Fohn) offer better climate conditions for some crops or orchards than the valley. In 2007, earring of triticale began two weeks earlier on the sunny slope over Banja Luka (450 m of altitude) than in the valley at 150 m of altitude (Nožinić et al., 2009).

Late frosts in the Spring period can do enormous damage to flowers in orchards in a short period. However frost intensity can vary depending on the microlocation characteristics. Zagreb area is covered with more meteorological stations then other regions. It is of great importance for better understanding of microclimate over a relatively small space, especially temperature inversion effects. The lowest minimum temperatures appear in the area of Lekenik (about 20 km on the south-east from Zagreb) and around the airport (10 km from the center of Zagreb). Good example of temperature differences happened on March 12, 2022 at 4 am; Zagreb Grič -3.7˚C, Zagreb Maksimir -6.1˚C, Zagreb airport -9.2˚C, and Lekenik -10.2˚C. As all mentioned stations share very similar latitude and height, these temperature differences are related to the night's period of inversion and are equalized during the day.

Fog, which mainly appears in the colder part of a year has a significant influence on ripening of crops and plant diseases. Water surfaces, rich relief forms and vegetation cover in the Dinaric and peri-Pannonian regions have a complex effect on the appearance and frequency of fog while the fog duration has a direct effect on the number of sunny hours. Thus, the region of central Sava River Basin records from 80-120 days with fog (Slavonski Brod, 120 days, mean for the period 1963-2020). Osijek (70 km north east from Slavonski Brod) has only 35 days with fog (1899-2020) because it is located in an open Pannonian lowland. Frequent fogs appear in the agricultural valleys around the Rivers Sana, Una and Vrbas. In the period 1997 - 2020, Prijedor had 87 days with fog, Banja Luka 58 and Bijeljina 49. Strong inversions in Sarajevo valley resulted with 29 foggy days in the december 1971. It was the first year of the "darkest" decennium (1971-1980) characterized with the smallest number of sunny hours at all stations and high rainfall in the summer months. The warmer part of the year shows smaller oscillations between the lowest and highest mean temperatures (Tab. 4-6). Although periods of extreme heat and drought have occurred in the past (1950), the past decennium will be remembered as the warmest one with seven extreme years. As the Agricultural Institute of the Republic of Srpska has its own certified seed production on 200 ha close to Banja Luka, the analyses of the warmer part of year will be focused on that station. The first year of the past decade (2011) was the driest in the period of measurements

(Tab. 8). It was the only year with the mean September temperature over 20˚C (Tab. 9). The drought caused many springs and water - courses in the Dinaric region to dry up. The next two years were characterized by a serious regional drought with record temperatures. Banja Luka valley is characterised with high maximum summer temperatures.

Though, Institute's hybrid maize was irrigated, the pollination was significantly reduced by frequent maximum temperatures over 35˚C. As a consequence of falling of flowers and pods at the temperatures over 32˚C, soybean yields have been significantly reduced too (Vratarić & Sudarić, 2008; Đorđević & Nožinić, 2013; Nožinić et al., 2022). The response of plant species to heat stress is different, sometimes bizarre and inexplicable. Contrary to expectations, after the extremely hot summer in 2021, the ripening of soybeans and corn was very late. If the warming continuos, heat stress sensitive crops (rye, buckwheat, spring oats, oilseed rape, trefoil, oil flax) will gradually "withdraw" from warm lowlands toward the mountain regions (Nožinić, 2008; Nožinić et al., 2011). Thanks to summer inversions, tropical nights in Banja Luka valley as well as other mountain valleys rarely appear, which is a certain advantage for some agricultural crops.

After three dry and warm summer seasons (2011, 2012, 2013) and hundred years after the rainiest year (2015), Banja Luka experienced a horrible flood in April and May 2014. Though the flood, frequent clouds and heavy rains (April - 214 l/m^2, May - 218 l/m^2) left the impression that it was a cool year, the mean annual temperature showed the opposite. Just 2019 was warmer than 2014 (Tab. 9). It is for the fact that no one remembers warm winters like the winter 2014. Great parts of Bosnia and Herzegovina shared similar problems in 2014. Thus, Tuzla coped with the highest amount of rain (339 l/m^2) in May 2014. Unlike other decades, all years' mean annual temperatures in the past decade were over 12˚C (Tab. 9). June had an additional increase of the temperature (0.7˚C) in relation to the previous decade, fixing its position with the months which have mean temperatures over 20˚C. June usually appears as the month with the highest amount of rain, although in some decenniums it can be May, July, October or November. The summer aridity of the past decade has mainly increased by moving the precipitation maximum in May and increase of summer temperatures. It seems the increased number of sunny hours have contributed to higher temperature and increased aridity. The locations (regions) with higher number of sunny hours need not be warmer than the locations with lower number of sunny hours. So, Wienna and Gospić have more sunny hours than Banja Luka and Zagreb parallely with lower temperatures in all months. However, the fact that all meteorological stations have recorded the most sunny hours in the past decade indicates that the temperature increase might be partly related to the regional increase of sunny hours (Tab. 14). Mean temperatures of the summer months in Banja Luka in the past decade reached mean temperatures characteristic for the north of the Adriatic coast. Banja Luka region will share the climate fortune of the of peri-Pannonian region. For higher rainfall (900 – 1,100 l/m^2), Banja Luka region could cope longer with a drought than Pannonian Basin. The climate change scenario for the whole Balkan region and the Middle Europe is very pessimistic (Eitzinger et al., 2009; Branković et. al., 2010; Gajić et al., 2010; Lalić et al., 2011; Branković et. al., 2012; Formayer et al., 2015; Lalić et al., 2021). According to the mentioned authors, the permanent increase of the temperatures will be continued by the end of this century, more in the summer than in the winter period. Summer precipitation could be reduced (35-40 l/m^2) in the Dinaric region and the Adriatic coast region in the period 2041-2070.

The Pannonian Basin is no longer in the optimal climate zone for field crop production. By the end of this century, Dinaric mountain valleys and plateaus could gradually take over primacy for field crop production from Pannonian lowlands. Lalić et al. (2011) foresee that in the 2080, Vojvodina could cope with reduced summer (June-September) rainfall, parallely with the increased summer temperature that could reach 24-25˚C. In the summer 2080 Novi Sad should receive 62 l/m^2 less than in 2010. If the trend of global warming continues, during the 22nd century, natural steppe vegetation in Hungary and Serbia could gradually turn to semi - desert vegetation. However, the global warming scenario does not exclude occasional visits of extremely cold winters or harsh frosts. Most likely, current decennium, which began with an atypically warm year, ought to be warmer than the previous one. Let us wait for 2031 year to describe new climate wonders in this decennium.

Table 2. The mean annual temperatures (˚C) in the periods of measurements

Station/period	1781-1810	1811-1840	1841-1870	1871-1900	1901-1930	1931-1960	1961-1990	1991-2020
Wien	9.5	9.1	9.1	8.9	9.1	9.4	9,7	11,0
Banja Luka	-	-	-	-	-	*10.8	10.6	12.0
Zagreb	-	-	-	11.1	11.3	11.4	11.7	-
Tuzla	-	-	-	-	9.5	9.7	9.8	11.1
Gospić	-	-	-	7.9	8.2	8.4	8.4	-

**(data by Šarić, 1977)*

Table 3. Precipitation (l/m^2) and Lang's rain factor (in the bracket) in the periods of measurements

Station/period	1871 - 1900	1901-1930	1931-1960	1961-1990	1991-2020
Banja Luka	1,041	1,106	1,031	1,028 (97)	1,062 (83)
Zagreb	929 (84)	955 (85)	913 (80)	885 (76)	*891 (70)
Tuzla	896 (97)	944 (99)	885 (91)	901 (92)	919 (83)

*The data for Zagreb are available for the period 1991-2014.

Table 4. Maximum and minimum mean monthly temperatures (˚C) in Zagreb in the period 1862-2014

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual
Maximum	7.5	8.8	11.5	15.8	20.9	24.5	25.0	26.5	21.4	15.4	12.5	6.9	13.8
In year	2007	1966	2012	2009	1868	2003	2012	1992	2011	2001 1907	1926	1915	2000 2014
Minimum	-7.1	-7.6	1.1	8.5	12.0	16.5	18.2	14.7	11.9	6.6	0.7	-7.1	9.5
In year	1864	1929	1932	1938	1876	1884	1940	1941	1912	1905	1908	1879	1940
Difference	14.6	15.7	10.4	7.3	8.9	8.0	6.8	11.8	9.5	8.8	11.8	14.0	4.3

Table 5. Maximum and minimum mean monthly temperature (˚C) in Tuzla in the period 1881 - 2020.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual
Max. temp.	5.4	7.7	10.8	15.8	18.2	22.3	23.9	23.3	19.4	14.6	11.8	5.9	12.2
In year	2007	1966	2001	2018	1945	2003	2012	1992	2011	1966	1926	1916	2019
Min. temp.	-10.5	-10.5	-2.0	6.2	8.9	14.3	16.6	15.5	10.9	5.5	-0.5	-5.2	7.9
In year	1893	1929	1907	1896	1919	1884	1913	1881	1912	1936	1908	1940	1940
Difference	15.9	18.2	12.8	9.6	9.3	8.0	7.3	7.8	8.5	9.1	12.3	11.1	4.4

Table 6. Maximum and minimum mean monthly temperature (˚C) in Banja Luka in the periods 1894-1913 and 1949-2020

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual
Maximum	6.2	7.7	11.2	16.2	19.8	24.1	25.2	24.6	21.4	16.6	12.2	6.9	13.4
In year	2007	1966	2001	2018	1908	2003	2012 2015	1992	2011	1907	1963	1910	2019
Minimum	-7.4	-9.6	1.6	7.2	12.2	17.0	18.3	17.8	10.4	6.9	0.4	-4.5	9.1
In year	1964	1956	1987	1997	1991	1962	1913	1965	1912	1905	1908	1899	1956
Difference	13.6	17.3	9.6	9.0	7.6	7.1	6.9	6.8	10.0	9.7	11.8	11.4	4.3

Table 7. The mean temperatures during the longest winter 1874/5

Station/year/month		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Year
Gospić	1874	-2.2	-1.5	0.6	10.1	9.0	17.5	20.4	15.5	14.5	8.3	-1.5	-1.2	7.5
	1875	-2.5	-6.3	-3.2	5.5	14.1	17.9	18.1	18.0	11.6	8.4	1.9	-6.1	6.5
Munich	1874	-0.9	-1.9	2.1	9.2	8.2	15.7	19.6	15.1	14.7	8.8	-0.2	-2.3	7.3
	1875	0.4	-6.0	-0.7	7.2	13.4	16.9	15.8	18.1	12.6	6.0	1.1	-3.5	6.8
Zagreb	1874	0.3	2.5	5.6	13.1	12.5	20.6	24.2	19.4	18.8	11.6	1.3	0.7	10.9
	1875	0.5	-3.2	1.3	10.0	17.3	22.0	21.6	21.9	15.5	11.0	5.1	-2.3	10.1
Ljubljana	1874	-0.6	1.0	3.2	11.5	10.7	18.1	21.4	17.3	15.8	9.6	-0.3	-1.2	8.9
	1875	-1.2	-3.9	-0.1	8.8	15.3	19.0	19.0	19.3	14.2	9.1	3.3	-4.5	8.2

Table 8. Mean decennial temperatures (˚C) in Banja Luka in the period 1951-2020

Year/month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Mean
1951-1960	-0.8	0.4	5.4	9.4	15.2	19.3	21.0	20.2	16.3	10.8	5.7	3.6	10.5
1961-1970	-2.3	0.1	5.8	11.4	15.2	18.9	20.0	19.3	16.0	11.1	7.3	-0.2	10.3
1971-1980	0.6	3.0	6.7	10.1	15.4	19.1	20.3	19.9	15.1	10.0	5.3	1.3	10.6
1981-1990	-0.4	0.7	6.2	11.4	16.3	18.8	21.3	20.1	16.8	11.5	5.1	2.1	10.9
1991-2000	0.7	2.5	6.9	10.8	16.3	20.1	21.7	21.7	16.7	11.5	6.4	1.5	11.4
2000-2010	1.1	3.3	7.4	12.1	17.3	20.7	22.6	21.7	16.1	11.9	7.3	2.2	12.0
2011-2020	2.2	3.7	8.0	13.1	16.5	21.5	23.4	23.1	17.8	12.4	7.6	3.2	12.7
1951-2020	0.2	2.0	6.6	11.2	16.0	19.8	21.5	20.9	16.4	11.3	6.4	2.0	11.2

Table 9. Mean monthly temperatures (˚C) in Banja Luka in the past decennium (2011-2020)

Year/month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Year
2011	1.9	1.7	7.1	13.0	16.0	21.2	23.1	23.7	20.2	11.0	3.1	3.9	12.2
2012	2.0	-2.8	9.3	12.7	16.1	23.0	25.2	24.4	18.9	12.5	9.9	1.3	12.7
2013	2.8	2.3	6.1	13.4	16.6	20.4	23.0	23.5	16.7	13.1	7.4	2.5	12.3
2014	5.6	6.5	9.6	13.1	15.8	20.3	21.7	20.6	16.4	13.5	8.9	4.0	13.0
2015	3.3	2.4	7.3	11.8	17.4	20.9	25.2	24.0	18.3	11.5	7.1	3.2	12.7
2016	2.3	7.6	8.0	13.5	16.2	21.5	23.3	20.5	17.8	10.6	7.4	0.4	12.4
2017	-3.6	5.5	9.7	11.7	17.5	22.9	24.4	24.0	15.7	11.9	6.9	4.6	12.6
2018	5.3	0.7	5.4	16.2	19.2	20.9	22.2	23.3	17.4	13.7	8.0	2.1	12.9
2019	1.3	5.3	10.0	12.6.	14.1	23.5	23.4	24.0	17.7	13.6	10.4	5.1	13.4
2020	1.5	7.4	7.8	12.6	15.9	20.6	22.3	23.3	18.4	12.8	6.4	4.7	12.8
2011-2020	2.2	3.7	8.0	13.1	16.5	21.5	23.4	23.1	17.8	12.4	7.6	3.2	12.7

Table 10. Maximum temperatures (˚C) in Banja Luka in the period 1961-2020

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Max. tem.	22.3	25.2	29.6	31.8	35.4	37.9	41.6	41.8	40.2	34.8	26.7	23.5
Year	2007	2008	1977	2013	1983	2007	2013	2017	2015	1982	2015	1989

Table 11. Decennial precipitation (l/m^2) in Banja Luka

Year/month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
1881-1890	68	44	68	94	89	90	79	95	92	170	73	70	1,031
1891-1900	61	51	76	90	129	112	110	94	79	97	79	74	1,051
1901-1910	68	66	78	120	100	121	84	80	103	111	95	88	1,113
1911-1920	61	41	76	95	115	120	130	97	93	121	91	88	1,129
1921-1930	52	59	53	109	102	162	74	85	108	84	121	66	1,075
1931-1940	72	64	82	103	107	104	74	89	98	137	97	71	1,098
1941-1950	69	60	51	69	71	118	69	42	90	88	119	73	919
1951-1960	65	76	64	88	113	131	104	93	72	92	85	93	1,075
1961-1970	79	78	85	94	95	119	84	92	77	58	91	108	1,060
1971-1980	65	59	54	87	100	108	126	108	86	92	100	68	1,053
1981-1990	67	55	99	80	91	109	77	82	75	73	80	85	972
1991-2000	69	59	84	86	90	116	103	59	111	97	118	102	1,091
2000-2010	78	66	81	98	91	124	74	90	120	74	85	97	1,078
2011-2020	76	81	75	84	135	76	73	76	100	88	74	80	1,017
1881-2020	67	60	73	93	99	118	91	85	93	100	95	83	1,055

Table12. Rainfall (l/m^2) in Banja Luka in the period 2011-2020

Year/month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
2011	52	29	34	38	63	37	113	9	26	62	5	121	589
2012	68	68	5	103	168	70	53	2	92	88	78	146	941
2013	94	116	89	63	120	54	27	36	70	68	156	0	893
2014	52	74	91	214	218	97	139	276	284	117	42	83	1,687
2015	111	91	79	54	118	61	21	23	75	143	86	8	863
2016	110	109	112	71	101	118	126	100	63	76	69	5	1,058
2017	87	100	124	148	92	35	38	43	134	99	106	142	1,148
2018	80	146	117	20	137	103	84	82	70	28	59	86	1,012
2019	85	21	43	105	225	123	59	49	83	24	90	71	978
2020	18	57	57	27	104	62	72	142	107	173	50	135	1,003
2011-2020	76	81	75	84	135	76	73	76	100	88	74	80	1,017

Table 13. Maximum and minimum precipitation (l/m^2) in Banja Luka since 1881

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual
Maximum	175	195	163	223	267	311	274	304	284	302	256	207	1777
In year	1915	1969	1932	1924	1899	1948	1915	1972	2014	1881	1925	1982	1915
Minimum	0	8	4	5	12	15	9	2	6	2	5	0	588
In year	1928	1998	1929	2007	1950	1950	1894	2012	1946	1995	2011	2013	2011

Table 14. Decennial number of sunny hours

Decennium/station	Wien	Zagreb	Banja Luka	Ljubljana	Munich	Osijek	Prijedor
1881-1890	2052	-	-	-	-	-	-
1891-1900	2096	2089	-	-	-	-	-
1901-1910	2007	1961	-	-	-	-	-
1911-1920	1896	1882	-	-	-	-	-
1921-1930	1967	1936	-	-	-	-	-
1931-1940	1873	1879	-	-	-	-	-
1941-1950	2109	2066	-	-	1781	-	-
1951-1960	1950	1910	-	1878	1727	-	-
1961-1970	1934	1937	1803	1845	1702	2024	-
1971-1980	1862	1835	1678	1794	1676	1967	-
1981-1990	1962	1918	1854	1867	1708	1990	-
1991-2000	1979	1985	1883	1941	1751	2080	-
2001-2010	2064	1986	1877	1915	1886	2006	-
2011-2020	2112	-	2082	*2007	*1849	-	1896
1961-2010	1960	1932	1817	1872	1745	2013	-

*The data for Munich and Ljubljana cover the preriod 2011-2015!

Conclusion

The methodology and results of this study represent a basis for more detailed analyses of climate changes as well as developing of agricultural strategies for mitigating the consequences of the global warming in future.

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Analiza klimatskih promjenja u peri - Panonskom basenu i Dinarskom regionu: osnova za buduće poljoprivredne strategije

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Sažetak

Cilj ovog rada je opis procesa klimatskih promjena na odabranim meteorološkim stanicama u Panonskom basenu i Dinarskom regionu. Materijal je prikupljen iz različitih izvora za kompletne periode merenja. Glavni metod rada je poređenje podataka između više stanica za različite periode. Opisani su i ekstremni vremenski događaji. Trendovi temperaturnog režima (povećanje temperature) u periodu merenja pokazuju sličnost na svim stanicama. Protekla decenija bila je najtoplijia sa naglim odstupanjem od prethodnih. Globalno otopljavanje praćeno je čestim pojavama ekstremnih vremenskih prilika. Ova analiza može poslužiti kao osnova za detaljnije proučavanje klimatskih promjena, kao i za razvoj poljoprivrednih i ekoloških strategija prilagođavanja na globalno otopljavanje u budućnosti.

Ključne riječi: klimatske promjene, ekstremni događaji, najtoplija decenija

Original scientific paper

Accumulation of heavy metals in root and shoot of red fescue grown at the flotation tailings dump

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Abstract

To examine the possibility of growing perennial grass species, red fescue (*Festuca rubra* L.) at the extremely unfavorable conditions, the experiment with fertilization was set up on flotation tailings dump in the Central Serbia. Flotation tailings are often completely without plant cover. Therefore, phytoremediation is one of the most economically and ecologically justified ways to recover such substrates. In the examined tailing, content of nitrogen, phosphorus and potassium was very low, while the content of all tested heavy metals (Cu, Cd, Ni, Pb, Zn) except Mn, was above the allowed amounts. As a consequence of high content of these elements in substrate, the concentrations accumulated in the roots of red fescue were at the levels that are toxic for most plant species. The contents of heavy metals were 50-80% higher in roots than in shoots of red fescue and 25-50% higher in the control plants comparing to fertilized one. The most accumulated element in plant material was Mn with concentration of 2725 mg/kg in root and 628 mg/kg in shoot of control plants respectively 2183 mg/kg in root and 911 mg/kg in shoot of fertilized plants. The translocation coefficient of all metals was less than 1, which means that the red fescue accumulates most of the adopted elements in the root.

Key words: red fescue, flotation tailing, heavy metals, phytoremediation

Introduction

The mining activities have a great impact on increasing concentration of heavy metals in the soil, which has a negative impact on human's and animal's health (Kumar Patra et al., 2021).

As a result, soils near to mine have altered mechanical, physical and chemical characteristics, very poor nutritional possibilities and increased content of heavy metals. Such soils have very low quality and are often completely bare, without any vegetation (Gibbs & Salmon, 2005). Due to the lack of vegetation, such soils are subject to fluvial and aeolian erosion, which spread the contamination in the form of dust over long distances. Therefore it is necessary to pay great attention to improve the quality of such lands. Phytoremediation has a positive effect on reducing the intensity of erosion and the content of heavy metals in tailings. It involves the sowing of plants that have the ability to grow on substrates of these characteristics and which can reduce the content of pollutants in the substrate through their transport and accumulation (Pusz et al., 2021). Plants that are tolerant to the increased metal content in the substrate affect the immobilization of these elements, and by covering the surface, affect the revival of degraded surfaces. Red fescue, thanks to low requirements in terms of substrate quality and growth ability on soils with increased content of lead (Pb), zinc (Zn) and cadmium (Cd), is potentially good candidate for species to be used for phytoremediation purposes (Gomez et al., 2020). The aim of this research was to investigate the possibility of growth of red fescue on the flotation tailings and its accumulation capacity on a substrate with increased content of heavy metals.

Material and Methods

For the purposes of the research, the experimental field was set up at the flotation tailing landfills of the mine where lead (Pb), copper (Cu) and zink (Zn) are extracted. The experiment was set up on April 1st, 2021 on the slope of the flotation tailings according to completely random block system with 3 repetitions. Sowing was performed on isohypses with row spacing of 50 cm. The area of each individual plot was 10 m^2. Red fescue K-14 from the Institute of Forage Plants in Kruševac was used. During sowing half of the experimental plots were fertilized with, NPK mineral fertilizer of the formulation 20:20:20 in the amount of 100 kg/ha and solid organic fertilizer based on chicken manure was added in the amount of 700 kg/ha (declared fertilizer formulation 4:4:4 NPK). The other half of plots were control plots without fertilization. The sampling of plants for testing the content of adopted metals in the roots and shoots was performed on October 27, 2021. On that occasion, a sod with about 2 kg of tailings was taken.

The microclimatic conditions at Rudnik during 2021 since the establishment of experiment are shown in Table 1 (Road Whether Information System, 2022). The total amount of precipitation during this period was 444.2 mm, while the average air temperature was 15.3°C. The total annual rainfall in this area is 951 mm, and the average annual air temperature is 10.1°C (RHI, 2021).

Table 1. Average air temperatures and total monthly precipitation during April-October 2021.

2021.	April	May	June	July	August	September	October
Temperature (°C)	6.35	14.2	19.7	23.6	17.8	16.2	9.58
Precipitation (mm/m^2)	26.9	120.5	63.6	121.7	58.4	41.9	11.2
Temperature average = 15.3°C				Total precipitation = 444.2 mm/m^2			

Chemical analysis of tailings was performed in the laboratory pH values were determined in water and 1M KCl, 1:2.5 w/v (ISO 10390:1994). Total organic C in the soil was determined by soil sample mineralization with boiling dichromate and sulphuric acid mixture and subsequent titration of excessive dichromate with Mohr salt solution (Pansu & Gautheyrou, 2006). N_{tot} (total nitrogen) in soil and plant samples was determined by semi-micro Kjeldahl method (ISO 11261:1995). Available phosphorus (P_2O_5) and potassium (K_2O) were extracted according to Egner et al (1960), by extraction with AL solution (mixture of 0.1M ammonium lactate and 0.4M of acetic acid). Available P_2O_5 was determined by molybdenum blue method via spectrophotometer (580 nm, Shimadzu UV-1900i). The K_2O concentrations have been determined by flame emission spectrophotometry on 766.5 nm, i.e. on the atomic absorption spectrophotometer Shimadzu AA-7000, according to calibration curve. The extracts for measuring the content of heavy metals (Pb, Zn, Ni, Cu, Cd, Mn) was obtained by digestion of 5 g of tailings in the presence of concentrated HNO_3 at 150ºC with the addition of 30% H_2O_2 (Jones & Case, 1990). After cooling down, the samples were filtered, and the heavy metal content was determined by measuring the absorbances of the extract using the atomic absorption spectrophotometer (Shimadzu AA 7000) and comparing them with the absorbances of standard solutions of known concentration.

The collected plant material was separated into roots and leaves and thoroughly washed with tap water. Afterwards it was transferred to an ultrasonic bath and then additionally washed with distilled water. After washing, the plant material was chopped and dried to constant weight in an oven at a temperature of 60°C. The total metal content of the plant material was determined after mineralization of 1 g of the sample with 10 ml of concentrated HNO_3 by heating to 150°C (Jones & Case 1990). After filtration, the absorbances of heavy metals (Pb, Zn, Ni, Cu, Cd, Mn) were measured using an atomic absorption spectrophotometer

(Shimadzu AA-7000). The concentrations of all metals were calculated according to standard curves obtained on the basis of the absorbances of a series of standard solutions of known concentration.

The data are calculated as the arithmetical means of three replications of each treatment,with the standard deviation (SD).

Results and Discussion

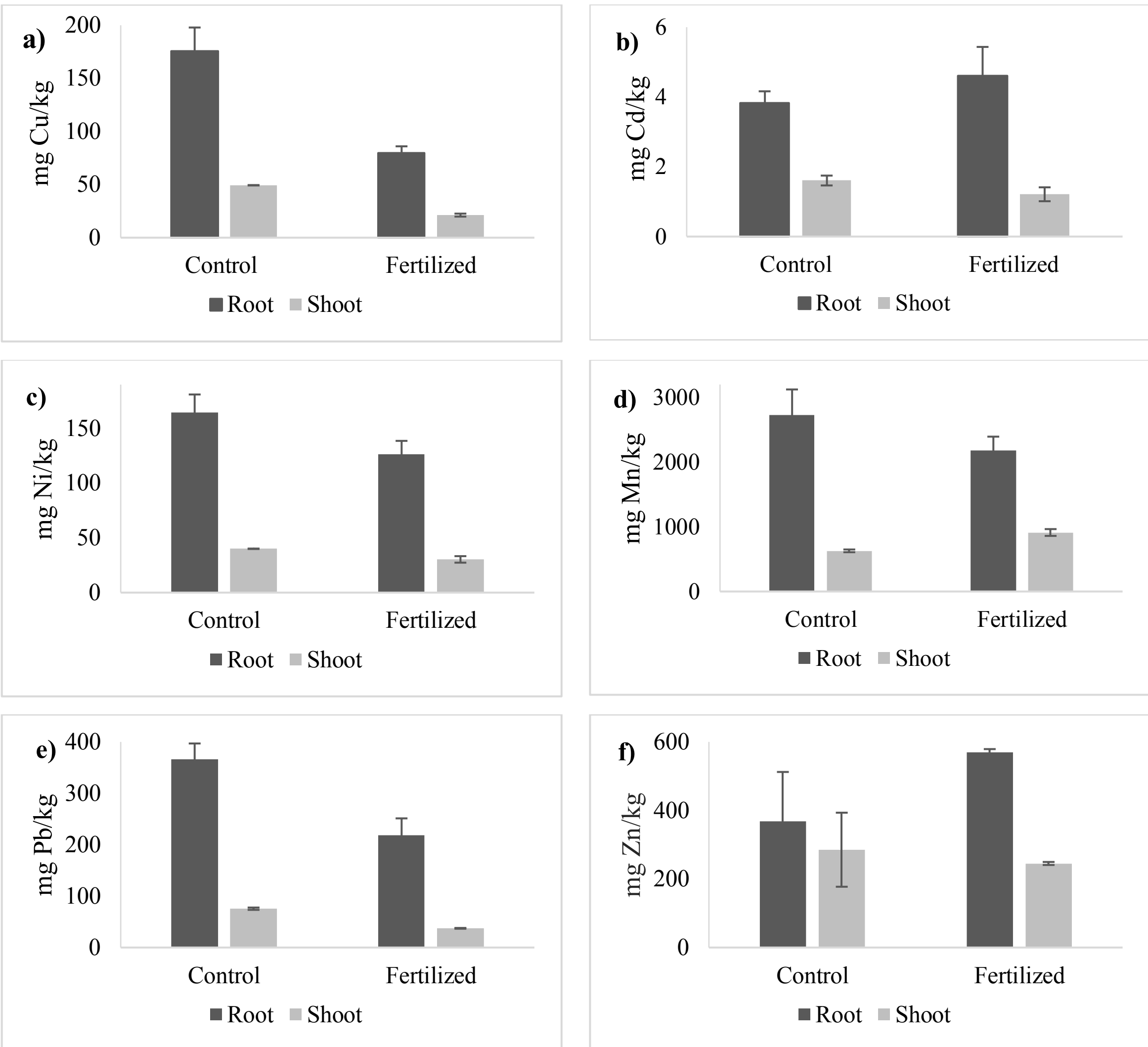
The chemical reaction of tailings is neutral–with very small differences in mean values between active (pH in H_2O) and substitutional acidity (pH in KCl). Such a small difference between active and substitutional acidity is the result of reduced presence of mineral and organic acids, as well as hydrolytic salts (Vukeljić, 2002; Andrejić, 2020). In our trial differences of pH were also very small (0,17 in fertilized and 0,26 in non-fertilized treatments). The total nitrogen content and contents of easily accessible forms of phosphorus and potassium in tailings was very low, below the optimal level for plant nutrition, which also indicates a deficiency of humic substances (Marchner, 1995).

Table 2. Chemical properties of substrate and maximum permitted amount (MPA) of elements in the soil (Official Gazette of RS, No 30/2018)

Parameter	Fertilized	Control	MPA (mg/kg)
pH in H_2O	7.02	6.85	/
pH in KCl	6.85	6.59	/
N (%)	0.002 ± 0,0001	0.005 ± 0,001	/
P_2O_5 (mg/100 g soil)	0.93 ± 0,0004	1.08 ± 0,006	/
K_2O (mg/100 g soil)	10.30 ± 0,86	8.49 ± 0,04	/
Organic C (%)	2.73 ± 0,80	3.71 ± 0,25	/
Pb (mg/kg)	665.4 ± 21,9	655.3 ± 0,86	85
Zn (mg/kg)	1112 ± 33,9	1217 ± 49,3	140
Ni (mg/kg)	203.6 ± 3,26	217.2 ± 8,47	35
Cu (mg/kg)	308.5 ± 5,07	443.4 ± 2,30	36
Cd (mg/kg)	6.40 ± 0,03	7.33 ± 0,63	0.8
Mn (mg/kg)	953.9 ± 29,4	884.6 ± 50,9	2000

Our research is in agreement with other authors Popović, 2015; Popović et al., 2008; 2011. Studies Lakić et al. (2020) of the content of microelements and heavy metals in degraded soil showed that it contained Cd in significantly higher value than the permitted limit.

The high content of organic carbon in tailings is the result of presence of the organic additive xanthate, which is used with the aim of improving the separation of lead and zinc ore (Shen et al., 2016). The content of all heavy metals, in tailings, with the exception of manganese was above the maximum permitted amounts for arable land (Table 2). The content of Zn in tailings was 10 times higher than the maximum allowed amounts, while the content of Ni and Cu was 3-4 times higher, and Pb about 6 times higher. The content of Zn, Ni, Cu and Cd was lower on fertilized plots compared to control ones, while the situation was reversed with the content of Pb and Mn. Very high concentrations of Pb, Zn and Cu were expected because these are the main elements extracted from the ore. Based on the data from Graph 2, it can be concluded that the red fescue accumulates all the elements in a much higher concentration in the root compared to the shoot. The content of heavy metals in the roots of plants is directly affected by their concentration in tailings and is within the toxic values for most plant species (Kabata-Pendias, 2011).



Graph 1. Content of heavy metals in the roots and shoots of the red fescue

Concentrations of Cu, Ni, Mn and Pb were lower in plants taken from fertilized plots, while the content of Cd and Zn in the roots of the red fescue was slightly increased compared to the control without fertilization. The cadmium content was higher in the roots than in shoots in both the fertilized (74% percent) and control plants (58% percent). In the case of fertilized plants, the cadmium content in the roots was 17% higher compared to the control plants. In the shoots of the fertilized plants 39% less of this element was accumulated in comparison with the control plants. The concentration of nickel in both treatments was 76% higher in the roots compared to the shoots. Control plants contained 23% more nickel in the roots compared to fertilized plants, and 24% more of this element in the shoots. The manganese content in the roots compared to its content in the shoots was 77% higher in control plants, and 58% higher in fertilized plants. In shoots of fertilized plants 31% more manganese was accumulated compared to control plants, while the content of this element in the roots of fertilized plants was 20% lower compared to non-fertilized plants. The concentration of lead in plant material is very high, which is in line with its concentration in tailings and is within the toxic values for most plant species (Kabata-Pendias, 2011). Most of the lead, as well as other elements, was accumulated in the roots of red fescue. In control plants, the lead content in the roots was 79% higher than in the shoots, while in fertilized plants this percentage was slightly higher (83%). Control plants accumulated 40% more lead in roots and 50% more in shoots compared to fertilized plants. The content of zinc in the roots of control plants was 22% higher than its content in the shoots, while in fertilized plants this difference was significantly higher and amounted to about 57%. The control plants of accumulated 35% less zinc in the roots compared to fertilized ones. Zinc concentration in the shoots was 14% higher in the control plants compared to fertilized plants. Based on standard deviation we conclude that there was no significant variation in the content of tested elements, except in the case of zinc. Zinc content in the control plants showed significant variation with SD ± 144 in the root and 108 in the shoot. Translocation coefficient (TF) was calculated for each tested element in both treatments. Translocation coefficient represents the ratio of the concentrations of the investigated element in the plant root and shoot (Baker, 1981; Karami et al., 2011). TF<1 was established for all elements in control and fertilized plants. These values of the translocation coefficient indicate a reduced transport of elements from the underground to the aboveground part of plants. Because of that red fescue can be classified as an excluder. Excluders are plant species that accumulate low concentrations of heavy metals and other harmful substances in their shoots, although they are present in the substrate in a much higher concentration (Alagić et al., 2014).

Conclusions

The content of all tested heavy metals (Pb, Zn, Cu, Ni, Cd), except Mn, in tailings was above the maximum allowed amounts. The concentration of Pb, Zn, Cu, Ni and Cd in tailings was lower on fertilized plots compared to non-fertilized ones, while the concentration of Mn was increased on fertilized plots. Heavy metals accumulated in plant organs were in quantities that are toxic to the most plant species. The increased concentration of heavy metals in plant organs is a consequence of their high content in the substrate. The concentration of most elements was significantly higher in control plants compared to plants fertilized with organic and mineral NPK fertilizers. Based on chemical analyses of samples of red fescue grown on the flotation tailings, we conclude that the highest concentration of all six tested elements was in the roots of plants. Red fescue can be classified as an excluder plant for all tested elements. This indicates on possibility of using of red fescue for phytoremediation. While the further research is needed, based on these findings we can conclude that fertilization with mineral and organic fertilizers can potentially have positive effects on reducing accumulation of heavy metals in roots and shoots of plants Therefore, the recommendation for further research is to assess the influence of organic and mineral fertilizers on the growth of red fescue and the accumulation of heavy metals on substrates with such unfavorable physical and chemical characteristics.

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Original scientific paper

The effects of fertilization treatments on phytoremediation properties of tall fescue (*Festuca arundinacea* Schreb.)

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Abstract

The goal of this research was to examine the influence of different types of fertilization treatments on phytoremediation qualities of tall fescue (*Festuca arundinacea* Schreb.). The experiment was set up in pots in the green house of the Faculty of Agriculture - University of Belgrade. The flotation tailings from the mine of lead, copper and zinc, which has very unfavourable chemical characteristics, was used as substrate. The pots were treated with 3 fertilization treatments (mineral fertilizer, organic fertilizer and mixture of this two fertilizers) and 2 rates of irrigation (50% FWC and 75% FWC). After each mowing the mowed biomass was measured and chemically analysed in order to determine the absorbed amounts of most important nutrients. The best result regarding the plant height of 56 cm was recorded in plants treated with organic fertilizer, while the ones treated with the mineral fertilizer showed the lowest growth (16.3 cm). Organic fertilizer treatment also showed the best biomass yield (6.1 g) while the plants treated with the mixture of organic and mineral fertilizer had the poorest biomass yield of 0.49 g. Nutrient accumulation showed significant variation with the different types of fertilization treatments. Highest amounts of nitrogen were recorded in plants treated with mineral fertilizer while the amounts of potassium and phosphorus were highest in plants treated with the organic fertilizer. Irrigation had no significant effects on the biomass yield nor the nutrient amounts in plant material.

Key words: tall fescue, phytoremediation, fertilizer, irrigation, yield

Introduction

The increase of industrial activities directly degrades the environment by increasing concentration of waste materials in the ecosystems (Xi et al., 2018). Activities such as ore extraction, smelting, agriculture, burning of fossil fuels, etc. have a big impact on environmental pollution (Yildirim & Sasmaz, 2017). The tailings left behind after the minerals have been extracted are characterized by high concentrations of heavy metals. By depositing large quantities of tailings on nearby soil mining directly raises the amount of heavy metals both in the soil and the surrounding waters (Kumar Patra, 2021).

The result is degraded soil of changed qualities with very low nutritive abilities and scarce vegetation. Phytoremediation is lately becoming increasingly recognized as a method for amending degraded areas (Sarwar et al., 2017; Mahar et al., 2016). Phytoremediation is a process which uses plants in order to stabilize and restore the degraded areas (Mendoza et al., 2015). Plant species most suitable for phytoremediation are ones that are both fast growing and form significant aboveground biomass (Hu et al, 2015). As grasses (Poaceae) are both fast growing and form large quantities of biomass they are suitable candidates to be used in phytoremediation. Many grass species are known to be tolerant of toxic materials present in soil (Kumar Patra et al., 2021). Khashij et al. (2018) found that the root extracts of tall fescue had a positive effect on the number of rhizospheric bacteria which increases the effects of bioremediation. Tall fescue is able to grow on substrates with increased concentrations of heavy metals including lead, copper, zinc nickel and cadmium (Lou et al., 2017; Kluk & Steliga, 2016).

The goal of this research was to assess the influence of different types of fertilizers and irrigation treatments on yield of tall fescue used in phytoremediation of degraded soils.

Material and Methods

Experimental conditions were semi controlled. The experiment was set up in the green house of the Faculty of Agriculture - University of Belgrade (Table 1). Tall fescue cultivar K-20, obtained from Institute for forage plants in Kruševac was grown in pots with a height of 10 cm and the diameter of 12 cm. The three experimental replications were set up on 06.10.2021. The experiment examined the effects of three different fertilization treatments (solid organic NPK fertilizer, solid mineral NPK complex fertilizer of 20:20:20 formulation and mixture of the organic and the mineral fertilizers) along with two irrigation regimes (50% field water capacity (FWC) and 75% FWC). The organic NPK (formulation 4:4:4) fertilizer was obtained by fermenting the poultry and the cattle manure mixed with plant and mineral bedding which is then dried, grinded and pelleted. Flotation tailings obtained from the lead, copper and zinc mine were used as substrate for growing the tall fescue. Tailings are characterized by very unfavourable chemical characteristics along with increased concentrations of heavy metals (Table 1).

Table 1. Chemical characteristics of substrate

Parameter	Concentration
pH u H_2O	6.58
pH u KCl	6.47
N (%)	0.002
P_2O_5 (mg/100g soil)	BDL*
K_2O (mg/100g soil)	0.56
Organic C (%)	2.78
Pb (mg/kg)	1892
Zn (mg/kg)	3544
Cu (mg/kg)	893
Cd (mg/kg)	7.33

*BDL – below detection limit

Before the setting up of the experiment the field water capacity (FWC) of tailings was determined in the laboratory using the porous plate method. FWC value represents the maximal amount of water which can be added during irrigation (Belić et al., 2014).The pots were filled with 1300 g of tailings after which a certain amount of fertilizer was added to each pot. Organic fertilizer in amount of 25 g was added to the first set of 6 pots and 5 g of mineral NPK fertilizer to the second set of 6 pots. The mixture of 12.5 g of organic and 2.5 g of mineral NPK fertilizer was added to the last set which also had 6 pots. The necessary amounts of fertilizers were calculated based on their nutritional content and the recommended amounts for 1 ha of soil. The required amount of fertilizers was determined based on mass percentage. Equal amount of 2% of pure fertilizers was added to each pot.

After the pots were filled with mixture of tailings and fertilizer, 0.67 g of seeds were sown in each pot. Seeds were put on wetted substrate and covered by 1 cm of sieved tailings. Half of the plants were irrigated by higher amounts of water (75% FWC) while the other half was watered by lower amounts (50% FWC). Every 4 days the amount of spent water was determined by measuring the mass of pots and the same amount of fresh water was added. Plant height was also measured at the same time. When the height of the plants reached around 20 cm the aboveground biomass was cut to the height of 5 cm which simulated the cutting in the field conditions. The mowed biomass was measured and dried in the oven at 60°C to constant mass. Dried samples were used to determine the contents of nitrogen, potassium and phosphorus in the laboratory. The Kjeldahl method was used to determine the content of nitrogen (Bremner, 1996). The content of potassium was determined using the flame emission spectrophotometry on the Shimadzu AA-7000. Plant material was mineralized in 10 ml of concentrated nitric acid (HNO_3) at 150°C (Jones & Case, 1990). Same extracts used for determining potassium content were used to detect phosphorus concentrations via spectrophotometric measurements of optical densities (Gee et al., 1954). The absorbances were measured using colorimeter (Iskra MA 9507). Potassium and phosphorus concentrations were read from the standard curve which was constructed using absorbances of standard solutions of known concentrations. The obtained data was processed using two-way factorial analysis of variance (fertilization, irrigation) in software Statistica 10. The nutrient content of plant material was shown using arithmetic means and standard deviations of 3 cuttings. Results of the experiment are shown cumulatively as a sum of reached heights and biomass and average values of absorbed nutrient in 3 cuttings.

Results and Discussion

There were statistically very significant differences in plant height and biomass yield between different fertilization treatments (Table 2). Plants fertilized with organic fertilizer showed the best results with the plant height of 56.3 cm and biomass yield of 6 g. The shortest plants were those fertilized with NPK mineral fertilizer while the plants fertilized with the mixture of organic and mineral fertilizer had lowest biomass yields. No statistically significant differences were detected between different irrigation regimes and the reached heights were 29.1 cm in less irrigated plants (50% FWC) and 31.9 cm in more irrigated plants (75% FWC). Similarly, the biomass yields were 2.61 g in less irrigated plants (50% FWC) and 2.49 g in more irrigated plants (75% FWC). Combined effect of the different fertilization and irrigation treatments on the plant height and biomass yield showed no statistically significant differences (tab 2). These results are in accordance with the previous research which found that the wheat grown on substrates contaminated by heavy metals showed the best results when grown with the addition of solid organic fertilizers and humic acids (Shtangeeva et al., 2004).

Table 2. Effect of fertilizers on the height and biomass of tall fescue

Fertilizer (A)	Plant height (cm)	Biomass (g)
O.	56.3	6.1
M.	16.3	1.03
O.+M.	18.8	0.49
Irrigation (B)		
50% FWC	29.1	2.61
75% FWC	31.9	2.49
ANOVA		
A	**	**
B	NS	NS
A×B	NS	NS

NS - non significant; * significant influence; ** very significant; O - organic fertilizer; M - mineral fertilizer; O+M mixture of organic and mineral fertilizers; FWC - field water capacity

Nutrient content of plant material varied significantly across different fertilization treatments (tab 3.). Plants fertilized with mineral NPK fertilizer had the highest nitrogen concentration of 5.47% in plants irrigated with more water (75% FWC) and 4.98% in plants irrigated with less water (50% FWC). Amounts of nitrogen varied significantly in different cuttings (SD = 1,75). The average nitrogen content in young plants in the early stage of vegetation is about 3.5% (Hannaway, 1999). In our samples nitrogen content was above the average content which is consequence of added fertilizers. Exception was the nitrogen in plants treated with organic fertilizer and lower water amount. While the recorded amount of nitrogen was lowest in plants treated with organic fertilizer, it was very stable and did not show significant variation. Phosphorus was accumulated in various amounts, from very small ones of 0.01% in plants treated with mineral fertilizer and irrigated with lots of water (75% FWC) to the 0.1% in plants treated with organic fertilizer and same amounts of water. The content of phosphorus was significantly lower than the average values for tall fescue that are about 0.37% (Hannaway, 1999). The higher phosphorus content in plants treated with organic fertilizer is in line with the results of Cheng et al., (2010) who, based on experiments with 13 different types of fertilizers, measured the highest phosphorus content in plants treated with organic fertilizer obtained after processing chicken manure. Standard deviations showed that the amount of phosphorus did not very significantly between different replications.

Table 3. Nutrient content of tall fescue

75% FWC	N (%)	P (%)	K (%)
O	3.51 ± 0.36	0.12 ± 0.003	6.99 ± 0.25
M	5.47 ± 1.75	0.01 ± 0.001	1.73 ± 0.74
O+M	3.96 ± 1.43	0.07 ± 0.002	1.35 ± 0.45
50% FWC			
O	3.31 ± 0.34	0.07 ± 0.002	6.19 ± 0.36
M	4.98 ± 1.75	0.02 ± 0.002	1.03 ± 0.5
O+M	4.56 ± 1.50	0.03 ± 0.004	0.97 ± 0.45

Legend: O - organic fertilizer; M - mineral fertilizer; O+M organic and mineral fertilizers combined; FWC - field water capacity

Plants treated with organic fertilizer accumulated largest amounts of potassium in the concentrations of 6.99 (75% FWC), and 6.19% (50% FWC) with low variations. Lowest potassium concentration of 0.97% was recorded in plants treated with the mixture of organic and mineral fertilizer and irrigated with lower amount of water (50% FWC). While irrigation treatment didn’t have significant effects on the nutrient content in the plants, it was noticed that the concentrations of all elements were lower in plants irrigated with lower amounts of water (50% FWC). Exception was the nitrogen in plants fertilized with the mixture of organic and mineral fertilizer where the concentration in plants watered with lower amounts (50% FWC) was slightly higher.

Conclusion

This experiment showed that in indoor conditions tall fescue has the ability to grow on substrates with increased amounts of heavy metals and poor chemical qualities such as tailings with the addition of certain amounts of organic matter. Best results regarding plant height and biomass yield were observed in plants treated with organic fertilizer while the plants treated with mineral fertilizer and mixture of organic and mineral fertilizer showed significantly poorer. Plant height and biomass yield were not affected by irrigation treatments. There were statistically significant differences in nutrient assimilation between different fertilization treatments, where the highest nitrogen concentration was recorded in plants fertilized with mineral NPK fertilizer, while the highest potassium and phosphorus concentrations were recorded in plants treated with organic fertilizer. Nutrient content in the plants was not affected by irrigation treatments.

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Original scientific paper

Influence of shading net on chlorophyll content, relative water content and weight of lettuce

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Abstract

The aim of this research was to determine the influence of light intensity on the growth and development of lettuce (Lactuca sativa L.), through changes in morphological and physiological parameters using a shading net with 50% density in autumn production. Cultivar Zeralda F1 is a type of butter lettuce that was used for late autumn production. Since the autumns have been warm for the last few years due to global warming, the use of shading net with 50% density in production of lettuce in greenhouse was tested. Chlorophyll content, relative water content (RWC) and weight of lettuce head were examined. The SPAD value of chlorophyll content and weight of lettuce head were shown to perform better in the shade net variant compared to non-shaded lettuce plants.

Key words: lettuce, shading net, chlorophyll content, RWC, weight

Introduction

Lettuce is used in the nutrition throughout the whole year, because there are a large number of varieties and hybrids that are successfully grown throughout the all seasons (Zdravković et al., 2014). During spring and autumn, lettuce is grown outdoors, while during winter it is grown in a protected area (greenhouses) (Ilić, 2018). Early spring, late autumn, and even winter lettuce production can be successfully achieved in high tunnels (greenhouses) (Gent, 2002). Depending on the location, lettuce production may be limited during the late spring and summer months due to unfavorable temperatures (day /night air temperature higher than 30/16 °C), but now even during autumn, considering current climate changes. High temperatures then optimal for the variety increase the risk of the formation of unwanted flowering, may lead to burns on the tops of the leaves and may lead to a change in the color of the main nerve on the leaf (Jenni and Yan, 2009). With these physiological disorders, the leaves may become bitter (Zhao and Corey, 2009) and the heads may lose their firmness (Fukuda et al., 2011). The technology of using shading (photoselective) nets in production is a new agro-technical concept that combines physical protection of plant species with different filtering of solar radiation (Ilić et al., 2015). In addition to affecting the amount of solar radiation, the nets can also transform radiation from direct to diffused light, allowing light to reach the inner leaves of the plant (Stamps, 2009). This prevents leaf burns, offers a moderate cooling effect and improves pest and disease control (Shahak, 2008). Shading nets can increase the proportion of scattered light and absorb several spectral ranges, affecting light quality (Perez et al., 2006; Stamps, 2009). The part of the light passing through the openings of these nets remains unchanged in quality, while the light reaching the surface of the net is spectrally modified and scattered (Appling, 2012). Therefore, the combination of light diffusion and spectral manipulation can alter the desired characteristics in plant species below the net (Ilić et al., 2018). This technique is increasingly used in production in protected areas (Rajapakse and Shahak, 2007; Shahak et al., 2008), however, its effects on yield and secondary metabolism of vegetables are not yet fully known (Fu et al., 2012).

Material and Methods

The experiment was set up under controlled conditions in greenhouse of the Faculty of Agriculture in Banja Luka. Greenhouse have 100 m2 surface without additional heating. Lettuce seedlings cv. Zeralda F1 produced from nursery production up to the stage with 4-5 fully developed leaves were selected for testing. The plants were planted in a nutrient substrate in pots with a volume of 1 L. The nutrient substrate "Fantazija", B&H was used for cultivation (mixture of black and white peat 60: 40%; pH of the substrate is 5.3-6.1 and contains 20 kg/m^3 of clay and 1 kg/m^3 PG mix). During cultivation, standard agrotechnical measures of protection and irrigation were applied using organic production principles. Lettuce was transplanted on September 15, 2020 and nurtured for up to 60 days until it reached technological matureness, after which the samples were forwarded to the laboratory of Faculty of Agriculture in Banja Luka for analysis. Plants were planted in two groups, the first group had 50 plants without shading net (control plants) and the second had 50 plants with shading net with 50% density.

In this study, the examined parameters were:

- chlorophyll content (SPAD value)

- relative water capacity (%)
- weight of lettuce head (g)

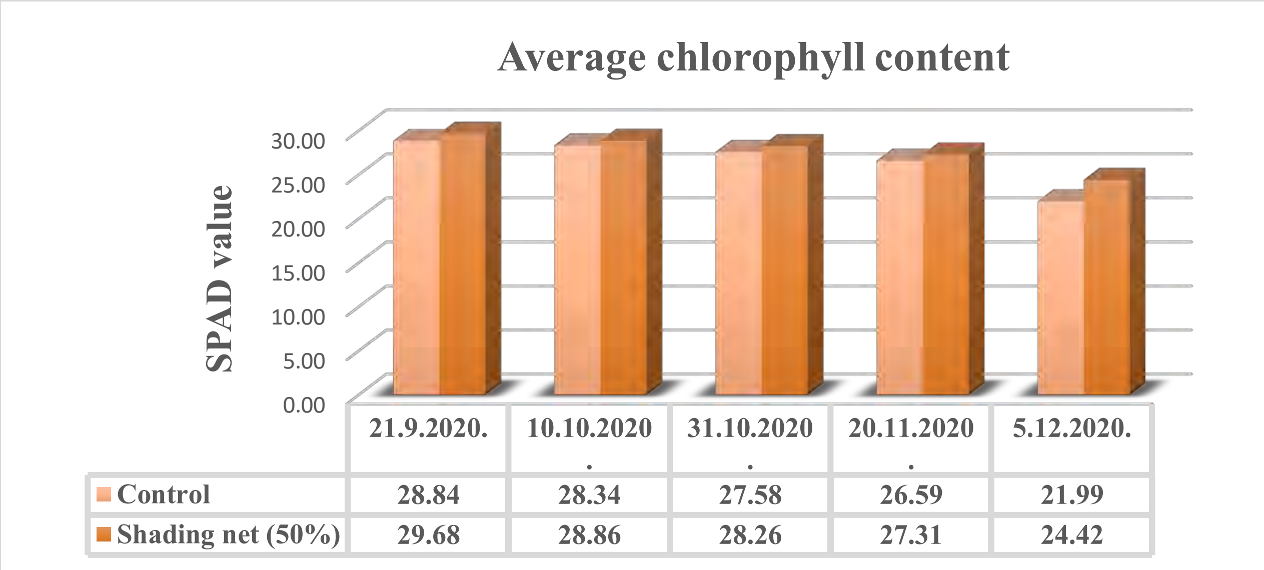
Leaf sampling was performed by taking and mixing leaves from all levels of the leaf rosette to obtain a representative sample composed of leaves of different ages. In the phase of technological maturity, i.e. in the phase of forming the lettuce head, in order to determine the mass of lettuce heads and leaves, the digital scale Constant was used. The SPAD meter was used for measures of chlorophyll content with the difference between the transmittance of a red (650 nm) and an infrared (940 nm) light through the leaf, generating a three-digit SPAD value (Uddling et al., 2007). To determine the relative water content of the leaf Relative Water Content, (RWC), lettuce leaves were weighed immediately after picking where the fresh leaf mass (FW) was measured. The weighed leaves were then placed in petri dishes filled with cotton wool and distilled water, after which they were left for 24 hours at room temperature in semi-darkness until the leaves were completely hydrated. The leaves were then weighed to give a leaf mass in full turgid weight (TW). The weighed leaves were placed in paper bags with tweezers and dried in an oven for 24 hours at 105°C. After 24 hours, the weight of the dry leaf (DW) was weighed. All weighed average leaf weights and mass of the lettuce (yield) were measured with an analytical balance with an accuracy of 0.0001 g. The final RWC value was calculated according to the formula for determining the RWC using the FW, TW, and DW values: RWC (%) =[(FW - DW)/(TW - DW)]x100 (Weatherley, 1950). Statistics were processed in excel using t-Test: Two-Sample Assuming Equal Variances.

Results and Discussion

Lettuce production depends on light (Dufault et al., 2009), namely light quality and light intensity which is also changed by situation with current climate changes (Ilić and Fallik, 2017). An important indicator of the influence of light is the content of chlorophyll in the leaf as well as RWC. Extremely strong light often decreases chlorophyll content in plant leaves because of the inhibition of chloroplast formation. In contrast, under weak light, the number of chloroplasts per unit leaf area in plant leaves drops, but the larger chloroplasts results in increased chlorophyll content in plant leaves (Fu et al., 2012).

The highest chlorophyll content for both variants was expected in the first measurement on 21.9.2020. since later daylight lasted shorter. The control had an average chlorophyll content of 28.84 SPAD value, while the shading net variant had a 29.68 SPAD value. The lowest chlorophyll content for both variants was at the last measurement when daylight was shortest

(Graph 1.), in control plants on average 21.99 SPAD value of chlorophyll content, and in plants below shading net 24.42 SPAD value, which is almost 2.5 SPAD value higher than the control.



Graph 1. Average chlorophyll content (SPAD value) in lettuce leaves

Limantara et al. (2015) presented study were they evaluated the chlorophyll content of green leafy vegetables collected from different supermarkets with SPAD value of lettuce of about 15, which is much lower in comparation with results in this reserach. Clemente et al. (2021) also examined chlorophyll content in lettuce set in twenty-three lines and six cultivars of lettuce from UFU's Biofortified Lettuce Breeding Program where the average SPAD value of chlorophyll content was 31.30. Their resuls are more similar to the results in this research.

Table 1. Average of relative water capacity (%) and average weight of lettuce head (g)

Variety	**RWC (%)**	**weight (g)**
Control	**90.69 ± 3.35**	**58.96 ± 19.95**
Plants under a green shade net of 50% density	**82.73± 6.47**	**60.88 ± 13.49**

Analysis of relative water capacity (RWC) under the shade netting with 50 percent shade and lettuce grown without shade netting indicated statistically and practically significant difference ($p<0.001$). The average RWC in control plants was 90.69%, while in plants below shading net it was lower (82.73%). Agüero et al. (2008) investigated postharvest changes in water status and chlorophyll content of lettuce (*Lactuca sativa* L.) and their relationship with overall visual quality and in which heads of butter lettuce were grown and harvested. Greenhouse lettuce heads were cultivated by applying “mulch” technology with a black plastic film separating each plant from the soil and were harvested at optimal maturity after reaching a marketable size (approximately 24 to 30 leaves per head) in which the average RWC was 48% , which is a much lower value compared to the results in both variants in Table 1. He et al. (2001) examined limitations to photosynthesis of lettuce grown under tropical conditions: alleviation by root - zone cooling where they examined leaf RWC (%) measured at pre - dawn and midday of lettuce plants grown at 20°C, where RWC in sunny and on a cloudy days was between 60.1% and 95.2%, which is similar to the results in Table 1. Analysis of head weight under the shade net with 50 percent shade and lettuce grown without shade netting indicated no statistically or practically significant difference ($p=0.574$). Plants without shading net had an average head weight of 58.96 g, while plants growing below shading net had an average head weight of 60.88 g. Wurr and Fellows (1991) examined the influence of solar radiation and temperature on the head weight of crisp lettuce and in their study the average weight of lettuce heads varied from a minimum of 426 g to 1.029 g where the shading net was used. Taking the study compared to the autumn production applied in this study, the mass of lettuce heads is very low (Table 1), and that the yield itself is not up to standard for producers.

Conclusion

Use of shading net of 50% density in autumn lettuce production in the greenhouse showed that the chlorophyll content below the shading net in the period of testing the plants was higher, which is unexpected, considering that the day is shorter and that use of nets is optimal during the summer. The average RWC in control plants was higher than the RWC of plants below shading net. Average weight of lettuce head was small compared to the average weight that would have a significance for producers in the autumn production in the greenhouse.

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Preliminary report

Influence of natural and enriched pyrophyllite on onion yield (*Allium cepa* L.)

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Abstract

The effect of onion *(Allium cepa* L.) watering with a suspension of water and pyrophyllite and a suspension of water and enriched pyrophyllite with nitrogen (N) from urea was investigated. Pyrophyllite originated from the site Parsovići, Konjic, AD Harbi, B&H. The suspension used in treatment 1 was composed of water and pyrophyllite (4 g/L water) and in treatment 2 of water and enriched pyrophyllite containing 12.5% N (4 g/L water). The treatments 1 and 2, compared to the control treatment with only water, contributed to the increase in mass of onion heads by 18.31% and 24.09% ($p<0.01$), respectively. Achieved results indicate that the amount of N in the soil was a limiting factor as well as on the justification of the use of enriched pyrophyllite which, at the same time, has the function of a soil conditioner

Key words: pyrophyllite, urea, watering, onion, yield

Introduction

Pyrophyllite is a type of phyllosilicate minerals, from the group of stratified silicates, based on a combination of tetradar (T) and octahedral (O) plates. The basic 2:1 structure (T-O-T), with silicon in tetrahedral sheets and aluminum in octahedral sheets characterizes pyrophyllite [$Al_2Si_4O_{10}(OH)_4$]. Poor cohesion between the layers, as a consequence of weak Van der Waals forces, causes pyrophyllite to have a tendency to easily disrupt the structure, ie. that the layers can easily split off along the basal plane. It got its name from the Greek words *piro*-fire and *phillon*-leaf, because when annealed it turns to be fanlike. The color varies depending on the content of oxides and ranges from white, gray-white, purple-white, to gray-green. The Mohs hardness is 1-1.5. It has buttery tuch like talk. It is rich in silicon and aluminum, which is why it belongs to the group of non-metallic aluminosilicate minerals. Pyrophyllite has a neutral electromagnetic charge, and a pH between 7.5-8.5. Its specific weight is 2.7-2.9 g/cm^3 (Adamović *et al.*2020).

It contains electrolytes: sodium, potassium, calcium, magnesium, iron, etc. necessary for the growth and development of plants and free ions that give it the ability to detoxify and acts as antioxidants. In the pyrophyllite shist ore (abbreviated PIR) dominant minerals are pyrophyllite, sericite and kaolinite. In a smaller percentage it also contains quartz, calcite, magnesite, dolomite, illite and montmorillonite.

The electro-neutral siloxane surface (basal surface of the silicate tetrahedron) points to a non-reactive surface, characteristic of pyrophyllite, in which isomorphic substitution does not occur under standard conditions. This type of "neutral surface siloxane" provides favourable adsorption domains for non-polar and semi-polar organic solutions. Minerals on neutral siloxane surfaces were found to have an unexpectedly high affinity for highly nonpolar materials, such as dioxins.

The most reactive surface functional group at the edge of the pyrophyllite surface is OH^- associated with two sites available for adsorption: Al (III) and Si (IV), which are located on octahedral and tetrahedral plates. At the edge of the octahedral layer is Al (III) H_2O, as Lewis acid. The hydroxyl group associated with this site may form a complex within the proton sphere at low pH or with hydroxyls at high pH. At the edges of tetrahedral plates,hydroxyl groups coordinate with Si^{4+} cations (Keren and Sparks, 1994).

Due to the listed characteristics of pyrophyllite, the properties of the soil conditioner (regenerator) are attributed to it. It contributes to the improvement of the physical, chemical and agrochemical properties of the soil. Improves soil structure, permeability, increases cation exchange capacity, increases soil's ability to retain nutrients and reduces rinsing and motility of heavy metals in the soil, improves the efficiency of mineral fertilizers, acts as a buffer, regulates soil pH. All of the above justifies the growing global interest in its use to improve and maintain soil productivity in the function of sustainable agricultural production (Maaz *et al.,* 2021, Murtić *et al.* (2020).

The research aimed to examine the influence of natural and enriched pyrophyllite on the development of the aboveground part of the plant, the yield of heads and the average mass of onion heads (*Allium cepa* L.), as well as its resistance to diseases (head rot, etc.).

Material and Methods

The experiment was performed at the location of the village Banja, Arandjelovac, Serbia. The soil on which the onion was grown was of the smonica type. At 0-30 cm of soil depth, the pH value (H_2O) was 5.06; humus content 2.53%, total nitrogen (N) 0.13%, and C/N ratio 11.3:1. NPK fertilizer (15:15:15) was added in the basic and pre-sowing soil preparation at 1.50 kg/ar. Urea (46% N) was added in the basic preparation in the amount of 1.00 kg/ar, and pre-sowing 2.00 kg/ar. In the previous year, the previous crop on the experimental plot was a tomato.

Table 1. Plan of the experiments

Treatments-way of watering		
Control H_2O	Exper. **treatment 1** PIR+H_2O[1]	Exper. **treatment 2** PIRO-N+H_2O[2]

[1]PIR: Pyrophyllite granulation <100 µm;
[2]PIRO–N: Modified pyrophyllite granulation <100 µm, enriched with 12.5% N from urea, applied to PIR by a special technological procedure.

In both experimental treatments pyrophyllite originated from the site Parsovići, Konjic, AD Harbi, B&H). The tested pyrophyllite contained 64.15% SiO_2, 15.10% Al_2O_3, 1.57% Fe_2O, 6.65% CaO, 1.06% MgO, with the low oxide content of Na, K, Ti, P, S and Ba (below 1%).Urea contained 45.84% N. The suspension in treatment 1 was composed of water and pyrophyllite (4 g PIR/L water) and in treatment 2 of water and enriched pyrophyllite (4 g PIRO-N/L water). Watering began on April 24, 2020, after the eruption of 2-3 feathers with 20 ml of water per plant on the control treatment, ie. with the same amount of suspension on experimental treatments 1 and 2. Watering was performed every other day and lasted until July 21, 2020. During the period June 14 – July 05, 2020, due to daily heavy rains (21 days), watering was not performed. During the experiment, the onion has been watered a total of 34 times. During the experiment, the development of the aboveground part of the onion (plant habitus, colour) and changes in plants that could indicate possible diseases was monitored. At the end of the experiment, the yield of onion heads was measured, and the average mass of the head was determined. The extraction of the onion heads was done on July 22, 2020. The extracted onion was placed to dry naturally for 24 hours. After drying, the soil was removed from the heads. The plumage on the heads were cut off with scissors at a height of 1 cm above the head. The mass of cleaned onion heads was measured with an electronic libra with a measurement accuracy of ±1 g. Statistical analysis was performed using a computer statistical package Statistica, version 10 (StatSoft, Inc., Tulsa, USA).

Results and Discussion

The area in which onions were grown was 1 m^2 for each treatment, each next to the other, and the number of plants according to the order of treatment was 26:25:26 (Pictures 1). During the experiment, no significant changes were observed in the appearance of the aboveground part of the onion (habitus and colour) that would indicate the existence of possible phytopathogenic diseases. Due to the small number of plants in the experiment, there was no objective possibility of sacrifice (premature harvesting of a certain number), which is why the quantification of leaf biomass of the above ground part of plants was not done. It should be noted that it was more developed in plants watered with a suspension of water and pyrophyllite enriched with urea (PIRO-N+H_2O) as well as with a suspension of water and pyrophyllite (PIR+H_2O) (Pictures 1). In these experimental treatments, the colour of onion plumage was a darker green compared to the control, which indicates a greater presence of phytoactive substances.

Pictures 1. Onions in the final stage of growth (from left to right treatment with H_2O; PIR+H_2O; PIRO-N+H_2O)

Watering of onion with PIR+H_2O suspension (treatment 1) and PIRO-N+H_2O suspension (treatment 2) compared to water treatment (control), contributed to the increase in onion mass by 18.31% and 24.09%, respectively (Table 2).Table 2. Yield and the average mass of onion

Table 2. Yield and the average mass of onion

Parameter	Control (H_2O)	Experimental treatment 1 (PIR +H_2O)	Eksperimental treatment 2 (PIRO-N+H_2O)
Area of onion plantations, m^2	1.0	1.0	1.0
Number of onion plants	26	25	26
Total mass of onion heads, g	2,361	2,686	2.930
Average mass of onion heads, g	90.81	107.44**	112.69**
SD (standard deviation)	±8.66	±8.59	±8.91
SEM (standard error of average)	±0.99	±0.98	±1.01
Variation interval, g	32 (75-107)	30 (91-121)	31 (94-125)

$^{**}p<0.01$

The identified differences were also statistically very significant ($p<0.01$). The mass of onion heads in **treatment 2** was higher than in **treatment 1** by 5.72 percentage points, and the differences were not statistically significant ($p<0.05$). Significantly better results of **treatment 2** indicate that the amount of nitrogen in the soil was a limiting factor, which points to the justification of its enrichment with urea nitrogen. Other additional effects of pyrophyllite (soil conditioner) found in similar studies with vegetable and field crops probably contributed to this (Picture 2).

Picture 2. Onion heads

Among other authors, Koleška (2019), Pašić (2019), Murtić *et al.* (2020) and other authors studied the possibility of using pyrophyllite originating from the site Parsovići, Konjic, AD Harbi, B&H in vegetables and crop production.

Koleška (2019), examined the influence of pyrophyllite on the biochemical parameters of two wheat cultivars (Brkulja and Spelta). The obtained results for most parameters differed depending on the granulation of pyrophyllite. In the cultivar Brkulja, it was determined that pyrophyllite with granulation of 5 mm had a more favourable effect on reducing the parameters of oxidative stress, as well as on increasing the concentration of protein and total chlorophyll. The most favourable ratio of fertilizer and pyrophyllite was determined in the variant in which in 20% of the added supplement the ratio of pyrophyllite to fertilizer was 50%:50%, or for some tested parameters pyrophyllite itself. Treatments with pyrophyllite in the Spelta variety induced smaller changes compared to the Brkulja variety. The addition of pyrophyllite with granulation of 100 µm reduced the activity of all stress marker enzymes compared to control samples, in which in 20% of the added supplement the ratio of pyrophyllite and fertilizer was 70%:30% or pyrophyllite itself.

Pašić (2019) investigated the influence of pyrophyllite in different relations with mineral fertilizers (NPK 15:15:15), on the height, growth and nutritional value of Bravo cabbage

(*Brassica oleracea* var. *capitata*). Treatments with a higher share of pyrophyllite significantly affected certain morphological properties of cabbage, increase in fresh and dry mass of roots and cabbage heads compared to the control treatment with mineral fertilizer without the addition of pyrophyllite. The application of pyrophyllite in all variants, during the two-year duration of this study, affected the increase in soil pH (H_2O) from 6.96 to 7.00 before the treatment to 7.10 to 7.44 after crop removal.

In an study conducted by Murtić *et al.* (2020) different ratios of mineral fertilizers and pyrophyllite (75%:25%; 50%:50%; 25%:75%), originating from the same site as in the previous two studies, were examined using its two granulations of 0.1 and 3 mm. The fertilization variant in which the ratio of fertilizer and pyrophyllite was 75%:25% proved to be the most efficient combination in terms of lettuce yield. The variant in which the ratio of used fertilizer and pyrophyllite was 50%:50% also had a positive effect on the increase of yield compared to the control variant. It was concluded that the use of pyrophyllite as a substitute for artificial fertilizers can significantly contribute to increasing the antioxidant capacity of lettuce, and thus its higher health safety. In the same experiment, pyrophyllite reduced the uptake and accumulation of heavy metals Cu, Zn, Pb and Mn in lettuce leaves. Similar, although preliminary, results of the investigation on the affinity of pyrophyllite modified with boric acid for adsorption of heavy metals (Cd^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+}) from an aqueous solution were determined by Trtić-Petrović (2019). In the paper of Halilović (2018), the optimization of the adsorption process of heavy metals from wastewater on pyrophyllite was performed. It was determined that Cr, Cu, Fe, Pb and Zn are adsorbed on pyrophyllite from a synthetic wastewater sample in the amount of 100%, while Mn of 80.37%, and Ni of 93.53%. Adsorption of metals from a real sample of wastewater (low concentrations) is lower and amounted 37.93% for Cu, 29.17% for Ni and 93.87 for Zn at a water pH 7.23, contact time of 30 min. and pyrophyllite mass of 1 g.

The results of the research obtained by Park *et al.* (2014), Kang *et al.* (2013), Monsif *et al.* (2019), Adamović *et al.* (2020), Šmitran *et al.* (2021) indicated that certain products based on pyrophyllite or similar clays can suppress the development of some microorganisms (*E. coli*, *S. aureus*, *Acinetobacter baumannii,* MS 2 bacteriophage and aerobic mesophilic bacteria). According to the research by Marković (2019), the ability of pyrophyllite with a granulation of 5 µm to absorb aflatoxin *in vitro* was very high (84.80%).

King (2020) cites the results according to which a pyrophyllite-based preparation added to plants in water suspension, beside the yields increase (19-59%) and the quality of plant products, contributes to their better vitality and health, and also acts as both an insecticide

and a fungicide.
In the preliminary research of Adamović and Stojanović (2019) performed in laboratory conditions, the possibilities of natural pyrophyllite enrichment with the nitrogen from urea (NPN) were investigated. The starting material was obtained which, with a small additional modification, can be used in agriculture and horticulture, fruit growing and viticulture, contribute to reduced consumption of expensive mineral fertilizers. Stojanović (2019a and 2019b) proposes natural and enriched pyrophyllite for remediation of contaminated soils.
This ability of products based on pyrophyllite and similar clays contributes to the increase of alternative solutions in the production of hygienically correct and safe food, and environmental protection (Bočarov-Stančić *et al.,* 2021a and 2021b).

Conclusion

Watering of onion with the suspension of water and pyrophyllite (PIR+H_2O) and suspension of water and enriched pyrophyllite (PIRO-N+H_2O) compared to the control treatment (H_2O) contributed to the increase in biomass of onion heads by 18.31% and 24.09%, respectively ($p<0.01$).
Significantly better results achieved in the treatment with enriched pyrophyllite (PIRO-N+H_2O) indicate that the amount of nitrogen in the soil was a limiting factor and the justification of the modification of pyrophyllite with urea nitrogen. This is probably due to other additional effects of pyrophyllite (soil conditioner) that have a suppressive effect on factors that can harm plant development (lower soil pH, the presence of heavy metals etc.).
The experiment was performed on a small area in field conditions, it is necessary to repeat the experiment, next time in the field and/or controlled conditions (in a greenhouse).

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Утицај природног и оплемењеног пирофилита на принос црног лука (*Allium cepa*)

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Сажетак

Приказани су резултати испитивања утицаја заливања црног лука (*Allium cepa*) суспензијом воде и пирофилита (природни пирофилитни шкриљац), односно пирофилита оплемењеног азотом (N) из урее на развој и принос лука. Пирофилит је потицао из налазишта Парсовићи, Коњиц, АД Харби, Б и Х. Суспензија огледног третмана 1 била је сачињена од воде и пирофилита (4g/L воде), а третману 2 од воде и оплемењеног пирофилита (4 g/L воде). Оплемењен пирофилит садржавао је 12,5% N.
Заливање лука суспензијом воде и пирофилита, односно суспензијом воде и оплеменјеног пирофилита у односу на контролни третман заливан водом, допринело је повећању масе главице лука за 18,31%, односно 24,09% ($p<0,01$).
Резултати указују да је количина азота у земљишту била лимитирајући фактор, и на оправданост употребе оплемењеног пирофилита. Овоме су допринела и друга својства пирофилита која имају супресиван утицај на чиниоце који штете развоју биљака.

Кључне речи: пирофилит, уреа, заливање, црни лук, принос

Original scientific paper

The influence of organic biostimulators on the growth and development of medicinal and aromatic plant species *Ocimum basilicum* L. and *Levisticum officinale* L.

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Abstract

Ocimum basilicum L. and *Levisticum officinale* L. are well known medicinal, aromatic and spicy herbs with remarkable application in cooking and medicine. In an attempt to improve their potted production and increase the yield of aboveground biomass, a vegetation experiment was conducted in the experimental field of the Institute for Medicinal Plants Research "Dr Josif Pančić" Belgrade, Serbia. Therefore, the aim of this study was to determine the effect of organic biostimulators on germination, growth and productivity of *O. basilicum* and *L. officinale* in potted production, conducted under semi-controlled conditions. Following treatments of seeds were applied: organic biostimulators Ecobooster 1 (in concentrations 10%, 30% and 50%) and Slavol S (in concentration 10%, according to manufacturer's recommendations), and control treatment (without biostimulators). A significantly higher number of seedlings was achieved in the treatments with biostimulators compared to control. The best effect on germination of *O. basilicum* and *L. officinale* seeds was achieved with Slavol S (91.3% and 72.5%, respectively) and Ecobooster 1 – conc. 50% (83.4% and 61.3%, respectively). The best effect on height of *O. basilicum* and *L. officinale* plants were achieved with Slavol S (26.2 cm; 14.4 cm, respectively) and Ecobooster 1 – conc. 30% (20.0 cm and 17.1 cm, respectively) and Ecobooster 1 – conc. 50% (21.7 cm; 19.4 cm, respectively), while the best effect on number of brunches showed Slavol S (2.3 and 5.1, respectively) Ecobooster 1 – conc. 10% (4.2 and 4.7, respectively) and Ecobooster 1 – conc. 30% (4.1 and 5.2, respectively). The best effect on the aboveground plant mass showed Ecobooster 1 – conc. 10% (5.03 and 1.74, g of absolute dry mass).

The obtained results confirmed that both organic biostimulators positively influence the observed morphological parameters in both plant species, particularly in the earlies phases of their development.

Key words: basil; lovage; Ecobooster 1; Slavol S.

Introduction

The great values of medicinal and aromatic plant (MAP) species in the traditional medicine and nutrition have been known for centuries. Nowadays, 70-80% of the human population predominantly uses herbs in the prevention and treatments of a number of diseases (Farnsworth et al., 1991), while a number of them are also enjoying status "functional food" (Kaul et al., 2012; Raina et al., 2014; Joshi et al., 2020). The market demands for MAP raw herb materials are growing, and so far it is mainly supplied from nature (Lubbe et al., 2011). Increased exploitation of MAPs from their natural habitats, untrained collectors and luck of collectors in rural areas, in addition to many legal restrictions, could be overcome only by cultivation of MAPs (Kuipers, 1997; Lange, 1998). The cultivation can be achieved in the urban area as well; in flower gardens, roof gardens, flower beds, etc. (Tuna et al., 2020).

Ocimum basilicum and *Levisticum officinale* are highly valued for their spicy, medicinal, fragrant and decorative characteristics. According to Stephens (1998), Bufalo et al. (2015) and Matlok et al. (2021), these two herbs can be also grown as potted plants, with satisfactory productivity, in case appropriate technology of their production is applied.

The aim of this study was to investigate the influence of organic biostimulators on the growth, development and yield of *O. basilicum* and *L. officinale*, grown as potted cultures.

Material and Methods

The experiment was initiated in the agricultural laboratory of the Institute for Medicinal Plants Research "Dr Josif Pančić" in Belgrade, in mid-February 2021, and it continued at the experimental field of the Institute, in Pančevo, South Banat, Serbia. The seeds of *O. basilicum* and *L. officinale* were soaked for 10 minutes in organic biostimulators, Ecobooster 1 or Slavol S. The Ecobooster 1 is composed of gibberellic acid, organic matter (2.8%), nitrogen (14%), phosphorus (2%) and potassium (5%), while Slavol S is composed of IAA (indole-3-acetic acid - an essential plant hormone that regulates its growth and development). The Ecobooster 1 was prepared in three concentrations, 10% (E_{10}), 30% (E_{30}) and 50% (E_{50}), while Slavol S (Sl) was prepared according to manufacturer's recommendations, in concentration of 10% (Table 1).

Table 1. The treatments applied on seeds of *O. basilicum* and *L. officinale*.

Treatment	Treatment preparation
E_{10}	10 ml solution of EcoBooster 1 + 90 ml of distilled water
E_{30}	30 ml solution of EcoBooster 1 + 70 ml of distilled water
E_{50}	50 ml solution of EcoBooster 1 + 50 ml of distilled water
Sl	10 ml solution of Slavol S + 90 ml of distilled water
Control	100 ml of distilled water

The seeds of *O. basilicum* and *L. officinale* were sown in Styrofoam containers, each with 160 cells previously filled with commercial substrate Cultivo SF. Then, the containers were transferred to a Grow box, at T 24°C and under Rh 70-80% and light intensity of 2100 lux. The rate of seed germination in each treatment was recorded on the 7 and 21 day following the sowing, after which the total germination rate per treatment was calculated according to following formula, proposed by Noman et al. (2018):

Total germination rate (%) = (number of germinating seeds / number of sown seeds) × 100

Figure 1. Potted plants production in non-heated greenhouse.

When first true leaf pair appeared (by the end of March), 30 uniform seedlings from each treatment were transplanted into 0.9 L vol. plastic pots (∅ 10 cm), which were transferred into the non-heated greenhouse (Figure 1), and left there at 30% shade, under the average daily T of 25°C and drip irrigation. When flower branches started their formation, which according to the BBCH scale suggested by Hess et al. (1997) corresponds to transition between the phase IV into phase V of plant development, the branches was counted and the plant height measured in all plants and for each treatment; this phase was assigned as ***the early growth*** (Figure 2).

Figure 2**.** Potted plants of *L. officinale* (left) *and O. basilicum* (right) in the early growth phase.

At the beginning of May, the potted plants were transferred outside the Greenhouse, where they were exposed to 40% shade and under the average daily T of 29.9°C and drip irrigation rate of 4 L water/h (Figure 3). The experiment lasted until the harvest (beginning of June), which happened when ca. 2/3 of plants were in the flowering phase. The plants height measuring and branches number counting were conducted on harvested plant material in the laboratory conditions; this phase was assigned as ***the harvest time***. Then, the harvested plant material was subjected to drying procedure (at 105ºC until the constant weight), to be able to compare the achieved yields between the treatments (g).

Figure 3. Potted plants transferred outdoors.

The obtained results were statistically analysed by the use of SPSS statistical software.

Results and Discussion

Application of organic biostimulators showed various effects on studied plant species, *O. basilicum* and *L. officinale.* The achieved effects were presented in Tables 2 and 3.

Regarding the incidence *O. basilicum* and *L. officinale* seedlings (Table 2), the most efficient was treatment with Sl; compared to the control, its application caused an increase in the incidence of *L. officinale* and *O. basilicum* seedlings by 72,62% and 19,50%, respectively.

Table 2. Incidence of seedlings observed on 21 day following the sowing (%).

Treatment	**Seedlings incidence** (%)	
	Ociumum basilicum	*Levisticum officinale*
$\mathbf{E_{10}}$	78.4	51.4
$\mathbf{E_{30}}$	79.1	55.5
$\mathbf{E_{50}}$	83.4	61.3
Sl	91.3	72.5
Control	76.4	42.0

The increase in the incidence of seedlings achieved in the treatments with Ecobooster 1, also depended on plant species and applied concentration. Compared to the control, the greatest increase in *L. officinale* seedlings was achieved with application of E_{50} (by 45.95%) and E_{30}

(by 32.14%), though the increase achieved with E_{10} was not negligible (by 22.38%). The increase in *O. basilicum* seedlings, observed in the treatments with E_{50}, E_{30} and E_{10} was siginificantly lower compared to the control, corresponding ones in case of *L. officinale* but was noticed (by 9.16%, 3.53% and 2.62%, respectively,).

Efficacy of organic biostimulators was also confirmed in other studies conducted on medicinal plants. Parađiković et al. (2019) assumed that chemical composition and essential hormones present in organic biostimulators were responsible for positive effects on germination of *O. basilicum* seeds, while in studies of Butola et al. (2005) and Eun et al. (1997), similar positive effects on seeds with the use of similar formulations were observed in *Hypericum perforatum* and *Foeniculum vulgare*, respectively.

Table 3. Growth and productivity parameters observed at different developmental stages of potted *O. basilicum* and *L. officinale* plants.

Plant species	**Treatment**	**Plant height** (cm)		**Branches** (number)		**Yield** (g)
		Early growth phase	Harvest time	Early growth phase	Harvest time	
O. bacilicum	E_{10}	15±3.36c	37.13±3.53a	4.2±1.19a	5.40±0.88a	5.03±1.16a
	E_{30}	20±2.16b	35.55±1.70b	4.1±0.96a	4.47±0.88b	4.46±0.75ab
	E_{50}	21.7±2.34b	34.57±3.14b	2.2±1.56b	5.14±0.50a	4.73±1.27ab
	Sl	26.2±2.24a	34.97±2.70b	2.3±1.16b	5.13±0.50a	4.38±0.73b
	Control	13±1.66d	34.93±1.44b	2.2±1.46b	3.53±0.96c	4.29±1.38b
L. officinale	E_{10}	13.7±2.25d	23.75±2.83ab	4.7±1.56b	6.33±0.94a	1.74±0.54a
	E_{30}	17.1±2.46b	22.65±2.26a	5.2±1.81a	4.83±0.82b	1.27±0.54ab
	E_{50}	19.4±3.67a	23.94±2.84a	3.1±1.36c	5.33±1.01bc	1.07±2.40c
	Sl	14.4±4.16c	24.99±2.08a	5.1±1.56a	5.73±1.39ab	1.72±0.66a
	Control	11.3±3.46e	22.43±2.86b	3.0±1.26c	4.93±0.93c	1.29±0.50bc

*Mean values marked with the same lowercase letters within the same column do not differ significantly ($p < 0.05$).

In the early growth phase, the examined organic biostimulators achieved significant positive effects on the growth of both medicinal plant species, compared to the control. In case of *O. basilicum*, the highest effect was achieved with Slavol S, which differed from all other treatments. Slightly less than tha Slavol S and slightly higher than tha E_{10}, was achieved by E_{50} and E_{30}, equally. Regarding the number of branches in this phase, treatments with E_{10} and E_{30} caused equally the highest branching in *O. basilicum*, while in the treatments with E_{50} and Sl the branching did not differ from the control (Table 3). ***In the early growth phase*** of *L. officinale* the effect of treatments on plant height differed among the treatments and all differed from the control; presented in descending order, the treatment efficiency was as follows E_{50} > E_{30} > Sl > E_{10}. Regarding the number of branches in the early growth phase of *L. officinale*, a positive effect compared to the control was observed in all treatments except in E_{50}, with the highest positive effect equally achieved by E_{30} and Sl (Table 3).

In the harvest phase of *O. basilicum*, the highest plant height was observed in treatment with E_{10}, which differed from all other, including control. Although all treatments had a positive effect on the branchingof *O. basilicum* and differed from the control. The highest effect with no difference between them, was achieved in treatments with E_{10}, E_{50} and Sl (Table 3). ***In the harvest phase*** of *L. officinale*, plant heights in all treatments differed from the control, and the highest were observed in Sl=E_{50}=E_{30}. On the other hand, apart that all treatments differed from the control, their effects on *L. officinale* branching, presented in descending order, were as follows $E_{10} > E_{30} >$ Sl $> E_{50}$ (Table 3).

According to Shirkhodaei et al. (2014), organic biostimulators with addition of organic fertilizer, prove to be capable to accelerate the growth and development of the aboveground mass of medicinal plant species *Coriandrum sativum*. Results of our study are in accordance with results of Zeljković et al. (2014), who also reported positive effect of applied biostimulators on early growth of *O. basilicum*. They also support some other findings that medicinal plants *O. basilicum* and *L.officinale* can be successfully grown in a protected area (Stephens, 1998; Bufalo et al., 2015; Gache et al., 2019). Nazmy (2020) studied the effects of chemical compounds similar to those in biostimulators used in our study, and achieved positive effect on the growth of *O. basilicum*. Gache et al. (2019) achieved better productivity of the aboveground biomass of *L. officinale* than it was achieved in our study; this could be attributed more to the larger pots they used, which enabled them higher productivity. Recent study of Złotek et al. (2020) presented various positive effects of biostimulators on morphological parameters of *L. officinale* but also on certain bioactive components specific for this medicinal plant species.

Conclusion

Medicinal plant species *O. basilicum* and *L. officinale* can be successfully grown as potted crops under semi-controlled conditions. Their productivity can be increased by applying appropriate organic biostimulators. In both medicinal plant species, all tested organic biostimulators had a positive influence on the incidence of seedlings and growth of the aboveground plant part, especially in the early growth phase. As medicinal and aromatic plants are known for their secondary metabolites, the carriers of their healing properties, further study should be focused on the influence of organic biostimulators on phytochemical composition of the obtained yields of *O. bacilicum* and *L. officinale.*

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Preliminary report

Extraction kinetics of phenolic acids during prolonged maceration time and vinification of Cabernet Sauvignon grape variety

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Abstract

Effects of maceration time on the phenolic acids content in red wine grape variety Cabernet Sauvignon (*Vitis vinifera L.*) was investigated in this study. In focus of this study were four derivates of hydroxybenzoic and hydroxycinnamic acids: gallic, syringic, caffeic and *p*-coumaric acids extracted into wine during five different maceration periods and using two different yeasts (FX10 and Qa23). The control wine was made according to technology of white wine, exactly separated must and solids immediately after crushing and destemming. Concentrations of these compounds were measured using UPLC-MS system (Agilent LCTQ 6495C Triple Quadrupole). The most abundant phenolic acid in wines fermented with yeast FX10, was *p*- coumaric acid and its maximal value was on 12th day of maceration. The highest extracted values during fermentation with Qa23 were obtained for syringic acid with maximal value at 12th day of maceration. Gallic and caffeic acids values also showed exponential increase during maceration but its extracted values were lower. A statistically significant difference was found comparing content of tested phenolic compounds in control wine and wines obtained after maceration for 3, 5, 7, 14 and 21 days using FX10 yeast strain ($p < 0,05$). Maceration which lasted 5, 7 and 21 days using Qa23 yeast strain also showed a statistically significant difference than control wine in term of phenolic acids concentrations ($p < 0,05$).

Key words: extraction, maceration time, phenolic acids, yeast strain.

Introduction

Phenolic compounds have been studied due to their beneficial effect on human health and their ability to prevent non-transmissible chronic diseases. Phenolic compounds also play a major role in some organoleptic characteristics of wine, such as colour and astringency (Muños Bernal et al., 2020). Red wine is rich source of phenolic compounds, including flavonoids (anthocyanins, flavan-3-ols, proanthocyanidins or condensed tannins and flavanols) and non-flavanoids (hydroxybenzoic and hydroxycinnamic acids and their derivatives, stilbenes and phenol alcohols) based on grape variety, growing techniques (viticulture) and winemaking conditions (Kocabey et al., 2016). Grapes contain non-flavonoid compounds mainly in the pulp, while flavonoid compounds are located mainly in the skins, seeds, and stems (Gomez Plaza et al., 2001). Extraction of phenolic compounds during maceration, during which phenols turn into wine from solid parts of grapes, depends on vinification conditions (Budić-Leto et al., 2008), while the maceration time has the greatest influence (Vrhovšek et al., 2002; Lisov et al., 2020). The production of ethanol during fermentation ~~also~~ affects ~~on~~ the extraction of phenolic compounds from the grape pomace into the wine together with punching down and pumping over, and these techniques guarantee the contact of wine with grape solids and improve extraction (Muños Bernal et al., 2020). All phenolic groups behave differently and have specific time for extraction in dependence of conditions during maceration and fermentation. Non-flavonoid compounds include hydroxybenzoic and hydroxycinnamic acids and their derivatives, are usually named low molecular weight phenols. Although being present in small amounts in wines they play an important role in sensory quality and may contribute to bitterness and harshness (Cabrita et al., 2008). Also these compounds have an important role in red wine color, even thay are colorless or slightly yellow, since they are known to enhance and stabilize the color of red wines by intra- and intermolecular reactions (Boulton, 2001). The aim of this research was to determine the influence of different maceration time (3, 5, 7, 14 and 21 days) on extraction of some phenolic acids into wine.

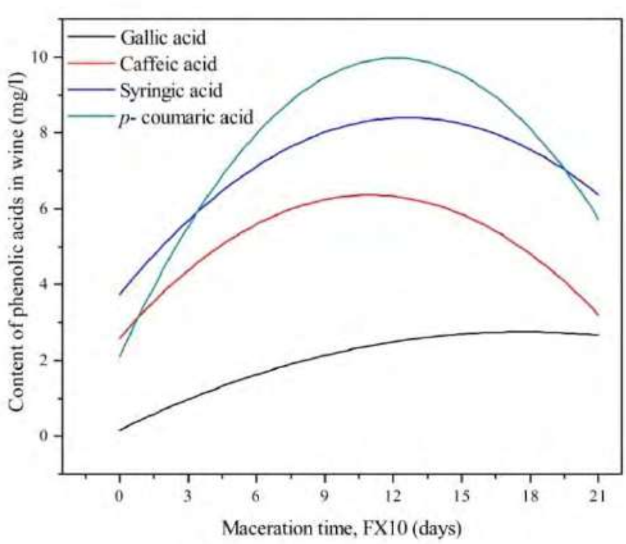
Material and Methods

Grape variety Cabernet Sauvignon was harvested in the state of technological maturity. Phytosanitary state: 100% health (visually), sugar in must 23% and total acid in must 6.8 g/l. Alcohol fermentation with maceration was carried out by microvinification method at temperature of 25±3°C using the pigeage system. Free sulfur dioxide 5g/hl was added to the grape pomace as $K_2S_2O_5$. First microvinification experiment was conducted with addition of yeast strain *Saccharomyces cerevisiae* in the amount of 20g/hl (Qa23, Lallemand, Canada).

Also it was used pure yeast strain *Saccharomyces cerevisiae* (FX10, Laffort, France) in the amount of 20g/hl for the second microvinification experiment. Each of these experiments included five different maceration time (3, 5, 7, 14, 21 day, respectively). Grape must was separated after certain time of maceration and fermented without seeds and skin. After the alcoholic fermentation, the wines were racked, bottled into 750 ml bottles and stored until analysis. The control samples presented wines made according to technology of white wine (without maceration). In order to identify and quantify the phenolic acids in wine samples of Cabernet Sauvignon variety, it was necessary to separate them from the accompanying components, concentrate and purify. Solid phase extraction has been experimentally found to be the most suitable. Determination of the amount of caffeic, *p*- coumaric, gallic and syringic acid was performed using the UPLC-MS (Agilent LCTQ 6495C Triple Quadrupole).

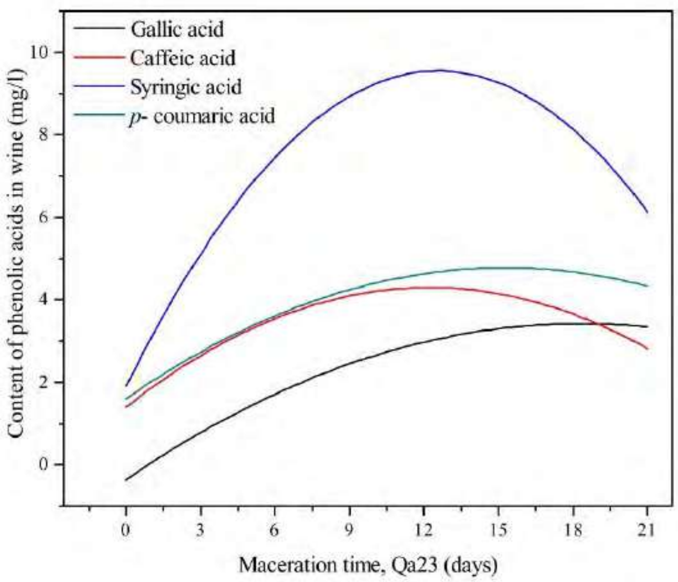
Results and Discussion

The most abundant phenolic acid in these Cabernet Sauvignon wines, which fermented with yeast FX10, was *p*- coumaric acid (Graph 1). Maximal extracted value for *p*- coumaric acid was at 12th day, after which there was a decline to the end of maceration. The lowest concentrations were measured for gallic acid with maximum at 17th day of maceration. Content of gallic acid increased progressively with prolonging contact of grape solids and must (Alencar et al., 2017; Francesca et al., 2014). A statistically significant difference was found comparing content of tested phenolic compounds in control wine and wines obtained after maceration for 3, 5, 7, 14 and 21 days ($p < 0,05$).



Graph 1. Extraction kinetics of phenolic acids during maceration and fermentation with FX10 yeast strain.

With Qa23 yeast strain, the highest extracted values were obtained for syringic acid with maximal value at 12th day of maceration (Graph 2). Similar to our results, the highest concentration was measured for syringic acid on days 13 and 20 in analysed wine of Aglianico di Taurasi grape variety (Francesca et al., 2014). As it was presented for the first experiment, gallic acid had lowest extracted values for wines which were fermented with Qa23 too, but trend of increase was similar to investigation of Ivanova Petropulos et al. (2016). In order to compare content of analysed phenolic acids in control and macerated wines, a statistically significant difference was obtained for control wine and wines which maceration lasted 5, 7 and 21 day using Qa23 yeast strain ($p < 0,05$).



Graph 2. Extraction kinetics of phenolic acids during maceration and fermentation with Qa23 yeast strain.

Extraction kinetics for all tested wines, independent of used yeast strain was exponentially (Kocabey et al., 2016; Lisov et al., 2020). According to Ivanova Petropulos et al. (2016), the content of total hydroxybenzoic acids as well as *p*- coumaric acid increased during winemaking and reached to the highest concentration in the wines after 9 days of maceration. In some research caffeic acid decreased during the maceration time what is contrary to our results (Alencar et al., 2017). But Plavša et al. (2012) showed a significant increase of hydroxybenzoic and hydroxycinnamic acids, including a caffeic acid, which agrees with our results.

Conclusion

During maceration content of all investigated phenolic acids increased with increasing length of pomace contact, although the times when they reached maximum differed somewhat and then gradually decreased. It was found that maceration time showed a significant influence on extraction of these tested phenolic acids and its necessary more than 12 days of maceration for maximal extraction of these compounds.

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Original scientific paper

Seed germination of *Calendula officinalis* L. under influence of different light conditions

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Abstract

The aim of this study was to examine germination rate and morphological characteristics of English marigold seedlings under influence of different light conditions. Seeds of *Calendula officinalis* L. were collected from the natural population of the Botanical garden of the University of Banja Luka. Experiment was set up in four replicates for each light treatment. Petri dishes with seeds were placed in growth chamber under artificial white (FLUO) and blue, red, and combination of blue/red (LED) light with 16h/8h photoperiod. Germination energy was tested after 7 days and germination of the seeds was tested after 14 days. Results showed significant difference in germination energy, germination rate, hypocotyl height, root length, and fresh weight. The highest average values of the germination energy and germination rate of the *Calendula officinalis* L. were recorded under red LED light (32%; 47%) while the lowest values were recorded under blue LED light (1%; 23%). The highest average values of hypocotyl height, root length and fresh weight were recorded also under red LED light (3,70 cm; 6,33 cm; 0,97 g) while the lowest values were recorded under combination of blue/red LED light (1,95 cm; 2,52 cm; 0,28 g). It can be concluded that the use of red LED light is recommended in the seed germination phase, not only for better germination but also for better morphological development.

Key words: germination, *Calendula officinalis* L., morphological parameters, LED light

Introduction

The key factors for plant growth and development are light and temperature. Plants have

photoreceptors that respond to the wavelength and light intensity (Zhang and Folta, 2012). The effect of light during plant growth and fertility process is undeniable. Among the various naturally occurring abiotic factors regulating plant development, light plays an important role in photosynthesis and photoperiodism, and only wavelengths ranging 400 to 700 nm can be used (Teklić, 2012).
Many of light sources which are used to enhance photosynthetic levels have very low energy use efficiency for growing plants. Solar light consists of electromagnetic radiation with wavelengths ranging 400 to 700 nm (violet, blue, green, yellow, orange and red). The changes in the light quality (wavelength) influences seed germination, seedling growth, photosynthesis, and flowering depending on the species and developmental stage (He et al., 2017).
In contrast to other light sources, lightemitting diode (LED) lighting system have various advantages, including the ability to set the desired spectral combination, permanence, specific wavelength, low heating and the electrical input (Lin et al., 2013). These advantages make them suitable light source for growing plants. The LEDs give wavelengths that can be matched with plant photoreceptors to provide optimal production and influence morphology and metabolism of plant (Morrow, 2008). Experimentations on impact of light variables on plant growth and development is attracting attention of plant scientists. Changes in different light attributes like intensity and duration, can influence all aspect of plant growth and physiology especially morphology and photosynthetic responses of the plants. Red and blue lights have great effects on plant growth because they contain the two main light spectra for photosynthetic CO_2 fixation in plants (Kasajima et al., 2008). It has been reported that red light is the most important light spectrum for growth and phytochrome responses in plants (Wang et al., 2016).
Red light is important for the development of the photosynthetic apparatus of plants and may increase starch accumulation in several plant species by inhibiting the translocation of photosynthates out of leaves (Paradiso et al., 2011). In contrast, blue light is important in the formation of chlorophyll, chloroplast development, stomatal opening, enzyme synthesis, activation of the photosynthesis, and photomorphogenesis (Kang et al. 2008; Demarsy and Frankhauser 2009).
Green light, in the process of seed germination of *Arabidopsis*, stimulates the early elongation of the stems, antagonizing the growth inhibition by light whereas the white and red light, in ferns can delay the chlorophyll loss due to senescence (Burescu et al., 2015). It has been reported that light is key factor regulating the seed germination in numerous plant

species. Jala (2011) found that seeds of *Nepenthes mirabilis* first germinated under white and red light, and the last germinated under green light, and the highest average speed of emergence was also recorded under red light.

Lal and Sachan (2017) investigated the effect of different colours of light (natural, red, blue, yellow, and green) on seed germination, hypocotyl growth, biomass production in *Vigna unguiculata* L. Walp., an important crops for grain and fodder purposes. Red light showed maximum % of germination (98%) at 84 hours while, green light showed almost no germination even after 96 hours. Blue light and yellow light caused significant reduction in % of germination to 71 and 56, respectively, at 84 hours. Root and shoot growth were highest in red light and the order of biomass production was red > yellow > natural > blue > green.

English marigold (*Calendula officinalis*) is an annual or biennial plant species that belongs to the Asteraceae family. It is native to North Africa and Southern Europe and represents as one of the most used ornamental plants in the gardens and green spaces. English marigold has different applications in horticulture industry: as a pot plant, garden plant, cut flower, food and medicinal plant. The aim of this study was to examine germination rate and morphological characteristics of English marigold seedlings under influence of different light conditions.

Material and Methods

Investigation was conducted in laboratory condition at the Faculty of Agriculture, University of Banja Luka. Seeds of *Calendula officinalis* L. were collected from the natural population of the Botanical garden of the University of Banja Luka. The experiment consisted of four light treatments: three different colors of light (blue, red, and combination of blue/red LEDs) as treatments, and white FLUO light, as control. The experimental design was completely randomized, with four replications of 25 seeds per experimental unit for germination test. Petri dishes were sterilized with 96% ethanol, and lined with moistened filter paper with 3 mL of purified water. One hundred surface sterilized seeds of *Calendula officinalis* L were placed in a series of five petri dishes for each colors of light.

These petri dishes were placed on shelves, exposed to different wavelength (white, blue, red, and combination of blue/red light) with 16h/8h photoperiod. Temperature during the research was constant (20±1°C). 2 ml of water per day was added to each petri dish. Seeds were kept under these conditions for 14 days. Germination energy was tested after 7 days and germination of the seeds was tested after 14 days. Both values are expressed as percentage (%). Also, after 14 days hypocotyl height, root length, and fresh weight were obtained. The obtained data were statistically analysed (LSD, F-test, t-test) using standard computer programs and VV-Stat paket (Vukadinović, 2017). Means comparison were performed using low significant differences procedure (LSD), with a significance level of 5% ($P<0.05$).

Results and Discussion

Statistically analyzed obtained results of germination energy, seed germination, number of cotyledons, hypocotyl height, root length, and fresh weight of *Calendula officinalis* L. under influence of different light conditions are presented in Table 1. and Table 2.

Treatment T_1-red LED light had the best result in all of three parameters. Germination energy and number of cotyledons were under very significant influence ($p=0.01$), while germination rate was under significant influence ($p=0.05$) of different light conditions.

The highest average values of germination energy of the English marigold was recorded in the treatment T_1 (32%), while the lowest value was recorded in the treatment T_2-blue LEDs (1%). Also, average value of germination rate was recorded in the treatment T_1 (47%), while the lowest value was recorded in the treatment T_2 (23%). The highest average development of hypocotyls were recorded in the treatment T_1 (11.50) and the lowest in the treatment T_2 (4.5).

Table 1. Influence of different light conditions on germination energy, seed germination, number of cotyledons of English marigold - *Calendula officinalis* L. seeds

Treatment variant	germination energy %	germination rate %	number of cotyledons
Control K	12^b	38^b	9.5^b
Treatmen T_1	32^a	47^a	11.50^a
Treatmen T_2	1^c	23^c	4.5^c
Treatmen T_3	10^b	26^c	5.75^c
Average	13.75	33.5	7.81
Analyses of variance - F	10.73822^{**}	4.032787^{*}	7.062718^{**}
LSD	germination energy %	germination rate %	number of cotyledons
0.05	12.2941	17.0185	3.7676
0.01	17.2366	*ns*	5.2822

(K-white FLUO light; T_1-red LED light; T_2-blue LED light; T_3-combination of red/blue light); means marked with different letters ^{a,b,c} significantly differ at $p=0.05$
ns=not significant

Data shown in Table 2. indicates very significant difference ($p=0.01$) between the average values of hypocotyl height, root length, and fresh weight of *Calendula officinalis* L. seeds under influence of different light conditions. Treatment T_1 - red LEDs, had the best result on all of three parameters. The highest average values of hypocotyl height and root length of

English marigold were in the treatment T_1 (3.70 cm; 6.33 cm), while the lowest average values of hypocotyl height and root length of English marigold were in the treatment T_2 (1.95cm; 2.52 cm). The same ratio was in fresh weight with the highest average values in the treatment T_1 -red LED light (0.97 g), than in control - white FLUO light (0.70 g). Lower average values were recorded in the treatment T_3 - combination red/blue LEDs (0.34 g) while the lowest average values were recorded in the treatment T_2 - blue LEDs (0.28 g) (Table 2.).

Table 2. Influence of different light conditions on hypocotyl height, root length, and fresh weight of English marigold - *Calendula officinalis* L. seeds

Treatment variant	hypocotyl height (cm)	root length (cm)	fresh weight (g)
Control K	3.00^b	5.18^b	0.70^b
Treatmen T_1	3.70^a	6.33^a	0.97^a
Treatmen T_2	1.95^c	2.52^c	0.28^c
Treatmen T_3	2.08^c	2.81^c	0.34^c
Average	2.68	4.21	0.57
Analyses of variance - F	11.43219^{**}	18.816^{**}	8.599304^{**}
LSD	hypocotyl height (cm)	root length (cm)	fresh weight (g)
0.05	0.7519	1.3111	0.3397
0.01	1.0541	1.8382	0.4762

(K-white FLUO light; T_1-red LED light; T_2-blue LED light; T_3-combination of red/blue light); means marked with different letters ^{a,b,c} significantly differ at p=0.05
ns=not significant

Higher light intensities during growth of soybean - *Glycine max* (L.) Merrill resulted in increases in photosynthesis rate, light saturation intensity, and specific leaf weight (Bowes et al., 1972). Blažević (2016) confirmed that blue LED light increased the germination energy of *Calendula officinalis* L. and *Tagetes patula* L. but energy rate was not under different light conditions. Astolfi et al. (2012) investigated used of LED lamps which emitted a continuous spectrum thanks to a mixture of blue, green, red and far-red LEDs. Their results showed that plant response to light quality seems to be related to the plant species. In *Fagus sylvatica* L. seedlings fresh and dry weight, shoot height and leaf area were greatest when plants were cultured under LED light, and lowest under fluorescent lamps. Also, they found that LED-induced reduction of chlorophyll contents in *Fagus sylvatica* L. and *Quercus ilex* L. leaves resulted in an increase of the carboxylase capacity in the same plant species suggesting an improvement of light use efficiency in these plants.

Behzadi et al. (2012) confirmed that exposed basil seeds with LED light with wavelength of 620-625 nm before sowing, induced plant growth density parameters, increasing of the internal energy on seeds and change increasing growth rate, chlorophyll content, fertility, and biological parameters.

Fraszczak (2013) exposed *Anethum graveolens* L. seeds with short term of red and blue light. Results showed that the values of plant fresh mass, area and height parameters were the highest for plants treated with red light.

Conclusion

The results of this research showed positive effects on germination energy, germinaton rate, and all morphological investigated parameters (hypocotyl height, root length, and fresh weight) in English marigold (*Calendula officinalis* L.) seeds exposed to red LED light, where the following conclusions were obtained:

- the highest percentage of germination was in the treatment with red light (T_1) of 47%, with an increase of 104% over the treatment with blue light T_2 (23%);
- the highest value for the average hypocotyl height was 3.70 cm on red light (T_1) in compare with the lowest on blue light (T_2) - 1.95 cm;
- the highest value for the average root length was 6.33 cm on red light (T_1) in compare with the lowest on blue light (T_2) - 2.52 cm;

Based on the obtained results we can conclude that the treatment with red LED light can enhance seed vigor and plant productivity of English marigold (*Calendula officinalis* L.). Blue light and combination of red/blue light had a similar results in average values of hypocotyl height and root length, and significantly reduced plant growth, in compare with red light. Also, we can conclude that the effects obtained may be geater if the seeds are preventively treated with a fungicide.

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Original scientific paper

Technological characteristics of Cabernet Sauvignon cv clones grown in conditions of Krnjevo vine area

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Abstract

Research was performed during 2020 and 2021 in vineyard of "Radovanović" winery, Serbia, GPS coordinates N 44º 25' 47" and E 21º 02' 14". The aim of the research is to determine technological characteristics of superior clones of Cabernet Sauvignon 169, 191 and 412 in grass inter-row covered vineyard. In the inter-row space grass cover was established from a mixture of two grasses (red fescue 60% and perennial ryegrass 30%) and a legume (white clover 10%). The most important grapes and berries structural indicators, sugar and total acids content, pH and glycoacidometric index in must were determined. During the two-year examination by mechanical composition of grapes and berries, clone 191 was favorable, followed by clone 169 and finally clone 412. Sugar content in must varied from 21.8–27.%, acid content from 6.3-7.9 g/ and pH 2.55-3.13.

Key words: Cabernet sauvignon,clone, grass cover, structural indicators, grape quality

Introduction

There are 7.4 mil ha of vineyards in the world, of which 56% are in Europe, followed by Asia 22.7%, America 13%, Africa 5.2% and Australia with Oceania 2.7%. The top four largest world wine producers include Italy, France and Spain, which together had for 33% of world grape and wine production. Cabernet Sauvignon ranks second in the total wine assortment, with a total of 341,000 hectares, or 4% of the world's area under vineyards (OIV, 2020). The OIV data from 2020 year on the distribution of Cabernet Sauvignon plantations in the world show that France is still in first place with 46,555 hectares, followed by Chile (42,409), the

United States (40,837) and China (40,300).

Many experiments have been carried out to better identify the influence of different floor covers in grapevine vegetative growth, yield, berry and wine quality (Nieddu et al., 2000, Mercenaro et al., 2014). In long experiment with Cabernet Sauvignon maintained for 17 years with or without interrow sward, Morlat and Jacquet (2003) reported that the permanent grass cover caused a considerable decrease in the number of vine roots in the interrow, mainly in the upper soil layers, but an increase close to the row. The amount of organic matter, nitrogen, exchangeable K_2O, pH, and soil moisture at field capacity increased under permanent grass cover, while bulk density and the mechanical resistance of the soil decreased. Today, cover crops are widely used in vineyard inter-rows combined with herbicide strips under the vines. Cabernet sauvignon 169, 191 and 412 clones are superior with low to medium fertility and production level. Clone 169 can use to produce balanced and well structured wines with round tannins, from 191 clon wines are colorful and well structured and wines from 412 clone are balanced and structured (Gatti et al., 2014).

Individual components of bunch (berries and stem) and berries (skin, mesocarp and seeds) is important for grape quality as processing materials for producing of high wine quality (Blesić, 2016). On average, 100 kg of grapes contain 3-5% of stem and 95-97% berries, while berry composition include 8-11% of skin, 2-4% of seeds and 85-95% of mesocarp (Marković et al., 2017; Blackford, 2021). Mechanical composition of grapes and berries parameters can vary under influence of temperature, insolation, microclimate in grapevine trunk (Keller, 2020), but also under application of different agrotechnical and ampelotechnical measures like irrigation, fertilization, grassing, defoliation etc. (Pržić, 2015; Marković, 2020; Cataldo et al., 2021).

Material and Methods

Research was performed during vegetation seasons 2020 and 2021 in vineyard of "Radovanović" winery, Serbia on Cabernet sauvignon cv. clones 169, 191 and 412. Experimental vineyard is located at altitude of 220 m, GPS coordinates N 44º 25' 47"and E 21º 02' 14". Row spacing is 2.4 m and 0.9 m between vines in row. Vineyards is characterized training system with height of 90 cm on which is Guyot pruning used. All experimental vines was uniformly pruned where was left spur with two buds and one arc with eight buds.

The experiment was established on the Cambisol soil type with following agrochemical properties of the soil (0-30 cm): pH_{H2O} 6.70 and pH_{KCl} 5.34, total organic carbon 0.938%, total nitrogen content 0.107 %, available phosphorus (P_2O_5) 3.95 mg 100 g^{-1} and potassium (K_2O) 22.8 mg 100 g^{-1}.

The inter-row space was sown with a following grass-legume mixture: 60% *Festuca rubra,* 30% *Lolium perenne* and 10% *Trifolium repens*. Nitrogen application was set up in the spring with 50 kg ha^{-1} KAN (27%N). The trial was set up in sampling of random plots of 10 m^2 in three replications for all clones.

Analysis of collected grape samples was done at laboratory of Faculty of Agriculture University of Belgrade. Berries were harvested at full maturity (fenolic maturity). Mechanical analysis of grape bunches and berries was done by Marković and Przić (2020). Bunches were measured for their weight, length and width, and pedicel from each berry was carefully cut off with scissors so that as little mesocarp as possible was left on stem. Number of berries per bunch was also determined and berry mass per bunch and mass of stems were measured on analytical balance. From each variety 100 representative berries were selected for purpose of mechanical analysis and after measuring mass of berries, berry skin and seeds were separated. Mass of seeds and skin of 100 berries was measured on analytical balance, number of seeds in 100 berries was determined by counting. Other parameters were obtained by computation.

Ripening parameters are shown through content of accumulated sugar in grape juice-must, total acids content and pH. Sugar content was determined by physicochemical methods using Oeshle mostwage, values were calculated using Dujardin-Salleron tables. The total acid content was determined by titration with n/4 NaOH and pH using pH-meter. In the paper all data were analyzed through average and standard deviation of parameters.

Results and Discussion

The surface of experimental plots (0-30 cm) is acidic. According to the content of total organic carbon, the surface layer of the soil is weakly in humus. The nitrogen content in the soil under the vineyard is generally low, while in the available P_2O_5 this soil is poor. In available K_2O, the land under the vineyard is generally well supplied.

The parameters of mechanical analysis of grapes and berries are important indicator of variety or clones oenological potential (Živković et al., 2016; Zdunić et al., 2019). During 2020, for most parameters of mechanical analysis, clone 169 stood out with higher values, while clone 412 had the lowest recorded values. The largest variations were found for the cluster mass for clone 169 (259.0 ± 39.54), followed by clone 191 (164.0 ± 29.61), while clone 412 had the smallest determined variations (127.2 ± 25.38). The same trend of variation was found for mass of all berries in bunch. The width and length of the cluster had the largest determined variations for clone 191, which indicates heterogeneity in cluster compaction. Clone 191 was also characterized by a higher percent of seeds in berry as well as stem in bunch with the least variation. Clone 412 was characterized by the biggest variation in the stem percent in the bunch. Participation of mesocarp in berry is parameter that affects grape juice randman. Clone 169 had the highest percent of mesocarps in berries (93.3%), while clones 191 and 412 had approximately same values of this parameter (91.3 and 91.45%).

Compared to the first reserach year, during the second year most parameters had higher values with a higher variation degree, with clone 191 standing out. Clone 169 in the second research year had lower cluster mass with higher variations compared to 2020 (120.3 ± 53.83). The same trend was found for clone 191 (134.1 ± 39.01). Clone 412 was more stable for this parameter with minor variations. In the second year, a lower stem percent in bunch and a higher percent of berries in bunch were determined for all clones. The percent of skin and seeds in berry was significantly higher in 2021, which indicates a higher oenological potential of clones in the second research year. This is indicated by significantly higher values of berry indicator, especially for clone 412. Mechanical composition of cluster afect wine quality, phenolic comosition and antioxidativ activity are more expresed in wines made from clone 169 (Maria Burin et al., 2011). This is according to Brighenti et al. (2011) and Qian et al (2022).

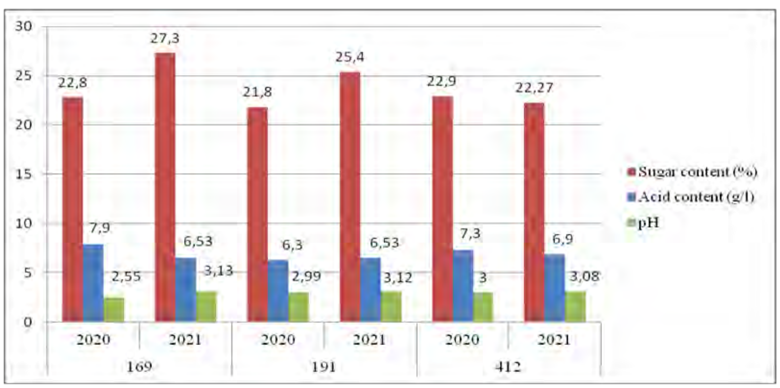
During two-year of research in the second year, higher values were determined for most parameters with higher variations. According to the values of examined parameters of mechanical composition of grapes and berries, clone 191 stood out on the first place, followed by clone 169 and finally clone 412. The results of the research are shown in Table 1 and they are in agreement with to Marković et al. (2017).

Table 1. Bunch and berry mechanical composition of Cabernet Sauvignon, clones 169, 191 and 412

Clone	**169**		**191**		**412**	
Parameters/Year	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
Bunch length (cm)	16.6±1.19	11.5±2.64	13.6±1.82	11.6±1.96	11.2±1.48	10.2±1.69
Bunch width (cm)	11.8±0.91	5.7±1.42	10.4±3.36	6.15±1.55	7.0±1.87	5.8±1.81
Bunch mass (g)	259.0±39.54	120.3±53.83	164.0±29.61	134.1±39.01	127.2±25.38	88.8±25.43
Average berries number in bunch	183.0*	124.3	142.0	138.9	112.2	112.0
Bunch stem mass (g)	9.6±2.41	3.9±1.87	8.2±1.1	5.0±1.89	4.6±1.52	3.1±0.99
Berries mass (g)	246.0±36.04	116.6±51.39	153.0±29.69	127.9±38.75	122.0±25.09	85.5±24.46
Mass of berry skin from 100 berries (g)	5.48	8.73	6.81	9.54	6.46	6.69
Mass of seeds from 100 berries (g)	3.08	3.82	4.37	5.08	3.71	4.36
Average seed number in 100 berries	115.0	150.0	167.0	153.0	135.0	131.0
Average berry skin mass from one bunch (g)	10.0	10.85	9.69	13.26	7.25	7.49
Average seed mass from one bunch (g)	5.62	4.75	6.22	7.06	4,17	4.89
% of stem in bunch	3.71±0.58	3.27±0.67	5.00±0.49	3.72±1.16	3.61±1.33	3.54±0.94
% of berries in bunch	96.3±0.58	96.73±0.67	95.0±0.49	96.27±1.16	96.38±1.33	96.46±0.94
% of epicarp in berry	4.28	9.70	5.32	10.84	5.43	8.98
% of seed in berry	2.41	4.24	3.41	5,77	3.12	5.86
% of mesocarp in berry	93.3	86.06	91.3	83.38	91.45	81.62
Berries indicator	70.6±11.54	125.01±59.99	86.8±16.22	110.6±28.44	88.2±18.5	134.24±32.5
Bunch structure indicator	9.24±1.1	5.02±2.23	5.80±0.99	4.25±1.24	6.94±1.51	4.69±1.31
Sugar content (%)	22.8	27.3	21.8	25.4	22.9	22.27
Acid content (g/l)	7.9	6.53	6.3	6.53	7.3	6.9
Glycoacidometric index	2.89	4.18	3.46	3.89	3.14	3.23
pH	2.55	3.13	2.99	3.12	3.0	3.08

*-values without standard deviations have a minimum SD (±0.0)

By examining of chemical parameters of grape juice, it was determined that clones 169 and 191 in the first reserach year had a higher sugar content (27.3 and 25.4%). Clones 169 and 412 had a lower total acid content in the second research year, while clones 191 had a higher content. The pH values in 2020 were lower with a variation from 2.55-3.12. All clones, based on the chemical parameters of grape juice analysis, meet the criteria for the production of premium red wines. Clones 169 and 191 had larger values variation of tested parameters during the two-year trial, while clone 412 was more stable with less variation in the obtained results. Lower values of most parameters during 2020 as well as greater variations can be attributed to the greater "sensitivity" of clones 169 and 191 to climatic conditions during maturation. During the vegetation in vineyard were applied same ampelotechnical measures to all clones. Precisely because of presented economic and technological differences, special attention is paid to varieties and clones selection for a specific geographical area in order to produce wine from the highest quality category (Popa et al., 2015; Morata, 2019; Pržić and Marković 2019). The results of the research are shown in Graph 1.



Graph 1. Qualitative parameters of grape juice

Conclusions

During 2020 year for most parameters of mechanical analysis, clone 169 stood out with higher values, while clone 412 had the lowest recorded values. The participation of mesocarp in berry indicates juice randman. Clone 169 was characterized with the highest percent of mesocarps in berry, while clones 191 and 412 had lower and approximately same values. In the second research year, skin and seeds participation in berry as well as the berry indicator had higher values, which indicates a higher oenological potential of clones in the second research year. During the two-year study by values of mechanical composition parameters of grapes and berries, clone 191 stood out on the first place, followed by clone 169 and finally clone 412.

Clones 169 and 191 in the first research year had higher sugar content, while clones 169 and 412 in the second year of research had a lower total acid content in grape juice. Examined clones, based on the chemical parameters of grape juice analysis had potential for the production of premium red wines.

Acknowledgements

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Original scientific paper

Heredity mode of duration of vegetative stage in onion (*Allium cepa* L.)

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Abstract

The duration of the vegetation period is a very important biological property of onion. In order to determine the ways of inheriting the duration of the vegetation period, the method of full diallel without reciprocals was applied, in order to obtain offspring of F1 and F2 generation. The field experiment with parents and hybrids of F1 and F2 generation was set up according to a random block system with five repetitions at the Institute for Vegetable Crops, Smederevska Palanka. The mode of inheritance of early maturity in this experiment was superdominance, observing all crossing combinations. The best general combiner in both generations was the AC 101 line. The highest significant value for SCA in both the F_1 and F_2 generations had the hybrid created by crossing Piroška x AC 101.

Key words: onion, inheritance, vegetation period

Introduction

Onion is one of the most widespread vegetable species grown from the far north to the equator, and depending on environmental conditions and purposes, different genotypes are represented. In the northern latitude countries, onion varieties have been grown for a long time, most often from seedling. In the climate conditions of Serbia, with medium and long days, onion varieties which belong to the group of hot or semi-hot are grown. In the conditions of the Mediterranean climate, with short-days, varieties that have a sweet taste and low dry matter content are grown (Lazić et al., 1993). Vegetative stage duration in onion is a trait that represents the number of days form sprouting to lodging (50%) of plants (Alahajkov, 1966). Duration of vegetative stage is an important biological trait and the adaptation of genotypes to certain ecological conditions, depends on this trait. Very often, one of the main breeding aims is early ripening (Galmarini, 1994). On the other hand, many scientist confirmed the direct connection among the vegetative stage duration and period of bulb formation, as well as the yield itself (Dawson and Abernethy 1979, Lazić et al., 1993). Determining the mode of inheritance is of extreme importance for breeders (Ortiz et al., 2001). By determining the combining abilities the choice of parental lines for obtaining good commercial onion varieties and hybrids is narrowed. Evaluation of parental lines is the start point for its further appliance in selection (Pavlović et al., 2015). The success of the selection depends on the gene determination of traits chosen as the aim of selection. Breeders can expect the positive answer in breeding with traits with high heterosis, which is important for the success in creating new varieties (Zdravković et al., 2010, Pavlović et al., 2011).

Material and Methods

Divergent genotypes of different geographical origin from the collection of onion germplasm of the Institute for Vegetable Crops were selected for this experiment. In a two - year study (2015 and 2016) in Smederevska Palanka, the diallel hybridization method was applied. Selected genotypes were: Makoi bronzi, Piroška, AC 101, Jasenički crveni and Bunkino beo. Vegetative stage duration with parents and hybrids of F_1 and F_2 was set up in random block system with five replications with 30 plants per replica. Standard growing technology in growing onion was applied. The evaluation of heredity mode of onion was performed by applying significance test of mean values of F_1 hybrids and F_2 generations comparing to parental average by Borojević (1986). Separation of genetic variance was performed by applying Hayman (1954) Mather and Jinks (1971) method and the analysis of combining abilities was performed by applying Griffing (1956), method 2, mathematic model 1, which includes parental and F_1 and F_2 generation.

Results and Discussion

The average value of the vegetation period in analysed parental genotypes varied from 92.33 days (Makoi bronzi) to 99.33 days (AC 101). Analysis of hybrids in F1 generation proved that the earliest hybrid had 85 days long vegetation (Jasenički crveni x Bunkino beo, Table 1). The latest hybrid was Piroška x AC 101 (107.33 days). In F2 generation of the same combination kept the primacy of the earliest and the latest hybrid combinations. Variability expressed through variation coefficient (Cv), in parental lines was from 3.19% (AC 101) to 12.05% for Bunkino beo. Variability in F1 hybrid was lower: 0.68% (AC 101 x Jasenički crveni) to 8.08% (AC 101 x Bunkino beo). Variability in F2 generation was form 6.27% (Makoi bronzi x AC 101) to 10.34% (AC 101 x Bunkino beo, Table1). In determinating the heredity mode for the duration of the vegetative stage in onion for three hybrid combinations in the F1 generation and two in the F2 generation, a statistically significant value was determined. This study proved the statistically significant values and the existence of three heredity modes of this trait: superdomination, partial and domination. All mentioned heredity modes were present in one of the hybrid combinations in the F1 generation, while in the F2 generation no partial domination was determined (Table 2). For these studied trait four hybrids had negative heterosis values, which practically represents the shortage of vegetative period in hybrids (F_1) comparing to parental average and this was the better perent. The highest percentage of negative heterosis had hybrid obtained by crossing lines Jasenički crveni x Bunkino beo (-10.37%). In F_2 generation no significant negative heterosis was found. (Table 1). Similar ways of inheriting the heterosis for vegetative stage duration was found by Panajotović (1986). Results of component analysis of genetic variance show that the value of additive component of variance (D) was lower than the dominant (H_1 and H_2) in both generations, which was the indicator of dominant gene action (Table 3).

Table 1: Mean value (□), standard error (Sx), variation coefficient (Cv), heredity mode, absolute and relative heterosis (Ha и Hr) for earliness of the researched lines of F_1 and F_2 onion hybrids

Genotype	**F_1**		F_2		Ha		**Hr**	
	$\bar{x}$ ±Sx	Cv (%)	$\bar{x}$ ±Sx	Cv (%)	F_1	F_2	F_1	F_2
Makoi bronzi (MB)	98.00±38.18	4.56	98.00±38.18	4.56				
MB x PR	96.67^{Pd+}±37.33	5.34	97.33±36.40	8.39	1.50	2.17	1.57	2.27
MB x AC 101	100.00±40.02	2.00	98.33±37.64	6.27	1.33	-0.33	1.35	-0.34
MB x JC	97.33±36.80	7.36	98.33±36.03	10.34	-0.17	0.83	-0.17	0.85
MB x BB	93.67±35.19	7.65	96.67±35.90	8.97	-1.67	1.33	-1.74	1.39
Piroška (PR)	92.33±35.46	5.60	92.33±35.46	5.60				
PR x AC 101	107.33^{Sd+}±42.64	2.95	109.00^{Sd+}±41.03	8.72	11.50^{**}	13.17^{**}	12.00	13.74
PR x JC	101.33±39.34	5.10	98.00±36.76	8.16	6.67^{*}	3.33	7.04	3.52
PR x BB	93.33±35.90	5.54	94.00±34.36	10.11	0.83	1.50	0.900	1.62
AC 101	99.33±39.28	3.19	99.33±39.28	3.19				
AC 101 x JC	97.67±39.60	0.68	98.33±36.64	8.81	-0.50	0.17	-0.51	0.17
AC 101 x BB	99.00^{d+}±37.20	8.08	99.33^{d+}±36.48	10.23	3.00	3.33	3.12	3.47
Jasenički crveni(JC)	97.00±35.30	10.82	97.00±35.30	10.82				
JC x BB	85.00±33.76	2.35	88.67±32.98	8.08	-9.83^{**}	-6.17	-10.37	-6.50
Bunkino beo (BB)	92.67±33.04	12.05	92.67±33.04	12.05				
	F_1 $lsd_{0.05}$ = 5.43 $lsd_{0.01}$ = 7.22		F_2 $lsd_{0.05}$ = 6.66 $lsd_{00.1}$ = 8.86					

In expression of this trait, great impact had recessive alleles, which is indicated by the F value, which was negative. This fact is supported by the ratio of the total number of dominant to recessive alleles (Kd / Kr = 0.800 and 0.732), which was less than one. Dominant and recessive alleles were not equally distributed in the parental lines ($H_2/4H_1$=0.181 and 0.196) (Table 2).

Table 2. Components of genetic variances for earliness of onion bulbs

Components	Values F_1	Values F_2
D	6.464	4.596
H_1	78.776	57.346
H_2	56.926	44.923
F	-5.025	-5.023
E	3.680	5.490
$H_2/4H_1$	0.181	0.196
$\sqrt{H_1/D}$	3.491	3.532
Kd/Kr	0.800	0.732
HU^2	0.482	0.396
$HŠ^2$	0.893	0.800

The average level of domination ($\sqrt{H_1/D}$ = 3.49 и 3.53) was higher that one, which proves that the inheritance way of earliness was superdomination, for all crossing combinations. The value for heritability in narrow sense was 0.45, and in wider 0.89. Similar values were obtained heritability in F_2 generation. High values of heterosis also points to a higher share of genetic factors in inheriting this trait. Variance analysis of combining abilities in our researches, the significant values for GCA and SCA in both crossing generations were determined. The calculated GCA values were 2.06 times (in F_1 generation) and 1.83 (in F_2 generation) higher than the SCA values, which points to dominant additive gene effect in inheriting the earliness (Table 4). Identical results were obtained by Panajotović (1986) и El-Sayed-Am et al., (1999).

Table 3. Variance analysis of combining abilities for earliness

Source of variation	Level of freedom	Sum of squares (SS)		Middle of squares (MS)		F-exp	
		F_1	F_2	F_1	F_2	F_1	F_2
GCA	4	159.981	119.092	39.995	29.773	10.867^{**}	5.365^{**}
SCA	10	193.545	161.989	19.354	16.198	5.258^{**}	2.919^{*}
Error	28			3.680	5.548		
GCA/SCA				2.066	1.837		

GCA $F_{0.05}$ = 2.71 $F_{0.01}$ = 4.07
SCA $F_{0.05}$ = 2.19 $F_{0.01}$ = 3.03

The best general combiner in both studied generations was line AC 101. It had significant positive values on both levels of significance in F_1 generation, while in F_2 generation only on level 0.05, (Table 4). The lowest level for GCA in both generations had line Bukino beo.

Table 4. GCA Values of parental lines for earliness

Parents	Value GCA F_1	**Range**	Se	Value GCA F_2	**Range**	**Se**
MB	0.485	2		0.533	2	
PR	0.438	3		0.009	3	
AC 101	3.20^{**}	1	1.025	2.961^{*}	1	1.259
JC	-0.704	4		-080	4	
BB	-3.419^{**}	5		-2.704^{*}	5	

$lsd_{0.05}$= 2.050 $lsd_{0.05}$=2.518
$lsd_{0.01}$= 2.727 $lsd_{0.01}$=3.349

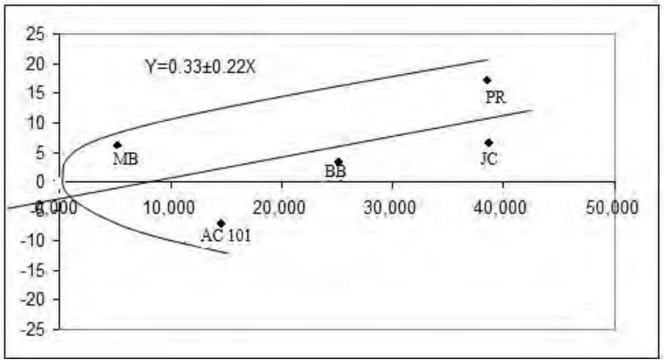
The highest significant value for SCA in both F_1 and F_2 generation had hybrid made by crossing Piroška x AC 101, while significant positive value at the level 0.05 had hybrid Piroška x Jasenički crveni. Significant negative value was determined in hybrid combination Jasenički crveni x Bunkino beo and negative value was determined in five more combinations, but not the significant values of SCA. In F_2 generation, only one significant positive value was determined for hybrid combination Piroška x AC 101 (Table 5).

Table 5. Values of SCA F_1 and F_2 generation for bulb earliness

Genotype	SCA F_1	Se	SCA F_2	Se
MB x PR	-0.968		-0.365	
MB x AC 101	-0.396		-2.317	
MB x JC	0.841		1.444	
MB x BB	-0.111		1.682	
PR x AC 101	6.980^{**}	2.292	8.873^{**}	2.815
PR x JC	4.880^{*}		1.634	
PR x BB	-0.396		-0.460	
AC 101 x JC	-1.539		-0.984	
AC 101 x BB	2.507		1.920	
JC x BB	-7.587		-4.984	

$lsd_{0.05}$ = 4.585 $lsd_{0.05}$ = 5.630
$lsd_{0.01}$ = 6.099 $lsd_{0.01}$ = 7.489

According to Vr Wr regression (Graph 1 and 2), regression coefficient b = 0.33 for F_1 generation and b = 0.35 for F_2 generation at level 0.05 significantly differs from one, which proves the presence of inter-allele interaction of genes in inheriting earliness of onion.

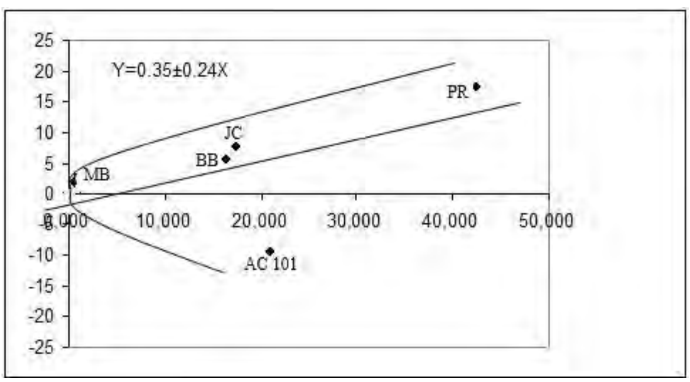


Graph 1. Vr Wr regression for earliness in F_1 generation

Regression line was distant from the limiting parabola, which proves that the impact of non-additive genes was higher (Graph 1 and 2).

The intersection of the expected regression line with the Wr axis was located above the coordinate start, which is an indicator of partial dominance as a way of inheriting this trait, (Graphs 1 and 2).

The arrangement of the scatter plot points indicates the genetic divergence of the selected parental genotypes. Since neither parent was at the intersection of the limiting parabola with the regression line, this indicates that not all parents have all the dominant or all recessive genes.



Graph 2. Vr Wr regression for earliness in F_2 generation

In F_1 generation the line Makoi bronzi was the carrier of the dominant genes, while lines Piroška and Jasenički crveni were the carriers of the recessive genes. In F2 generation, the picture was very similar except for the *Jasenički crveni* genotype, which now carried approximately the same number of recessive and dominant alleles (Graph 2).

El-Sayed-AM et al. (1999) in their research found that the additive genetic variant is responsible for controlling the early maturity of onions.

Conclusion

Our results proved that the earliest parental line was Makoi bronzi and the latest AC 101. Analysis of hybrids of F_1 generation proved that the earliest hybrid had the vegetative period of 85 days (Jasenički crveni x Bunkino beo). The latest hybrid was Piroška x AC 101 (107.33 days). In F_2 generation, combinations kept the primat of the earliest and the latest hybrid combinations. Heredity mode of earliness in this experiment was the superdomination in all crossing combinations. The best general combiner in both researched generations was line AC 101. The highest significant value of SCA in F_1 and F_2 generation had hybrid Piroška x AC 101.

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Način nasleđivanja dužine vegetacionog perioda kod crnog luka (*Allium cepa* L.)

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Sažetak

Dužina vegetacionog perioda predstavlja jako važnu biološku osobinu crnog luka. Kako bi utvrdili načina nasleđivanja dužine vegetacionog perioda u ovom eksperimentu primenjen je metod punog dialela bez recipročnih ukrštanja, radi dobijanja potomstva F1 i F2 generacije. Poljski ogled sa roditeljima i hibridima F1 i F2 generacije postavljen je po slučajnom blok sistemu u pet ponavljanja u Institutu za povrtarstvo, Smederevska Palanka. Način nasleđivanja ranostasnosti u ovom eksperimentu je superdominacija, posmatrajući sve kombinacije ukrštanja. Najbolji opšti kombinator u obe generacije ispitivanja bila je linija AC 101. Najvišu signifikantnu vrednost za PKS i u F_1 i F_2 generaciji imao je hibrid nastao ukrštanjem Piroška x AC 101.

Ključne reči: crni luk, nasleđivanje, vegetacioni period

Review paper

Court garden in Sremski Karlovci – natural and cultural heritage of Serbia

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Abstract

The purpose of the research of „Court garden in Sremski Karlovci“ was to find out when exactly it was designed, what was the original project and architecture of Garden, to find out historical names, historical plants of Garden, and to make an inventory of dendroflora in 2022. In this paper landscape-architectural, horticultural, dendrological, natural, cultural, historical and other values of the garden protected as natural monument, were represented. Archival material from archives, museums, documentation of the Institute for Nature Conservation of Vojvodina province, of Cultural Heritage Preservation Institute, the current urban planning, cadastral maps, plans, old photographs, postcards, e sources, etc. has been investigated. The Court garden was designed in the first half of the 19th century. A 117 species and lower taxa (21 coniferous and 96 deciduous) were recorded. Based on the analysis of the current state of the garden, values, historical genesis and current needs, it is possible to propose a measures of the protection, renewal and development.

Key words: Court garden, natural monument, dendroflora, historical and field analysis

Introduction

Natural monoument Court garden is located in Serbia, in Vojvodina, settlement of Sremski Karlovci - historic town of great architectural and cultural-historical value. The historical gardens and parks of Serbia are valued as natural monouments protected by the Law of Nature Conservation (“Official Gazzette or the Republic of Serbia” No. 36/2009, 88/2010, 91/2010, 14/2016, 95/2018, 71/2021). Sremski Karlovci is a historic town of great architectural and cultural-historical value protected by the Law on Cultural Heritage (“Official Gazzette or the Republic of Serbia” No. 71/94, 52/2011, 99/2011, 6/2020, 35/2021). Court garden is located on the left bank of the Strazilovo stream. It belongs to the Serbian Patriarchate. The garden was built on the site of the utilitary garden of the Metropolitanate of Karlovci. Mićić (2004) presents Court garden date back to early 18th century. On the initiative of Metropolitan Josif Rajačić, a park with a flower garden and a brick greenhouse was built. The total area is 7.29 ha. The first preserved data on woody species date back to 1957, when the forestry engineer Lazar Maširević, noticed about 200 species and lower taxon units in the garden and inside the greenhouse (private documentation of Atanacković). Ninić Todorović, 1986 records 137 species and 24 lower taxonomic units (varieties and forms).

Material and Methods

Archival material from different periods of historical genesis from archives, museums, documentation of the Institute for Nature Conservation of Vojvodina province, of Cultural Heritage Preservation Institute, the current urban planning, cadastre, old photographs, postcards, etc. has been investigated. Analysis was carried out in the field in the aim to: perform the audit of boundaries of the park and total area, prepare complete list of dendroflora. Historical gardens were presented in the UNESCO Venice Charter (1964). The Florence Charter (ICOMOS, 1982) is basic document in the field of preservation of historical gardens. The Florence Charter classified historic gardens as “living monuments” with a particular public interest from a cultural point of view, independently from the historic style, the design, the surface and the property (public or private).

Results and Discussion

Landscape-architectural and cultural-historical values

Court garden is Natural Monument, in the boundaries of Important Plant Area (IPA). Local ecological corridor stream of Strazilovo flows through the Garden. The database is compiled and maintained by the Institute for Nature Conservation of Vojvodina province. Gardens are important elements green infrastructure providing ecosystem services. Court garden is located in the old nucleus of the settlement of Sremski Karlovci, border with the slopes of Natural Park Fruska gora covered with vineyards and forests. Park greenery is covering 7.29 ha.

Original plan of Court garden dated back in 1844 was presented in internet (https://www.delcampe.net/en_GB/collectables/search?search_mode). The original name was Metropolitanate of Karlovci Garden of Contemplation. The historical data collected provide information about the park’s structure and design. Park was created by combining elements of the geometric design (formal garden) with the lime tree alley ending with fountain and a green area covered with more or less circular paths and lawns with smaller groups of mostly deciduous trees (informal garden).

Figure 1: The lime trees alley (Mladjenovic, 2021.)

The layout of paths in the northwestern part of the space that today still be seen on old postcards original plan testified about original design. Some paths from the original plan are preserved. These are primarily the lime trees alley at the main entrance, the path in front of the greenhouse, The path in the center of space connects the lower and upper plateau partially covered with macadam and stone steps and paths and bridges on the stream. Alleys gained special significance during the Baroque as dynamic and grandiose space-forming garden design elements (Szilágyi et al, 2020). Over time the informal layout of the paths in the garden, and especially on the lower plateau in front of the greenhouse, has been completely abandoned. Of the plants on the ground floor in front of the greenhouse, only yews (*Taxus baccata* L.) remained.

Figure 2: Orangery in the Court garden

Figure 3:An example of a similar orangery from the beginning of the 19th century, England

The original plan represents the space with the historic buildings like orangery, a complex of buildings in the central part of the space and a building at the end of the Linden Alley and fountain. Orangery was constructed from brick and it has a north-facing wall and tall windows faceing south. It had a heating system inside. The old wine cellar, in the center of Garden, under the upper plateau, and the authentic gate with part of the fence at the main entrance to the garden has been preserved. All of these objects are still presented, but their condition requires renewal. Old stone benches have not been preserved. Solitary specimens of exotics further beautified the garden, and many species were brought into the greenhouse in winter. Postcards by private collectors Jasna Atanacković and Branislava Arsenijević are a testimony to the appearance of the Court garden from the time before the First World War. The Court garden used to be decorated with exotic species such as palm trees, agaves, and araucaria, which were taken out in summer in wooden containers. Old postcards showing the mentioned exotic plants were given by Mićić, 2004.

Figure 4: Original plan "Plan des Lustgarten siene Excllenz zu Carloviz. 1844. (https://www.delcamp.net/en_GB/collectables/search?search_mode)

Figure 5: Old cadastre map from the 19th century (https://maps.arcanum.com/en/map/europe-19centurysecondsurvey/?layers=158%2C164&bbox=2215511.29183435%2C5651413.340841524%2C2224602.5208860184%2C5654681.023800716)

Figure 6: Map of Natural Monument „Court garden" (the Institute for Nature Conservation of Vojvodina province)

Dendroflora

The park has various species and indigenous dendroflora, allochthonous species and exotics.

Mostly deciduous tress are represented. In total, 117 species and lower taxa (21 coniferous and 96 deciduous) was noted by field analysis in 2022.

Dendroflora of the Court Garden in Sremski Karlovci in 2022:

Family: *Ginkgoaceae* Engl.

1 *Ginkgo biloba* L. – Ginkgo

Family: *Pinaceae* Spreng. ex F.Rudolphi

2 *Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr. – White Fir

3 *Abies cephalonica* Loudon – Greek Fir

4 *Abies nordmanniana* (Steven) Spach – Caucasian Fir

5 *Pseudotsuga menziesii* (Mirb.) Franco – Common Douglas-fir

6 *Pseudotsuga menziesii* subsp. glauca (Beissn.) A.E.Murray – Common Douglas-fir

7 *Picea abies* (L.) H. Karst – Norway Spruce

8 *Picea omorika* (Pancic) Purk. – Serbian Spruce

9 *Cedrus atlantica* (Endl.) Manetti ex Carrière – Atlas Cedar

10 *Pinus wallichiana* A.B.Jacks. – Blue Pine

11 *Pinus nigra* J.F.Arnold – Black Pine

12 *Pinus sylvestris* L. – Scots Pine

Family: *Cupressaceae* Gray

13 *Juniperus × pfitzeriana* (Späth) P.A.Schmidt

14 *Juniperus virginiana* L. – Eastern Juniper

15 *Cupressus pyramidalis* O.Targ.Tozz.

16 *Cupressus sempervirens* subsp. horizontalis (Mill.) A.Camus

17 *Chamaecyparis lawsoniana* (A.Murray bis) Parl. – Lawson's Cypress

18 *Platycladus orientalis* (L.) Franco – Oriental thuja

19 *Thuja plicata* Donn ex D.Don – British Columbia red cedar

Family: *Taxaceae* Gray

20 *Taxus baccata* L. – European Yew

Family: *Magnoliaceae* Juss.

21 *Magnolia liliiflora* Desr.

Family: *Berberidaceae* Juss.

22 *Berberis aquifolium* Pursh – Oregon grape

Family: *Ranunculaceae* Juss.

23 *Clematis vitalba* L. – Traveller's-joy

Family: *Platanaceae* Lindl.

24 *Platanus* × *hispanica* Mill. ex Münchh. – London Plane

Family: *Ulmaceae* Mirb.

25 *Ulmus minor* subsp. minor – Field Elm

26 *Ulmus pumila* L. – Siberian Elm

Family: *Cannabaceae* Martinov

27 *Celtis australis* L. – European Nettle Tree

28 *Celtis occidentalis* L. – Common Hackberry

Family: *Moraceae* Gaudich.

29 *Morus alba* L. – White Mulberry

30 *Morus alba pendula* (Risso) Sudw.

31 *Broussonetia papyrifera* (L.) L'Hér. ex Vent. - Paper Mulberry

32 *Maclura pomifera* (Raf.) C.K.Schneid. – Osage-orange

Family: *Fagaceae* Dumort.

33 *Fagus* × *taurica* Popl. – Crimean beech

34 *Castanea sativa* Mill. – Roasted chestnuts

35 *Quercus rubra* L. – Northern Red Oak

36 *Quercus robur* L. – Pedunculate Oak

37 *Quercus robur* L. 'Fastigiata'

38 *Quercus petraea* (Matt.) Liebl. – Sessile Oak

39 *Quercus pyrenaica* Willd. - Pyrenean Oak

Family: *Betulaceae* Gray

40 *Betula pendula* Roth – Silver Birch

41 *Alnus glutinosa* (L.) Gaertn. – Black Alder

42 *Carpinus betulus* L. – Hornbeam

43 *Carpinus orientalis* Mill. – Oriental Hornbeam

Family: *Corylaceae* Mirb.

44 *Corylus colurna* L. – Turkish Hazel

45 *Corylus avellana* L. – Common Hazel

Family: *Juglandaceae* DC.ex Perleb

46 *Pterocarya fraxinifolia* (Poir.) Spach – Caucasian Wingnut

47 *Juglans regia* L. – Persian Walnut

Family: *Salicaceae* Mirb.

48 *Populus tremula* L. – Eurasian Aspen

49 *Populus alba* L. – White Poplar

50 *Populus nigra* f. italica (Münchh.) A.Andersen

51 *Salix alba* L. – White Willow

Family: *Malvaceae* Juss.

52 *Tilia tomentosa* Moench - Silver lime

53 *Tilia cordata* Mill. - Small-leaved Lime

54 *Tilia platyphyllos* Scop. – Large-leaved Lime

Family: *Malvaceae* Juss.

55 *Hibiscus syriacus* L. – Common Hibiscus

Family: *Rosaceae* Juss.

56 *Spiraea × vanhouttei* (Briot) Carrière

57 *Kerria japonica* (L.) DC.

58 *Rosa canina* L.

59 *Rubus fruticosus* L. - European blackberry

60 *Prunus laurocerasus* L. – Cherry-laurel

61 *Prunus mahaleb* L. – Saint Lucie Cherry

62 *Prunus cerasifera* Ehrh. – Cherry Plum

63 *Prunus cerasifera* var. Atropurpurea Jaeg.

64 *Cydonia oblonga* Mill.- Quince

65 *Chaenomeles japonica* (Thunb.) Lindl. ex Spach – Japanese quince

66 *Crataegus monogyna* Jacq. – Common Hawthorn

67 *Torminalis glaberrima* (Gand.) Sennikov & Kurtto

68 *Pyrus communis* L. – European Wild Pear

Family: *Hydrangeaceae* Dumort

69 *Philadelphus coronarius* L.- Sweet Mock-orange

70 *Deutzia scabra* Thunb.

Family: *Fabaceae* Lindl.

71 *Cercis siliquastrum* L. - Judas-tree

72 *Gleditsia triacanthos* L. – American Honey-locust

73 *Styphnolobium japonicum* (L.) Schott – Pagoda Tree

74 *Robinia pseudoacacia* L. – Black Locust

75 *Caragana arborescens* Lam. – Siberian pea-tree

Family: *Anacardiaceae* R.Br.

76 *Cotinus coggygria* Scop. – Smoke Bush

Family: *Simaroubaceae* DC.

77 *Ailanthus altissima* (Mill.) Swingle – Tree of Heaven

Family: *Rutaceae* Juss.

78 *Ptelea trifoliata* L. – Common hoptree

Family: *Sapindaceae* Juss.

79 *Koelreuteria paniculata* Laxm. – Goldenrain Tree

80 *Acer negundo* L. – Manitoba Maple

81 *Acer negundo* 'Variegatum' - Variegated Box Elder

82 *Acer platanoides* L. – Norway Maple

83 *Acer campestre* L. – Field Maple

84 *Acer pseudoplatanus* L. – Sycamore Maple

85 *Acer atropurpureum* Dippel

86 *Acer tataricum* L. – Tatar Maple

Family: *Cornaceae* Bercht. & J.Presl

87 *Cornus mas* L. - European Cornel

88 *Cornus sanguinea* L. – Common Dogwood

Family: *Araliaceae* Juss.

89 *Hedera helix* L. – Common Ivy

Family: *Hippocastanaceae* DC.

90 *Aesculus hippocastanum* L. – Horse-chestnut

Family: *Celastraceae* R.Br.

91 *Euonymus europaeus* L. – European Spindle

92 *Euonymus verrucosus* Scop.

Family: *Vitaceae* Juss.

93 *Parthenocissus quinquefolia* (L.) Planch.

94 *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch.

Family: *Apocynaceae* Juss.

95 *Vinca minor* L. - Lesser Periwinkle

Family: *Oleaceae* Hoffmanns. & Link

96 *Jasminum nudiflorum* Lindl.

97 *Chrysojasminum fruticans* (L.) Banfi

98 *Ligustrum vulgare* L. – Common Privet

99 *Ligustrum ovalifolium* Hassk.

100 *Forsythia suspensa* (Thunb.) Vahl – Weeping Forsythia

101 *Forsythia x intermedia* Zabel

102 *Syringa vulgaris* L. - Common Lilac

103 *Fraxinus ornus* L. – Manna Ash

104 *Fraxinus excelsior* L. – Common Ash

105 *Fraxinus excelsior* subsp. excelsior

106 *Fraxinus excelsior* 'Diversifolia'

107 *Fraxinus pennsylvanica* Marshall

Family: *Caprifoliaceae* Juss.

108 *Symphoricarpos albus* (L.) S.F.Blake – Snowberry

109 *Symphoricarpos orbiculatus* Moech – Coralberry

110 *Lonicera tatarica* L.

Family: *Viburnaceae* Raf.

111 *Sambucus nigra* L. – Common Elder

Family: *Bignoniaceae* Juss.

113 *Catalpa bignonioides* Walter - Southern Catalpa

Family: *Paulowniaceae* Nakai

114 *Paulownia tomentosa* Steud. - Empress Tree

Family: *Asparagaceae* Juss.

115 *Ruscus aculeatus* L. - Butcher's Broom

116 *Ruscus hypoglossum* L. - Spineless Butcher's Broom

117 *Yucca filamentosa* L. - Adam's Needle

The park is dominated by deciduous trees. The most common species are: *Styphnolobium japonicum* (L.) Schott, *Broussonetia papyrifera* (L.) L'Hér. ex Vent., *Tilia tomentosa* Moench, *Gleditsia triacanthos* L., (*Ailanthus altissima* (Mill.) Swingle, *Acer campestre* L. and *Fraxinus ornus* L..

The solitary trees *Corylus colurna* L., *Pterocarya fraxinifolia* (Poir.) Spach and *Tilia tomentosa* Moench stand out for their great age and beauty. In parks in Vojvodina *Fraxinus excelsior 'Diversifolia'*, *Castanea sativa* Mill., *Torminalis glaberrima* (Gand.) Sennikov & Kurtto and *Thuja plicata Donn ex D.Don* which are present in the Court Garden are rare.

Invasive dendroflora species have also been registered in the garden, the retention of which is not recommended. These are: *Ulmus pumila* L. – Siberian Elm, *Celtis occidentalis* L. – Common Hackberry, *Morus alba* L. – White Mulberry, *Broussonetia papyrifera* (L.) L'Hér. ex Vent. - Paper Mulberry, *Maclura pomifera* (Raf.) C.K.Schneid. – Osage-orange, *Gleditsia triacanthos* L. – American Honey-locust, *Robinia pseudoacacia* L. – Black Locust, *Ailanthus altissima* (Mill.) Swingle – Tree of Heaven, *Acer negundo* L. – Manitoba Maple, etc.

In an effort to turn the ornamental garden into a public park, after World War II, decorative plants were cut down from a significant area of the upper plateau and a sports complex was built, and many plants have been lost from the garden, like a several species from the following genera: *Abies* sp., *Picea* sp., *Pinus* sp., *Juniperus* sp., *Thuja sp., Ulmus* sp., *Quercus* sp., *Populus* sp., *Cedrus* sp., *Cryptomeria* sp., *Taxodium* sp., *Larix* sp., *Liriodendron* sp., *Parrotia* sp., *Zelkova* sp., *Morus* sp., *Hydrangea* sp., *Laurus* sp., *Albizia* sp., *Gymnocladus* sp., *Laburnum* sp., *Wisteria* sp., *Elaeagnus* sp., *Arbutus* sp., *Agave* sp., etc., are not presented in the Garden any longer.

Conclusion

Based on the analysis of the present conditions of the park, values, historical data and results in this paper, it is possible to propose measurement of renewal. Present condition of park is the basis for the planning and design process. In relation to the time when the park was formed there is an obvious inadequate layout of paths, open space and land area under trees and bushes, so the concept of the park is disturbed. Identifying the historical compositional elements, including a system of paths, botanical species to be protected, safeguarded and replanted should be considered the first step for future management planning process. It is important to preserve the existing linden trees alley and all valuable trees. The orangery in Court garden is one of the very few remaining buildings of that type in Vojvodina. All buildings and garden architectural elements require renewal and development.

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Original scientific paper

Economic benefits of raspberry growing in a protected area

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Abstract

This research examined an economic analysis of raspberry production cv. Heritage in the greenhouse. As domestic experiences with the cultivation of floricane raspberry cultivars are very modest, the aim of the paper is to show the possibilities and point out the advantages of growing the raspberry cv. Heritage in a protected area on an agricultural farm in Milićevo village (Požega municipality). The calculation of production was done for two years, 2018 and 2019. Total production costs, total revenues and cost-effectiveness of production are shown. The yield in the orchard of 0.2 ha was 4,650 kg in 2018 and 5,145 kg in 2019. In 2018, the purchase price was 5 €/kg for raspberries of class I and 1 €/kg for raspberries class II, and in 2019 4 €/kg for raspberries of class I and 1.4 €/kg for raspberries of class II. In 2018, was generated a profit of € 10,200, and in 2019 a profit of € 8,183.The advantage of growing raspberries in a protected area, shows the great interest of producers in this type of production, both because of the quality of the products and because of the change in the climatic characteristics in Serbia.

Key words: raspberry, yield, revenue, costs

Introduction

In recent years, not only primocane but also floricane raspberry cultivars have been introduced into production and they are grown in the open field or in a protected area of the tunnel and semi-tunnel type. The advantages of floricane raspberry cultivars are the expansion of the growing area in less suitable areas for primocane cultivars, then the extended harvest season from two to three months and in the period when there are far fewer fresh raspberries on the market, the possibility of machine harvesting. The disadvantages of floricane raspberry cultivars are reflected in lower yields, lower fruit value (most varieties darken when frozen), frequent immaturity of large quantities of fruit (especially if the cold autumn occurs early), mandatory irrigation, shade during flowering in warmer regions (Milivojević et. al., 2011).

Breeders in Europe and the USA are working very intensively on creating better floricane varieties of raspberries. The most represented in the production are the cultivars: Heritage, Polka, Polana, Autumn Bliss and Erika (Nikolić and Milivojević, 2015). Serbia has the potential for continuous production of raspberries in the period from six to seven months, ie from the beginning of May to the end of October or the middle of November, combining primocane and floricane cultivars and different altitudes where this production would take place. Such a chance has not been realized so far, which does not mean that it will not in the future.

As domestic experiences with the cultivation of floricane raspberry varieties are very modest, the aim of this paper is to show the possibilities and point out the advantages of growing the floricane raspberry cv. Heritage in a protected area.

Material and Methods

The experimental orchard is located on the family farm in Milićevo village (12 km away from Požega) in a protected area, in greenhouses (tunnels). The orchard was established in 2017. Canes were trained to narrow ribbons with spacing 2 m × 0.33 m (3300 canes) placed in 4 greenhouses.

For the purposes of this paper, data from the production of the raspberry cv. Heritage was used. It is grown in the USA, Poland, and sporadically in Serbia (Vojvodina-Deronja, Mladenovac, Arandjelovac, Lazarevac, Kraljevo, Prijepolje and Požega). In recent years, it is also grown in Spain, Morocco and Mexico, and in Chile, this is the dominant cultivar. It ripens late, belongs to the late autumn cultivar. Heritage in the protected area has two yields, the first yield in early June, and the second in late August, depending on climatic conditions and orographic factors. Shoots are numerous, strong, erect and yielding. The fruit is medium-sized (3-3.5 g), fringed conical, red, firm and sweet and sour, easy to pick. It is used mainly for fresh consumption, and the rest for freezing and processing. It tolerates slightly heavier soils well, but in case of poor drainage, root rot develops. Drip irrigation was a regular agromeliorative measure in the process of raspberry production in our experiment.

The harvest was done on two occasions, summer and autumn. The yield was calculated and expressed through the total yield per harvest, yield per seedling, yield per shoot as well as fruit mass per fruiting branch. Economic analysis of production was performed using the calculation method. The calculation of production was done for two years 2018 and 2019. Total production costs, total revenues and cost-effectiveness of production (Ep) are shown.

Results and Discussion

The total number of raspberry plants is 3300 on an area of 0.2 ha, grown in four greenhouses. One plant has three canes with eight fruiting branches per cane. There were about 5-6 fruits per fruiting branch. The harvest was done on two occasions, summer and autumn. Yield, expressed through total yield per harvest, yield per seedling, yield per shoot as well as fruit mass per fruiting branch, is shown in Table 1 for 2018 and 2019.

Table 1. Yield of raspberry cv. Heritage in 2018 and 2019

		Total Yield (kg)	Yiled/Plant (g)	Yield/Cane (g)	Fruit mass/Fruiting branch (g)
2018	Summer yield	1653	501	167	20,9
	Autumn yield	2996	908	302,7	37,8
	∑	4649			
2019	Summer yield	2084	631	210	26,30
	Autumn yield	3061	928	309	38,65
	∑	5145			

As it is shown in Table 1., in both experimental years, the highest yield was achieved in the summer harvest. Harvesting raspberry Heritage in a greenhouse, with certain shade nets, has far greater advantages: the fruit is dry (reduced level of gray rot of the fruit), has no burns (in high temperatures), can be harvested in bad weather (rain). Production is significantly more expensive, but due to the earlier ripening of the fruit, higher prices are achieved on the market, which covers the increased production costs. The knowledge so far indicates that very high yields are achieved and better quality of fruits is obtained, they are firmer and uniform in size.

Calculations in the production of raspberry Heritage

Investments in raspberry production are as specific as the production itself owing to the influence of climatic factors, the discrepancy between the production time and working hours, the biological nature of certain working processes and the like. Biological processes, due to their cyclical and seasonal character, have an impact on the realization of investments in certain periods of the year (Sredojević et al., 2013; Galić et al., 2014). Based on the production results and costs during the production, calculations of raspberry production for the observed period of two years were made.

Table 2 shows the calculation of costs in 2018 for the area of one greenhouse and it amounts to € 1960.5. On an area of 0.2 ha (4 greenhouses) the total costs are 1960.5 € x 4 = 7 842 €. The yield of raspberries in 2018 was 4,650 kg. In total, 72% (3,348 kg) were in class I, and 28% (1,302 kg) in class II. Revenue € / 0.2 ha: 3,348 kg x € 5 = € 16,740 1,302 kg x € 1 = € 1,302 Total: € 18,042.

PRODUCTION VALUE - PRODUCTION COSTS: € 18,042 - € 7,842 = € 10,200

The profit in 2018 amounted to € 10,200.

Table 2. Cost calculation in 2018

Type of cost	Unit of measurement	Requirement per tunel	Price per unit of measurement (€)	Total cost (€)	
A. LABOUR COSTS					
Protection and fertilization	workday	2	20.00	40.00	
Binding shoot and tightening wire	workday	2	20.00	40.00	
Removal of young shoots	workday	1	20.00	20.00	
Harvesting	workday	60	20.00	1200.00	
Pruning (pinching) removal of old shoots	workday	1	20,00	20.00	
TOTAL: 1320.00					
B. MACHINERY COSTS					
Protection	workday	/	manually	/	
Other works	workday	2	100	200.00	
TOTAL:					200.00
C. MATERIAL COSTS					
Nitrogen fertiliser (CAN)	ml	250	7.3	7.30	
Complex fertiliser (NPK)	kg	100	0.17	17.00	
Plant protection products	Integral protection program			70	
Irrgation	spring platoon			/	
Pump mixture				20	
TOTAL:					114.50
D. OTHER COSTS					
Unforeseen expenses				300.00	
COSTS A + B + C + D		A. Labour costs		1320.00	
		B. Machinery costs		200.00	
		C. Material costs		114.50	
		D. Other costs		300	
		TOTAL:		**1960.50**	

Table 3. shows the calculation of costs in 2019 for one greenhouse.

On an area of 0.2 ha (4 greenhouses) the total costs are 1995.5 € x 4 = 7 982 €. The yield of raspberries in 2019 was 5,145 kg. 67% (3,447 kg) of that, was in class I, and 28% (1,698 kg) was in class II. Revenue € / ha: 3,447 kg x € 4 = € 13,788 1,698 kg x € 1.4 = € 2,377 Total: 16 165 € PRODUCTION REVENUE - PRODUCTION COSTS: € 16,165 - € 7,982 = € 8,183

Profit in 2019 amounted to € 8,183.

Table 3. Cost calculation in 2019

Type of cost	Unit of measurement	Requirement per tunel	Price per unit of measurement (€)	Total cost (€)	
A. LABOUR COSTS					
Protection and fertilization	workday	2	20.00	40.00	
Binding shoot and tightening wire	workday	3	20.00	60.00	
Removal of young shoots	workday	2	20.00	40.00	
Harvesting	workday	60	20.00	1200.00	
Pruning (pinching) removal of old shoots	workday	1	20,00	20.00	
TOTAL: 1360.00					
B. MACHINERY COSTS					
Protection	workday	/	manually	20	
Other works	workday	2	100	200.00	
TOTAL:					220.00
C. MATERIAL COSTS					
Nitrogen fertiliser (CAN)	ml	500	8.5	8.50	
Complex fertiliser (NPK)	kg	100	0.17	17.00	
Plant protection products	Integral protection program			70	
Irrgation	spring platoon			/	
Pump mixture				20	
TOTAL:					115.50
D. OTHER COSTS					
Unforeseen expenses				300.00	
COSTS A + B + C + D	A. Labour costs		1360.00		
	B. Machinery costs		220.00		
	C. Material costs		115.50		
	D. Other costs		300		
	TOTAL:		**1995.50**		

According to Kljajić et al. (2017), raspberry production in the region of Arilje, provides an average income of 9,300 €/ha, but on an open field. Use of irrigation, limited to smaller areas in private hands, gave positive results in terms of high and uniform yield of quality raspberry (Cecić et al., 2007; Gajic et al., 2013). Also, there is the fact that the level of profitability that is realized through production depends on the achieved the volume of production and purchase price, but can be increased by reducing production costs, increasing the yield per unit area, as well as better organization of production and distribution (Mihajlovic, 2014). Raspberry plantation as an investment should be realized in a form that will ensure the maximum efficiency of exploitation, i.e. as high level of benefit per unit of invested funds as possible (Kljajić et al., 2017). The methods, through which the economic efficiency of investment is expressed in agricultural holdings, hold an important place not only in agriculture but also in the organization of sustainable development of a company at micro-level (Subić, 2010).

The economy in the production of raspberry variety Heritage in a greenhouse

Production efficiency is calculated by the ratio between the value of production and the total costs incurred in the production process.

REVENUE-TO-COST RATIO (RCR) = TOTAL REVENUES / TOTAL COSTS

Depending on the obtained revenue-to-cost ratio can be:

- Not efficient RCP <1
- At the borderline of efficiency RCP = 1
- Efficient RCP> 1

Revenue-to-cost ratio in the production of raspberry variety Heritage in 2018:

Ep = 18,042 € / 7,842 € = 2.30

Revenue-to-cost ratio in the production of raspberry variety Heritage in 2019:

Ep = 16 165 € / 7 982 € = 2.02

Since the revenue-to-cost ratio in both years of testing is higher than 1, the production of raspberry variety Heritage in the protected area is financially efficient. Results achieved by Hanson et al. (2011), say that relative cultivar characteristics (harvest season, yield, berry quality) were similar in the field and tunnels, but the tunnel environment tended to increase plant vigor, yield, and fruit quality and suppress several diseases. According to Kljajić et al. (2017) raspberry production is economically justified and very profitable. But also, they point out that raspberry production has certain disadvantages because of the high sensitivity of fruits, low durability, and low transportability. It is estimated that almost 40% of the yield is lost during the transportation period between the farm and the final consumer. A large part of this loss occurs due to poor post-harvest handling, including transport. By reducing the amount of hand contact and applying appropriate packaging techniques, the percentage of loss will be reduced too. Therefore, the proximity to processing facilities is very important as well as quick and organized transport in order to prevent loss of quality (Sarić, 2009).

Conclusion

The production of raspberry cv. Heritage in the greenhouse is economical, as indicated by revenue-to-cost ratio, 2.30 in 2018 and 2.02 in 2019. According to the established economic indicators, raspberry greenhouse production on a farm in the region of Požega is economically viable and highly profitable. The profit on 0.2 ha in 2018 amounted to € 10,200, and profit in 2019 amounted to € 8,183. Fresh raspberries are available for six to seven months, which is an advantage on the market, and the most important problem in this type of production is that Serbia produces a small amount of fresh raspberries Heritage, to be exported to other countries, where the price would be much higher.

By observing the results of the economic analysis of raspberry production may be concluded that this production achieves high profitability.

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Original scientific paper

Evaluation of potential of four entomopathogenic nematodes to control box tree moth (*Cydalima perspectalis* Walker)

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Abstract

Box tree moth - *Cydalima perspectalis* Walker, 1859 (Lepidoptera: Crambidae) is native to South East Asia and has been recently introduced to Europe and Bosnia and Herzegovina, where it damages box trees. Insceticide application in urban areas where box trees are common part of ornamentals, might be not solution. Biological control agents, entomopathogenic nematodes, present an environmentally sound solution for control of wide range of pests. In this study we tested in laboratory and field conditions susceptibility of larvae of *C. perspectalis* to local strains of four species of entomopathogenic nematodes *Steinernema feltiae, S. carpocasae, S. kraussei* and *Heterorhabditis bacteriophora*. In laboratory condition aplied range of concentrations of 50-500 infective juveniles per larva caused mortality of 80-100%. However, in the filed conditions observed mortality was 13-30% revealing that foliar application of EPN might require interaction with ecological factors to achieve similar results like in favorable laboratory conditions.

Key words: biological control, *Steinernema*, *Heterorhabditis*, ornamental plants

Introduction

Box tree moth - *Cydalima perspectalis* Walker, 1859 (Lepidopreta: Crambidae) is a pest of ornamentals plants from family of Buxaceae. It is native to subtropical parts of India, China, Japan and Korea (Hizal *et al.*, 2012). In Europe it was introduced in 2007 and firstly reported from Germany from the vicinity of the port of trans-shipment in Weil am Rhein (Baden-Württemberg) (Billen, 2007). It is suspected that it has been introduced by imported reproductive material of ornamental *Buxus* (Krüger, 2008). It has spread around the continent within a few years (Hizal et al., 2012). First report from Bosnia and Herzegovina is from 2014 (Ostojić et al., 2015).

C. perspectalis is an oligophagous insect that life cycle completes on *Buxus* plants. Moths are active in the late evening and during a night. After mating the female lays eggs in clusters on the lower leaf surface. Larval development lasts for several weeks during which period it feeds on leaves and bark after defoliation of leaves. Small larvae feed aggregated in groups and older ones solitary. It overwinters as a third larval instar within spun cocoon between a few leaves. Boxs trees are ornamental shrubs very popular in decoration of public and private areas. Buxus has low requirements for soil quality and easiness of trimming to any shape made it widely used. However, in areas where *C. perspectalis* is present, *Buxus* plants are affected and without control measures Box trees dying within one season. Application of insecticides is often disliked or even prohibited on public areas. Management of this pest in Japan is based on chemical insecticides, such as pyrethroids (Wan et al., 2014). Non pesticide control include mechanical measures collection of larvae, that is not effective since is highly labor intensive. Pheromon attractants did not give satisfactory level of efficacy (Korycinska & Eyre, 2011). In Europe there is no natural enemies of the pest. Entomopathogenic bacteria *Bacillus thuringiensis* var. *kurstaki* is effective only when it is digested by the larva (Korycinska & Eyre, 2011) while entomopathogenic fungi *Beauveria bassiana* does not cause mortality (Wan et al., 2014). Entomopathogenic nematodes (EPN) of families Steinernematidae and Heterorhabditidae together with their symbiotic bacteria are lethal to many insect pests (Lacey et al., 2012). Their application as biological control agents of pests has started to grow after development of technologies of *in vitro* mass rearing (Ehlers & Shapiro-Ilan,, 2005). Their easy application by convention pesticide application equipment, safety to farmers and food consumers, low level or no negative effect on non target organisms, ability to active search for their hosts, exemption of registration lead to popularity of their use for pests control (Ehlers & Shapiro-Ilan, 2005). The only stage of EPN that can be found outside a host is a third stage juvenile, an infective stage called infective juveniles (IJ). EPN are generally applied to control soil dwelling pests but there are examples of successful control of foliar pests Tomato Leaf Miner-*Tuta absoluta,* Red Palm Weevil - *Rhynchophorus ferrugineus* and Codling Moth - *Cydia pomonella* (Lacey et al., 2012). The aim of this study was to test local strains of four species of entomopathogenic nematodes *Steinernema feltiae, S. carpocasae, S. kraussei* and *Heterorhabditis bacteriophora* from Bosnia and Herzegovina in laboratory and field conditions against larvae of *C. perspectalis.*

Material and Methods

Laboratory assay

For laboratory assay larvae of different instars of *C. perspectalis* were colected from infested box trees. Five larvae were placed per 5.5 cm diameter petry dish, lined at the bottom with filter paper. Four species of EPN *S. feltiae, S. carpocasae, S. kraussei* and *H. bacteriophora* local strains were used for test of mortality of the insect larvae. Nematodes were reared on the last larval instar of Grate wax moth (*Galleria mellonela*). Harvested invective juveniles were placed in refrigerator at 6±2°C, until application. The nematodes (IJ) were applied in four doses 250, 500, 1000 and 2000 infective juveniles per petry dish. There was no treatment wihith 250 IJ for *S. kraussei.* The nematodes were applied in 350 µl water suspension. Each treatment had five replicates. The control group was was treated also with 350 µl water suspension. The insects were offered diet that consisted of buxus tree leaves. Mortality of *C. perspectalis* larvae was assessed at 24 hours and 5 days after treatment.

Field trial

For the field trial infested fence of box tree, height 80 cm and 40 cm width, in privat garden in Banja Luka was used (44° 45' 53" 17° 12' 46"). The same nematode species in the field trial were used like in laboratory experiment. The nematodes dissolved in one liter of water were applied at the dose of 500,000 IJ per 1 m length of the fence with backpack sprayer. Nematode application was done in the late afternoon. Each treatment contained five replicates. Next morning 20 larvae were collected from each replicate, placed in the petri dish and observed for mortality after 48 hours. All data were subject to analysis of variance (ANOVA). Differences are reported as significant of not according to Duncan'c multiple range test ($p<0,05$).

Results and Disscusion

This is the first report on the susceptibility of local strains of EPN from Bosnia and Herzegovina against *C. perspectalis,* and the first report of susceptibility of this pest to the species *S. kraussei*. Results of laboratory assay of larval mortality exposed to different doses are presented in Figure 1. Two nematode species *S. feltiae* and *H. bacteriophora* after 24 hours did not caused mortality at any dose. *S. carpocapsae* at the dose of 2000 IJ caused 100% mortality and at other doses there was no mortality. *S. kraussei* caused mortality at 80, 100 and 100% at the doses 500, 100 and 2000 IJ respectevely. After 5 days all nematodes caused 100% mortality for all treatments except for *S. carpocapsea* at the rate of 250 IJ, and *H. bacteriophora* at the rate of 500 IJ. There was no observed mortality in control group, but presence of cannibalism was observed. In the field experiment the highest mortality of larvae after 48 hours was caused by *S. kraussei* at the rate of 30%, three other nematodes *S. feltiae, S. carpocasae,* and *H. bacteriophora* caused mortality of 18,3, 16,7 and 13.3, % respectively. There was significant difference between treatments in laboratory test (ANOVA: F(4,29)=873.4475, p=0), and field trial (ANOVA: F(4,19)=8,76191, p=0,001142).

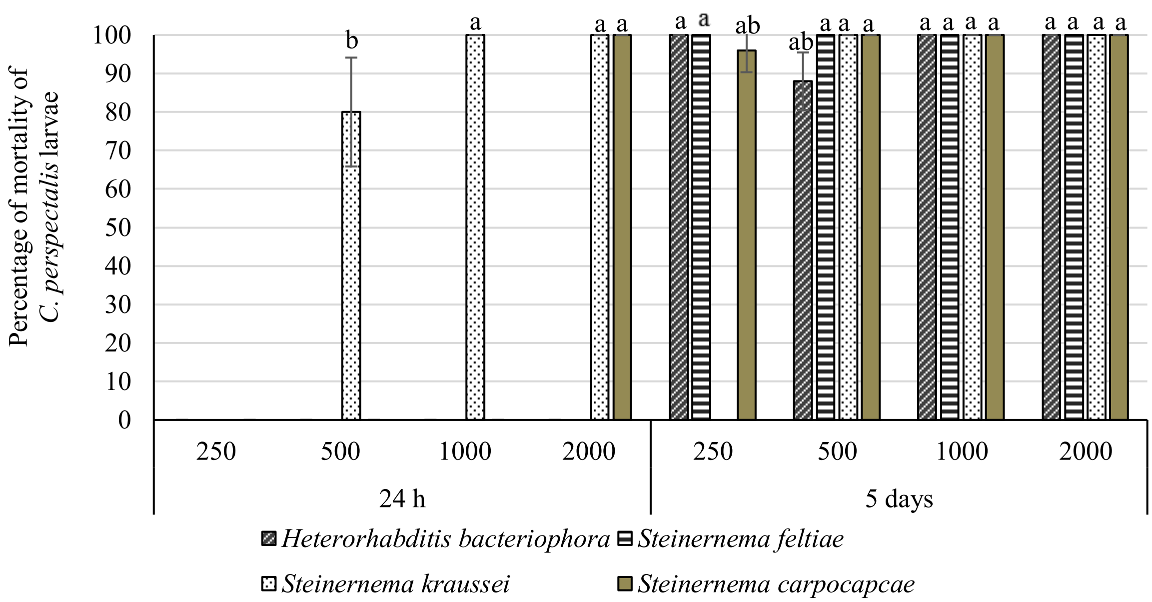

Figure 1. Laboratory assay on susceptibility of *C. perspectalis* larvae in exposed to entomopathogenic nematodes *H. bacteriophora, S. feltiae, S. kraussei* and *S. carpocapsae*

There are a few studies on application of EPN against *C. perspectalis* (Gőtig & Herz, 2018; Choo et al., 1991). In these studies two species of EPN *S. carpocasae* and *H. bacteriophora* were applied in laboratory conditions against 2nd and 4th larval instars. Mortality of 100% was obtained with concentration of 80-100 IJ per larva for *S. carpocasae,* while 10-40 of *H. bacteriophora* IJ per larva caused 94-99% mortality. Results of our study reveal also high susceptibility of local strains of EPN to different larval instars of *C. perspectalis*. The lowest concentration of 50 IJ per larva caused mortality 80-100% five days after application, while 24h after application mortality was observed only with *S. kraussei* (80-100%). There are no reports in literature of susceptibility of *C. perspectalis* to *S. kraussei.* EPN species and strains usually used in tests are those that are commercially available world wide, for which large scale production technologies have been developed (Van de Linden et al., 2021).

In this study in the field trial (Figure 2.) with concentration of 500.000 IJ/m^2 low mortality was observed (13-30%). In the study of Gőttig & Herz (2018) similar results were obtained. 625.000 IJ/m^2 caused 11-16 mortality when *S. carpocapsae* was applied, while 10 million IJ/m^2 caused 96% mortality seven days after application. Higher dosage result in higher mortality since more nematodes give more chance that some of them will enter a host (Arthur et al., 2004; Sunnanda et al., 2014). Host instar and larval size play also important role in patogenicity of EPN. Bigger the host, bigger are the openings through the IJ will enter the body (trachea, mouth, anus), Also bigger the larva will eat more leaves and that gives the higher chance that foliar applied EPN will enter the host. Potential of self-propagation within the host should not be underestimated since it would bring prolonged effect of control. Beside ability of EPN to infest a host tested in laboratory conditions, ecological factor might play important role in pathogenicity in field conditions. Temperature and moisture are the most important ecological factors affecting effectives of EPN against foliar pests (Glazer et al., 1992; Williams & Walters, 2000; De Waal et al., 2018). Therefore, providing optimal conditions, like at least 95% of relative humidity, temperature above 10°C are prerequisite for successful control of target pests. Application during light rainfall would give optimal conditions for EPN activity. Moreover, adding adjuvant to tank spray will increase efficacy of nematodes.

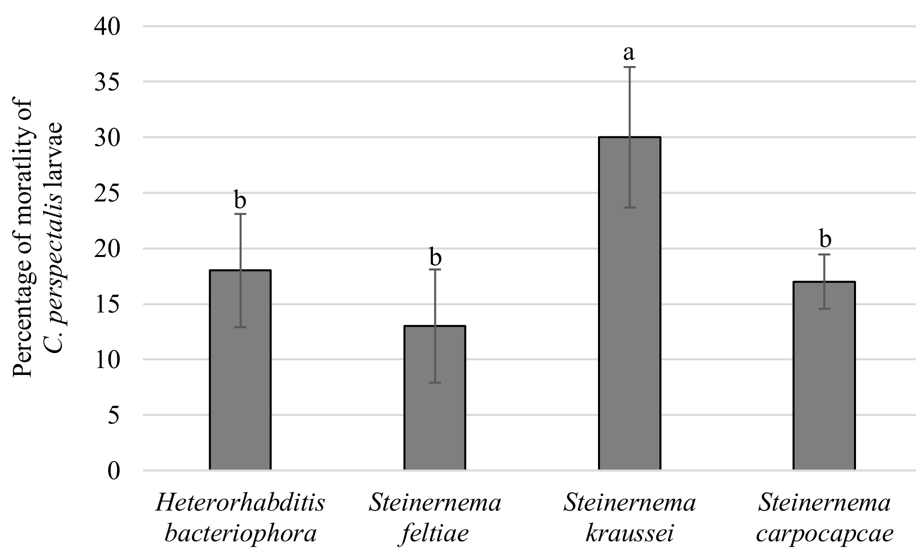

Figure 2. Field trial on susceptibility of *C. perspectalis* larvae in exposed to entomopathogenic nematodes *H. bacteriophora, S. feltiae, S. kraussei* and *S. carpocapsae*

This study reveals potential of EPN application against foliar pets *C. perspectalis* but field trial results revel that they are much less effective compared to insecticides application and another biological control agent *Bacillus thurigiensis*. Application of insecticides and *B. thunigiensis* have disadvantages since they might require restriction of access to human beings for some time after application. That could be motivation to explore further improving of factors that reduce EPN efficacy since EPN have advantages over two other solution now available for control.

Conclusions

In this study local strains of four EPN species were applied against mixture of instars of *C. perspectalis* in laboratory and field conditions. In laboratory conditions all species caused high mortality (80-100%) five days after application. In the field condition lower level of control was obtained 13.3-30%, where *S. kraussei* was the most effective. Foliar environment present harsh conditions for EPN, and ecological factor such moisture and temperature must be suitable. Improving ecological factors within the canopy of box tree might be the solution to exploit potential of EPN against *C. perspectalis* shown in laboratory conditions.

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Original scientific paper

Control of *Cydia pomonella* L. in apple orchards using spinetoram, pyriproxyfen and chlorantraniliprole

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Abstract

Codling moth occurs regularly in Republic of Serbia every year. It is one of the most important pests, particularly in apple orchards. It often causes permanent and high damages, that result in premature fruit drop, larvae-eaten fruit, and difficult preservation during storage, primarily due to untimely and inadequate protection. In 2021, the trials were carried out according to the standard EPPO methods with the aim to establish the level of apple protection against the codling moth, using three plant protection products based on insecticides pyriproxyfen (100 g a.i./L, EC), spinetoram (250 g a.i./kg, SG) and chlorantraniliprole (200 g a.i./L, SC). In apple orchard (variety Granny Smith) at Budisava locality (Vojvodina, Serbia), the products were foliar applied by backpack sprayer at a concentration of 0.04%, 0.10% and 0.02%, respectively. The experiment was set up in four replications in randomized block design. The efficacy of the insecticides was performed according to Abbott, and significance of differences (ANOVA) for the confidence interval of 95%.

Eleven days after application the efficacy ranged from 90.5-94.4% for all applied products, while the chlorantraniliprole achieved the highest efficacy. After 24 days, the products showed good efficacy ranged from 85.4-97.1% and the highest efficacy was achieved by spinetoram. In both estimates, the number of damaged apples in the variants where insecticides were applied was significantly lower than in the control. Finaly, the applied insecticides showed high efficacy for the protection of apple fruits from *C. pomonella* in agricultural production in Vojvodina province.

Key words: Apple, *C. pomonella*, Insecticide, Efficacy.

Introduction

Apple is the most important pome fruit in Serbia, grown on 26,089 ha, which makes it the second biggest grown fruit by the area of cultivation, just behind the plum (Statistički godišnjak, 2019). New agriculture technology in fruit growing requires efficient protection in order to ensure high yields and product quality with good economic aspects, without harmful pesticides residues in fruits used for consumption. Apple production has become a high-tech business and requires great dedication. The most important diseases of apples are:apple scab (caused by *Venturia ineaqualis*), apple powdery mildew (caused by *Podosphaera leucotricha*), apple leaf spot (caused by *Alternaria mali)*, blossom blight (caused by *Monilia laxa*), apple gray mold (caused by *Botrytis cinerea*) and fire blight of apple (caused by *Erwinia amylovora*). Also, the most important pests of apples are codling moth (*C. pomonella*), aphids (Aphididae), apple leaf miner (Lyonetiidae), European red mite (*Panonychus ulmi*), the fall webworm (*Hyphantria cunea*), the apple blossom weevil (*Anthonomus pomorum*) and mites (Acarina) (Stamenković, 2000). The codling moth (*C. pomonella*) cause great economic loss. It is widespread in almost all orchards in our country. The expansion of *C. pomonella* has been observed especially in the last few years. If suitable ways of protection are not implemented, damages can reach up to 100% (Kereši et al., 2019). *Cydia pomonella* completes 2-3 generations per year. Caterpillars burrow into the fruit, most often in places where two fruits, a leaf and fruit, touch or next to a stalk or sepal. The control of the codling moth should be adjusted to each orchard, and the strategy primarily depends on the abundance that needs to be established and monitored. Due to the necessity of insecticides used for the protection of apples from codling moths, there are justified reasons for their use. Preference should be given to toxicological and ecotoxicological more favorable insecticides. However, due to the frequent use of insecticides, in many apple orchards, there has been reduced effectiveness of some insecticides for the control of *C. pomonella*. Most of them belong to the group of organophosphates and pyrethroids. The aim of this study was to determine the efficacy of insecticides based on pyriproxyfen 100 g a.i/l EC, spinetoram 250 g a.i./kg WG and chlorantraniliprole 200 g a.i./l SC for the control of codling moth in apple orchards, as well as to determine are there any significant differences in efficacy between these products.

Materials and Methods

The trial was performed in a private apple orchard, on the Granny Smith apple variety, at the locality Budisava (Vojvodina, Serbia).The experiment was set up according to standard OEPP methods: for experimental design and data analysis (Anonymus, 2012), for the effectiveness of insecticides for codling moth (Anonymus, 2004) and for phytotoxicity (Anonymus, 2014). The experiment was set up according to a random block design in four replications. The size of a basic plot consists of four trees. Preparations based on pyriproxyfen (100 g a.i./L EC) at the concentrationof 0.1%, spinetoram (250 g a.i./kg WG) 0.04% and chlorantraniliprole (200 g a.i./L) with the concentration of 0.02%.

The treatment was performed foliar, with a back atomizer "Solo" with water consumption of 1000 l/ha. The treatment was performed on May 24, 2021 when the apples were in phase BBCH 72 (fruit size up to 20 mm). Two evaluations of the applied insecticide effects were performed. The evaluation was performed on the number of damaged apple fruits, out of 300 examined apples per repetition. The first assessment was performed 11 days after treatment (June 4, 2021), and the second 24 days after treatment (June 17, 2021). The results of the experiment are presented through absolute and mean values for the number of damaged apple fruits. Also, the standard deviation (Sd+) was determined, as well as the significance of the differences (LSD 5%) (ANOVA). Efficacy (E%) was calculated according to Abbott (Wentzel, 1963).

Results and Discussion

The results of the product's effectiveness for the control of codling moth (*C. pomonella*) in apple orchards are shown in Tables 1 and 2. The number of damaged apple fruits, 11 days after application of products is significantly lower compared to the control. The efficiency of insecticides ranged from 90.5 to 94.4%, depending on the insecticide used. The insecticide based on pyriproxyfen (90.5%) showed the lowest efficiency, while the insecticide based on chlorantraniliprole (94.4%) showed the highest efficiency. The effectiveness of the tested products 24 days after application ranged from 85.4% to 97.1%. The lowest efficiency was achieved for pyriproxyfen (85.4%), and the highest for spinetoram (97.1%). Also, the number of damaged apple fruits, 24 days after application, is significantly lower compared to the control. It was also stated that the tested products did not cause phytotoxic changes on the leaves, young shoots, or fruits on the Granny Smith apple variety, at the Budisava region.

In 2013, the effectiveness of insecticides from the groups of avermectins, diamides, and neonicotinoids for the control of *Cydia pomonella* was assessed at the localities of Radmilovac and Vinča. High efficiency was obtained with the mixture based on emamectin benzoate and mineral oil (96.2-100%), the mixture of chlorantraniliprole and cyantraniliprole (92.0-95.6%), which is in accordance with our research. Thiacloprid-based insecticide showed lower efficiency (82.8-85.7%), while acetamiprid (54.6-55.1%) showed very low efficiency in both localities (Tamaš et al., 2014), which indicates reduced susceptibility of *C. pomonella* to insecticides belonging to the neonicotinoid group.

Experiments for the efficacy of new insecticides for the control of *C. pomonella*, were carried out by Bosch et al. (2017), on 20 field codling moth larvaepopulations collected from three different Spanish apple production areas. According to them, spinetoram and chlorantraniliprole provided high efficacy ranging from 88.1-95.1 and 88.5-89.2, respectively, which is similar to results obtained in this research. Depalo et al. (2016) also indicated spinetoram as a valuable candidate to be used in multi-strategy IPM control programs in pome and stone fruit orchards. In particular, spinetoram can be used as an ovicide spray against 1st larval generation due to the fact that its residual activity provides a satisfactory level of protection, for approximately 10 days after treatment (Depalo et al., 2016).

Mortality rates of codling moth (*C. pomonella*) neonates from a laboratory colony ranged from 34-39 %, when treated with pyriproxyfen (Reuveny and Cohen, 2004). That indicates that larvae are more tolerant to pyriproxyfen. The number of available insecticides against codling moth has fallen drastically in the European Union (EU) since the re-registration of active substances covered by Directive 91/414/EEC, and some of the products most widely used to control codling moth are no longer available.

Table 1.The number of damaged apple fruits and the efficacy of insecticides in control of *C. pomonella* 11 days after treatment

Insecticide (%)	repetition				∑	$\bar{x}$ ±Sd	E %
	I	II	III	IV			
pyriproxyfen (0.1)	2.0	7.0	5.0	3.0	17.0	**4.25**±2.21 a	**90.5**
spinetoram (0.04)	7.0	0.0	3.0	2.0	12.0	**3.00**±2.94 a	**93.3**
chlorantraniliprole (0.02)	2.0	4.0	0.0	4.0	10.0	**2.50**±1.92 a	**94.4**
Control	48.0	42.0	39.0	51.0	180.0	**45.0**±5.50 b	/
LSD 5%	4.17						

$\bar{x}$ – average number; ±Sd - standard deviation; E %- efficacy; F=147.74; p<0.01.

Table 2.The number of damaged apple fruits and the efficacy of insecticides in control of *C. pomonella* 24 days after treatment

Insecticide	repetition				∑	$\bar{x}$ ±Sd	E %
	I	II	III	IV			
pyriproxyfen (0.1)	11.0	5.0	7.0	7.0	30.0	**7.50**±2.52 a	**85.4**
spinetoram (0.04)	5.0	0.0	1.0	0.0	6.0	**1.50**±2.38 a	**97.1**
chlorantraniliprole (0.02)	6.0	4.0	2.0	1.0	13.0	**3.25**±2.22 a	**93.4**
Control	69.0	41.0	52.0	44.0	206	**51.5**±12.6 b	/
LSD 5%	8.56						

$\bar{x}$ – average number; ±Sd - standard deviation; E %- efficacy; F=52.09; p<0.01.

Conclusion

Based on the performed tests and the obtained results for the control of the codling moth (*Cydia pomonella*) in the apple orchard at the Budisava locality, the following conclusions can be drawn:

- The efficacy of the tested insecticides, 11 days after treatment ranged from 90.5 to 94.4%, with the highest efficacy shown by chlorantraniliprole.
- The efficacy of the tested insecticides 24 days after application ranged from 85.4% to 97.1%, with the highest efficacy shown by spinetoram. In both evaluations, the number of damaged apple fruits in the variants where insecticides were applied was significantly lower compared to the control.
- The obtained results indicate a high sensitivity of *C. pomonella* to the tested products at the locality of Budisava (Vojvodina).

Acknowledgement

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Original scientific paper

Dissipation and residues of emamectin benzoate in paprika

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Abstract

In this study, the dissipation and residues of insecticide emamectin benzoate in paprika fruits grown in a greenhouse were evaluated. Plant protection product based on emamectin benzoate was applied at the recommended rate. For the analysis of insecticide residues, the QuEChERS based method, followed with HPLC analysis was validated in accordance with SANTE/12682/2019. The results indicated that emamectin benzoate degrades rapidly in paprika fruits in greenhouse conditions and exhibited first-order kinetics dissipation, with a half-life of 0.6 days. The lowest residue level indicates that the pre-harvest interval for emamectin benzoate, after its application in the recommended rate in the paprika, is appropriately prescribed. Based on these results, it has been proven that, if pesticides based on emamectin benzoate are used in accordance with good agricultural practice, produced paprika fruits could be classified as "zero pesticide residues" products.

Key words: emamectin benzoate, paprika, dissipation dynamic, residues

Introduction

Emamectin benzoate is an efficient semi-synthetic pesticide (Feely et al., 1992). It is a macrolide substance produced by modifying the chemical structure of the avermectin B1, derived from the natural fermentation products of actinomycete *Streptomyces avermitilis* (Caldas, 2015). Emamectin benzoate (Fig 1) is a mixture of (4R)-5-O-demethyl-4-deoxy-4-(methylamino) avermectin A1a + (4R)-5-Odemethyl-25-de(1-methylpropyl)-4-deoxy-4-(methylamino)-25-(1-methylethyl) avermectin A1a, (9:1) (Van der Velde-Koerts, 2014).

It shows up to 1600 times higher insecticidal activity and high efficiency on a wide range of

Lepidopteran species (Čeković, 2006). It leads to paralysis and the death of pests via the activation of a glutamate-gated chloride channel (GluCl) in the invertebrate nerve and muscle cells. Like abamectin, emamectin benzoate is significantly less toxic to other non-target insects belonging to the different orders, particularly to beneficial insects, due to its short half-life in leaves, which makes it suitable for use in integrated plant protection agricultural systems.

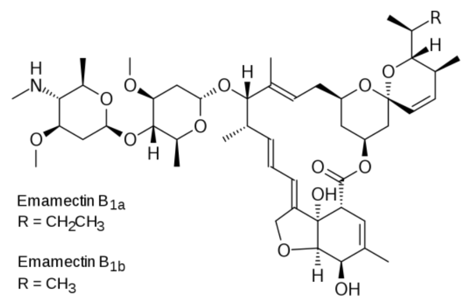


Figure 1. Emamectin benzoate - Chemical Structure

Insecticides based on the emamectin benzoate, which belong to the avermectin group, are also used for the control of Cotton bollworm in paprika. This substance leads to disturbance of nerve impulses which trigger off paralysis. These insects are not able to feed just within a few hours after ingestion which leads to their death (Jansson et al., 1997).

It has a significant control effect on Lepidoptera, Homoptera, Coleoptera, and other pests (Willis et al., 2003; Willis et al., 2005). In the Republic of Serbia, the insecticide based on emamectin benzoate is registered for the control of the Cotton bollworm (*Helicoverpa armigera*) in the paprika crop. Moreover, it is widely used for the pest control of fruit, vegetables and field crops as an alternative to highly toxic pesticides.

However, the careless use of emamectin benzoate could cause environmental pollution and serious harm to other, non-target organisms. Considering the production of high-quality products and healthy food, with the aim of environmental protection and human health, in addition to the proper and rational application of plant protection products, it is necessary to timely, regularly monitor, control, and analyze pesticide residues in food, especially fruit and vegetable that are mostly consumed fresh. The aim of this paper was to evaluate the behavior and the dissipation dynamics of insecticide emamectin benzoate in paprika grown in a greenhouse, after its application in the recommended rate and to calculate its half-life. For this purpose, a high-performance liquid chromatography (HPLC) method followed with QuEChERS based method has been applied for the determination of residues of emamectin benzoate in paprika.

Material and Methods

Field trials

The field trial was carried out in the greenhouse (Fig 2), at the locality Bačko Gradište (Republic of Serbia), at the end of July/beginning of August 2021. The experiment was conducted according to the standard EPPO methods (Anonimus (2004a). Plant protection product based on emamectin benzoate (9.5 g a. i./kg, SG) was foliar applied at the rate of 2 kg/ha, with water consumption of 300 l/ha. The treatment was conducted in order to control the presence of *Helicoverpa armigera* in the paprika crop in the ripening stage. The experiment was set up in four replications, as a randomized block system.

To evaluate the emamectin benzoate residues in the paprika fruits, approximately 1.0 kg of representative vegetable samples from field experiments were randomly picked and delivered to the laboratory, according to the FAO/WHO recommendations (FAO, 1986). The samples were taken 1 h after the insecticide application and daily for one week. Also, the untreated samples were also collected and used as a control.

Figure 2. Field experiment

Analytical procedure

The samples were analyzed according to the QuEChERS method described by Anastassiades et al. (2003). Residues were analyzed by HPLC using an Agilent 1100 HPLC system (USA), with photodiode array detector, and C_{18} Zorbax XDE (50 mm × 4.6 mm, 5 μm film thickness) column. The column was kept at room temperature, flow rate of mobile phase (acetonitrile/water, 98/2, v/v) was 0.510 ml/min and injection volume was 20 µl. Emamectin benzoate was detected at wavelength 254 nm. The retention time of emamectin benzoate was about 6.52 min.

The method was validated through linearity, precision, the limit of detection and quantification (LOD, LOQ), matrix effect, and accuracy. A five-point standard solution and matrix-matched standard solutions were prepared at the concentration of 0,010-2,95 µg/ml. The recovery assays were performed to estimate the accuracy and precision of the method. The recovery experiments were conducted by three replicates at three spiked levels (0.01, 0.10 and 0.69 mg/kg). The accuracy and the precision under these conditions for repeatability were expressed as the average recovery and the relative standard deviation (RSD).

Data calculation

The dissipation kinetics of emamectin benzoate in paprika fruits was determined by plotting residue concentration against time (Deng et al., 2020). In order to describe the dissipation rate of active ingredient of the pesticides in crop, the following first-order kinetic equation was used:

$$C_t = C_0 \times \exp^{(-k \times t)}$$

where C_t is the residual pesticide concentration (mg/kg) at time t (days) after application; C_0 is the initial pesticide concentration; and k is the pesticide dissipation rate constant. The half-life equation is:

$$t_{1/2} = \ln(2)/k$$

In this equation $t_{1/2}$ is the pesticide dissipation half-life (days), which was used for all pesticides with significant models (R^2>0.5). The model parameters of C_0 and k were calculated from all experimentally determined residue values of each active pesticide ingredient in the products (C_t) and the corresponding number of days after pesticide application (t).

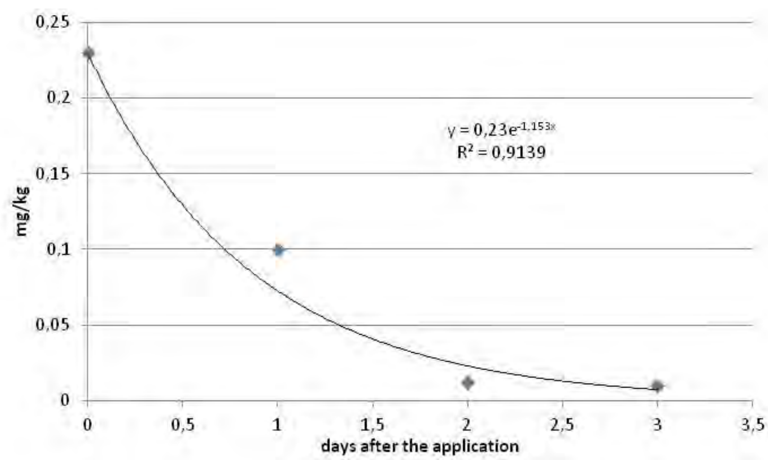
Results and Discussion

For the analysis of the emamectin benzoate residues in paprika fruits, the method validated in accordance with the requirements of the SANTE/12682/2019 standard were applied (Table 1).

Table 1. Validation parameters

Linearity, 0,027-2,95 µg/ml	Precision, RSD	Recovery	LOD	LOQ	ME
r^2=0.9629	1.08%	88.28-92.05%	0.005 mg/kg	0.01 mg/kg	115.4%

Thus, validated method was used for the analysis of emamectin benzoate residues in paprika fruits (Graph 1), in order to determine the safeness of its use in the paprika. The highest amount of emamectin benzoate was determined 1h after the application (0.23 mg/kg). In the sample collected 24h after the treatment, the average amount of emamectin benzoate in paprika fruits was 0.1 mg/kg, with a loss of 56.52%. In the samples collected on the second day after treatment, the amount of active ingredient was reduced to 0.012 mg/kg, indicating that only 5.22% of the initial amount remained. Further analysis revealed a decrease in the content of emamectin benzoate, and on the third day after treatment the average content in paprika fruits was 0.01 mg/kg, while four days after the application residues of emamectin benzoate were not determined, i.e. they were below LOD. Pre-harvest interval (PHI) of emamectin benzoate in paprika in Serbia is three days, while the maximum residue level (MRL) of this compound in paprika fruits is 0.02 mg/kg. In this study, the MRL level was achieved on the second day after insecticide application.



Graph 1. Dissipation of emamectin benzoate residues (mg/kg) in/on paprika

First-order kinetics has been extensively used to describe the dissipation rate of pesticide's active ingredient in crops. In this study, the results show a gradual decrease of emamectin benzoate in paprika fruits, with a correlation coefficient of 0.9139. The half-life of emamectin benzoate in paprika fruits was 0.6 days.

There are numerous papers reporting the degradation of emamectin benzoate in various crops. Lei Wang et al. in 2012 studied the degradation of emamectin benzoate in apples, cabbage and soil, using QuEChERS and LC-ESI-MS/MS. The half-life was 1.34–1.72 days for cabbage, 2.75–3.09 days for apple and 1.89–4.89 days for soil. The final residues of emamectin benzoate were 0.001-0.052 mg/kg in cabbage, 0.003-0.090 mg/kg in apples and 0.001-0.089 mg/kg in soil, which is below the MDK value. A similar study showed DT50 of emamectin benzoate in cabbage and soil of one day (Shuaigang et al., 2012), while the half life of emamectin benzoate in rice was 2.04–8.66 days, in water 2.89–4.95 days and in soil 3.65–5.78 days (Minghui et al., 2011). The dissipation dynamics and residues level of emamectin benzoate in tea leaves, as well as the transfer of residues during tea brewing, showed DT50 of 1-1.3 days (Zhao et al., 2016). In eggplant, emamectin benzoate residues were below LOD (0.05 mg/kg) after 3 and 5 days of the treatment, with a half-life of 1.53 to 1.57 days, after its application in the recommanded rate (Vinothkumar et al., 2018).

Conclusion

In this study, the dissipation rate of emamectin benzoate in paprika fruits, after its application at recommended dose, were evaluated. The results indicated that emamectin benzoate degrade rapidly in paprika fruits in greenhouse production and exhibited first-order kinetics dissipation, with a half-life of 0.6 days. Furthermore, the prescribed PHI of three days for emamectin benzoate in paprika is appropriate.

Based on these results, it has been proven that, if pesticides based on emamectin benzoate are used correctly, in accordance with good agricultural practice, produced paprika fruits could be classified as "zero pesticide residues" products.

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Original scientific paper

The content of metals and metalloids in bulbs of different genotypes of *Allium* species

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Abstract

In this research, we examined the effect of 15 genotypes of selected *Allium* species: *A. sativum* L. (10 genotypes), *A. ampeloprasum* L. var. *ampeloprasum* (3) and *A. cepa* L. (2), on the content of metals and metalloids in bulbs. Determination of the content of elements was performed using the method of atomic absorption spectroscopy and ICP-OES method (mg/kg of dry matter).

This research found that all tested genotypes differed statistically significantly in the content of Na, K, Ca and Mg both from each other and within the species. The highest content of Na, K, Ca and Mg was established in *A. sativum*, while the lowest content were observed in onion (*A. cepa*). Genotypes of *A. ampeloprasum* var. *ampeloprasum* contained moderate amounts of Na, K, Ca and Mg. The content of certain metals was the same in all genotypes of the tested *Allium* species and was < 0.01 mg/kg (Hg) and < 0.5 mg/kg (Hg, Co, Ni and Mo). The species *A. ampeloprasum* var. *ampeloprasum* showed the highest affinity for Cd accumulation in bulbs, followed by *A. sativum* and *A. cepa*. According to the content of Fe and Zn, *Ljubičasti sredbrenjak* as onion variety, it stood out in relation to all genotypes of tested *Allium* species. The highest content of Cd, Mn and Se was noticed in genotypes of *A. ampeloprasum* var. *ampeloprasum*. The presence of potentially toxic elements (Pb, Hg, As) was also determined in the bulbs of tested species, but in the safe levels. Considering the fact that species of the genus *Allium* show the ability to accumulate elements that are important for human health, especially Fe, Zn and Se, future research should be directed to enriching popular species from this genus

with these elements by applying simple and cost effective agrotechnical measures, such as biofortification.

Key words: Allium species, genotypes, metals, metalloids

Introduction

The genus *Allium,* from family Alliaceae, is one of the largest plant genera, which includes more than 800 species (Fritsch et al., 2010). All members of this genus are annual, biennial or perennial geophytes with underground stems known as bulbs (Li et al., 2010). The most economically important species from this family are: onion (*Allium cepa* L.), garlic (*A. sativum* L.), chives (*A. schoenoprasum* L.), shallots (*A. cepa* var. *aggregatum*), leek (*A. ampeloprasum* L. var. *porrum*) and bunching or Welsh onion (*A. fistulosum* L.).

Onion a very commonly used vegetable, ranks third in the world production of major vegetables, with 104 million tons per year. It is grown in almost all countries of the world, and the largest producer is China with a share of 25% in total world production. Garlic is the second most important species of the genus *Allium* with a production of 28 million tons per year, and China is also the world's largest producer with a 75% of total world production. In Europe, total production of onions is around 10 million tons and the largest producers are Russia, the Netherlands, Ukraine and Spain, while, in the case of garlic, the largest producers are Spain, Ukraine and Russia with total production with 870 thousand tons (FAO, 2020).

Allium species contain a large number of different compounds that have a positive effect on human health. These species are source of numerous bioactive compounds together with many vitamins (B1, B2, C, E, K), micro and macronutrients (especially K and S). Recent research has shown that onion and garlic extracts can prevent cardiovascular and other diseases, so they are increasingly used in traditional medicine (Zeng et al., 2017). Therapeutic effects as well as the smell and taste of *Allium* species are associated with a high content of essential oils in edible parts (Block, 1985).

The chemical composition of plants, especially the content of macro and microelements, as well as their dislocation in plant tissues largely depends on the chemical composition of the soil. Research conducted by Gambelli et al. (2021) indicates that agroecological conditions, but also varieties, significantly affect the mineral composition of garlic.

In general, the dry matter of plants contains about 45% of C, 45% of O, 6% of H, 1.5% of N and 2.5% other elements. Depending on the importance for the plants, all elements are divided into essential (C, H, O, N, P, K, Ca, Mg, S, Fe, B, Mn, Cu, Zn, Cl, Mo and Ni), beneficial (Na, Si, Co, Se, and more recently Al) and others (all other elements found in plants) (Kastori, 2006). In addition to mentioned elements, toxic elements, in the literature often known as heavy metals, can often accumulate in plant tissues, as: Hg, Pb, As, Ni, Cd. Research by Soudek et al. (2009) performed on four *Allium* species (onion, garlic, leek and chives), which are related to the absorption of toxic elements from nutrient solution and their distribution and accumulation in plant parts, showed that increasing their content through nutrient solution, increases their content in plant tissues. The same authors point out that toxic elements have mainly accumulated in the root system of plants. Considering the total amount of accumulated Cd, 75% was contained in the root, while in the case of Co this percentage varied from 40-90% (Soudek et al., 2009). According to Ke et al. (2011) the presence of heavy metals in the soil negatively affects the germination and growth of garlic.

Actually, toxicity is mainly related to the amount of an element, but this range varies greatly for each individual element. In the case of fresh vegetables, the maximum allowed concentration prescribed by Regulation (28/2011) for Pb is 1 mg/kg, Cd 0.05 mg/kg, Hg 0.02 mg/kg and As 0.3 mg/kg of fresh matter.

Research conducted by Vadalà et al. (2016) which analyzes the concentration of elements in different varieties of garlic from Spain, Tunisia and Italy indicate that the concentrations of the considered elements can be used as geographical indicators to distinguish the origin of garlic samples. Namely, it was determined that samples of garlic from Tunisia and Spain had a high level of Ni, while a relatively high content of Se was detected in the garlic variety *Nubia Red Garlic* from Italy. As *Allium* species have a high ability to accumulate Se in edible parts, they can be used as an effective tool to increase Se levels in human nutrition. In this case, the doses of selenium fertilizers as well as the methods of their application should be studied in order to avoid negative effects. In this work was examined the effect of 15 genotypes of three *Allium* species: *A. sativum* L. (10 genotypes), *A. ampeloprasum* L. var. *ampeloprasum* (3 genotypes) and *A. cepa* L. (2 varieties), on the content of metals and metalloids in bulbs. Determination of the content of elements: sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), mercury (Hg), cadmium (Cd), iron (Fe), zinc (Zn), cobalt (Co), nickel (Ni), manganese (Mn), chromium (Cr), molybdenum (Mo), lead (Pb), aluminum (Al), arsenic (As) and selenium (Se) was performed using the method of atomic absorption spectroscopy (AAS) and ICP - OES method. The aim of the research was to determine the mineralogical composition of selected species of the genus Allium, with special reference to elements that are useful or toxic to human health.

Materials and Methods

Source of plant material

Plant material and experimental field Rimski Šančevi used in this study were provided by Institute of Field and Vegetable Crops (Novi Sad). In the experiment were used following genotypes: *A. sativum* – autumn garlic (JBL 3/17, JBL 7, JBL 8/17, Ranko, Bosut)*, A. sativum* – spring garlic (Sedef, PBL 37-3, PBL 95, PBL 101, Labud), *A. ampeloprasum var. ampeloprasum* - elephant garlic (Živa, 30A, 17A) and *A. cepa* - onion (Ljubičasti srebrenjak and Kupusinski jabučar). Standard agrotechnical measures were applied during experiment. Bulbs of autumn garlic, elephant garlic and Ljubičasti srebrenjak were planted in October of 2018, and harvested in Jun of 2019. Nevertheless, the crops that consisted rest of the *Allium* species were formed in March of 2019, while plant material was collected in July of the same year. For purposes of chemical analysis each genotype counted 10 samples of healthy, properly formed bulbs.

Chemicals

All of the chemicals used in this research were of analytical purity grade. Nitric Acid (67-69 % for trace metal analysis, VWR BDH Prolabo chemicals, Canada) and hydrogen peroxide (30% for traces analysis, VWR BDH chemicals, France) have been used for the digestion of samples

Sample preparation technique for content determination of metals via AAS and ICP-OES methods

Solid samples of the plant material, previously minced, were prepared using the microwave digestion method to investigate the level of metals. Respectively, triplicates of samples were weighted 0.4 g and transferred into cuvettes. Subsequently, 7 mL of nitric acid (67-69% HNO_3) and 1.5 mL of hydrogen peroxide (30% H_2O_2) were added. In the same manner, blank was prepared, excluding the sample.

Vessels then stood for 10 minutes, before they were sealed and relocated to microwave oven (TITAN MPS Microwave Sample Preparation System). Digestion lasted for 56 minutes at constant temperature and pressure that was evenly amplified from 0 to 30 bar (Banule and Ajwa, 1999; Block 1995; Liang et alm 2019). The content of cuvettes cooled down before transferred to 50 mL volumetric flasks and diluted with distilled water up to mark. All samples were made in triplicate

Atomic absorption spectroscopy (AAS)

The toxic metal concentration (Pb, Cd, As) was determined by electrothermal atomization using a graphite furnace Atomic absorption spectroscopy (GF-AAS), PinAAcle 900T. Adjusted wave lengths for elements were respectively 283.3 nm, 228.8 nm and 193.7 nm. The accuracy and precision, LOD (limits of detection) and LOQ (limits of quantification) of the method were tested by the above mentioned standard reference material. By validating the method, it was determined that all validation parameters are satisfied, that the method is accurate and that it can be used to determine the mentioned metals.

Determination of mercury using Mercury Analyze instrument

In order to establish the content of Hg, samples of onion genotypes underwent examination on Mercury Analyze instrument, FIMS 100, Perkin Elmer, serial number: 101S14121001. SRM used for this purpose is: Reference Standard Hg - Mercury standard traceable to SRM from NIST in HNO_3 (5%), 10 mg/L, Perkin Elmer, N9300253, CL9-136HGY1.

Inductively coupled plasma optical emission spectrometry (ICP-OES)

Instrument used to analyze the remaining content of metals and metalloids was ICP-OES (Optima 8000), whilst SRM utilized: Instrument calibration standard 2, 100 µg/mL, Perkin Elmer, Ag, Al, Ca, Co, Cr, Fe, K, Mg, Mn, Mo, Na, Ni, Se, Zn, catalogue number N9301721, lot: CL3-191MKBYl.

Chemical analyses of soil

A Pye glass electrode pH—meter—potentiometer (W.G. Pye, Cambridge) was used to measure the pH value (in 001 M KCl). The humus content was determined by oxidation with the KMnO4 solution (according to Kotzman), and total nitrogen content by the Kjeldahl method. Available P and K were determined by extraction with Al solution, and P and K by colorimetry with molybdate and flame photometry, respectively (Egner et al., 1960). The EDTA extractable concentrations of heavy metals were determined by the EDTA extraction protocols for IRMM BCR reference materials CRM-484 (Milenković et al., 2015).

Statistical analysis

The obtained results were analysed according to the model of the one-factor analysis of variance, and the individual comparison of groups was performed by the subsequent LSD test ($p<0.05$ and $p<0.01$). The data were processed using various mathematical and statistical softwares (Excel 2010, DSAASTAT) and results are presented in the tables.

Results and Disscusion

Chemical properties of soil

Since soil and its richness in chemical composition play a significant role in defining the chemical composition of plants that grew on it (Banuelos and Ajwa, 1999; Něměček, 2001), Table 1 shows the results of basic agrochemical properties of soil, while Table 2 shows content of total and content of accessible microelements and potentially toxic elements, detected in soil.

Table 1. Basic agrochemical properties of soil

Basic properties								
pH (KCl)	**pH (H_2O)**	**$CaCO_3$ %**	**Humus %**	**Total N %**	**P_2O_5 (Al)**	**K_2O (Al)**	**Total salts %**	**EC (mS/cm)**
7.44	8.22	16.59	0.98	0.06	1.78	9.5	0.03	0.34

According to obtained results of the basic agrochemical analysis, the soil on which *Allium* species were grown was: the carbonate, alkaline reaction, poor in humus content and poor in content of accessible P and K (Table 1).

Table 2. The content of total and easy accessible microelements and toxic elements in soil (mg/kg)

Element	Cu	Zn	Mn	Fe	Mo	Co	Pb	Cd	Cr	Ni
Total content (HNO_3)	19.93	56.23	368.33	23264.96	10.27	17.79	30.41	0.61	28.6	41.97
Accessible content (EDTA)	0.86	0.132	2.784	5.024	0.464	0.464	2.07	0.106	0	0.932

The results of the content of elements in the tested soil shown in Table 2 indicate that their total content is appropriate, while their accessibility for the plant is lower, which is attributed to the pH value as well as the mechanical composition of the soil.

Elemental content of plant material

The content Na as beneficial element for plants, and the content of K, Ca and Mg as essential elements for growth determined in *Allium* genotypes was shown in table 3.

The obtained results indicate that the highest content of Na and K was observed in genotypes of *A. sativum*, and the lowest in *A. cepa*. Namely, the content of Na was the highest in genotypes

JBL 7, *Ranko* and *Bosut*, while the the highest content of K was achieved in *Ranko*, *Bosut* and *JBL 8/17*. In the case of both elements, the contents achieved in the mentioned genotypes were statistically significantly higher compared to contents observed in other genotypes of all tested *Allium* species (Table 3).

The content of Ca and Mg in tested species was as follows in ascending order *A. cepa* < *A. ampeloprasum* < *A. sativum*. Genotype *Bosut* (*A. sativum*) had the highest content of Ca (183.5 mg/kg) and that content was statistically significantly higher compared to other genotypes of *A. sativum*. Observing the content of Mg, genotypes *Bosut* and *Ranko* was contained statistically significantly higher content of Mg than other *A. sativum* genotypes. The lowest content of Ca (68.0 mg/kg) and Mg (97.9 mg/kg) was observed in variety *Kupusinski jabučar* (*A. cepa*) (Table 3).

Table 3. The content of N, K, Ca and Mg in bulbs of selected genotypes of *Allium* sp. (mg/kg of dry matter)

***Allium* species**	**Genotype**	**Element**			
		Na	**K**	**Ca**	**Mg**
A. sativum	Sedef	31.46	3133.1	142.6	183.6
	PBL 37-3	16.15	3077.7	130.9	197.1
	PBL 95	15.83	3628.5	164.4	200.5
	PBL 101	67.73	3514.5	137.8	202.4
	Labud	36.59	3827.2	95.6	200.7
	JBL 3/13	32.40	2811.5	106.0	224.6
	JBL 7	50.61	3560.8	121.0	209.8
	JBL 8/17	25.39	4145.0	128.8	228.0
	Ranko	72.85	4018.4	128.0	265.4
	Bosut	84.68	3992.5	183.5	348.8
A. ampeloprasum	30 A	17.84	2899.5	167.0	285.1
	Živa	16.61	2505.7	100.8	314.6
	17A	17.49	2871.2	74.1	228.7
A. cepa	Ljubičasti srebrenjak	19.49	1378.6	107.4	105.0
	Kupusinski jabučar	44.21	1517.4	68.0	97.9
	$\bar{x}$	**36.6**	**3125.4**	**123.7**	**219.5**
	Min	**15.8**	**1378.6**	**68.0**	**97.9**
	Max	**84.7**	**4145.0**	**183.5**	**348.8**
	LSD 0.05	12.50	410.5	11.4	19.1
	LSD 0.01	17.44	770.2	16.2	25.7

The content of microelements (Fe, Zn, Ni, Mn, Mo), beneficial (Co, Se, Al) and potentially toxic elements (Hg, Cd, Cr, Pb, As) determined in bulbs of genotypes of selected *Allium* species was shown in table 4.

Table 4. The content of microelements and potentially toxic elements in bulbs od selected genotypes of *Allium* species (mg/kg per dry matter)

***Allium* species**	**Genotype**	**Element**												
		Hg	**Cd**	**Fe**	**Zn**	**Co**	**Ni**	**Mn**	**Cr**	**Mo**	**Pb**	**Al**	**As**	**Se**
A. sativum	Sedef	< 0.01	0.017	1.56	4.40	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.3	0.5
	PBL 37-3	< 0.01	0.021	0.69	4.86	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.3	2.18
	PBL 95	< 0.01	0.015	0.74	4.38	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.3	0.5
	PBL 101	< 0.01	0.016	1.33	6.92	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	0.66	< 0.3	0.5
	Labud	< 0.01	0.015	0.56	4.66	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.3	1.95
	JBL 3/13	< 0.01	0.017	5.35	5.54	< 0.5	< 0.5	1.51	< 0.5	< 0.5	0.41	5.93	< 0.3	1.62
	JBL 7	< 0.01	0.024	3.47	3.75	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	0.52	17.43	< 0.3	2.62
	JBL 8/17	< 0.01	0.029	4.44	4.05	< 0.5	< 0.5	0.58	< 0.5	< 0.5	0.21	2.99	< 0.3	2.79
	Ranko	< 0.01	0.013	10.07	8.00	< 0.5	< 0.5	1.48	0.59	< 0.5	< 0.1	8.09	< 0.3	0.5
	Bosut	< 0.01	0.025	11.62	8.83	< 0.5	< 0.5	2.59	< 0.5	< 0.5	< 0.1	15.55	< 0.3	0.5
A. ampeloprasum	30 A	< 0.01	0.032	6.49	4.39	0.86	< 0.5	4.29	< 0.5	< 0.5	< 0.1	6.58	< 0.3	6.39
	Živa	< 0.01	0.022	6.57	4.28	0.79	< 0.5	3.68	< 0.5	< 0.5	< 0.1	8.95	< 0.3	4.57
	17A	< 0.01	0.037	6.72	3.56	0.99	< 0.5	2.47	< 0.5	< 0.5	< 0.1	6.42	< 0.3	4.45
A. cepa	Ljubičasti srebrenjak	< 0.01	< 0.01	405.71	10.53	0.88	< 0.5	2.26	< 0.5	< 0.5	< 0.1	1.97	< 0.3	0.5
	Kupusinski jabučar	< 0.01	< 0.01	2.82	1.46	0.78	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	4.66	< 0.3	1.46
	$\bar{x}$	**< 0.01**	**0.022**	**31.21**	**5.31**	**0.86**	**< 0.5**	**2.30**	**0.59**	**< 0.5**	**0.38**	**7.20**	**< 0.3**	**2.07**
	Min	**-**	**0.013**	**0.56**	**1.46**	**0.78**	**-**	**0.58**	**0.59**	**-**	**0.21**	**0.66**	**-**	**0.50**
	Max	**-**	**0.037**	**405.71**	**10.53**	**0.99**	**-**	**4.29**	**0.59**	**-**	**0.52**	**17.43**	**-**	**6.39**
	LSD 0.05	-	0.005	2.11	3.26	-	-	0.46	-	-	0.09	2.92	-	0.8
	LSD 0.01	-	0.010	3.42	5.48	-	-	1.39	-	-	0.14	4.10	-	1.5

The highest content of Fe (11.62 mg/kg) and Zn (8.83 mg/kg) was observed in garlic genotype *Bosut*, while the onion genotype *Kupusinski jabučar* had the lowest content of Fe (2.82 mg/kg) and Zn (1.46 mg/kg). All genotypes of elephant garlic had Fe and Zn in contents that did not differ statistically significantly.

The Mn content was ranged from < 0.5 mg/kg in most garlic genotypes to 4.29 mg/kg in elephant garlic genotype - *30A*. Similar results were observed in the case of Se, the lowest content (0.5 mg/kg) was noticed in garlic genotypes, while the highest content was achieved in genotype *30A*. In fact, all selected *A. ampeloprasum* genotypes (*30 A*, *Živa* and *17A*) had a statistically significantly higher content of Mn and Se compared to all tested *Allium* genotypes (Table 4).

The results indicate that in all tested *Allium* genotypes was achieved same content of Hg (< 0.01 mg/kg), Ni (< 0.5 mg/kg), Cr (< 0.5 mg/kg), Mo (< 0.5 mg/kg), As (< 0.3 mg/kg), while same content of Co (< 0.5 mg/kg) was observed only in *A. sativum* genotypes. The content of Cd in all *Allium* species was varies from < 0.01 to 0.037 mg/kg which is below of toxicity levels recommended by the WHO/FAO (0.2 mg/kg) (WHO 1986, 1989; Elbagermi et al., 2012). The content of Pb was < 0.1 mg/kg, except in the case of garlic genotypes, *JBL 3/13*, *JBL 7* and *JBL 8/17* which were contained 0.41, 0.52, 0.21 mg/kg, respectively. The safe value for Pb, in vegetables prescribed by the WHO is 0.3 mg/kg (Elbagermi et al., 2012). The highest content of Al was noticed in garlic genotypes *JBL 7* (17.43 mg/kg) and *Bosut* (15.55 mg/kg). In general, all genotypes analized in this stady had average content of Al 7.2 mg/kg which is in accordance with the values obtained in bulb crops (7.4 - 9.9 mg/kg) in similar studies (Liang, 2019).

Conclusion

Allium species have been valued in nutrition and folk medicine for centuries, due to their rich chemical composition. In this study, it was found that the genotypes of selected *Allium* species (*A. sativum*, *A. ampeloprasum* var. *ampeloprasum* and *A. cepa*) had a moderate content of the examined elements. Namely, genotype of *A. sativum Bosut* had the highest content of essential elements (K, Ca, Mg, Fe, Zn, Mn) and beneficial element (Na). All tested genotypes of *A. ampeloprasum* var. *ampeloprasum* (*30A*, *Živa* and *17A*) contained a similar level of all studied elements. Significantly higher content of iron and zinc was found in the onion varieties *Ljubičasti srebrenjak*. Content of toxic elements (Hg, As, Cd) was within safe limites recommended by WHO. Only in the case of garlic genotypes *JBL 3/13*, *JBL 7* the content of Pb was more than prescribed by the WHO.

Given that species of the genus Allium show a good ability to accumulate elements important for human health, especially Fe, Zn and Se, future research should focus on agro-technical measures that increase their content in plants (biofortification).

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Original scientific paper

Application of new technologies in transfer of knowledge and information in agriculture

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Abstract

The aim of this paper is to analyze how the application of information communication technologies facilitates the distribution of knowledge and information to farmers in Serbia. The obstacles in this process are the level of computer and media literacy of farmers and agricultural extension officers. Extension officers are in one of the most important "channel" for transfer of information, but other actors can participate in this process such as various agricultural development organizations, professional organizations or individuals. Desk research method is used in the paper. The research results indicate that information important for the improvement of agricultural production are efficiently distributed to agricultural producers using mobile telephony, personalized e-mail and Internet.

Key words: knowledge and information transfer, agriculture, ICT, agricultural extension service

Introduction

Global population growth, especially in developing countries, and the growth of living standards and consumer demands in highly developed countries exert pressure on agriculture to produce increased quantities of final products and high-quality raw materials for food industry. The improvement of agricultural production depends on the application of modern technical and technological achievements. Traditionally, agricultural extension service (AES) has had the role of a "channel" for information transfer from creators of knowledge to agricultural producers.

Recently, farmers have had access to an abundance of information from various sources such as state and local government, organizations engaged in the improvement of agriculture and rural development, professional and non-governmental organizations. In order to select important information, farmers need to have adequate techniques and knowledge necessary to collect and classify the obtained information. At the same time, this process is hampered by various limiting factors, depending on the availability and access to technology, such as owning a computer, a "smart phone" or a similar device, and Internet access, which can be a significant prerequisite, especially in remote rural areas.

If the key conditions are met, the second set of conditions includes sufficient knowledge to select and interpret available information. To improve production and farming, farmers are expected to use different channels of communication, such as farm registration or application for subsidies, which require implementation of adequate skills and knowledge.

Because of the lack of communication systems in rural areas, and a relatively low level of computer literacy among the total and rural population, assistance in transfer of information to farmers is necessary. Individuals and organizations take on the role of channels that select, adapt and further distribute information to farmers.

The paper analyzes the model of distribution of selected information and new knowledge to farmers and the role individuals and organizations have in this process. The level of computer literacy, especially among the rural population, was analyzed as one of the preconditions of the process. Special emphasis was placed on the importance of AES in the transfer of knowledge to users using various information and communication channels.

Computer and media literacy

Literacy has traditionally been associated with the ability to read, understand texts and write. With the establishment of mass media, such as radio and television and more recently information and communication technologies (ICT), a new type of literacy, often called computer or internet literacy is required.

Computer literacy can be defined as person's ability to use basic computer applications in performing everyday tasks, or the ability to process texts, create tables, send and receive electronic mail, and use the Internet (SORS, 2013). Observed in this way, computer literacy indicates a person's ability to use basic computer applications, necessary in performing most tasks. Computer literacy in Serbia was first recorded in 2011 within the Census of Population, Households and Dwellings among residents older than 15 years. Computer literate persons are all persons capable of performing the four listed activities. Partial computer skills include the ability to perform one, two or three of the listed activities. Computer illiterate persons are all persons who cannot use a computer, i.e. they cannot perform any of the stated tasks.

More than half of the population of Serbia over the age of 15 is computer illiterate (51.0%), which indicates an unsatisfactory level of computer literacy. The share of persons with partial computer skills in total population over the age of 15 does not vary depending on the region, but shows significant variation in other indicators. The only region where the participation of computer literate persons is higher than that of computer illiterates is the Belgrade region (Table 1). Almost one third (32.5%) of computer literate people are from the Belgrade region. The regions of Šumadija and Western Serbia and Southern and Eastern Serbia have a higher share of computer illiterate persons in the population older than 15 years than the national average.

Table 1. Structure of the population over the age of 15 according to computer literacy, by regions and type of settlement (%)

	Computer literate persons	Persons with partial computer skills	Computer illiterate persons
	Total population		
Republic of Serbia	34.2	14.8	51.0
Belgrade Region	48.1	13.9	38.0
Vojvodina Region	34.9	15.8	49.3
Region of Šumadija and Western Serbia	27.8	14.8	57.4
Region of Southern and Eastern Serbia	26.9	14.4	58.7
	Population in “other” settlements		
Republic of Serbia	19.8	14.3	65.9
Belgrade Region	28.1	15.9	56.0
Vojvodina Region	24.4	15.9	59.7
Region of Šumadija and Western Serbia	17.4	13.6	68.9
Region of Southern and Eastern Serbia	15.1	12.9	72.0

Source: 2011 Census of Population, Households and Dwellings in the Republic of Serbia, SORS, Belgrad

If these indicators are analyzed on the territory of “other” settlements,[1] the situation is noticeably more worrying. Less than one fifth of the rural population is computer literate (19.8%), while Belgrade Region and Vojvodina Region are above average. In rural areas of all regions, the number of computer illiterate persons is two or more times higher than the number of computer literate persons. With the increase of average age, the number of computer illiterate persons increases both in the total population, and in the population in “other” settlements. That is why it is interesting to compare these data with those on the age of farmers in Serbia.

Indicators of age structure of the holders of family holdings (farm holders) show the dominance of the older population. The share of farm holders under the age of 35 is the lowest in all regions. In Šumadija and Western Serbia and Southern and Eastern Serbia, the

[1] Statistical reporting in Serbia uses the division of settlements into “urban” and “other”. If “urban” means urban settlements, it can be implicitly assumed that “other” settlements are rural.

majority of farm holders are above 65 years of age, while in Vojvodina and Belgrade most farm holders are between 55 to 64 years of age (Bogdanov and Babović, 2014). When combined with data on the age structure of farm holders, the indicators on computer literacy of the total and rural population indicate low computer literacy of persons who make decisions on agricultural production in Serbia.

The dominance of new media and the Internet imposes greater demands on the population in terms of acquiring new skills. With the appearance of ICT, a new type of literacy emerges. Originally referred to as Internet literacy, the term media literacy is used today. Media literacy can be defined as *the ability of an individual to access, analyze, evaluate and create (and share) media content*. Accordingly, there are four components that jointly constitute a skills-based approach to media literacy (Livingstone, 2004).

One of the basic elements of media literacy is the ability to access and use information, i.e. the person's ability to recognize when and what information is needed, to find, evaluate and apply it in an efficient manner. Access to information implies the ability to find information regardless of its format (photo, graphic, video, audio, text, multimedia, etc.) and the medium through which it is communicated (radio, print media, television, internet, social networks). The assessment of information includes its interpretation and analysis, but also the determination of credibility, objectivity and reliability. Only such information can be a solid basis for making the right decisions. The level of media literacy is not monitored systematically, and conclusions are made on the basis of individual research. In that sense, the media literacy of the population of the Republic of Serbia, including the rural population, cannot be estimated. Since the core of media literacy lies in the computer literacy, it can be assumed that the number of media literate farmers is extremely low. Nevertheless, they face the need to access and apply new knowledge and information in the production process.

Farmers encounter an abundance of information on agricultural production. However, they only need certain information related to the type of production they are engaged in, or even on certain aspects of this production, which usually varies depending on the season or time of the year. They search for specific information and knowledge which, if applied in the production, can contribute to its improvement and overall economic success. Because agriculture is time consuming, farmers often do not have sufficient time to analyze all available information, select the relevant information and interpret them successfully. Considering the established level of computer and (indirect) media literacy, it is evident that this process should be taken over by certain institutions or to an extent by individuals.

Specifics of ICT application in agriculture

Farmers face various obstacles in the process of improving their production. Research shows that one of the key difficulties is the access to information. Farmers with the low financial status single out access to (1) loans, (2) higher market prices, and (3) reliable and relevant information as the key barriers (Bell, 2015). Other research has shown similar results, which indicate that managers of agricultural cooperatives in Serbia single out (1) access to loans, (2) local community support and (3) better market information as the most important difficulties (Simmons et al., 2010).[2] Access to information has been recognized as one of the weak points in the process of improving agricultural production. ICT is emerging as an important tool in this process.

Information and communication technologies include various technical resources used to manage information and facilitate communication, including computers, information technology, telephones, electronic media, and all types of audio and video signals (Rohila et al., 2017). Media used in knowledge and information transfer in agriculture can be systematized into five categories: radio and television, video content, mobile phones, the Internet and various social groups, such as Facebook, Twitter, and so on (Bell, 2015).

AES uses three groups of methods when communicating with farmers: individual, group and mass methods. The first two groups of methods imply the *face to face* contact of extension officers with one or a group of farmers, while the third group of methods involves the distribution of information to a large number of users through mass media. One of the goals of extension work is to cover as large target group as possible, so the optimism about the use of ICT in agricultural extension is not surprising. Despite the advantages of using these methods, research shows that it is not easy to introduce ICT in agricultural extension work, especially in developing countries. Most of the ICT agricultural extension projects were implemented as pilot projects funded by international organizations. These projects were short-lived and after the pilot period, most of the projects were never implemented on a larger scale (Rohila et al., 2017) due to insufficient equipment and training of farmers, but also of extension officers.

In addition to professional competence, manifested as pedagogical, program and communication competence, agricultural advisors should also have ICT competence in order to "find, adopt and apply the latest knowledge in the field of biotechnology" (Bojović and

[2] One of the most important obstacles was the adoption of a new law on cooperatives, but this is a factor specific to the development of agricultural cooperatives, and not to the entire agricultural production.

Tanasković, 2016). The research showed that extension officers in Serbia lack ICT and communication competence, which is gradually being overcome by organizing professional development programs.

Regardless of the limitations, the application of ICT in the dissemination of information in agriculture is extremely important because it drastically increases the coverage of the target group and enables the distribution of information to a large number of users. Different media can also be combined to achieve the best possible results in disseminating information and practical knowledge. For example, the proliferation of "how to" videos on Youtube is a simple example of the power of ICT in improving agriculture (Bell, 2015). Viewing such a message does not require highly specific user knowledge, and the content can be educational and help farmers solve problems.

One of the problems in using ICT in distributing knowledge and information is the lack of feedback from users about receiving and understanding the message, which is especially present in print media, radio and television. Therefore, it is often necessary to use a number of different media to disseminate single information. Some authors, on the other hand, believe that certain forms of ICT, such as the Internet, enable timely feedback (Aromolaran et al., 2016) but this is not always the case. Research in developing countries has shown that, even with the application of ICT, information still flow one-way, i.e. that there is a limited scope of interaction with farmers (Rohila et al., 2017).

Models of knowledge and information distribution in agriculture

Distribution of information in the field of agricultural production can be conducted in many different ways. One of the most common forms of information exchange is among farmers. This traditional form of dissemination of information has taken on a new dimension with the application of ICT. An interesting form of information distribution to farmers was organized in the municipality of Požega, where one farmer created an Internet platform with text message (SMS) service that allows a large number of farmers from the local community to be informed about various activities of local and state authorities in the field of agriculture and the available subsidies for agricultural inputs (https://www.agroklub.rs/poljoprivredne-vesti/putem-sms-a-obavestava-poljoprivrednike-o-konkursima-i-novinama-u-agraru/57635/). The idea was to provide relevant information to farmers in a timely manner and in a simplified way, in order to make it as understandable as possible to users. The creator of this platform selects information of importance for a certain group of farmers, simplifies, i.e. interprets them in an understandable way and distributes them to subscribed users. Significant help is given not only through the selection of information, but also the analysis of information and systematization of the existing knowledge, which saves time for the farmers who are engaged in agricultural production and do not have enough time to perform these tasks.

The use of mobile phones, or providing information on a certain topic via text (SMS) messages, has become extremely common. Establishing contacts with farmers, especially in remote rural areas, is a challenge. Only about 10% of the rural population can be contacted directly, using available technologies (Bell, 2015). As mobile telephony is one of the fastest growing forms of communication, it is often used in the process of informing farmers.

Number of mobile phones users in the world is increasing dramatically. Globally, the number of mobile phone users per 100 inhabitants increased from 67 to 108 from 2009 to 2020, and in 2016, for the first time, the number of mobile users exceeded the total number of inhabitants on the planet (https://data.worldbank.org/indicator/IT.CEL.SETS.P2). The number of mobile phone users is greater in developed than in underdeveloped countries. By reducing the prices of phones and increasing the number of functions they can perform, mobile phones are becoming an extremely important communication tool between extension officers and farmers, but also between farmers themselves (FAO, 2017). Because they can be used to interact through voice, text, and photography, mobile phones are becoming an almost unavoidable channel of communication in the transfer of knowledge and information in agriculture.

There are several specialized organizations in Serbia that use ICT to contact farmers. These organizations are most often focused on one segment of agricultural production or a group of information that farmers are particularly interested in. Informing farmers by sending text (SMS) messages is a widespread practice in Serbia. Information is most often aimed at exercising the right to subsidies, which indicates that this area is of special importance for farmers. Specialized systems in Serbia inform farmers about various forms of incentives in agriculture[3] at a certain fee. Some information are available and free on the websites of these organizations, but if farmers seek an answer to a particular question or some specific information there is a fee or annual membership for such services. This can be considered as providing extension services in agriculture in broader terms. In addition to the right to subsidies, farmers are interested in prices and the possibility of marketing agricultural products. Some organizations, such as Info Tim Logistika (www.infotim.rs), send information to their customers in the form of SMS messages or e-mails, and provide specially prepared reports or market research.

[3] For example http://subvencije.rs/sms-obavestenja/ (all Internet addresses were accessed in January 2022)

Internet is one of the most important forms of ICT used to distribute information in agriculture. The number of people using the Internet per 100 inhabitants in the period from 2009 to 2019 increased from 70.1 to 89.1 in high income countries and from 16.3 to 50.4 in low and middle income countries (https://data.worldbank.org/indicator/IT.NET.USER.ZS). With the increase in the total number of Internet users, an increase in the number of users among the rural population is also expected. The prevalence of Internet is lower in "other" areas than in urban areas in Serbia (70.5% and 85.5%, respectively). In addition, the number of people using the Internet in rural areas is increasing more slowly than in urban areas. As the most common reason why there is no internet connection at home, the respondents stated that they did not need the Internet (76.2%), and only 12.5% stated that they did not have sufficient skills (SORS, 2019).

Farmers can find different information on the Internet, but it requires certain skills. As previously mentioned, searching the data on the Internet is a new form of literacy which is expected from extension officers and, to some extent, from farmers (Aromolaran et al., 2016). Farmers face obstacles in using the Internet, such as absence of availability or an ability to work on computers or other smart devices, or a low level of education. One of the limiting factors to which less attention is paid is English language skills. For example, one website dedicated to agricultural production proposes five applications which can facilitate work and improve performance in agriculture, from crop protection, calculation of the required amount of fertilizer, information related to organic production, to various methods of pest control (https://www.agromedia.rs/blog/agrokutak/5-najboljih-aplikacija-za-modernog-poljoprivrednika/). Four of the five proposed applications are free, but all five are in English, which significantly reduces the potential number of users. On the same website, almost a year later, three applications that may be interesting to agricultural producers were proposed in Serbian (https://www.agromedia.rs/blog/agrokutak/3-najkorisnije-poljoprivredne-aplikacije-na-srpskom-jeziku/), which indicates the fact that language skills were an important obstacle that might have prevented Serbian farmers from using the previously proposed applications.

One of the applications in Serbian used in organizing agricultural production is Agro-Assist by DuPont Pioneer, which allows farmers to monitor meteorological data, applied technology and achieved results on their plots through an electronic field book (https://www.agroklub.rs/poljoprivredne-vesti/besplatno-preuzmite-agro-assist-aplikaciju/23266/). In this case, information available to farmers is systematized in a way that should facilitate decision-making. This example shows that ICT service providers can be different organizations and individuals, such as companies in the pre-farm sector, agricultural organizations or individuals.

Professional organizations can also be a significant source of information. One of the examples for such practice is the Association of Beekeeping Organizations of Serbia, whose website abounds in information on recommendations for improving beekeeping, honey control, wholesale and retail sales of final products, and other information relevant to beekeepers. What sets this website apart from others is that the information is grouped and only information important for beekeeping production is selected, which enables producers to access the information they need.

Conclusion

Economic success of agricultural producers depends on the level of application of modern technology in production. Agricultural extension service (AES) uses ICT in the distribution of new knowledge and information, but insufficient media literacy of both farmers and extension officers is a limiting factor. In addition to AES, the transfer of knowledge to farmers is performed by other organizations that collect, process, and distribute information to farmers depending on their needs. Information delivery according to specific requests is given through SMS messages and personalized e-mails. Farmers' associations can also play a significant role in this process. They collect information related to one or several topics and deliver them to their members. In specific situations, accessing, processing and distribution of information can be done by individuals with adequate knowledge or skills and funding.

The limiting factor in the ICT use in agriculture is the level of technical knowledge required to analyze the abundance of available information of most participants in this process. Therefore, further directions should be reflected not only in finding new channels of information transfer, but also in education of all persons involved in the process. It is important to include knowledge related to the use of ICT in regular education of agricultural extension offices. Farmers' organizations and educational institutions should be involved in formal and informal education of farmers in order to give them the opportunity to be educated and trained to access, evaluate and implement available knowledge, which would contribute to the increase of their autonomy in the process of improving agricultural production.

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Original scientific paper

External price parities of wheat and maize in the Republic of Srpska

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Abstract

The aim of this research was to examine the changes in external price parities for wheat and maize, i.e. the relation between the prices of inputs (seeds, mineral fertilizers and fuel) and purchase prices of observed crops. The research was based on secondary data published by the Institute of Statistics of Republic Srpska for the period from 2010 to 2020. Considering the source of data, the desk research method was applied. The external price parities for wheat and maize are calculated, and additionally determined the basic indicators of descriptive statistics. The average price of wheat was 0.34 BAM/kg with a coefficient of variation of 13.09%, while the average price of maize was 0.33 BAM/kg and with a coefficient of variation of 14.65%. The research results showed that the purchase prices of wheat and corn had a slower growth compared to the prices of selected inputs. Compared to other inputs, the prices of mineral fertilizers had more pronounced variations, especially UREA (16.38%). In the analyzed period, maize price had a negative compound annual growth rate as well as euro diesel price. The external parities showed an imbalance of wheat and maize prices in relation to variable inputs prices which indicate an unfavorable economic position of agricultural producers in the Republic of Srpska.

Key words: wheat, maize, prices, external parities, Republic of Srpska

Introduction

Price policy and prices, as an instrument of this policy, play a significant role in agriculture and agriculture development. Generally, the basic factor that determines the conditions of business and financial position of agricultural entities (producers, cooperatives and other agricultural enterprises) is price. Pejanović (2000) emphasizes that the experiences of developed countries have shown the price policy of agro-food products as the most appropriate instrument of influencing the development of agriculture. Additionally, Vlahović et al. (2010) highlighted the use of different methods of price formation and regulation in developed countries in order to control of spontaneous activity of market laws as well as to route them in the desired direction. Each country leads certain control of policy prices of agricultural products to protect producers and consumers commonly by using three elements: *level of prices, parity prices and long-term level of prices* (Božić et al. 2011). Price parity is an important indicator not only of the economical position of some products as well as for the income of primary agricultural producers. Stabile price parities ensure high agricultural production without major variation as well as certain accumulation for agricultural producers *(Stojanović-Poparić, 2017*).

The price policy for basic agricultural products in the Republika Srpska (Bosnia and Herzegovina), has changed in recent decades. Bearing in mind that today's the Republic of Srpska, Bosnia and Herzegovina belonged to the former common state, a state that had a centrally planned system of prices control of strategic agricultural products. Božić et al. (2011) following Marković et al. (1982) highlighted that starting from the 80s last century, the long-term program for the development of agro-industrial production during the stabilization period and the Low of Social Price Control System (1985) defined the price policy, according to which prices are freely formed under the influence of market laws and world market prices, with some social intervention (Ranđelović, 2001). Law on the System of Social Price Control also considered the specifics of agriculture, provided the formation of protective prices for main agricultural products, and for that, the basic element was *external parity* (Mataga, 1985). After the 1990s, prices continued to be freely formed in the market of the former common state, under the influence of supply and demand, import and export. Prices of strategic agro-food products were not regulated by systematic laws and clear mechanisms, but mainly by regulations at the entity level (Bajramović et al., 2008). Similarly was in Serbia, where prices of strategic agricultural and food products were not regulated by systematic laws and sufficiently efficient mechanisms, but only by regulations and decisions at the national level. Due to that, the synergetic effect did not exist for agricultural policy measures related to market regulation, which together with the inefficient and inconsistent implementation of mechanisms of market support reduced the production-economic effects of the sector (Bogdanov, 2008).

In the Republic of Srpska, the agricultural products prices policy was determined through the Law on Agriculture (Official Gazette of the Republic of Srpska, no. 70/06; 20/07; 86/07; 71/09), which contains prescribing special forms of protective prices (guaranteed, indicative and minimum price threshold) to stabilize the market and protect the interests of consumers. The Government of the Republic of Srpska, through certain bylaws, prescribed minimal purchase prices for cereals (mercantile wheat and rye) in the amount of 270 BAM/t in 2001 and 2002 for the needs of the Republic Directorate for Commodity Reserves and for the producers as well. The last year in which the Government prescribed the purchase price of wheat was 2009, after which with the adoption of the new Law on Intervention Procurement (Official Gazette of Republika Srpska, Low of Social Price Control System no. 74/17), the Directorate for Commodity Reserves in the Republic of Srpska ceases to function (Mrdalj, 2020).

Many authors have dealt with the topic of internal and external parities in wheat and maize production. Mutavdžić et al. (2016) investigated the changes in maize and wheat prices and parities for the period from 1994 to 2004 in Serbia to predict future trends in the production of these cereals. Munćan and Božić (2018) examined the influence of input and output prices on the effectiveness of maize production, based on collected data from 2005 to 2017 through a survey of 25 family farms, size 10-20 ha of arable land, placed in Južni Banat as a part of Vojvodina region. The authors pointed out that the deterioration of external price parities led to a significant increase of variable costs and a reduction of gross margin in maize production during the observed period.

Ostojić et al. (2009) analyzed the parity of wheat and maize in the Republic of Srpska for a period from 2006 to 2008. Results of this research pointed out favorable wheat price parities in 2008, with a positive effect on increasing sown areas in 2009 by 9.5%. This was not the case with maize, i.e. unfavorable maize price parities in 2008 influenced the decrease of sown areas in 2009 by 8.8%. Vlahović et al. (2010) examined external price parity between selected inputs (mineral fertilizers and euro diesel) and basic crops (wheat and maize). Authors indicated the faster growth of input prices than output prices, which led to an imbalance in parities and finally to harming the production of basic agricultural products. As a consequence of price parity imbalance, authors emphasized the visible decline of accumulative and reproduction ability of primary agricultural production. This statement was also confirmed by the research of Jalić et al. (2021) who did an ARIMA time-series forecasting and concluded that it is expected a downward trend of wheat production in Bosnia and Herzegovina.

As already mentioned, the problem of external price disparities is present in wheat and maize production in the Republic of Srpska, especially emphasized since these cereals in sown areas are considered as the most important. The research in this paper will not deal in depth with the causes of the growth of external price parities. The aim of this research is to determine the level, variation and relation between output-input prices in wheat and maize production in the Republic of Srpska for a period of time from 2010 to 2020.

Material and Methods

The desk research method was applied to analyze the price parities of wheat and maize and their production inputs for 11 years period. Production quantity and price trends were shown through graphs and trend lines for the period 2010-2020. Basic descriptive statistics as the interval of variation, mean and coefficient of variation were calculated for the input and output prices. The data was collected from the Institute for Statistics of the Republic of Srpska, Statistical bulletins and Statistical Yearbook. The external parities were calculated between the purchase price of wheat and maize and the basic inputs (wheat and maize seed, euro diesel, and fertilizer type KAN, UREA and NPK 15:15:15). Generally, price parities are important indicators of the economic position of individual production lines as well as the income levels of primary agricultural producers. External parities show how many units of input can be purchased by selling one unit of output.

$$\text{Parity} = \frac{Output\ price}{Input\ price}$$

The coefficient of variation was obtained from the relation between standard deviation and arithmetic mean, while the Compound Annual Growth Rate is calculated by the formula:

$$\text{CAGR} = \sqrt[n-1]{\frac{y_t}{y_1}} - 1$$

Where:

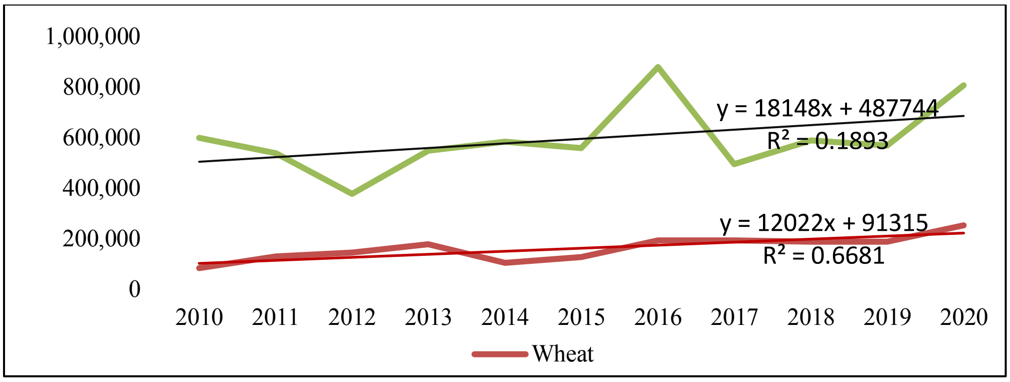
CAGR – Compound Annual Growth Rate

Y_t – value from the last year

Y_1 – value from the first year

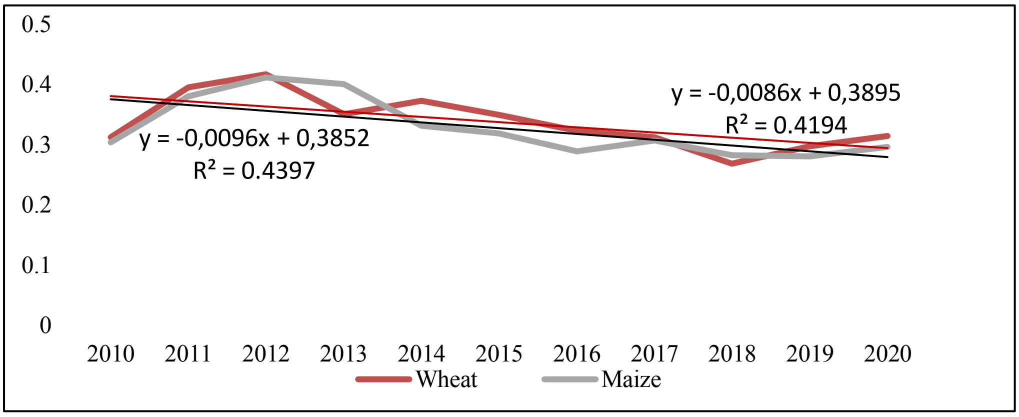
Results and Discussion

In order to understand the wheat and maize producers' economic position, an analysis of the production for the same period was done. The graph below represents the annual production of wheat and maize in tons.



Graph 1. Wheat and maize production (t) in the Republic of Srpska (2010-2020)

Produced quantity of wheat and maize increased annually in the analyzed period with a few variations. The compound annual growth rate for wheat production in the period is 11.62% and for maize 3.03%. The lowest level of wheat production was achieved in 2010 (86,647 t) and the most was produced in 2020 (254,093 t). Maize was the least produced in 2012 with an amount of 378,714 t. The highest production was achieved in 2016 (880,997 t). Maize is, for sure, the most important agricultural crop in the Republic of Srpska.



Graph 2. Wheat and maize prices (BAM[4]) in the Republic of Srpska (2010-2020)

Prices of wheat and maize increased in the first three years of the observed period. After the initial growth, from 2012 to 2018 and 2019 respectively, prices had a downward trend (Graph 2). In the last two years, the analyzed prices of these cereals have again reached the level of 2010. The highest prices of wheat and maize were recorded in 2012 (0.42 BAM/kg for wheat, and 0.41 BAM/kg for maize). The lowest wheat price was recorded in 2018 (0.27 BAM/kg) and the lowest maize price in 2019 (0.28 BAM/kg). The average price of wheat was 0.34 BAM/kg and for maize 0.33 BAM/kg. The compound annual growth rate for these cereals are very low (wheat 0.08% and maize -0.25%). It means that the prices are almost equal comparing the first and the last year of the analyzed period (Table 1). The wheat price

[4] 1 BAM =1.95583 EUR, Central Bank of Bosnia and Herzegovina

coefficient of variation (C_v) was 13.09% and maize 14.65%. The relevance of these coefficients of variation is harmonized with the Mićić and Bosančić research (2012) who claimed that the most acceptable coefficients of variation for statistics are between 10% and 20% and that in certain cases coefficients of variation of 5-10% and 20-30% are accepted. Mutavdžić et al. (2016) analyzed the wheat and maize prices in Serbia for time series 1994-2004. The authors stated that the average maize price was 107.96 EUR/t with an interval of variation between 76.92 and 189.74 EUR/t. Wheat price was 117.95 EUR/t with an interval of variation between 77 and 159 EUR/t. Regarding the coefficient of variation for observed cereals, the same authors recorded higher values of this statistical indicator compared to the values in research presented in this paper. The wheat price coefficient of variation was 20.35% and maize of 29.62%. Munćan and Božić (2018) confirmed the tendency of increase of input prices used in maize production, especially by maize seed price. Maize seed and NPK 15:15:15 price grew according to the compound annual growth rate of 2.54% and 2.53%. The lowest positive growth is registered for wheat seed, UREA and KAN (0.11%; 0.51%, 0.67%). The negative CAGR is only calculated for euro diesel, -0.65%. Vlahović et al. (2010) in their research for the period 2006-2009 pointed out very unfavorable economical conditions for crop producers in Serbia. The wheat market was characterized by significant variations of prices before and after harvesting. Maize prices were significantly below an acceptable level for producers. The increase in input prices did not lead to an increase in purchased wheat prices. The highest price growth for the analyzed period achieved maize seed and NPK. The same authors state that the wheat to NPK 15:15:15 parity was 2.12 and wheat to euro diesel 1.4, while maize NPK 15:15:15 1.7, and wheat - euro diesel 1.1, in 2009.

Table 1. Basic statistical indicators of input and output prices in the Republic of Srpska (2010-2020)

Element	Unit	Interval of variation		Mean	(C_v)	CAGR
		Minimum	Maximum			
Wheat	BAM/kg	0.27	0.42	0.34	13.09%	0.08%
Maize	BAM/kg	0.28	0.41	0.33	14.65%	-0.25%
Wheat seed	BAM/25 kg	17.48	20.71	18.73	6.01%	0.11%
Maize seed	BAM/25,000 grains	52.14	67.00	60.24	9.38%	2.54%
Euro diesel	BAM/l	1.64	2.42	2.07	12.77%	-0.65%
NPK 15:15:15	BAM/kg	0.73	0.94	0.88	8.63%	2.53%
KAN	BAM/kg	0.45	0.69	0.54	15.56%	0.67%
UREA	BAM/kg	0.62	0.96	0.73	16.38%	0.51%

Source: Author's own calculation.

Observing the prices of chosen variable inputs, it is evident, that their prices were higher than output prices. The average wheat seed price was 18.73 BAM/25 kg; maize seed - 60.24 BAM/25,000 grains; euro diesel - 2.07 BAM/l; NPK 15:15:15 - 0.88 BAM/kg; KAN - 0.54 BAM/kg and UREA - 0.73 BAM/kg. The values of coefficient of variation indicated the highest variation for UREA and KAN prices (coef. of variations 16.38%; 15.56%), then euro diesel (12.77%). The relative variation of maize seed prices was 9.38%, the prices of NPK 15:15:15 fertilizer 8.63% and wheat seed price had the lowest variation of all inputs 6.01%. The increase in fertilizers prices was also determined by Gugic et al. in their research from 2014. They stated that the price of fertilizers in 2011 was 83.9% higher than in 2005.

By analyzing the wheat input-output parities in the period 2010-2020, it is obvious the imbalance of wheat prices in relation to variable input prices. The average parity of wheat and wheat seed was 2.24. This means that wheat producers were supposed to sell 2.24 kg of wheat to purchase 1 kg of seed. The compound annual growth rate of this parity was 0.03% and could be considered quite stable (Table 2). At the level of the multi-year average, the wheat price of 6.17 kilograms was equal to 1 liter of euro diesel. The CAGR of this parity was negative -0.73%. On average, 1.58 kg of KAN and 2.16 kg of UREA were equal to the price of one kilogram of wheat. The CAGR of parity between wheat and KAN and UREA was 0.59% and 0.43% per year, which indicated the tendency of an increase in fertilizer prices according to these growth rates. The average parity price for wheat to NPK was 2.65 with the tendency of increasing by 2.45% per year (Table 2). Ostojić et al. (2009) also indicated an imbalance between wheat price and seed price, as well as fertilizer prices. In 2007 agricultural producers had to set aside 2.77 kg of wheat for 1 kg of seed, i.e. 2.87 KAN, while in 2008. 2.61 kg of NPK 15:15:15 and 2.15 of UREA.

Table 2. Wheat input-output price parities (2010-2020)

Year	Wheat – Seed (kg – kg)	Wheat – Euro diesel (kg – l)	Wheat – KAN (kg – kg)	Wheat – UREA (kg – kg)	Wheat – NPK (kg – kg)
2010	2.24	6.11	1.45	2.06	2.35
2011	1.99	5.69	1.59	2.19	2.29
2012	1.96	5.80	1.65	2.31	2.26
2013	2.36	6.73	1.74	2.51	2.49
2014	2.04	6.11	1.53	2.02	2.33
2015	2.13	5.44	1.64	1.96	2.43
2016	2.29	5.07	1.57	2.01	2.92
2017	2.37	5.79	1.45	2.02	3.02
2018	2.65	8.04	1.67	2.29	3.51
2019	2.37	7.42	1.57	2.20	2.54
2020	2.25	5.68	1.54	2.15	2.99
Average	2.24	6.17	1.58	2.16	2.65
CAGR	0.03%	-0.73%	0.59%	0.43%	2.45%

Source: Author's own calculation.

The following table presents the maize input-output parities in the period 2010-2020. Since the Institute of Statistics of the Republic of Srpska has only evidence about the prices of 25,000 grains package of maize seed, it was not possible to compare the prices of seeds and mercantile maize per kilogram. To buy one package with 25,000 grains producers had to set aside 188.72 kg of maize in average (Table 3). The compound annual rate of maize/seed price was positive, 2.80 % per year. It means the tendency of increasing seed prices. Munćan and Božić (2018) in their research recorded the extreme growth of maize seed price in relation to maize price, which was confirmed through the growth rate of 8.57%. Ostojić et al. (2009) pointed out that agricultural producers could buy 1 kg of maize seed by selling 64% more maize. The same was for NPK 15:15:15, whereby agricultural producers should have sold more of 71.5% of maize to buy 1 kg of this fertilizer. Significant price disparity in maize production is also confirmed in research by Vlahović et al. (2010). For one litter of euro diesel in the period between 2010 and 2020 the agricultural producer had to sell in average 6.35 kg of maize per year. The parity price of maize/euro diesel fluctuated between 5.68 and 7.87. The average annual parity change rate of maize price and euro diesel price was negative 0.39. This parity is the only favorable for maize producers in the Republic of Srpska since the price of euro diesel decreased according to this average change rate per year. The average parity price of maize and mineral fertilizers has deteriorated during the observed period, which is negative for cereal producers. The average parity price index between maize and KAN was 1.63; UREA 2.22, and NPK 2.74. The value of the CAGR of maize and mineral fertilizers was positive, i.e. the prices of KAN, UREA and NPK grew with an average annual change rate of 0.92%; 0.77% and 2.29%.

Table 3. Maize input-output price parities (2010-2020)

Year	Maize – Seed [5](kg – kg)	Maize – Euro diesel (kg – l)	Maize – KAN (kg – kg)	Maize – UREA (kg – kg)	Maize – NPK (kg – kg)
2010	171.53	6.28	1.49	2.12	2.42
2011	137.41	5.91	1.65	2.27	2.38
2012	127.11	5.88	1.67	2.34	2.29
2013	143.82	5.89	1.52	2.20	2.18
2014	186.35	6.87	1.71	2.27	2.62
2015	197.25	5.97	1.80	2.15	2.67
2016	217.57	5.68	1.76	2.25	3.27
2017	204.39	5.89	1.47	2.06	3.07
2018	230.01	7.64	1.59	2.18	3.33
2019	234.34	7.87	1.67	2.34	2.69
2020	226.11	6.04	1.63	2.29	3.18
Average	188.72	6.35	1.63	2.22	2.74
CAGR	2.80%	-0.39%	0.92%	0.77%	2.79%

Source: Author's own calculation.

[5] 25,000 grains

Vlahović and Radojević (2020*)* claim that the consequence of unfavorable parities is the reduced use of mineral fertilizers, plant protection products and quality seeds, which of course causes a reduced yields in production. Comparing the calculated values of average and CAGR wheat/ maize parity price, it can be concluded that the parity values between maize and chosen variable inputs were more unfavorable for agricultural producers in the Republic of Srpska in the observed period.

Conclusion

The absence of an active price policy and the determination of prices in agriculture of the Republic of Srpska solely on basis of supply and demand criteria has resulted in the growth of external price parities to the detriment of the domestic agricultural sector in the last decade (2010-2020), in the case of wheat and maize production. The results show that wheat production recorded growth and maize significant variations in the observed period. The increase of wheat price is almost unnoticeable (CAGR = 0,08%), while in the case of maize its price has decreased (CAGR = - 0.25%). In relation to output prices, input prices increased, with the exception of euro diesel prices during the observed period. The prices of wheat and maize recorded a coefficient of variation of 13.09%, i.e. 14.65%. The highest value of the coefficient of variation of input prices was for UREA fertilizer 16.38%, then KAN 15.56%, and euro diesel 12.77 %. The determined external parities of output and input prices in the observed period indicated their growth, which in wheat production was most pronounced with NPK, i.e. with an average value of CAGR = + 2.45%, while for euro diesel the average value of CAGR = –0.73%. In maize production, average CAGR input/output parity prices are higher than wheat, with the exception of euro diesel. The highest positive value of CAGR = + 2.80% in maize production was recorded in maize/seed price parity, while the growth rate of maize price and euro diesel price was negative, i.e. CAGR= – 0.39%. Generally, positive values of the compound annual growth rate indicate that the external parities are getting worse for producers, but on the other side indicate a better position for producers and traders of variable inputs. Based on all the above mentioned, it can be concluded that wheat and maize producers in the Republic of Srpska in the period 2010-2020 faced a significant increase in input prices, except for euro diesel, which greatly affected their unfavorable economic position. It is difficult to predict the movement of wheat and maize prices, as well as input prices in the domestic market, but one thing is for sure, if this tendency continues, as research results in this paper shown, it will negatively affect the size of sown areas under these crops, i.e. the production itself, and finally the income of domestic agricultural producers.

Since the research in this paper was focused on the analysis of external parities based on statistical prices data, a new opportunity for further research was opened to analyze the causes of price disparities between the two most important field crops and their inputs in the Republic of Srpska. Furthermore, research on this topic and its contribution should lead to the determination of long-term level of input and outut prices of wheat and maize on the domestic market.

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Eksterni pariteti cijena pšenice i kukuruza u Republici Srpskoj

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Abstract

Cilj ovog rada bio je da se ispitaju promjene eksternih pariteta pšenice i kukuruza, to jeste odnosi između cijena inputa (sjeme, mineralna đubriva i euro dizel) i prodajne cijene analiziranih kultura. Istraživanje je bazirano na sekundarnim podacima od 2010. do 2020. godine objavljenim u Zavodu za Statistiku Republike Srpske. S obzirom na izvor i prirodu podataka, sprovedeno je istraživanje za stolom. U radu su izračunati eksterni pariteti cijena pšenice i kukuruza kao i osnovni pokazatelji deskriptivne statistike. Prosječna cijena pšenice iznosila je 0,34 KM/kg sa koeficijentom varijacije od 13,09%, dok je prosječna cijena kukuruza bila 0,33 KM/kg i koeficijentom varijacije od 14,65%. Rezultati istraživanja su pokazali da su otkupne cijene pšenice i kukuruza imale sporiji rast u odnosu na cijene odabranih inputa. U poređenju sa ostalim inputima, cijene mineralnih đubriva su imale izraženije varijacije, posebno UREA (16,38%). U analiziranom periodu cijene kukuruza su imale negativnu stopu promjene, kao i cijene dizela. Eksterni pariteti su pokazali nepovoljne paritete cijena pšenice i kukuruza u odnosu na varijabilne cijene inputa što svakako ukazuje na nepovoljan ekonomski položaj poljoprivrednih proizvođača u Republici Srpskoj.

Key words: pšenica, kukuruz, cijene, eksterni pariteti, Republika Srpska

Original scientific paper

Impact of climate conditions of Vojvodina on soybean yields

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Abstract

Due to the constant increase in air temperature, prolongation of the vegetation period and large fluctuations in precipitation, especially in the last two decades, crop production is facing extreme oscillations, both in terms of yield and production results.

Therefore, the subject of research in this paper is the influence of climate conditions on the formation of soybean yield in the territory of Vojvodina in the period from 1971 to 2020. The research was conducted on the basis of SORS (Statistical Office of the Republic of Serbia) data on realized yields and data of the Republic Hydrometeorological Service of Serbia on basic climate parameters (atmospheric precipitation and air temperature).

According to obtained data, the aim of this paper is to examine the impact of atmospheric precipitation and air temperature on the soybean yields in the territory of Vojvodina, by using correlative and regression analysis.

The research established that there is a great statistical significance of atmospheric precipitation in vegetation on the formation of soybean yields, while the air temperature in vegetation has no statistical significance.

Key words: soybean yield, impact, precipitation, temperature

Introduction

Favorable climate conditions are necessary for the rational performance of field production in addition to land and selected agricultural techniques. The region of Vojvodina is characterized by changing climate conditions both in terms of atmospheric precipitation and in terms of oscillations in air temperature. These changing climate conditions are the most common cause of poor yields and poor quality of field crops.

According to the results of the Statistical Office of the Republic of Serbia (Survey on the structure of agricultural farms in 2018), the total number of family farms in Vojvodina was about 127,000 and they own about 1.43 million ha of arable land and gardens. Also, according to the same source, a total of about 74,000 hectares of arable land is irrigated, of which almost 21,000 hectares are vegetable gardens. For this reason, the impact on unfavorable climatic conditions, which occur in some years, is almost negligible.

Traditionally, in the region of Vojvodina, according to the results of the Statistical Office of the Republic of Serbia, corn and wheat are the most important field crops and are grown on average over 60% of arable land. However, in the last decade in particular, more and more important areas are used for soybean cultivation. Soybean production in Serbia varies significantly from year to year as a result of varying sown areas and yields (Balešević-Tubić et al., 2008). Also, the trend of permanent increase of soybean areas is noticeable. This is supported by the fact that in the last ten years soybeans have been grown on an average of 12% of available arable land, with an average annual growth of 3.47%, achieving average yields of 2.79 t/ha, which ranged from 1.73 to 3.57 t/ha.

These achievements in soybean production are impressive, but there are challenges ahead which may slow the rate of improvement. Possibly the greatest challenge to increased productivity is global warming and climate change (Burton, Miranda, 2013.). Having in mind the evident significant oscillations of the realized soybean yields in the territory of Vojvodina, the aim of this paper is to examine the influence of atmospheric precipitation and air temperature on the formation of soybean yields in the territory of Vojvodina.

Material and Methods

Two basic data sources were used for research in this paper. Data on soybean yields trends in the observed period were taken from the Statistical Office of the Republic of Serbia (SORS), while data on atmospheric precipitation and average air temperatures, both during the year and during the growing season (April-September), were taken from the Republic Hydrometeorological Service of Serbia for the period 1971-2020. year, for seven measuring points in the area of Vojvodina (Palić, Sombor, Rimski Šančevi, Kikinda, Zrenjanin, Vršac, Sremska Mitrovica).

The observed fifty-year period is divided into five ten-year subperiods, as follows:

-1971-1980;

-1981-1990;

-1991-2000;

-2001-2010;

-2011-2020 year.

Based on the available data, their scope and quality, the research methodology was defined. After the general statistical processing, which calculated the indicators of descriptive statistics, the interdependence of soybean yields and two climate factors (atmospheric precipitation and air temperature during the growing season) was determined. Correlative analysis determined the significance of individual factors for the achieved level of soybean yields, while regression analysis confirmed the dependence and determined the form of dependence of the observed climatic factors and yields.

The obtained results were tested with Pearson correlation coefficients, at the significance level of 5% and 1%. The analysis of the collected data and the determination of their interdependence was performed by applying analytical statistics using the *IBM SPSS Statistics Subscription* software package.

Results and Discussion

Climate conditions are one of the most important natural resources and along with the soil they form the starting point of any agricultural production. Climate and its benefits for human, animal and plant life are assessed on the basis of the values of climatological elements and parameters, which are obtained by statistical processing of multi-year series of data obtained by meteorological measurements and observations. Vojvodina is characterized by a moderate continental climate, with the existence of certain specifics, which are mainly related to a large range of precipitation and extreme temperatures.

Atmospheric precipitation

Atmospheric precipitation, as the most important source of water for plants, shows its greatest importance in the vegetation period, although in the colder part of the year they provide moisture reserves in the surface layer of the soil, which is necessary for plants at the beginning of the vegetation period. Although precipitation is necessary for plants during the entire vegetation period, in certain periods of development, the so-called critical periods, the absence of water causes irreversible damage, which ultimately manifests itself through low yields and poor yield quality. According to the data of measurements in meteorological stations in the period 1971-2020. year, the average annual amount of precipitation was 612.3 mm, and ranged from 277.05 mm in 2000 to 940.38 mm in 2010 (Table 1), with a variation of about 21% throughout the observed period.

Table 1. Quantity and distribution of atmospheric precipitation in the area of Vojvodina in the period year 1971-2020.

Period	Average	Coefficient of variation	Interval of variation	
			Min	Max
Average annual quantity of atmospheric precipitation				
1971-80	604.21	12.32	445.57	686.85
1981-90	551.56	14.18	441.14	711.14
1991-2000	597.91	25.21	277.05	854.81
2001-10	708.81	22.19	470.15	940.38
2011-20	599.01	18.53	412.67	795.85
1971-2020	612.3	21.35	277.05	940.38
Average atmospheric precipitation in vegetation period				
1971-80	377.81	18.25	252.28	509.00
1981-90	323.97	16.91	242.42	423.94
1991-2000	349.56	30.69	143.58	503.53
2001-10	429.01	32.34	234.60	605.28
2011-20	356.52	27.35	241.10	575.98
1971-2020	366.76	28.36	143.58	605.28

Despite the fact that the region of Vojvodina has an average of 612 mm of precipitation, a special problem is the uneven distribution of precipitation, especially in the summer months, when due to high temperatures and high evaporation there is a significant lack of water necessary for plants.

If we take into account the fact that in the vegetation period there is an average of 366.76 mm of precipitation, and that according to a large number of studies (Glamočlija, 2004, Pejić, 2008, Popović, 2010) soybean water needs are about 450 mm, it can be claimed that soybean production in the territory of Vojvodina is very much faced with a lack of water, especially in critical periods of vegetation (June, July, August) when the daily needs of soybeans are 3-4 mm.

Unfavorable weather conditions caused the formation of small and insufficiently filled seeds with a low 1000-seed mass. Moreover, low seed moisture during harvest increased mechanical seed damage, thus causing harvest losses (Kostić at al., 2018).

Air temperature

Heat is one of the most important agroecological factors that affects the intensity of photosynthesis, respiration, transpiration and absorption of both water and minerals, regulates the duration and course of plant development phases, which directly determines the height and quality of yields. High temperatures have an extremely unfavorable effect on plants, and especially in dry periods, when there is an obvious lack of water, the effect of these two climatic factors have extremely unfavorable consequences. The average annual temperature in the observed fifty-year period was 11.59°C, and ranged from a minimum of 9.80°C in 1980 to 14.15°C in 2005. Mean maximum temperatures appear in July with the average of about 21.5°C, while the lowest average temperatures are in January, and amount to about -1.3°C. The average temperature in the vegetation period (April-September) for the observed period is average of 18.6°C (Table 2).

Table 2. Average annual air temperature and air temperature in the vegetation period for the area of Vojvodina in the period year 1971-2020.

Period	Average	Coefficient of variation	Interval of variation	
			Min	Max
Average annual air temperature				
1971-80	10.75	5.00	9.80	11.41
1981-90	10.95	4.58	9.98	11.80
1991-2000	11.39	7.19	10.35	12.97
2001-10	12.24	6.52	11.38	14.15
2011-20	12.63	3.52	11.74	13.27
1971-2020	11.59	8.36	9.80	14.15
Average air temperature in the vegetation period				
1971-80	17.08	3.77	16.05	18.07
1981-90	17.84	2.35	16.96	18.51
1991-2000	18.33	4.91	17.07	19.85
2001-10	19.10	6.92	17.74	22.44
2011-20	19.45	3.35	18.38	20.57
1971-2020	18.36	6.55	16.05	22.44

Based on the available data, it can be noticed that in the last 50 years there has been a change in climatic conditions. Namely, the average annual temperature increased by about 1.9°C, while in the vegetation period it increased by about 2.4°C, which is especially important if we consider the coefficient of variation, which for the entire observed period is only 6.32%.

Yields of soybean

Realized soybean yields largely depend on climatic impact and soil, as well as on applied agricultural techniques (variety selection, basic and additional land cultivation, crop measures and care) and economic conditions for production.

The average realized soybean yields are expressed per unit area, both for individual subperiods and for the observed period as a whole and were statistically processed by the method of descriptive statistics (Table 3).

Table 3. The realized yields of soybean in Vojvodina in the period year 1971-2020.

Period	Average	Coefficient of variation	Interval of variation	
			Min	Max
1971-80	1.58	34.59	0.52	2.34
1981-90	1.94	16.28	1.53	2.45
1991-2000	1.92	25.81	1.24	2.66
2001-10	2.49	15.16	1.73	3.11
2011-20	2.79	19.19	1.74	3.57
1971-2020	2.14	29.68	0.52	3.57

The average yield in the observed fifty-year period was 2.14 t/ha and ranged from a minimum of 0.52 t/ha in 1974 to 3.57 t/ha in 2014. Yield trends in the entire observed period were accompanied by significant variation (Cv = 29.68%). The highest (2.79 t/ha) yields were achieved in the last subperiod, while in the first subperiod (1971-1980) the average yield was only 1.58 t/ha, with significant variation (Cv = 34.59%), which can be attributed primarily to the low level of technical and technological development of soybean production (low production intensity, poor genetic potential of the range, underdeveloped means of mechanization, etc.) ...

Although soybean production is strongly influenced by climatic and other production conditions, thanks to a good assortment and increased investments in production, it can be said that the average yield, and thus the total soybean production, is growing from year to year. (Kostić at al., 2020)

Interdependence of soybean yields on atmospheric precipitation and air temperature during the vegetation period

In order to determine the significance of atmospheric precipitation and air temperature in the vegetation period on the height of the achieved soybean yields, a correlative analysis and testing of the obtained results was performed. The relative dependence of the properties was measured by the Pearson Correlation coefficient, at the significance levels of 5% and 1%, respectively.

Table 4. Interdependence of realized yields and atmospheric precipitation and air temperature in the vegetation period

		Atmospheric precipitation in the vegetation period	Air temperature in the vegetation period
Soybean	Pearson Correlation	.475**	.275
	Sig. (2-tailed)	.000	.053
	N	50	50
**. Correlation is significant at the 0.01 level (2-tailed).			

The obtained Pearson Coefficients (Table 4) indicate that:

- precipitation in the vegetation period has a positive, statistically very significant impact on the level of realized soybean yields ($p < 0.01$);
- average air temperature in the vegetation period has impact that is not statistically significant ($p > 0,05$).

In order to confirm and determine the form of dependence of the realized soyabean yields on the examined climatic factors: atmospheric precipitation and air temperature in the vegetation period, regression analysis was performed. The term regression analysis means a set of statistical procedures for examining the form of dependence between two or more characteristics (Mutavdžić, B., 2009).

Table 5. Regression model of realized yields, atmospheric precipitation and air temperature in the vegetation period

N=50	Regression Summary for Dependent Variable: Yield of soybean R = .572 R Square = .328 Adjusted R Square = .299 F = 11.455 (Sig. = .000) Std. Error of the Estimate = .539				
	B	Std. Error	Beta	T	Sig.
(Constant)	-2.100	1.225		-1.714	.093
Atmospheric precipitation in the vegetation period	.003	.001	.504	4.197	.000
Air temperature in the vegetation period	.170	.064	.320	2.666	.010

Based on the determined equation of soybean yields formation and accompanying statistical indicators (Table 5), it can be concluded that the observed climatic factors contribute to the change in realized yields with 32.8% (R^2 = 0.328). Also, regression analysis determined that atmospheric precipitation in the vegetation period has a greater and positive impact on the level of yields (Beta=.504) compared to the temperature in the vegetation period (Beta=.320). Otherwise, the standardized Beta coefficient is used as a basis for examining the influence of a factor, because it neglects the units of measure, which in this case are different. The influence of atmospheric precipitation in the vegetation period, as the first independent variable, statistically significantly contributes to the formation of soybean yields ($p <0.01$), while the second independent variable, air temperature in the vegetation period, has no statistical significance ($p> 0.05$). The whole regression model can be accepted as statistically very significant, because the F-ratio = 11.445 was estimated with $p <0.01$.

Conclusion

The analysis of the climate conditions of Vojvodina in the last fifty years (1971-2020) shows certain changes, which are reflected in the constant increase of the average air temperature by almost 2°C, while the even more extreme increase is related to the vegetation period. The determined increase of the average temperature in the vegetation period is almost 2.5°C. Therefore, it can be expected that the main threat to soybean production in the coming period will be the increase in temperature, which with the absence of moisture in critical periods of development and drought in summer, can have a very negative impact on the level of yields. Correlation and regression analysis of soybean yields in the observed period depending on atmospheric precipitation and air temperature in the growing season, found that these climate conditions contribute to the change in soybean yields by 32.8%, and that the impact of atmospheric precipitation in the growing season is statistically significant, while the impact of air temperatures in vegetation is not statistically significant.

Therefore, it can be concluded that the soybean yield, despite the technical and technological development of agricultural production, still depends very much on climate conditions, and above all on atmospheric precipitation.

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Original scientific paper

Evaluation of criteria when selecting agricultural machinery suppliers

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Abstract

The aim of this paper is to apply a modern decision-making method to select the given criteria that would help in selecting the most favourable supplier of sowing equipment. For that purpose, DEMATEL (*The Decision Making Trial and Evaluation Laboratory*) is a suggested method for multi-criteria decision-making, i.e. its *fuzzy* logic. The reason for this is the use of professional judgment of experts in a given field of analysis where the *fuzzy* logic of decisions tried to approach human thinking. The paper focus is the equipment for sowing of an agricultural farm in the municipality of Bijeljina, and the obtained results show the influence of certain criteria that are crucial in the selection of suppliers. Also, the benefit of the research stems from the observation of shortcomings, i.e. improving the quality of the subject of work according to certain processed criteria.

Key words: multicriteria decision making, DEMATEL method, fuzzy logic, agricultural mechanization

Introduction

Modern agricultural production is inconceivable without the use of adequate agricultural machinery, which should provide the most productive production process within the given agro-technical deadline. Particularly sensitive periods from the agrotechnical point of view in the production process are certain plant species at the time of sowing. In that case, the correct choice of a particular seed drill is an essential measure of a well-run production process. The important thing of every business entity is that the supplier should be someone they can rely on. As Aguezzoul (2012) points out, choosing a supplier is a strategic purchasing decision that affects the overall success of any company. According to some authors, improper selection and evaluation of potential suppliers can overshadow the performance of supply chains within an organization (Jafarnejad, Salimi, 2013). The choice of suppliers is influenced by many factors (Puška, 2015). It is extremely important to identify the most important factors that influence the choice of suppliers. Using previous research, some of the authors cite a number of different factors that influence supplier selection. Thus, Liao and Kao (2011) highlight 29 different factors, while Aguezzoul (2012) as many as 36.

Considering the subject of this paper, 8 economic and social criteria were selected (delivery costs, payment flexibility, quality of delivered goods, applied production technology, supplier reputation, innovation in production, distance of suppliers, importance for the local community) and their importance was confirmed based on some of the earlier research by several authors (Bai and Sarkis, 2009; We et al., 2013; Jain et al.,, 2013; Liu, 2010; Mwikali and Kavale, 2012 etc.). Multi-criteria decision-making methods are mainly used to assess the criteria needed to select suppliers. Decisions are made by assessing alternatives according to defined criteria (Rozman et al., 2017), which in turn can be qualitative and quantitative (Rozman et al., 2016). In addition to the use of classical methods of multi-criteria decision-making, their fuzzy methods (variants) are increasingly being used. The reason for that lies in the fact that some of the given criteria can be qualitative, as is the case in this paper. Thus, in the subject area, we have previous research that has just used this method (Govindan et al., 2013; Stević et al., 2019, Nedeljković et al., 2021a; Nedeljković et al., 2021b, Nedeljković, 2022, etc.). Therefore, the aim of the paper is to use a modern decision-making method to select the set criteria that would lead to the selection of the most favourable supplier, which in this case is sowing equipment on a family farm in the municipality of Bijeljina.

Material and Methods

The source of data in this paper is the relevant literature in the field of analysis as well as expert assessment by five experts in the observed field. The experts filled in the questionnaire, giving certain weights to the analyzed criteria and according to the given *fuzzy* scale. The DEMATEL method was used in the research, and the reason for its use lies in the fact that it represents an adequate method of multicriteria analysis when it comes to the subject area. This is confirmed by its considerable use in previous research (Gharakhani, 2012; Govindan et al., 2013; Govindan, Chaudhuri, 2016; Shaik, Abdul-Kader, 2018; Hsu, Yeh, 2017; Jarosz, 2019; Yildirim and Koca, 2021 itd.). DEMATEL (*The Decision Making Trial and Evaluation Laboratory*), one of the MCDM (*Multi-Criteria Decision Making*) methods, was developed in 1972 by the Battelle Memorial Institute of Geneva Research Center. The DEMATEL method allows separating a factor into cause and effect groups and identifying the most important criteria from the group of all criteria indicated as crucial in the decision-making process according to stakeholder needs. (Chang et al., 2011) Also, one of the reasons for the application of the DAMATEL method is its popularization in solving the problem of determining the weight of certain criteria needed for the decision-making process. Using fuzzy logic, this method attempts to bring the final decision closer to human thinking. Chen (2000) extended this method using triangular fuzzy numbers that replace numerical language scales for grading and weighting. The steps used in the application of this method are listed in the chapter research results, where through a case study in the paper it tries to make a selection of given criteria that are important in decision making.

Results and Discussion

Further in the paper, the procedures (steps) of the applied methodology are presented, through which the results of the obtained research are shown.

Generate the fuzzy direct- relation matrix

In order to identify the model of the relations among the n criteria, an n × n matrix is first generated. The influence of the element in each row exerted on the element in each column of this matrix can be represented a fuzzy number. If multiple experts' opinions are used, all experts must complete the matrix. Arithmetic mean of all of the experts' opinions is used to generate the direct relation matrix z.

$$z = \begin{bmatrix} 0 & \cdots & \tilde{z}_{n1} \\ \vdots & \ddots & \vdots \\ \tilde{z}_{1n} & \cdots & 0 \end{bmatrix}$$

The table below indicates the direct relation matrix, which is the same as pairwise comparison matrix of the experts.

Table 1. The direct relation matrix

	Shipping costs	Payment flexibility	Quality of goods	Technology used	Reputation	Innovation	Location (distance)	Significance for local community
Shipping costs	(0.000,0.000,0.000)	(5.000,7.000,8.600)	(3.800,5.800,7.800)	(5.000,7.000,7.800)	(5.800,7.800,9.000)	(5.000,7.000,7.800)	(4.600,6.600,8.600)	(4.600,6.600,8.200)
Payment flexibility	(5.400,7.400,8.600)	(0.000,0.000,0.000)	(4.200,6.200,8.200)	(5.000,7.000,8.200)	(4.200,6.200,8.200)	(5.400,7.000,7.800)	(5.000,7.000,9.000)	(5.400,7.400,8.200)
Quality of goods	(6.200,8.200,8.600)	(6.200,8.200,9.000)	(0.000,0.000,0.000)	(5.000,6.600,7.800)	(5.400,7.400,9.000)	(6.200,8.200,9.000)	(6.200,8.200,9.000)	(5.800,7.800,8.600)
Technology used	(1.800,3.000,5.000)	(1.800,3.800,5.800)	(1.000,2.600,4.600)	(0.000,0.000,0.000)	(1.800,3.800,5.800)	(4.200,6.200,8.200)	(2.200,3.800,5.800)	(3.800,5.800,7.800)
Reputation	(3.800,5.800,7.800)	(3.800,5.800,7.800)	(3.000,5.000,7.000)	(5.400,7.400,9.000)	(0.000,0.000,0.000)	(5.800,7.800,9.000)	(4.200,6.200,8.200)	(5.800,7.800,9.000)
Innovation	(1.400,2.600,4.600)	(1.400,3.000,5.000)	(1.000,1.800,3.800)	(3.800,5.800,7.800)	(1.800,3.800,5.800)	(0.000,0.000,0.000)	(1.800,3.800,5.800)	(3.000,5.000,7.000)
Location (distance)	(3.400,5.400,7.400)	(3.000,5.000,7.000)	(2.200,4.200,6.200)	(5.000,7.000,9.000)	(3.000,5.000,7.000)	(5.800,7.800,9.000)	(0.000,0.000,0.000)	(4.600,6.600,8.600)
Significance for local	(1.400,2.600,4.600)	(1.400,3.400,5.400)	(1.400,3.400,5.400)	(4.600,6.600,8.600)	(3.000,5.000,7.000)	(5.400,7.400,9.000)	(2.600,4.200,6.200)	(0.000,0.000,0.000)

community								

Source: Authors' calculation

The following table shows the fuzzy scale used in the model.

Table 2. Fuzzy Scale

	Linguistic terms	L	M	U
1	Very Low	1	1	3
2	Low	1	3	5
3	Medium	3	5	7
4	High	5	7	9
5	Very high	7	9	9

Source: According to Kiani Mavi et al., 2016; Mijajlović et al., 2020.

Normalize the fuzzy direct-relation matrix

The normalized fuzzy direct-relation matrix can be obtained using the following formula:

$$\tilde{x}_{ij} = \frac{\tilde{z}_{ij}}{r} = \left(\frac{l_{ij}}{r}, \frac{m_{ij}}{r}, \frac{u_{ij}}{r}\right)$$

where

$$r = \max_{i,j}\left\{\max_{i}\sum_{j=1}^{n} u_{ij}, \max_{j}\sum_{i=1}^{n} u_{ij}\right\} \qquad i,j \in \{1,2,3,\ldots,n\}$$

Table 3. The normalized fuzzy direct-relation matrix

	Shipping costs	Payment flexibility	Quality of goods	Technology used	Reputation	Innovation	Location (distance)	Significance for local community
Shipping costs	(0.000,0.000,0.000)	(0.082,0.115,0.141)	(0.062,0.095,0.128)	(0.082,0.115,0.128)	(0.095,0.128,0.148)	(0.082,0.115,0.128)	(0.075,0.108,0.141)	(0.075,0.108,0.134)
Payment flexibility	(0.089,0.121,0.141)	(0.000,0.000,0.000)	(0.069,0.102,0.134)	(0.082,0.115,0.134)	(0.069,0.102,0.134)	(0.089,0.115,0.128)	(0.082,0.115,0.148)	(0.089,0.121,0.134)
Quality of goods	(0.102,0.134,0.141)	(0.102,0.134,0.148)	(0.000,0.000,0.000)	(0.082,0.108,0.128)	(0.089,0.121,0.148)	(0.102,0.134,0.148)	(0.102,0.134,0.148)	(0.095,0.128,0.141)
Technology used	(0.030,0.049,0.082)	(0.030,0.062,0.095)	(0.016,0.043,0.075)	(0.000,0.000,0.000)	(0.030,0.062,0.095)	(0.069,0.102,0.134)	(0.036,0.062,0.095)	(0.062,0.095,0.128)
Reputation	(0.062,0.095,0.128)	(0.062,0.095,0.128)	(0.049,0.082,0.115)	(0.089,0.121,0.148)	(0.000,0.000,0.000)	(0.095,0.128,0.148)	(0.069,0.102,0.134)	(0.095,0.128,0.148)
Innovation	(0.023,0.043,0.075)	(0.023,0.049,0.082)	(0.016,0.030,0.062)	(0.062,0.095,0.128)	(0.030,0.062,0.095)	(0.000,0.000,0.000)	(0.030,0.062,0.095)	(0.049,0.082,0.115)
Location (distance)	(0.056,0.089,0.121)	(0.049,0.082,0.115)	(0.036,0.069,0.102)	(0.082,0.115,0.148)	(0.049,0.082,0.115)	(0.095,0.128,0.148)	(0.000,0.000,0.000)	(0.075,0.108,0.141)
Significance for local community	(0.023,0.043,0.075)	(0.023,0.056,0.089)	(0.023,0.056,0.089)	(0.075,0.108,0.141)	(0.049,0.082,0.115)	(0.089,0.121,0.148)	(0.043,0.069,0.102)	(0.000,0.000,0.000)

Source: Authors' calculation

Calculate the fuzzy total-relation matrix

In step 3, the fuzzy total-relation matrix can be calculated by the following formula:

$$\tilde{T} = \lim_{k \to +\infty} (\tilde{x}^1 \oplus \tilde{x}^2 \oplus \ldots \oplus \tilde{x}^k)$$

If each element of the fuzzy total-relation matrix is expressed as $\tilde{t}_{ij} = (l''_{ij}, m''_{ij}, u''_{ij})$, it can be calculated as follows:

$$[l''_{ij}] = x_l \times (I - x_l)^{-1}$$

$$[m''_{ij}] = x_m \times (I - x_m)^{-1}$$

$$[u''_{ij}] = x_u \times (I - x_u)^{-1}$$

In other words, the normalized matrix the inverse is first calculated, and then it is subtracted from the matrix I, and finally the normalized matrix is multiplied by the resulting matrix. The following table shows the fuzzy direct-relation matrix.

Table 4. The fuzzy total-relation matrix

	Shipping costs	Payment flexibility	Quality of goods	Technology used	Reputation	Innovation	Location (distance)	Significance for local community
Shipping costs	(0.047,0.158, 0.591)	(0.121,0.267, 0.738)	(0.093,0.221, 0.664)	(0.145,0.320, 0.847)	(0.138,0.291, 0.781)	(0.153,0.336, 0.864)	(0.123,0.277, 0.784)	(0.137,0.311, 0.840)
Payment flexibility	(0.128,0.267, 0.717)	(0.046,0.165, 0.617)	(0.099,0.227, 0.672)	(0.146,0.321, 0.856)	(0.116,0.271, 0.774)	(0.160,0.338, 0.868)	(0.130,0.284, 0.793)	(0.149,0.323, 0.844)
Quality of goods	(0.149,0.299, 0.742)	(0.148,0.305, 0.772)	(0.042,0.153, 0.577)	(0.159,0.344, 0.882)	(0.143,0.310, 0.812)	(0.186,0.383, 0.915)	(0.157,0.324, 0.820)	(0.168,0.356, 0.879)
Technology used	(0.049,0.140, 0.516)	(0.048,0.156, 0.545)	(0.032,0.121, 0.481)	(0.033,0.132, 0.551)	(0.051,0.165, 0.574)	(0.101,0.234, 0.682)	(0.058,0.166, 0.580)	(0.090,0.215, 0.654)
Reputation	(0.099,0.233, 0.695)	(0.098,0.239, 0.719)	(0.077,0.201, 0.645)	(0.145,0.313, 0.854)	(0.045,0.166, 0.643)	(0.158,0.333, 0.871)	(0.111,0.260, 0.769)	(0.148,0.314, 0.841)
Innovation	(0.040,0.124, 0.481)	(0.040,0.134, 0.503)	(0.029,0.101, 0.443)	(0.087,0.204, 0.627)	(0.048,0.153, 0.541)	(0.032,0.126, 0.525)	(0.049,0.154, 0.546)	(0.074,0.191, 0.607)
Location (distance)	(0.086,0.212, 0.654)	(0.079,0.212, 0.671)	(0.060,0.176, 0.601)	(0.129,0.286, 0.810)	(0.084,0.224, 0.707)	(0.147,0.311, 0.826)	(0.039,0.150, 0.611)	(0.121,0.277, 0.793)
Significance for local community	(0.046,0.145, 0.539)	(0.045,0.161, 0.569)	(0.040,0.140, 0.517)	(0.109,0.243, 0.709)	(0.072,0.192, 0.620)	(0.124,0.265, 0.728)	(0.068,0.183, 0.616)	(0.036,0.142, 0.575)

Source: Authors' calculation

Defuzzify into crisp values

The CFCS method (*Converting Fuzzy data into Crisp Scores*) has been used to obtain a crisp value of total-relation matrix. The steps of CFCS method are as follows:

$$l_{ij}^n = \frac{\left(l_{ij}^t - \min l_{ij}^t\right)}{\Delta_{min}^{max}}$$

$$m_{ij}^n = \frac{\left(m_{ij}^t - min\ l_{ij}^t\right)}{\Delta_{min}^{max}}$$

$$u_{ij}^{n} = \frac{(u_{ij}^{t} - min\ l_{ij}^{t})}{\Delta_{min}^{max}}$$

So that

$$\Delta_{min}^{max} = \max u_{ij}^{t} - \min l_{ij}^{t}$$

Calculating the upper and lower bounds of normalized values:

$$l_{ij}^{s} = {m_{ij}^{n}}\Big/{(1 + m_{ij}^{n} - l_{ij}^{n})}$$

$$u_{ij}^{s} = {u_{ij}^{n}}\Big/{(1 + u_{ij}^{n} - l_{ij}^{n})}$$

The output of the CFCS algorithm is crisp values.

Calculating total normalized crisp values:

$$x_{ij} = \frac{[l_{ij}^{s}(1 - l_{ij}^{s}) + u_{ij}^{s} \times u_{ij}^{s}]}{[1 - l_{ij}^{s} + u_{ij}^{s}]}$$

Table 5. The crisp total-relation matrix

	Shipping costs	Payment flexibility	Quality of goods	Technology used	Reputation	Innovation	Location (distance)	Significance for local community
Shipping costs	0.228	0.332	0.284	0.39	0.358	0.405	0.349	0.383
Payment flexibility	0.329	0.238	0.289	0.392	0.342	0.408	0.355	0.392
Quality of goods	0.355	0.365	0.22	0.412	0.375	0.446	0.387	0.419
Technology used	0.203	0.219	0.181	0.202	0.232	0.304	0.234	0.285
Reputation	0.3	0.309	0.266	0.387	0.244	0.405	0.334	0.386
Innovation	0.184	0.195	0.159	0.273	0.217	0.193	0.219	0.258
Location (distance)	0.279	0.282	0.241	0.362	0.298	0.383	0.227	0.352
Significance for local community	0.21	0.227	0.201	0.315	0.261	0.336	0.253	0.215

Source: Authors' calculation

Set the threshold value

The threshold value must be obtained in order to calculate the internal relations matrix. Accordingly, partial relations are neglected and the network relationship map (NRM) is plotted. Only relations whose values in matrix T is greater than the threshold value are depicted in the NRM. To compute the threshold value for relations, it is sufficient to calculate the average values of the matrix T. After the threshold intensity is determined, all values in matrix T which are smaller than the threshold value are set to zero, that is, the causal relation mentioned above is not considered. In this study, the threshold value is equal to 0.2980.298

All the values in matrix T which are smaller than 0.2980.298 are set to zero, that is, the causal relation mentioned above is not considered. The model of significant relations is presented in the following table.

Table 6. The crisp total- relationships matrix by considering the threshold value

	Shipping costs	Payment flexibility	Quality of goods	Technology used	Reputation	Innovation	Location (distance)	Significance for local community
Shipping costs	0	0.332	0	0.39	0.358	0.405	0.349	0.383
Payment flexibility	0.329	0	0	0.392	0.342	0.408	0.355	0.392
Quality of goods	0.355	0.365	0	0.412	0.375	0.446	0.387	0.419
Technology used	0	0	0	0	0	0.304	0	0
Reputation	0.3	0.309	0	0.387	0	0.405	0.334	0.386
Innovation	0	0	0	0	0	0	0	0
Location (distance)	0	0	0	0.362	0	0.383	0	0.352
Significance for local community	0	0	0	0.315	0	0.336	0	0

Source: Authors' calculation

Final output and create a causal relation diagram

The next step is to find out the sum of each row and each column of T (in step 4). The sum of rows (D) and columns (R) can be calculated as follows:

$$D = \sum_{j=1}^{n} T_{ij}$$

$$R = \sum_{i=1}^{n} T_{ij}$$

Then, the values of D+R and D-R can be calculated by D and R, where D+R represent the degree of importance of factor i in the entire system and D-R represent net effects that factor *i* contributes to the system.

The table below shows the final output.

Table 7. The final output

	R	D	D+R	D-R
Shipping costs	2.088	2.73	4.818	0.641
Payment flexibility	2.168	2.745	4.913	0.577
Quality of goods	1.842	2.979	4.821	1.137
Technology used	2.733	1.861	4.594	-0.872
Reputation	2.326	2.631	4.958	0.305
Innovation	2.881	1.699	4.58	-1.182
Location (distance)	2.357	2.423	4.781	0.066
Significance for local community	2.69	2.018	4.708	-0.672

Source: Authors' calculation

The following figure shows the model of significant relations. This model can be represented as a diagram in which the values of (D+R) are placed on the horizontal axis and the values of (D-R) on the vertical axis. The position and interaction of each factor with a point in the coordinates (D+ R, D-R) are determined by coordinate system.

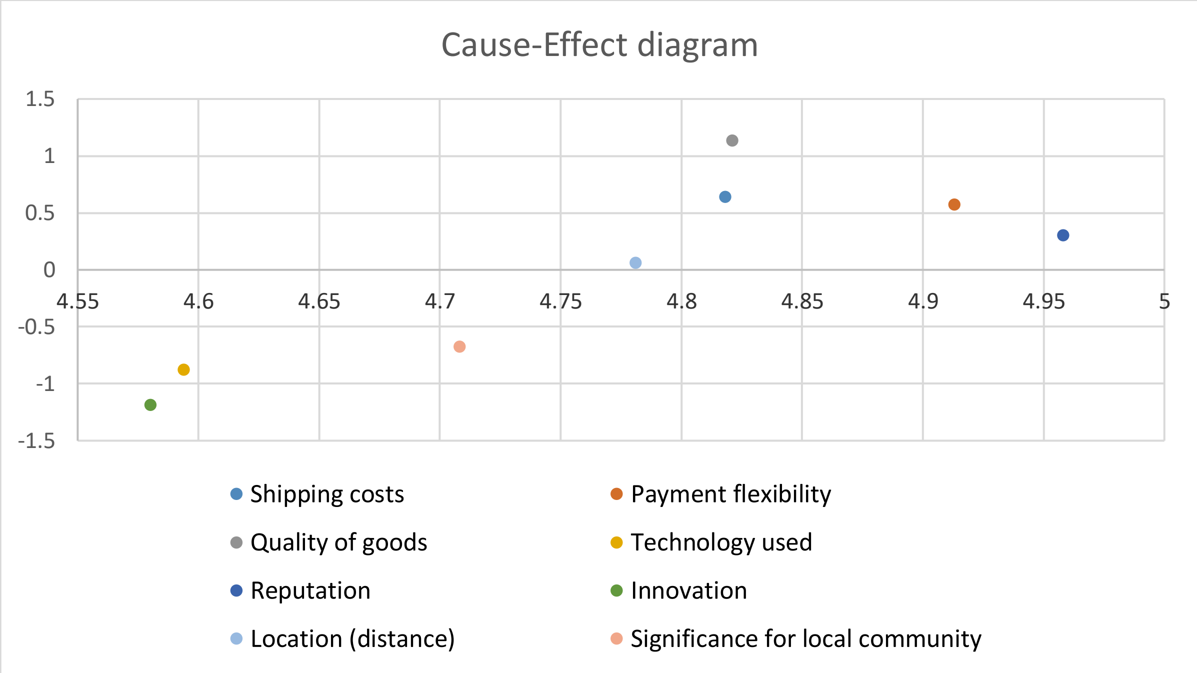

Figure 1. Cause-effect diagram

Interpret the results

According to the diagram and table above, each factor can be assessed based on the following aspects:

Horizontal vector (D + R) represents the degree of importance between each factor plays in the entire system. In other words, (D + R) indicates both factor i's impact on the whole system and other system factors' impact on the factor. In terms of degree of importance, Reputation is ranked first followed by Payment flexibility, Quality of goods, Shipping costs, Location (distance), Significance for local community, Technology used and Innovation. In this study, Shipping costs, Payment flexibility, Quality of goods, Reputation, Location (distance) are considered to be as a causal variable, Technology used, Innovation, Significance for the local community are regarded as an effect. The vertical vector (D-R) represents the degree of a factor's influence on system. In general, the positive value of D-R represents a causal variable, and the negative value of D-R represents an effect. In terms of degree of importance, Reputation is ranked in first place and Payment flexibility, Quality of goods, Shipping costs, Location (distance), Relevance to the local community, Technology used and Innovation, are ranked the next. In this study, Shipping costs, Payment flexibility, Quality of goods, Reputation, Location (distance) are considered to be a causal variable, Technology used, Innovation, Significance for the local community are regarded as an effect.

Conclusion

From the above we can conclude that the process of choosing a supplier is a complex problem for rational decision makers, and that the rationality in its decision making can be increased by applying modern methods of multi-criteria decision making. In previous research, fuzzy logic is imposed as a solution when it comes to selection among qualitative criteria. In addition to the applicative significance that was elaborated in the last step of this paper, this research brings with it a good basis for improving the characteristics of some of the given criteria, which could be the subject of some new research in the coming period.

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Ocjena kriterijuma prilikom izbora dobavljača poljoprivredne mehanizacije

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Sažetak

Cilj rada je da se primjenom savremene metode odlučivanja izvrši selekcija zadatih kriterijuma koji bi pomogli u odabiru najpovoljnijeg dobavljača opreme za sjetvu. Tom prilikom korišćenja je DEMATEL (*The Decision Making Trial and Evaluation Labaratory*) metoda višekriterijskog odlučivanja, odnosno njena *fuzzy* logika. Razlog za to je upotreba ekspertske ocjene stručnjaka iz date oblasti analize gdje se *fuzzy* logikom odluka pokušala približiti ljudskom razmišljanju. Predmet rada predstavlja oprema za sjetvu (sijačica) jednog poljoprivrednog gazdinstva na području opštine Bijeljina, a dobijeni rezultati pokazuju uticaj pojedinih kriterijuma koji su odlučujući kod izbora dobavljača. Takođe, korist istraživanja proizilazi i iz uočavanja nedostataka, odnosno unapređenja kvaliteta predmeta rada po pojedinim obrađenim kriterijumima.

Ključne riječi: višekriterijumsko odlučivanje, DEMATEL metod, fuzzy logika, poljoprivredna mehanizacija

Original scientific paper

Climate change adaptation measures in agriculture: the perspective of different experts groups

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Abstract

Climate change has a significant impact on agriculture and there are many measures proposed for its mitigation and adaptation. The selection of the most suitable measures for given local conditions is crucial for proper planning, implementation, and sustainability. In this paper, we present the results of the evaluation and prioritization of 24 measures done by 10 experts in a three-step multi-criteria procedure. First, experts individually assessed seven criteria for evaluating the measures: level of responsibility, category, implementation timeframe, multi-functionality, technical skills, cost-benefit ratio, and mitigation effectiveness. Assessment of criteria and calculation of their priorities is performed by using the analytic hierarch process (AHP), well known multi-criteria method. Second, experts are grouped into three clusters according to their backgrounds, and their priorities within each cluster are aggregated into group priorities of criteria. Finally, criteria priorities of each cluster are used to rank proposed measures as alternatives by the TOPSIS method. Results obtained for three groups of experts show differences in prioritization of criteria, as well as in the ranking of adaptation and mitigation measures. The applied methodology proved that planning of adaptation/mitigation measures requires the inclusion of different experts in the assessment and evaluation process, but also careful joint analysis of the results since wrong decisions can influence financial feasibility and applicability of measures in the practice.

Key words: climate change, agriculture, adaptation measures, planning, multi-criteria analysis, AHP, TOPSIS

Introduction

Climate changes are evident all over the world and governments, institutions, scientists, and practitioners are engaged in finding the most suitable mitigation and adaptation strategies and measures. This is a difficult task because there are many factors influencing the impact in different sectors. In the case of agriculture, the most influencing are latitude, topography, and other local circumstances (Vitasse et al., 2018). When it comes to measures, their applicability depends largely on infrastructure, the local community, characteristics of the region, limiting factors, access to information, agricultural systems, and farmers' knowledge and experience (Niles et al., 2015, Pandey, et al., 2018).

Identification of suitable measures usually includes a comprehensive study of observed and predicted climate change impacts, adaptation measures, discussions with advisory services, scientific literature, and expert knowledge (Stričević et al., 2020).

Following this, a team of experts consisting of one each in field crop farming, vegetable growing, orcharding, viticulture, plant protection, water and soil management, and animal husbandry, identified twenty-four adaptation measures as most suitable for Serbia: (1) use of multi-purpose reservoirs for water supply; (2) antifrost irrigation systems; (3) agricultural technologies (crop density, sowing date, fertilizer application, improved monitoring of invasive species); (4) use of drainage ditches for irrigation; (5) restoration of wetlands and vegetation and afforestation to prevent flooding or erosion; (6) construction of irrigation systems; (7) improved water erosion control practices; (8) antihail netting; (9) irrigated farming; (10) improved water use efficiency in irrigated farming where the water resource is insufficient; (11) careful selection of fruit trees, rootstock and terrain; (12)construction and proper management of drainage systems; (13) drought- and heat-tolerant crops/hybrids; (14) installation of shading nets to save water and lower temperature; (15) flood protection levees; (16) water cups to prevent rotting in the event of heavy rainfall; (17) new crops/hybrids that tolerate high temperatures; (18) small water reservoir/tank on farm; (19) agricultural insurance; (20) enhanced crop rotation; (21) irrigated high-yield crops/hybrids (to improve the efficiency of water, nutrient and energy use); (22) windbreak trees; (23) reduced tillage; and (24) late-blooming fruit trees.

Experts also identified criteria and indicators for the evaluation of the adaptation measures (Table 1.).

Table 1 Criteria and indicators for the evaluation of adaptation measures (Stričević et al., 2020)

Criterion	Indicator	Assigned value	Description
C1 Level of responsibility	Farm/Stakeholder (**F/S**)	1	The measure can be implemented by the farmer independently of the government
	Local government (**LG**)	2	Local government to create conditions for the implementation of the measure
	Government (**G**)	3	The measure of public interest and needs to be implemented at a higher level
C2 Category	Agronomic (**Agro**)	1	Requires research and innovation in agronomic sciences, new technologies, and environmental monitoring in agriculture
	Management (**Manage**)	3	Development of institutional, management, and organizational skills to improve the standard of living and the system
	Infrastructure (**Infrast**)	2	Requires design and building of physical structures and infrastructures
C3 Implemen-tation timeframe	Short term (**ST**)	1	The measure can be implemented promptly or already in place for 0 – 5 years
	Medium term (**MT**)	3	Measure planned and can be implemented in 5-10 years
	Long term (**LT**)	2	Measure planned and preparations underway for implementation after 10 years
C4 Multi-functionality	Individual (**S**)	1	Measure effective only on farm
	Broader public (**R**)	3	Measure multifunctional and relevant to the broader public
C5 Technical skills	Low (**L**)	1	Skills inadequate, training required in climate change risks and mitigation measures
	Medium (**M**)	2	Knowledge and skills exist, but additional training is required to implement new technologies
	High (**H**)	3	High level of knowledge, regular updates, and familiarity with scientific advances required
C6 Benefit/cost	**B:C>1**	3	Benefit exceeds cost
	B:C≈ 1	2	Benefit equal to investment
	B:C<1	1	Cost exceeds investment
C7 Effectiveness of mitigation measure	Yes (Y)	3	Full mitigation of the adverse effect
	No (N)	1	Adverse effect not mitigated but production still profitable
	Partial (P)	2	Adverse effect partly mitigated, relevant to the environment and cost-effectiveness

In (Stričević et al., 2020) a novel framework is proposed for efficient evaluation of adaptation measures and planning strategies in Serbian agriculture. It was based on two well-known multi-criteria methods, the analytic hierarchy process (AHP) (Saaty, 1980) and TOPSIS (Hwang and Yoon, 1981). The extension of the framework is presented here, aimed to analyze the perspective of different groups of experts on the importance of climate change-related adaptation measures in national agriculture.

Material and Methods

The methodology applied in this paper is adopted from Stričević et al. (2020), and the same multicriteria methods (AHP and TOPSIS) are used in a modified group context. Ten experts evaluated the measures through a two-stage multicriteria evaluation process. First, experts used AHP to evaluate seven criteria (Table 1). Individually obtained weights are aggregated in the second stage into the group weights and used to rank adaptation measures by the TOPSIS method. Notice that both methods and aggregation procedures are scientifically sound and used worldwide, including many types of research in Serbia published nationally and internationally (e.g. Suvočarev et al., 2008; Srđević and Srđević, 2019; Srdjevic et al., 2020a,b).

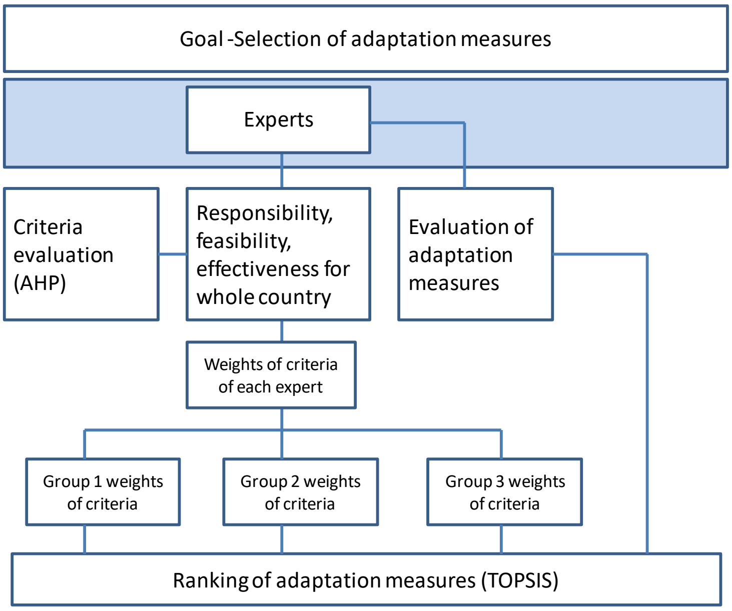

Figure 1. Methodology to obtain a perspective of different experts' groups on the importance of various adaptation measures in Serbia

Here we used modified methodology (Figure 1): before aggregation of the individual weights, experts were grouped according to their professional background into three groups. So, in the second step, three sets of weights of criteria (aggregated weights for Group 1, Group 2, and Group 3) were obtained and, together with evaluation of measures vs criteria, used in TOPSIS to rank the measures. In the end, three different rankings provide the perspective of different experts.

Note that all experts had the same importance within the group and that results of the evaluation of measures vs criteria are taken from Stričević et al. (2020). Complex supporting mathematical apparatus used to evaluate criteria, aggregate individual weights, and rank measures is not provided here due to the limitations in the length of the paper; interested readers are referred to the reference paper above.

Results and Discussion

Based on the evaluation of criteria vs goal (Selection of adaptation measures), ten sets of individual weights of criteria (Table 2.) are calculated using standard AHP prioritization methodology within the first step of the methodology.

Table 2. Weights of criteria for each expert (Strićević et al., 2020)

Group	**Individual**	**Weight**						
		C1	C2	C3	C4	C5	C6	C7
Group 1	Expert 1	0.100	0.103	0.128	0.153	0.203	0.028	0.285
Group 2	Expert 2	0.036	0.061	0.079	0.032	0.158	0.293	0.340
	Expert 3	0.044	0.395	0.290	0.137	0.035	0.026	0.073
	Expert 4	0.047	0.061	0.249	0.097	0.067	0.240	0.240
Group 3	Expert 5	0.031	0.251	0.034	0.249	0.041	0.262	0.133
	Expert 6	0.050	0.234	0.075	0.194	0.059	0.200	0.189
	Expert 7	0.076	0.198	0.030	0.165	0.021	0.270	0.240
	Expert 8	0.062	0.085	0.041	0.099	0.151	0.308	0.254
	Expert 9	0.133	0.331	0.033	0.118	0.089	0.162	0.136
	Expert 10	0.043	0.262	0.071	0.055	0.067	0.293	0.210

Before the calculation within step two has begun, experts were grouped according to their professional background: Group 1 is made by one representative of agricultural inspection; Group 2 consisted of three meteorological experts; and Group 3 of six agronomists. Aggregated individual weights within three groups are presented in Table 3.

Table 3. Weights of criteria by groups

Group/criteria	C1	C2	C3	C4	C5	C6	C7
Group 1	0.100	0.103	0.128	0.153	0.203	0.028	0.285
Group 2	0.054	0.145	0.228	0.096	0.092	0.155	0.231
Group 3	0.063	0.225	0.047	0.140	0.064	0.260	0.201

Results indicate significant differences in the weights of most of the criteria. For example, the weights of criterion C5 are 0.203, 0.092, and 0.064 for Group 1, 2, and 3 respectively.

Table 4. Ranks of criteria by groups

Group/ranking of criteria	C1	C2	C3	C4	C5	C6	C7
Group 1	6	5	4	3	2	7	1
Group 2	7	4	2	5	6	3	1
Group 3	6	2	7	4	5	1	3

Considering the ranking of criteria (Table 4), the importance of criterion C6 (Benefit/cost) is viewed oppositely by Group 1 (rank 7) and Group 3 (rank 1). Similarly, Group 2 and Group 3 have seen quite different importance of criterion 3 (ranks 2 and 7, respectively). Almost consensus of the groups can be seen on the ranking of C1 and C7.

The final step of the methodology was to use aggregated weights of criteria per group, given in Table 3, as input to method TOPSIS to rank the adaptation measures. To illustrate the different perspectives of three groups of experts, ten top-ranked adaptation measures per group are presented in Table 5.

Results show that Group 2 and Group 3 have identical and Group 1 very similar ranking of first top four adaptation measures. Five measures (1, 3, 22, 23, 24) ranked as 5-10 by Group 1 and 2 are the same, but have different rankings. Experts in agronomy (Group 3) identified as important also measures 12 and 21; the other two groups have not recognized their value in adaptation planning in Serbia.

Table 5. Ten top-ranked adaptation measures per group

Rank	Group 1	Group 2	Group 3	All experts one group
1	8	5	5	8
2	19	8	8	21
3	2	2	2	4
4	6	19	19	22
5	24	1	12	23
6	22	3	1	2
7	23	6	21	24
8	11	24	6	3
9	3	22	24	1
10	1	23	23	5

Interesting discussion on how the grouping of experts influence the final results can be provoked if the adaptation measures ranking by Groups 1-3 are compared with the ranking obtained if all experts are included into one group as in (Stričević et al., 2020) and presented in the last column of Table 5.

Conclusion

The decision-making process is always complex and challenging, especially if it is organized as a participative procedure and should result in recommendations with wider economic, social and environmental impact. Implications of such decisions in the agricultural sector can make local, regional, and national economies more or less vulnerable to future climate changes and influence financial feasibility and applicability of mitigation/adaptation measures in the practice. The methodology applied in this paper proved that planning of adaptation/mitigation measures in the agricultural sector requires the inclusion of different experts in the assessment and evaluation process. The perspective of different experts on the same problem can create different solutions, and send a confusing message to the policymakers. Results of the evaluation process must be thus carefully analyzed from many aspects and with many interested parties (not only experts).

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Professional paper

The analysis of business behaviour in terms of the crisis of three agricultural companies from Croatia, Serbia and Slovenia

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Abstract

The aim of this paper was to analyse how, in crisis conditions, to direct the cash flow of income to the business process that brings the greatest benefit. Therefore, the production of wheat and sunflower for three agricultural companies from different countries (Croatia, Serbia, and Slovenia) during one year, considering other factors: inflation, interest rates, cash flow and net present value, was analysed. The obtained results indicate that the current interest rate is within normal limits and that no significant price increase is expected due to it. Furthermore, the occurrence of inflationary pressures as a consequence of market shocks in cooperation with interest rates causes an additional increase in market prices. In such conditions, it is necessary to consider the value of money, i.e. the income that agricultural companies have at their disposal, and decide on future production. The results showed that the net present value is positive for all crops and that there are no major mutual oscillations, except for the agricultural producer from Slovenia, for whom sunflower production is much more profitable.

Key words: crisis, budget, interest rate, inflation, net present value

Introduction

Uneven economic cycles that are frequent in the markets cause economic shocks. Economic shocks have an impact on prices, interest rates, employment, and market aggregates (Hungary, 2010). Current market events during the COVID pandemic and the war in Ukraine have returned the issue of capital flows to the centre of interest of scientific and professional circles, especially in terms of their sensitivity to macroeconomic shocks. The economic crisis as a consequence of economic shocks reveals some aspects of economic trends and social interactions that are not visible in normal and stable conditions (Stiglić, 2004). The crisis, whether caused by the shortcomings of the market or the state, is manifesting itself negatively in all economic and financial parameters and has a worrying effect on all subjects of the market and public sector, i.e. on all citizens in the country (Jonung, 2009). The primary cause of cyclical fluctuations is changing the amount of money in circulation, which inevitably leads to disruption of the price system and, consequently, to the wrong direction of production. This is where there is room for consumer behaviour. Agricultural companies in the stages of procurement of raw materials (seeds, fertilizers, pesticides, etc.) for sowing represent consumers. They should decide on how much money they will spend on the purchase of raw materials, decide which crops to plant, or consider decisions on whether to invest the surplus money in fixed assets or not. Intertemporal choice is the choice of consumption over time (Varian, 2008). Most people can spend their income now, or save some - rationally for the future. Observing consumer behaviour over time, ie how consumers distribute income concerning consumption, shows the movement of supply and demand in the market (Varian, 2008). Consumer behaviour is limited by budget, spending preference, inflation, interest rates, and taxes (Silberg and Sun, 2000). When choosing how many units of a product to consume, the consumer is faced with one limitation, and that is his income (Ronald, 2009). Intertermpolar choice can be explained in the simplest form in the way that the consumer has a certain amount of money and wants to get two goods. On the one hand, he must consider the available budget, and on the other hand, look at the prices of these goods and finally make the most optimal combination of purchases. So, the consumer has a certain amount of money at his disposal, m and he can afford a combination of goods x_1, x_2, x_3... which do not cost more than m. This set of goods that the consumer can afford is called a budget set (Varian, 2008). The combination of goods that the consumer wants to procure represents different levels of his preference. The theory of indifference is a fundamental concept of studying consumer behaviour that starts from the assumption that the consumer is indifferent in choosing a combination of goods if each of them brings him the same satisfaction (Samuelson, 1992). Consumers have the choice to purchase certain goods at a certain moment or to wait for the future period and a more favourable moment for shopping. The initially available amount of income with higher present value allows the consumer more spending opportunities in each period if he can borrow and lend money at favourable interest rates, or at constant interest rates, which contributes to higher consumption (Crotty, 2009).

Budget constraints are a very important factor in consumer behaviour because each purchase is determined by the funding limit. At a certain moment, the consumer has a certain amount of money at his disposal, but also the possibility to borrow at the expense of future income. The goal of consumers is to find a basket of products and the amount of consumption that will give them maximum satisfaction with disposable income (Stojanović, 1997). The profitability of today's investment in some future period can be shown by the current value. Present value is the only correct way to express the value of future income in current monetary value. Regardless of consumer preferences for consumption in different periods, cash flow that has a higher current value should always be preferred to one with a lower current value.

This research was conducted in a period when the market is facing uneven economic cycles and constant price growth. Therefore, the aim was to analyse how, in such circumstances, to direct the cash flow of income to the business process that brings the greatest benefit. Therefore, the production of wheat and sunflower for three agricultural companies from different countries during one year, considering other factors: inflation, interest rates, cash flow and net present value, was analysed.

Material and Methods

Farmers have a limited budget, operate in a market where interest rates vary, the price of raw materials also varies, and inflation is present. This research analysed the period for year 2021 and the current market conditions and forecasts for 2023. With regard to current consumption, a projection of the value of production volume for 2023 was made. The case study method was used to analyse the business of three agricultural companies: the crop producer Novi Agrar Ltd. from the Republic of Croatia, Matijević Ltd. from the Republic of Serbia and the Agricultural Combine Ptuj Tovarna from the Republic of Slovenia. Those companies, among other products, produce agricultural crops, wheat and sunflower, and given the current market circumstances, market prices, supply and demand ratio make a calculation for the production of crops whose investments are positive.

In order consider consumer behaviour, further analysis included annual inflation and interest rates in working capital as the cost of new borrowing on a one-year basis. For the business process to be profitable, the cash flow of income in the present and future periods must be higher than the flow of payments in the present and future periods. According to Varian (2008), this statement can be expressed in mathematical terms in the following way:

The ratio of cash flow and payment flow increased by the interest rate

$$M_1 + \frac{M_2}{1 + r_1} > P_1 + \frac{P_2}{1 + r_1}$$

Where:

M_1, M_2 - cash flow in each period

P_1, P_2 - payment flow in each period

$1 + r_1$ - interest rate in each period.

Cash flow movements are necessary to calculate the net present value. The net present value represents a measure that shows how much is being created today or how much value is being added by investing. It is calculated when the investment cost is deducted from the present value of future cash flows. According to Varian, the equivalent way to measure the value of an investment is to use the notion of net present value. The net present value is calculated by calculating the net cash flow in each period (M_1 - P_1, M_2 - P_2) and then discounting that flow to the present time according to the following formula:

Calculation of net present value (NPV)

$$NPV = M_1 - P_1 + \frac{M_2 - P_2}{1 + r}$$

If this mathematical expression is compared with the previous mathematical expression, it can be seen that the investment is justified if and only if the net present value is positive.

Results and Discussion

The comparative overview of production costs and income of three different agricultural companies in year 2021 is presented in the Table 1. Here, three agricultural companies and two types of agricultural crops they produce were compared. The agricultural combine Ptuj Tovarna sells its products at the highest price, but at the same time has the highest production costs. On the other hand, Novi Agrar Ltd. makes the biggest profit in production which also has the lowest production costs.

Table 1. Comparative overview of producer and sales prices in year 2021

Novi Agrar Ltd.					
Crop	Production in year 2021 (kg)	Production cost per kg (in Euro)	Production cost (in Euro)	Income (in Euro)	Profit
Wheat	244,000.00	0.19	46,360.00	58,560.00	12,200.00
Sunflower	147,000.00	0.39	57,330.00	72,030.00	14,700.00
Total	**391,000.00**		**103,690.00**		**26,900.00**

Matijević Ltd.					
Crop	Production in year 2021 (kg)	Production cost per kg (in Euro)	Production cost (in Euro)	Income (in Euro)	Profit
Wheat	227,000.00	0.17	38,590.00	47,670.00	9,080.00
Sunflower	161,000.00	0.41	66,010.00	77,280.00	11,270.00
Total	**388,000.00**		**104,600.00**		**20,350.00**
Agricultural combine Ptuj Tovarna					
Crop	Production in year 2021 (kg)	Production cost per kg (in Euro)	Production cost (in Euro)	Income (in Euro)	Profit
Wheat	213,000.00	0.26	55,380.00	61,770.00	6.390,00
Sunflower	156,000.00	0.43	67,080.00	79,560.00	12.480,00
Total	**369,000.00**		**122,460.00**		**18.870,00**

* prepared by the author according to the data from the survey questionnaire

Due to unstable market conditions, interest rates and inflation were introduced in the further analysis, which according to Crotty (2009) are the most important parameters necessary for calculating the current value of money, cash flow and net present value. The data in the Table 2. show these values for each agricultural producer according to the product (wheat, sunflower).

Table 2. Comparative presentation of the costs of new borrowing on the market

Novi Agrar Ltd.					
Crop	Interest rate (in %)	Inflation (in %)	Cash flow for one year	New borrowing cost increased by interest rate	Net present value of today's money
Wheat	3.50	12.6	51,181.44	47,982.60	3,090.67
Sunflower	3.50	12.6	62,954.22	59,336.55	3,495.33
Matijević Ltd.					
Crop	Interest rate (in %)	Inflation (in %)	Cash flow for one year	New borrowing cost increased by interest rate	Net present value of today's money
Wheat	3.10	7.5	44,094.75	39,786.29	4,178.91
Sunflower	3.10	7.5	71,484.00	68,056.31	3,324.63
Agricultural combine Ptuj Tovarna					
Crop	Interest rate (in %)	Inflation (in %)	Cash flow for one year	New borrowing cost increased by interest rate	Net present value of today's money
Wheat	3.40	4.6	58,928.58	57,262.92	1,610.89
Sunflower	3.40	4.6	75,900.24	69,360.72	6,324.49

* prepared by the author according to the data from the survey questionnaire

The highest annual inflation on agricultural products affects consumers the most in Croatia and it amounts to 12.6%, while the lowest annual inflation is in the market in Slovenia and amounts 4.6%. This means that the money available to agricultural companies in the current year is worth less than the percentage of inflation compared to the same period last year. On the other hand, inflation is not the only factor influencing the market. Since it is about agricultural companies who have a long-standing market tradition, it is necessary to consider other factors that affect the continuation of the flow of reproduction for the next year. The interest rate ranges from 3.10% to 3.50%. The lowest borrowing costs are present on the market in Serbia, i.e. domicile agricultural companies borrow there the most favourably in relation to other markets. According to the survey, all agricultural companies are ready to invest the same amount of money in the next production process that they invested in the previous period. Accordingly, to Samuelson (1992), if the current inflation rate, which will raise the prices of raw materials, is considered, it could be assumed that the selling price of wheat and sunflower will also increase. Finally, that burden will be passed on to end customers. According to the data from Table 2, the state of the budget curve according to the individual value of wheat and sunflower were projected (Figure 1).

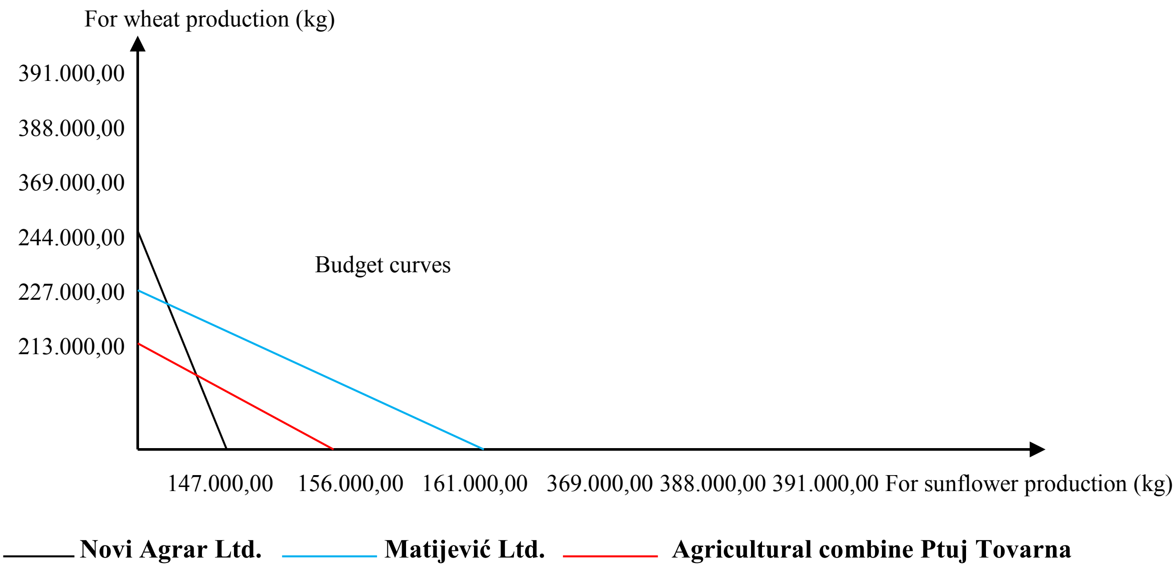

Figure 1. Budget curve

Budget curves show how much raw material can be bought for available funds. According to the values from Table 1, Novi Agrar Ltd. has a total of 103,690.00 Euros at its disposal and it can invest these funds at the same time for wheat production worth 244,000.00 Euros and sunflower production worth 147,000.00 Euros. However, he still has the choice to decide for wheat production alone in the amount of 391,000.00 Euros, so the budget curve becomes steep, or only sunflower production in the same value, so the budget curve becomes flatter. He also has the option to save the money for the next period if he thinks that the money will be worth more then. The same possibility applies to other agricultural companies.

The decision on the choice of future production is made by companies according to the projections of supply and demand in the market. Varian (2008) emphasised that the goal of any production is to maximize profits, the final decision is influenced by the value of income that has some real value today, but the question arises if it is put into cash flow, how much will its future value be worth today. The answer to the question lies in the calculation of the net present value according to formula 2. The data from Table 2 show that the net present value of all companies is higher than zero, which means that investments in wheat and sunflower production are justified, i.e. profitable. For the Agricultural combine Ptuj Tovarna, the most profitable is the investment in the production of sunflower, which is almost 6 times more profitable than the investment in wheat. There is not so much difference with other agricultural companies. They have approximately the same positive net present value for the production of both crops so that in the future they can decide for the same production ratio.

Conclusion

The paper investigates the intertermolar choice in the production of wheat and sunflower for three agricultural companies as well as the application of the optimal choice of future production. Companies are limited by the budget and the choice of which culture to produce. But not everything is so simple on the market. Often, due to insufficient financial resources, it is necessary to borrow more. The price of indebtedness is expressed through interest. Lower interest rate implies lower prices. The results of the paper showed that the current interest rate is within normal limits and that no significant price increase is expected due to it. On the other hand, the occurrence of inflationary pressures as a consequence of market shocks in cooperation with interest rates causes an additional increase in market prices. In such conditions, it is necessary to consider the value of money, i.e. the income that agricultural companies have at their disposal, and decide on future production. The results showed that the net present value is positive for all agricultural crops and that there are no major mutual oscillations, except for the agricultural producer from Slovenia, for whom sunflower production is much more profitable. Therefore, it can be concluded that through intertermpolar choice, an analysis can be made that shows a better understanding in making business decisions.

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Original scientific paper

Impact of fat levels in a meal on pheasant chicks´ growth under controlled brooding conditions

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Abstract

The paper presents research results relating to the growth of pheasant chicks in the initial phase of brooding, in the period between the first and forty-second day, affected by the level of fat in the mixtures used to feed pheasant chicks. The research objective was to establish the extent to which the level of fat affects pheasant chicks´ body mass, the number of dead chicks, the feathering of chicks, and the uniformity of a flock. The body mass of little pheasant chicks at the moment of release into the hunting ground is very important for the degree of survival in the period of adaptation to natural conditions. The research was conducted at the pheasant farm of the Niš Hunting Association under controlled conditions and in accordance with hunting ethical principles. The research included 200 pheasant chicks divided into four groups of 50 heads, one control group, and three experimental groups. Each group of pheasant chicks was fed with a standard mixture technologically recommended for that age group, and enriched with different % of lard, 0,5% (I), 1,5% (II), and 2,5% (III). When one considers the current data in relevant reference literature one can conclude that brooding pheasant chicks under controlled intensive conditions requires a well-balanced meal and high-fat levels in the third and fourth weeks of life (day 20-30), immediately before their intensive growth and accelerated feathering.

Increased weight gain of 13.94 g in the period between the first and third measurement indicates that the share of added lard with 1.5% in the mixture is considered rational and has a positive effect on chicks´ body mass, weight gain, flock feathering, reduced mortality and flock uniformity.

Key words: pheasant, chicks, feeding, fat

Introduction

Pheasants are birds that inhabit our hunting grounds. It belongs to the genus of birds that live in nature.They become sexually mature before they are one year old. Once a year these hens make a nest on the ground, they lay from 10 to 18 eggs, and if the nest gets destroyed they build a new one with fewer eggs. After hatching, the young are capable of following their mother.

First pheasants in Europe appeared in Italy, and then in France, England, Czechoslovakia, and other countries. They were brought to our country in 1880, to the Niš hunting ground of Toponica (Gajić, 1994). In the later period, pheasants as game birds started to spread and take the central place in almost all Serbian hunting grounds. In recent years, in addition to foxes, cats, and hawks, animals that pose the greatest threat to pheasants, the human factor has been a serious competitor to take the lead as the greatest enemy of this poultry. The pheasant diet mostly consists of plants. Thus, their meal mostly includes heads of cereal plants, clover, buds, berries, and often seeds (Djordjević et al, 2008). However, the menu can also consist of insects, worms, frogs, field mice, and snails. In addition to food, these animals also swallow small bits of gravel which serve as gizzard stones and help their stomachs digest food, i.e. break it down (Djordjević et al, 2009).

Unfortunately, in recent years the population of pheasants has been declining, mainly due to habitat loss and uncontrolled hunting. It is believed that around 80% of caught pheasants are only several months old and they do not have offspring. Furthermore, unfavourable weather conditions, intensive cultivation of crops, and the use of pesticides are additional causes for their reduction. For that reason, we have developed a system of artificial breeding of pheasants and their subsequent release into the hunting ground as adult birds (Popović et al, 2010). The technology of pheasant brooding under controlled conditions, i.e. indoors, is almost completely perfected, starting from the production of fertile eggs at the pheasant farms of parent flocks, over controlled and balanced diet consisting of complete concentrated nutrients, prevention medicine, to hatching eggs in modern incubators, hatcheries and breeding grounds for brooding pheasant chicks from the moment of hatching until they are ready to be released into the nature (Ipek et al, 2007; Pekeč et al. 2006, Popović & Stanković, 2009). New and modern technology has influenced the percentage of fertilised eggs as well as the percentage of hatching per year. Thus, the number of bred pheasants increases steadily (Hoodless, 2001; Kokoszynski et al. 2002). The purpose of this research was to establish to what extent the level of fat affects body mass, the number of dead chicks, chicks´ feathering, and the uniformity of the flock in the first 45 days of life of pheasant chicks.

Material and Methods

Animals: The research was conducted on the pheasant farm *Vinik* owned by the Niš Hunting Association. It was conducted under controlled conditions and in accordance with hunting ethical principles. At the *Vinik* pheasant farm, rooms with artificial brooders have been built in two rows, with six brooders in each row. The floor is decked and covered with sand. Rooms are connected by passages that can be closed. They are also connected with concrete outlets with eaves, also divided into six segments. Outlets are connected with rewilding aviaries. When pheasant chicks are 20 days old the wire is lifted and they can go through. The research included 200 pheasant chicks, divided into four groups of 50 heads, one control, and three experimental groups.

Experimental measurements: Measuring pheasant chicks´ body mass, which was the first parameter, was done every morning before feeding by resorting to a precision digital scale with an accuracy of 0.001g. The first measurement of pheasant chicks was done immediately after hatching, and there were three more control measurements over a period of 15 days. Measurement was done on random samples. The second parameter was food conversion calculation which was done three times over a period of 15 days, while food consumption was controlled every day immediately before the next meal by emptying the feeders and measuring the remaining food with a precision digital scale with an accuracy of 0.001g. The third monitored parameter was the uniformity of pheasant chicks. It can be expressed by a percentage of chicks whose body mass deviates ±10% from the average mass. The feathering of pheasant chicks was the fourth parameter. Feathering and uniformity are key parameters for pheasant chicks up to 45 days of age because those chicks leave the breeding ground to encounter the natural ambiance for the first time and they need to possess the properties of thermoregulation.

Statistical analysis: All statistical analyses were done by using the SPSS Statistics 25 software. The obtained results have been shown in tables and graphs by calculating the mean value, and then by using the t-test we established the variable of quantitative properties.

Diet of pheasant chicks: Each group of pheasant chicks was fed with a standard mixture with 28% of protein (Table 1), technologically recommended for that age group, which contained different % of lard: 0,5% (I), 1,5% (II) and 2,5% (III) (Table 2).

Table 1. Complete mixture for pheasant chicks from 0 to 6 weeks of age

N°	NUTRIENT	PERCENTAGE IN THE MIXTURE (%)			
		C group	I group	II group	III group
1	Corn	32,43	32,43	32,43	32,43
2	Soybean meal (SP 44%)	20,00	20,00	20,00	20,00
3	Soybean semolina (SP 18%)	18,73	18,73	18,73	18,73
4	Fish flour (SP 64%)	1,58	1,58	1,58	1,58
5	Sunflower meal (SP 33%)	7,00	7,00	7,00	7,00
6	Fodder yeast	10,00	10,00	10,00	10,00
6	Dicalcium phosphate	2,77	2,77	2,77	2,77
7	Fodder chalk	0,85	0,85	0,85	0,85
8	Fodder salt	0,24	0,24	0,24	0,24
9	Lysine	0,092	0,092	0,092	0,092
10	Methionine	1,31	1,31	1,31	1,31
11	Premix	1,00	1,00	1,00	1,00

Table 2. shows the results of the analytical analysis of lard percentage in mixtures for experimental groups after lard addition.

Table 2. Complete mixture for pheasant chicks from 0 to 6 weeks old with lard addition

N°	NUTRIENT	AMOUNT IN THE MIXTURE			
		C group	I group	II group	III group
	ADDED lard* (%)	**-**	**0,50**	**1,50**	**2,50**
1	Metabolic energy (MJ/kg)	12,66	12,76	12,96	13,26
2	Raw protein (%)	28,00	28,00	28,00	28,00
3	Cellulose (%)	4,97	4,97	4,97	4,97
4	Fat (%)	9,56	9,56	9,56	9,56
5	Ash (%)	7,52	7,52	7,52	7,52
6	Calcium (%)	1,20	1,20	1,20	1,20
7	Phosphorus total (%)	1,10	1,10	1,10	1,10
8	Phosphorus usable (%)	0,70	0,70	0,70	0,70
9	Salt (%)	0,30	0,30	0,30	0,30
10	Lysine (%)	1,70	1,70	1,70	1,70
12	Methionine+cystine (%)	2,11	2,11	2,11	2,11

**lard*

Results and Discussion

Insufficient natural reproduction of pheasants requires their breeding in strictly controlled conditions with a very intensive diet (Djordjević et al, 2011). Table 1 shows the results of an analytical analysis of the chemical composition of complete mixtures used to feed pheasant chicks up to 6 weeks of age. In addition to the chemical composition of meals, one should also consider the type and physical form of the feed by firstly using the granulated feed, then pelleted feed, and finally grain feed. In addition to concentrated food, it is mandatory to give them green mass and insect larvae, all for maximum adaptation to the natural conditions (Djordjevic et al, 2011). Pheasant chicks´ growth is very intensive which requires an adequate diet. According to Nadaždin (1996), the weight of pheasant chicks at the moment of hatching is 21.0 g, two weeks later it is 85 g and after the 6th week, it is 380 g. The daily growth of pheasant chicks goes from 1 g during the first days of their lives up to 15 g after eight weeks.

Nadaždin (1996) established experimentally that the total growth of pheasant chicks from 0 to 15 days old was 64.0 g, from 16 to 42 days old 295 g, i.e. for the period between 0 and 42 days old, it is 359 g on average. According to the same author, the daily growth of pheasant chicks between 0 and 15 days old is 4.26 g, from 16 to 42 days old is 10.93 g, i.e. for the whole period, it is 8.54 g. With a 2:1 feed conversion, feed consumption per head is between 2 g/day in the first week of life and 30 g/day at the end of the brooding period. For the whole eight-week period of feeding the total consumption of concentrate per chick is around 800g.

Table 3. Production results of pheasant chicks

Group	Number (n)	Body mass $\overline{X}$ (g)			Feed conversionX (kg/kg)			Chicks´growth X (g/dan)			Flock uniformity (%) (±10)			Flock feathering (%)	Deaths	
	heads	Age in days			Age in days			Age in days			Age in days			Age in days	Age (day 45)	
		15	30	45	15	30	45	15	30	45	15	30	45	45	heads	%
C	50	51,29	155.58	320.03	1.76	2.54	3.12	2.08	6.95	10.96	96.31	97.04	99.67	98,50	11	2.23
I	50	51,78	162.06	335.93	1.80	2.61	3.19	2.11	7.35	11.60	96.33	97.09	99.72	98.50	12	2.24
II	50	55,97	179.25	388.27	1.86	2.71	3.32	2.39	8.22	13.94	98.80	99.29	99.33	99.80	9	1,85
III	50	53,22	161.28	336.21	1.53	2.30	2.85	2.21	7.24	11.63	96.67	98.21	96.69	96.00	16	3.12

Table 3 shows an average mass of pheasant chicks from control and experimental groups on days 15, 30, and 45 of pheasant brooding average feed conversion of pheasant chicks in control and experimental groups on days 15, 30, and 45 of pheasant brooding, the average growth of pheasant chicks in control and experimental groups on days 15, 30 and 45 of pheasant brooding, uniformity of flocks of pheasant chicks in control and experimental groups on days 15, 30 and 45 of pheasant brooding, feathering of flocks and number of dead pheasant chicks at the end of the brooding period, on day 45.

The results show that experimental group II achieved the best results and reactions after consuming the feed enriched with lard in the amount of 1.5%. This reaction relates to the weight of pheasant chicks which was greater by 21.3% in comparison to the control group, 15.6% greater than the experimental group I, and 15.4 % greater than the experimental group III. Similar results were obtained by Kuzniacka et al (2010) and Kokoszynski et al (2012). By observing feed conversion one can observe that the mixture, enriched with 1.5% of lard is to be considered rational in the feed of pheasant chicks up to 45 days old which can reduce costs, which was confirmed by Krawczyk et al (2002).

The results of uniformity of pheasant chicks presented in Table 3 have been expressed as a percentage of chicks whose body mass deviated ±10% from average and it has been established that the lowest variability in pheasant chicks was in group II and the largest in group III. It is certain that the diet and the composition of the mixture, first and foremost the presence of lard, improve the absorption of liposoluble vitamins and the utilisation of metabolic energy in food, which affects the quality of plumage and feathering, which can be seen in the experimental group II and the highest in group 3, which indicates that too much fat can have harmful effects. The number of dead chicks is the smallest in the experimental group II, only 1.85%.

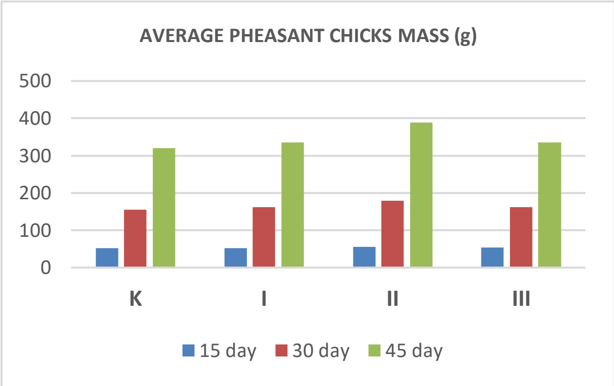
Table 4. The value of *p*, the statistical significance of the mass of pheasant chicks at the end of the research period of 45 days

Comparison	Difference	p	P	Significance
I-C	15.90	4	0.010	**
I-II	52,34	4	0.004	*
I-III	0.28	4	0.957	NS
C-III	16.18	4	0.011	**
C-II	68.24	4	0.003	*
II-III	33.23	4	0.009	*

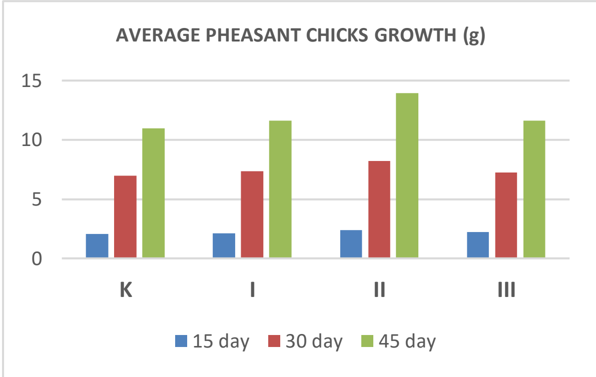
*NS- no statistical significance, *-the significance of differences at the level of 95%, **- the significance of difference at the level of 99%*

Table 4 shows the average mass of pheasant chicks in control and experimental groups on days 15, 30, and 45 days of brooding. There are significant differences in the mass of pheasants on days 15 and 30 and on the 45th day of brooding in the experimental group of pheasant chicks which were fed with food enriched with 1.5% of lard. At the end of the brooding period, the pheasant chicks in the O-II group (388.27 g) had a significantly larger mass in comparison to pheasant chicks from other groups ($p<0,05$).

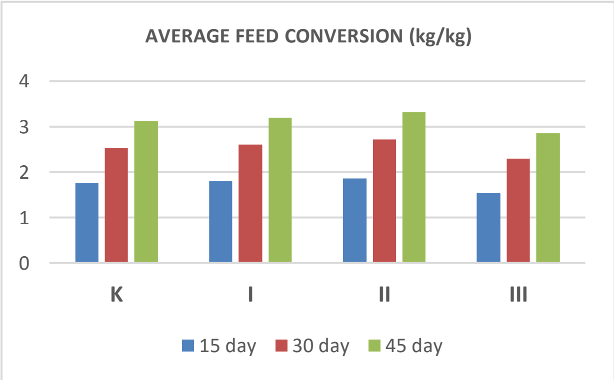
A graphical representation of quantitative properties of pheasant chicks for the period of 45 days was given in Graphs 1,2,3,4



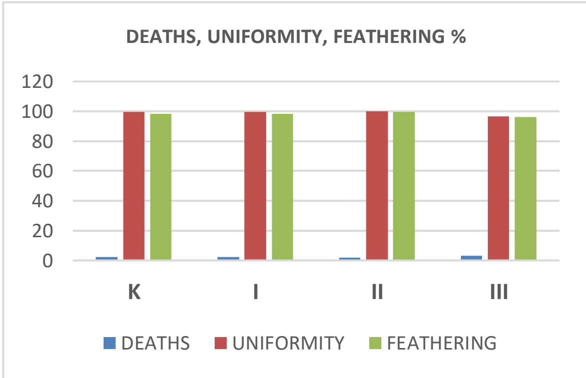
Graph 1. Average pheasant chicks´ mass in g



Graph 2. Average pheasant chicks growth,



Graph 3. Average feed conversion, kg/kg



Graph 4. Performances of pheasant chicks, %

Conclusion

The technology of pheasant brooding under controlled conditions, i.e. indoors, has been almost completely perfected, starting from the production of fertile eggs on pheasant farms of parent flocks, over a controlled and balanced diet consisting of complete concentrated nutrients. Unfavourable weather conditions, intensive cultivation of crops, and the use of pesticides are also reasons for the reduced number of pheasants. For that reason, one needs to resort to artificial breeding of pheasants and their subsequent release into the hunting ground as adult birds. By going through the current relevant reference literature one can conclude that brooding of pheasant chicks under controlled intensive conditions requires a well-balanced meal and high levels of fat in the third and fourth weeks of life (days 20-30), and immediately before their intensive growth and accelerated feathering. The obtained gain of 13.94 g in the period between the first and third measurement indicates that the percentage of 1.5% of added lard is considered rational and it has a positive effect on the body mass, growth, feathering, decreased percentage of deaths, and uniformity of the flock.

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Original scientific paper

Hoof trimming as factor affecting milk production in high-producing dairy cows

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Abstract

The milking cow uses a number of factors. The most important are breed, age of the cattle head, period of lactation, climatic conditions, microclimatic conditions in the facilities, dietary conditions and maintenance of healthy conditions of the animal. One of the most important factors used in the production results of dairy cows is the negative hoof status. The aim of this study was to examine the production results of two groups of dairy cows aged 3 years (second lactation) whose processing and care of hoofs affected the level of productivity. The total number of analyzed animals, in the free breeding system, was 100 dairy cows in the second lactation at the cattle farm "Lazar" in Blace. The first group (1) consisted of 50 dairy cows of the Simmental breed with which the hooves were circumcised twice during the year, at the age of 3 years. The second group consisted of 50 (2) heads of dairy cows of the Simmental breed with uncircumcised hooves, aged 3 years. The analysis included the total amount of milk produced during lactation, during which a weekly control of milk, proteins and milk fats was performed and in that way their quantitative and qualitative production results were monitored. For data analisys, the statistical method ANOVA was used, which examined the effect of several independent variables on a single variable. The correlation between the observed parameters and the Pearson correlation coefficient were also determined. Statistical processing of data and review of stable lists of production heads showed that the first group of animals showed a higher level of production of milk, milk fat and milk proteins compared to the second group of cows. It was proved that there are differences between the groups and that this difference is statistically significant at the level of $P<0.001$. All correlations are statistically significant at the $p<0.001$ level.

Key words: hoof trimming, care, dairy cows, milk

Introduction

Healthy extremities and good mobility are of great importance for the welfare and good productivity of cattle, especially dairy cows. In a closed system of stable keeping (stables with a system of tied keeping or stays with boxes in which a free system of keeping cows is implemented), it is very important to take care of proper, preventive care of the hooves. Healthy extremities and good mobility are essential for the well-being and good productivity of cattle, especially dairy cows. If the hooves are not cared for in an appropriate way, numerous diseases of the locomotion system occur (Hristov et al., 2011).

Given the frequency of occurrence, economic losses caused by various pathologies of the locomotion system are very significant. They are reflected in the premature exclusion of the cow from production, reduction of milk production and treatment costs. There are numerous data in the scientific literature in the world that indicate that lameness causes a significant reduction in the amount of milk (Bicalho et al., 2008). However, data related to the composition of milk in lameness are represented to a much lesser extent. In Serbia, there is not enough recent scientific knowledge about the frequency of lameness and its impact on the milk yield of cows, as well as the most significant causes and predisposing factors of lameness on our farms. According to recent data from the scientific literature (Ettema et al., 2007), there are six key external complex risk factors: cow comfort, cow hygiene, social and physical integration of heifers and dried cows, movement of cows on the farm, nutrition, appropriate preventive shortening of hoof horn (Hristov et al., 2011).

Good hoof care can save significant financial resources, because the reduced incidence of lameness in cows means less veterinary interventions, lower costs, fewer cows culled and higher milk production (Bicalho et al., 2008). Lameness is most often a consequence of impaired morphological-functional integrity of the musculoskeletal system of the extremities. The causes of lameness can be numerous mechanical insults, in combination with predisposing factors, which act continuously over a longer period of time (Zemljič, 2009).

Many researchers believe that the prevention and treatment of lameness is a problem of recent times, but this is still not the case. Greenogh (2007) states in his publication that in ancient Rome in the first century they wrote about the problems of oxen with hooves: "animals will limp less after hard work if their feet are washed in plenty of cold water, as well as if the ankles, the crowns of their hooves and the parts between the hooves are rubbed with axle grease". Today, the Simmental breed is present on all continents. According to the data given in the paper by Panić and Vidović (2006), and based on the data of the World

Simmental Federation Database, Simmental cattle, with about 42,000,000 head, make up a large group of pied cattle in the world. The Simmental breed is mainly bred in the area of central Serbia as a breed with combined production characteristics. The milk yield of the Simmental breed in different countries, depending on the quality of the stock, growing conditions and selection program, varies considerably and ranges from 4000 kg to over 5000 kg with 3.6 - 4% milk fat in the milk type (Skalicki et al., 2007). Particularly phenotypic and genetic progress is recorded by the population of Austrian Simmental, whose average production per population of 230,000 cows is 6,128 kg of milk with 4.42% milk fat and 3.43% protein (Medić et al., 2006).

Four main factors have been defined that are "triggers" for the development of hoof lesions. The first and most important cause is the diet of cows (Tomlinson et al., 2004), the second cause is hormonal changes during calving (Tarlton et al., 2002), the third factor is external trauma (Shearer et al., 2006), and the fourth factor, infection with various microorganisms is reported. The composition of milk may also indicate the existence of health or nutritional problems in the milk. A high percentage of fat and low milk production indicate a health disorder or poor diet, while a low percentage of milk fat may be related to rumen dysfunction, metabolic disorders or poor ration composition (Pešić et al., 2020). It has been noticed that variations in the amount of fat in milk are greater in breeds that have more fat in milk, and the milk of cows of the same breed often varies more in the amount of fat than the total milk of different breeds (Ostojić, 2007).

The average value of protein in the milk of domestic pied breed cows is 3.27% with individual variations of 2.60 - 4.10%. Christ et al. (2011) found a statistically significant decrease in protein content with an increase in BSC in whole milk. The most important prerequisite for high cow production is proper nutrition, as well as well-groomed and healthy hooves. When trimming the hooves, special attention should be paid to the well-known "white line". It is recognizable and serves as a kind of border: up to which it is possible to remove excess hooves without the danger of catching the "living part". Also, all cows should have their own skin care equipment, but also hoofs trimming equipments to prevent infection (Fjeldaas et al., 2006). The aim of this paper was to determine in what way and to what extent the pruning of hoofs trimming affects the production performance of dairy cows and the qualitative characteristics of the produced milk.

Materials and Methods

Animals: The research in was conducted on the farm of lactating cows "Lazar" in two time periods: 60 days at the beginning of lactation and 60 days at the end of lactation. There were 550 cows with offspring on the farm. For this experiment, 100 lactating cows of the Simmental breed in the second lactation, aged 3 years, were selected.

We divided the animals into two groups of 50 animals each. The first group consisted of 50 heads with circumcised hooves conducted twice during the year. The second group consisted of 50 heads with uncircumcised hooves. All cows had pedigrees, HB numbers were marked with ear tags, and around their necks, they wore transponders (type Westphalia), which were used to monitor and read the activities of animals, both in the barn and in the milking parlour.

Ambient conditions: Intensive milk production is organized on the farm, which represents a high level of exploitation of animals in the facility. The animals in the facility were exposed to the same microclimatic conditions, with identical positions with respect to light, ventilation, water, food, and the performance system.

Hoof correction: For correction-pruning of hoofs on the farm, a box from the domestic manufacturer "INPAK" was used. After fixing the animals and extremities, the procedure of correction of the hooves was started. After the correction of the hoofs, the disinfection of the hooves was started. Control measurements: During 12 months, a weekly control of skimmed milk, protein and milk fat content in milk was performed, and in that way quantitative and qualitative production results of the throat were controlled.

Statistical analysis: All statistical analyzes were performed using the program SPSS Statistics 25. The obtained results are presented in a table, by calculating the mean values and standard error, and using statistical method ANOVA the variability of quantitative traits was tested.

Results and Discussion

The appendices contain summary statistics of the examined milk yield parameters: milk quantity, milk fat content and protein content. For all parameters, statistical indicators are presented: number of cows, quantity of milk, average milk production, minimum milk production, maximum milk production, milk fat (MM) production per kg, milk fat (MM) production in %, protein production in kg, protein production in %.

Table 1 shows the production results of dairy cows, i.e. the total milk production, milk fat production and milk protein production during the exploitation period during lactation II. Based on the data from Tables 1 and 2, we can conclude that there are statistically significant differences in terms of all three parameters of milk production in relation to the trimming of hooves in the lactation period, which was recalculated to a standard period of 305 days, in favor of cows hoofs trimmed.

Table 1. Total milk production, milk fat of milk proteins of dairy cows (II lactation-Simmental breed-2019) calculated on a standard lactation of 305 days.

PARAMETERS		N	Total amount of milk, l /standard lactation 305 days / throat	SD	SE	95% Confidence Interval for Mean		Production interval	
						Lower Bound	Upper Bound	Min.	Max
Milk, l	with trimmed hoofs	50	7113.98	346.16	48.95	7015.60	7212.35	6693.00	7912.00
	with untrimmed hoofs	50	6348.74	552.20	78.09	6191.80	6505.67	5121.00	7442.00
	total	100	6731.36	598.42	59.84	6612.61	6850.10	5121.00	7912.00
Milk protein, kg	with trimmed hoofs	50	242.61	12.44	1.76	239.07	246.15	226.89	270.35
	with untrimmed hoofs	50	204.60	18.64	2.63	199.30	209.89	165.33	237.83
	total	100	223.60	24.77	2.47	218.69	228.52	165.33	270.35
Milk fat, kg	with trimmed hoofs	50	273.90	17.96	2.53	268.79	279.00	240.71	322.74
	with untrimmed hoofs	50	230.06	20.26	2.86	224.30	235.82	186.92	281.72
	total	100	251.98	29.12	2.91	246.20	257.76	186.92	322.74

Source: Stable lists of 50 Simmental breed-farm "Lazar" Blace

Table 2. Display of the degree of statistical significance

PARAMETERS		Mean Square	F	Sig.
Milk, l	Between Groups	14639806.44	68.93	.000
	Within Groups	212381.86		
	Total			
Milk protein, kg	Between Groups	36127.74	143.85	.000
	Within Groups	251.14		
	Total			
Milk fat, kg	Between Groups	48043.77	131.09	.000
	Within Groups	366.47		
	Total			

Based on the data from Tables 3 and 4, we can conclude that there are statistically significant differences in terms of all three parameters of milk in relation to the circumcision of the hooves in the first 60 days of lactation, in favor of cows whose hooves were circumcised when it comes to total production of milk and milk proteins, and in favor of cows in which the hooves are uncut when it comes to milk fat.

Table 3. Results of production of milk, milk fat and milk proteins of dairy cows (II lactation-Simmental breed-2019) in the first 60 days of the lactation period.

PARAMETERS		N	Total amount of milk, l / in the first 60 production days / throat	SD	SE	95% Confidence Interval for Mean		Production interval	
						Lower Bound	Upper Bound	Min.	Max
Milk, l	with trimmed hoofs	50	2001.20	174.29	24.64	1951.66	2050.73	1767.00	2355.00
	with untrimmed hoofs	50	1789.12	112.09	15.85	1757.26	1820.97	1511.00	1964.00
	total	100	1895.16	180.58	18.06	1859.32	1930.99	1511.00	2355.00
Milk protein, kg	with trimmed hoofs	50	68.25	6.12	.86	66.51	69.99	59.90	80.54
	with untrimmed hoofs	50	57.73	5.06	.71	56.29	59.17	47.14	66.76
	total	100	62.99	7.69	.77	61.47	64.52	47.14	80.54
Milk fat, kg	with trimmed hoofs	50	52.99	4.82	.68	51.61	54.36	45.68	61.72
	with untrimmed hoofs	50	64.98	6.08	.86	63.25	66.71	50.62	77.91
	total	100	58.98	8.13	.81	57.37	60.60	45.68	77.91

Source: Stable lists of 50 Simmental breed-farm "Lazar" Blace

Table 4. Display of the degree of statistical significance

PARAMETERS		Mean Square	F	Sig.
Milk, l	Between Groups	1124448.16	52.36	.000
	Within Groups	21471.46		
	Total			
Milk protein, kg	Between Groups	2765.70	87.58	.000
	Within Groups	31.57		
	Total			
Milk fat, kg	Between Groups	3595.44	119.114	.000
	Within Groups	30.18		
	Total			

Based on the data from Tables 5 and 6, we can conclude that there are statistically significant differences in terms of all three parameters of milk in relation to the circumcision of the hooves in the last 60 days of lactation, in favor of cows whose hooves were circumcised.

Table 5. Results of production of milk, milk fat and milk proteins of dairy cows, (II lactation-Simmental breed-2019) in the last 60 days of the lactation period

PARAMETERS		N	Total amount of milk, l / in the last 60 production days / throat	SD	SE	95% Confidence Interval for Mean		Production interval	
						Lower Bound	Upper Bound	Min.	Max
Milk, l	with trimmed hoofs	50	1299.00	115.87	16.38	1266.07	1331.93	1125.00	1498.00
	with untrimmed hoofs	50	1128.30	203.61	28.79	1070.43	1186.16	802.00	1520.00
	total	100	1213.65	185.80	18.58	1176.78	1250.51	802.00	1520.00
Milk protein, kg	with trimmed hoofs	50	44.30	4.09	.57	43.14	45.47	38.14	51.53
	with untrimmed hoofs	50	36.46	7.30	1.03	34.39	38.54	25.90	51.98
	total	100	40.38	7.08	.71	38.98	41.79	25.90	51.98
Milk fat, kg	with trimmed hoofs	50	106.08	9.66	1.36	103.33	108.82	91.35	123.44
	with untrimmed hoofs	50	40.99	8.09	1.14	38.69	43.29	30.74	60.56
	total	100	73.53	33.881	3.38	66.81	80.26	30.74	123.44

Source: Stable lists of 50 Simmental breed-farm "Lazar" Blace

The obtained results shown in Tables 5 and 6 clearly indicate a higher level of production, the amount of milk, milk fat and milk proteins in dairy cows in which hoof trimming was performed, as well as the statistical significance between the examined groups.

Table 6. Display of the degree of statistical significance

PARAMETERS		Mean Square	F	Sig.
Milk, l	Between Groups	728462.25	26.54	.000
	Within Groups	27444.06		
	Total			
Milk protein, kg	Between Groups	1536.95	43.86	.000
	Within Groups	35.03		
	Total			
Milk fat, kg	Between Groups	105913.14	1332.82	.000
	Within Groups	79.46		
	Total			

Further analysis of the data determined the degree of correlation of the variables, on which occasion Pearson's correlation coefficient was used.

Table 7. Correlation of independent variables with dependent

Correlations	Hoofs	Total amount of milk, l /standard lactation 305 days / throat			Total amount of milk, l / in the first 60 production days / throat			Total amount of milk, l / in the last 60 production days / throat		
		Total	Protein	Fat	Total	Protein	Fat	Total	Protein	Fat
Hoofs	1	-.643**	-.771**	-.756**	-.590**	-.687**	.741**	-.462**	-.556**	-.965**

**hoofs- 1: with trimmed hoofs 2: with untrimmed hoofs*

Based on the results from Table 7, it can be concluded that there is a statistically significant correlation between hoof processing and milk parameters. All correlations are statistically significant at the .001 level. During lactation, which was recalculated to a standard period of 305 days, the results show a positive correlation between hoof trimming and milk parameters: total milk production, milk protein and milk fat.

During the first 60 days of lactation, the results show a positive correlation between hoof trimming and milk parameters: total milk and milk protein production, while the correlation between hoof trimming and milk fat parameters is negative. In the last 60 days of lactation, the results show a positive correlation between hoof trimming and milk parameters: total milk production, milk proteins and milk fat.

Conclusion

The condition of hoofs is of immense significance for an animal´s health and the productivity of an individual head. Animals that go through regular hoof trimming have healthy hoofs, consume more food, and move better.

As a result, their production is increased as well as production profitability. Only healthy, properly fed and nurtured dairy cows can calve every year and, depending on their genetic potential, produce the maximum amount of milk with exhibited qualitative and quantitative properties. Every milk producer must bear in mind that they shall secure easier and more productive work only if they respect the health and well-being of their animals and if they resort to good breeding practice. As a result, they shall obtain larger amounts of milk and a better quality of the produced milk which leads to a better purchase price, higher profits and a better life standard on the farm.

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Original scientific paper

Analysis of the frequency distribution of metabolic parameters in a pooled sample in early lactating cows

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Abstract

The use of a pooled blood sample can be of great importance in assessing the metabolic status of cows. Pulling of samples allows a single sample to be obtained from a large number of individual samples. The aim of this paper was to examine the frequency distribution characteristics of a large number of pooled samples. The experiment included 60 pooled samples consisting of 10 individual samples. Samples were selected by random selection, and originated from 100 cows in early lactation (0-60 days of lactation) from which blood was taken and 30 individual samples were made from each cow. Laboratory analysis includes determination of beta-hydroxybutyrate, non-esterified fatty acids, cholesterol, triglycerides, glucose, albumin, total protein, urea, Ca, P, total bilirubin and aspartate aminotransferase. The mean values and standard deviations are similar to the reference values of cow metabolites. There is a significant correlation between the values of the pooled sample and the calculated mean values of the individual samples participating in the pool. By visual analysis of frequency distributions (histogram and QQ-plot) we conclude that there is a distribution that corresponds to the normal frequency distribution. The KS-test confirmed the normality of the frequency distribution for all metabolites from the pooled samples except for BHB, TGC and AST. The normality of the distribution of pooled samples and their mean value and variability similar to the reference values indicate that there are good preconditions for the use of pool samples in the assessment of the metabolic status of the herd in early lactation.

Key words: cow, early lactation, pooled sample, metabolic profile

Introduction

Compton's metabolic profile has been used for forty years to examine the health status of cows and meet their food needs. Metabolic profile is defined as a series of specific laboratory tests that determine metabolic values after blood collection in cows in the dry period, early lactation and peak lactation. With the development of science, the number of parameters determined in the metabolic profile became larger. Today, metabolites can be divided into those that reflect the energy status of cows, protein status of cows, functional status of the liver, mineral status, etc. (Cincović et al., 2012, 2021; Belić et al., 2018; Đoković et al. 2019; Delić et al., 2020). Assessment of energy status - Peripartal metabolic stress is decided by negative energy balance, consumption of fat for energy purposes of peripheral tissue and consumption of glucose for udder and milk production. This metabolic rearrangement leads to decreased glucose concentration and increased concentration of non-esterified fatty acids (NEFA), the overuse of which leads to the formation of ketone bodies in the liver, and the concentration of beta hydroxybutyrate (BHB) in the blood increases. This metabolic rearrangement with the increase of ketones and fatty acids causes ketosis, inflammatory disorders, insulin resistance and reduced productivity of cows. Assessment of protein status - Assessment of protein status is much more complex than assessment of energy status in cows in the peripartum period, and it is especially difficult to separate the impact of energy balance from protein balance due to their common metabolic relationship.

The following are used as indicators for the assessment of protein status: concentration of total proteins, albumin and urea in the blood. Parameters that indicate necrosis of the liver parenchyma (hepatocyte necrosis syndrome) are reflected in the activity of the following enzymes: alanine aminotransferase (ALT), aminotransferase (AST), etc. In cows, the concentration of bilirubin and the activity of liver enzymes increase in the peripartum period. The concentration of triglycerides and the concentration of cholesterol in the serum decreases, due to the accumulation in the liver, i.e. reduced production of VLDL. The value of many metabolites correlates with the degree of fatty liver in cows. Estimation of ion concentrations - Metabolism of minerals in the peripartum period has changed significantly, and of special pathophysiological significance is the study of the concentration of calcium (Ca), magnesium (Mg) and phosphorus (P). Hypocalcaemia, hypomagnesaemia and hypophosphatemia are of great clinical importance in the peripartum period. In everyday practice, it is necessary to determine the metabolic profile of cows during different lactation periods, which represents an additional cost.

The use of a pooled blood sample can be of great importance in assessing the metabolic status of cows. Pulling of samples allows a single sample to be obtained from a large number of individual samples. Thus, instead of creating a larger number of metabolic profiles, only one metabolic profile is made, based on which the metabolic and health status of the herd is assessed, but not individual animals. Only when it is determined that there are certain deviations at the level of the pooled sample, the analysis of individual samples is performed. In previous studies, reference ranges of weeded samples as a function of the percentage of cows with inadequate metabolic status and differences in the value of weeded specimens in healthy herds and herds with a history of metabolic diseases were determined (Van Saun, 2008; Hussein et al., 2013). The use of a pooled sample in everyday practice is possible if that sample shows certain characteristics of frequency distribution, such as the normal theoretical distribution (Hald, 1998). The aim of this paper was to examine the frequency distribution properties of a large number of pooled samples.

Material and Methods

The experiment included 60 pooled samples consisting of 10 individual samples. Samples were selected by random selection, and they came from 100 cows in early lactation (0-60 days of lactation) from which blood was taken and 30 individual samples were made from each cow that were mixed with each other. Laboratory analysis includes determination of beta-hydroxybutyrate-BHB, non-esterified fatty acids-NEFA, cholesterol-CHOL, triglycerides-TGC, glucose-GLU, albumin-ALB, total protein-TPROT, UREA, Ca, P, total bilirubin-TBIL and aspartate aminotransferase–AST. Reagents from Biosystem (Spain) and Rayto-Chemray spectrophotometer (China) were used. Frequency distribution analysis was performed by measuring indicators of central tendency and variation. The correlation between the values of the metabolites in the pooled sample and the mean values of the individual samples participating in the pooled was determined by Pearson's correlation coefficient. Frequency distributions were graphically represented, and in order to determine the normality of frequency distribution, QQ plots were graphically presented comparing the ideal normal distribution and the distribution of the pooled sample.

Results and Discussion

Mean values of metabolites in the group of pooled samples, their deviation, and reference values (median and quartiles) are shown in Table 1. The obtained mean values and standard deviations are similar to the reference value for cows in Vojvodina, region of Northern Serbia

(Belić and Cincović, 2020). The mean values of the pool correlate almost ideally (R^2> 0.98) with the calculated mean value of the individual samples participating in the pool for all examined parameters (results not shown). By visual analysis of the frequency and QQ plot distributions (Figs. 1-12 and 13-24), we conclude that there is a distribution corresponding to the normal frequency distribution. The KS-test confirmed the normality of the frequency distribution for all metabolites from the pooled samples except for BHB, TGC and AST.

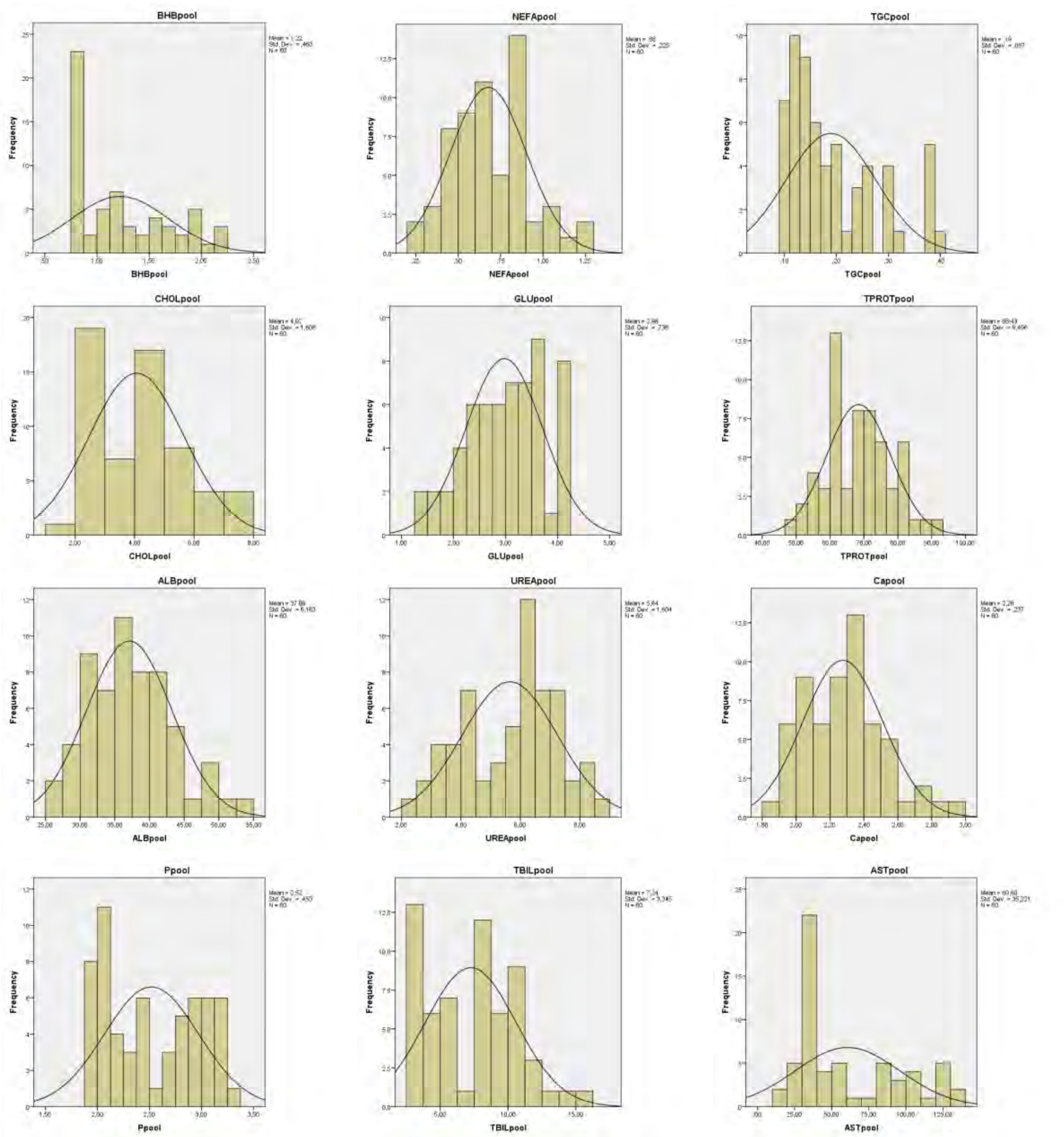
All the above indicates that there are good preconditions for the use of a pool of samples in the assessment of the metabolic status of the herd. There are several reasons for this. In order for the sample pool to be used adequately, it must correlate with the values of the arithmetic mean from the individual samples. Normal distribution of metabolite frequency distribution in the population will lead to normal distribution of values in the pooled sample, with the frequency distribution of the pooled sample showing less variability and less flatness, but this depends on the number of samples in the pool (Mary-Huard et al., 2007). Using a pool of samples, unique herd data measuring its central tendency were obtained, but data on metabolic variability in the herd were lost. Caudill (2010) summarizes principles which make it possible to characterize a population distribution of individuals using pooled samples: (1) measured values of pooled samples are comparable to means of individual samples; (2) the variance among individuals can be obtained from the variance among means of randomly sampled individuals; and (3) all that is needed to completely characterize a normally distributed population is an estimate of the mean and variance of the population of individuals.

Table 1. Descriptive statistics for frequency distribution of pooled sample

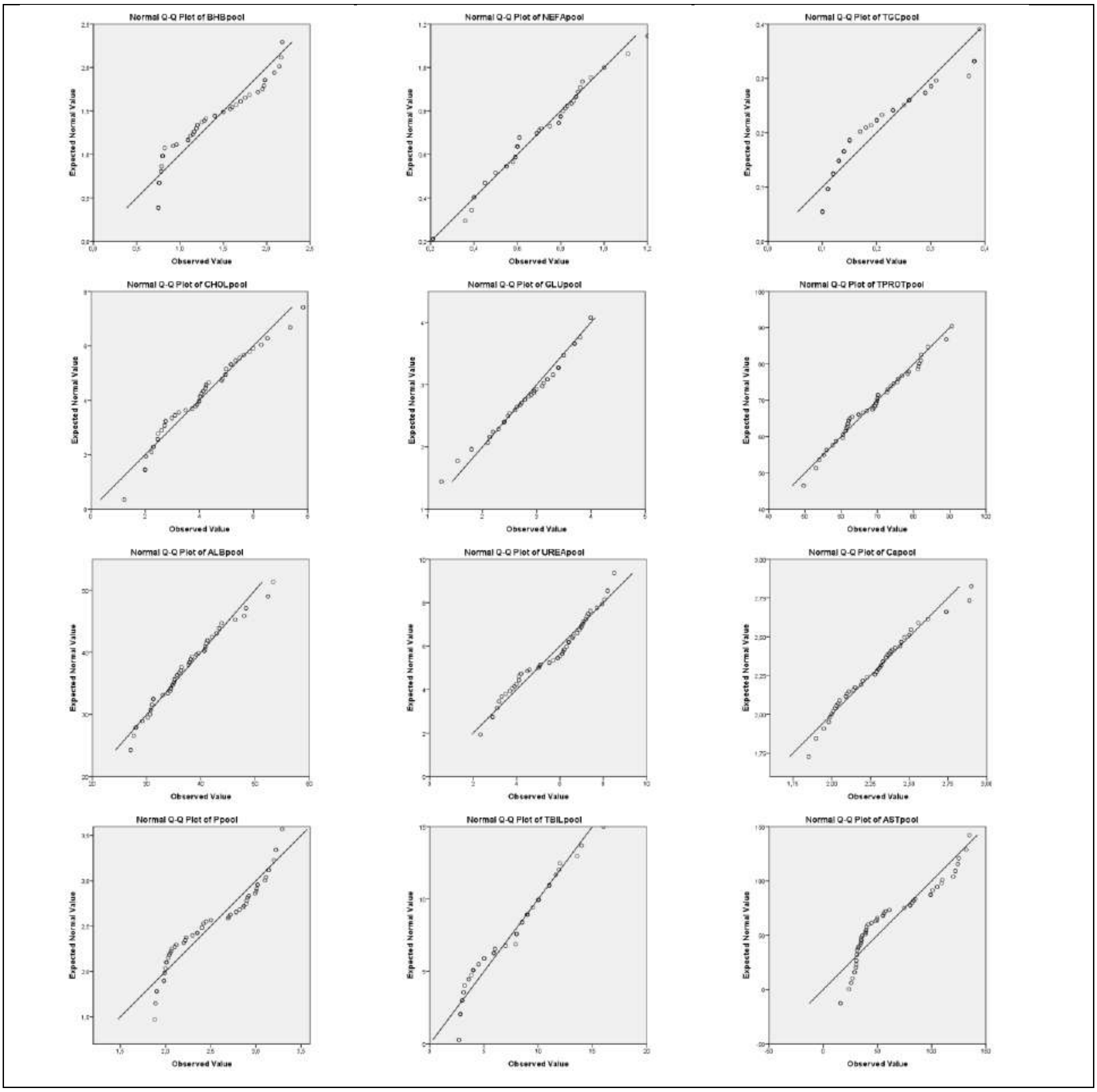
		BHBpool (mmol/L)	NEFApool (mmol/L)	TGCpool (mmol/L)	CHOLpool (mmol/L)	GLUpool (mmol/L)	TPROTpool (g/L)
Mean		1,2217	0,6778	0,1895	4,0668	2,9757	68,4335
Median		1,1350	0,6100	0,1500	4,0200	3,1000	69,3600
Std. Deviation		0,46287	0,22452	0,08707	1,60765	0,73785	9,49646
Skewness		0,686	0,208	1,032	0,538	-0,506	0,233
Std. Error of Skewness		0,309	0,309	0,309	0,309	0,309	0,309
Kurtosis		-0,834	-0,276	-0,030	-0,319	-0,402	-0,544
Std. Error of Kurtosis		0,608	0,608	0,608	0,608	0,608	0,608
	25	0,8000	0,5125	0,1200	2,6350	2,4175	61,2500
Percentiles	50	1,1350	0,6100	0,1500	4,0200	3,1000	69,3600
	75	1,5950	0,8450	0,2450	5,0000	3,5000	75,1650
KS test of normality		0,026	0,221	0,011	0,480	0,577	0,534

Table 1: Continued

		ALBpool (g/L)	UREApool (mmol/L)	Capool (mmol/L)	Ppool (mmol/L)	TBILpool (µmol/L)	ASTpool (IU/L)
Mean		37,0867	5,6445	2,2755	2,5198	7,2450	60,6033
Median		36,3000	6,1000	2,2900	2,4100	8,0000	43,1500
Std. Deviation		6,16261	1,60404	0,23715	0,45334	3,34504	35,22053
Skewness		0,538	-0,264	0,519	0,126	0,322	0,750
Std. Error of Skewness		0,309	0,309	0,309	0,309	0,309	0,309
Kurtosis		0,060	-0,995	0,184	-1,501	-0,623	-0,886
Std. Error of Kurtosis		0,608	0,608	0,608	0,608	0,608	0,608
Percentiles	25	31,6500	4,1025	2,0900	2,0525	4,0000	31,6000
	50	36,3000	6,1000	2,2900	2,4100	8,0000	43,1500
	75	40,9500	6,8750	2,4325	2,9175	9,8750	95,5000
KS test of normality		0,659	0,263	0,973	0,281	0,282	0,012



Figures 1-12. Frequency distribution of metabolites in pooled sample (histograms) and line of theoretical normal frequency distribution



Figures 13-24. Q-Q plots of metabolites in pooled sample (circle) and line of theoretical normal frequency distribution

Conclusion

In this study, an ideal correlation was found between the values of metabolites in the pool of the sample and the mean values of individual samples participating in the pool. The mean value of the pool distribution and the standard deviation are similar to the frequency distribution of the reference values.

The frequency distribution of pooled blood samples of cows in early lactation has a normal frequency distribution. All the above confirms that pooled blood serum samples can be used to assess the metabolic status of a herd of cows in early lactation.

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Review paper

An analysis of Simmental breed production results in the municipality of Loznica before and during the COVID19 pandemic

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Abstract

The COVID19 pandemic exerted a strong impact on various sectors of the economy, including agriculture. This paper aimed to show whether the pandemic had also affected the situation in animal husbandry in the municipality of Loznica and to what extent. According to the official data, there were 886,000 cattle heads in our country in 2020, which is the historical minimum in livestock production. The long-term negative trend in the number of Simmental cattle heads in the municipality of Loznica has affected the total milk production, as well as the average milk production per cow. The COVID19 pandemic made a great impact on the agricultural production of this area. Exports and imports of goods were also impeded, which affected the cattle breeding process. At the beginning and in the course of the pandemic, the general interest in cultivating the land declined. The presented results show that the trend in decreasing the number of monitors was followed by the trend of decreasing the amount of produced milk, year in and year out. Also, the analysis of the results indicates that the value of milk fat content ranged from 3.85% to 4.16%. The highest value of milkfat content was in 2021, and the lowest value was recorded in 2018. The protein content had the lowest value in 2021 (2.90%,), and the highest value was measured in 2018 (3.06 %). The results are characterized by small variability between the years, because the research was conducted in the same area, on cows of the same breed composition and a similar diet. This trend of livestock production in the municipality of Loznica is a consequence of inadequate and non-standardized cattle nutrition due to the limited activities of agricultural producers following the COVID19 pandemic.

Key words: milk, production, milkfat, proteins, pandemic

Introduction

Agricultural production has a significant impact on Serbia´s economy, and concerning the total agricultural production animal husbandry is the dominating branch. Over the last year, the burning topic in our country as well as in the entire world was the COVID19 pandemic which will become synonymous with the beginning of the third decade of the 21st century (National Assembly of the Republic of Serbia, 2020). It has marked the year 2020 and it has entered with a relentless step into 2021, while it remains uncertain for how long it will be a part of our everyday lives and recently acquired behaviours. On the one hand, the epidemic has cast a shadow over and virtually stopped tourism, hospitality, hairdressing and beauty salons, shopping malls, sport and recreation activities, while on the other hand, it has enormously increased the demand and purchase of staple food (Vuković et al, 2021).

On the one hand, consumers are in a panic to obtain certain products, transport has been impeded and its price has increased, while on the other hand primary agricultural producers face multiple challenges in their response to the increased demand, with their access to seeds, protective equipment and transport seriously hampered (Virijević Jovanović et al, 2021). The majority of households in Serbia are engaged in agricultural production (90%). More than half of them (52%) are engaged in the processing of agricultural products, while only 5% are engaged in rural tourism. The most frequent forms of production are market gardening, dairy production, followed by sheep breeding, production of berries and pomes, cereals, jams, juices, preserves, poultry, pig production, and to a somewhat lesser extent production of nuts, spices and herbs, honey and cattle breeding.

Cattle breeding is an important animal husbandry branch and its share in the value of agricultural production is between 50% and 60% (Petrović D.M., et al, 2004). Cattle are the single largest producers of milk and meat, i.e., of staple food for human consumption and raw material for the food industry consumed by all population categories in the world. The Simmental breed is the most popular mixed breed in the world intended for the production of meat and milk. Nevertheless, it should be noted that Simmental breed milk production is lagging in our region in comparison to the world´s average, first and foremost due to poor diet and management. The Simmental breed is the finest beef cattle with marbled high-quality meat (Petrović, D.M. et al 2005).

In addition to various other economic sectors, agriculture was also under a strong impact of the COVID19 pandemic (Ostojić et al. 2021). Due to various external and internal issues, our cattle breeding industry has been facing a negative trend, i.e. a decrease in the number of cattle heads. One of the main reasons is an increasing migration of population from rural to urban areas, as well as the negative impact of the pandemic in our country and worldwide (Janković et al, 2021). The decreasing number of cattle heads on the one hand, and increased prices of cattle fodder on the other consequently led to increased meat prices. As to milk, the repurchase was mostly unhindered, but the amount was slowly decreasing. The usual flow of goods was slowed down at the beginning of the pandemic, and in some instances almost completely stopped. The reason was the shutdown of cattle products processing facilities because the virus had affected employees. This analysis aimed to show if and to what extent the pandemic influenced the state of cattle breeding on the territory of the Municipality of Loznica.

Number of cattle heads in the Republic of Serbia and the Municipality of Loznica in the period between 2018 and 2021

According to official data, there were 886.000 cattle heads in the breeding process in 2020. This is a historical minimum of cattle breeding, which is by 1,44% lesser than in 2018, while in 2019 there were 878.000 cattle heads in Serbia. It should be noted that at the beginning of 2020 there were 898.000 cattle heads in our country, whereby 12.000 young cattle in fattening were slaughtered for the purpose of export to Turkey.

Table 1. Trends in the number of cattle heads in the Republic of Serbia

Year	Cattle head number	Percentage of decrease or increase based on the number from the previous year
2018	899.000	100%
2019	878.000	-2,34%
2020	898.000	+2,23%
2021	886.000	-1,44%

Source: The Archive of PSSS Loznica

The latest available statistical data indicate that in the Republic of Serbia there were 886.000 cattle heads of all categories and breeds in 2020, while on the territory of the Municipality of Loznica there were 9.006 cattle heads, which makes 1,01% of the total cattle population on the territory of our country. By observing the period behind us, one can assert that the number of cattle heads was increasing between 1990 and 1991, then again between 1994 and 1995, as well as between 1995 and 1996.

The statistical data also indicate that there was an increase between 1997 and 1999, the period followed by a negative trend which lasted until 2004. The total number of cattle heads was increasing between 2007 and 2009, while after 2009 one could observe a continual decrease.

Table 2. Trends in the number of cattle heads in the Municipality of Loznica

Year	Number of cattle heads	Percentage of decrease based on the number from the previous year
2018	9.635	100,00%
2019	9.446	-1,97%
2020	9.143	-3,21%
2021	9.006	-1,50%

Source: The Archive of PSSS Loznica

The perennial negative trend in the number of cattle heads of Simmental breed on the territory of the Municipality of Loznica had an impact on the total milk production, as well as on average milk production per cow.

Conducting several zootechnical measures to improve cattle breed composition resulted in qualitative, but not quantitative results. The most significant measure is by far the application of artificial insemination.

Table 3. Trends in the number of cattle heads per breed on the territory of the Municipality of Loznica

Breed	Number of cattle heads within a specific breed	Percentage of participation of a breed in the total number of cattle heads
Brown Swiss Cattle	1	0,011%
Bush Cow	2	0,220%
Holstein-Friesian cattle	598	6,640%
Mixed breed	1133	12,580%
Montafon Cattle	1	0,011%
Simmental Breed	7271	80,730%

Source: The Archive of PSSS Loznica

The low number of Simmental cattle heads has had a negative impact on milk quotas when it comes to the territory of the Municipality of Loznica in the context of Serbia. Stopping the decline in the number of cattle heads, improving breeding conditions along with intensive selection with purebred as well as applying ameliorative crossbreeding of Simmental breed and dairy breeds are some of the measures to be applied by the Agriculture Regional Advisory Service and local self-government as a priority in Loznica municipality´s agricultural policy.

In this manner, the milk production capacity is significantly improved which is especially important for the affirmation of dairy cattle breeding and increase in the number of cattle heads. Furthermore, monitoring the effects of selection of Simmental breed, as well as crossbreeding Simmental and Red Holstein would lead to significant progress.

Table 4. Trends in the number of Simmental cattle heads on the territory of the Municipality of Loznica

Year	Number of cows	Percentage of decrease based on the numbers from the last year
2018	5.045	100,00%
2019	4.500	-10,81%
2020	4365	-3,00%
2021	4250	-2,64%

Source: The Archive of PSSS Loznica

As presented in the table above, the number of cattle heads in the Municipality of Loznica decreases year in and year out. Such a situation in cattle breeding in this region of Serbia has been influenced by two factors: demography and the COVID19 pandemic.

Demography: On the territory of the Municipality of Loznica one faces a decreasing number of young farmers. Like all other parts of our country, this region along the Drina river faces an increasing drain of young population and their migration to bigger cities or even abroad. Villages remain inhabited mostly by the elderly population and a fewer number of young people who were born and grown on larger farms. Very few young people started some form of agricultural production from the ground up. In villages around Loznica, one can encounter mostly smallholder farms that are massively closing down in these turbulent times. Those are mostly farms comprising up to three cows and a few hectares of arable land. It is in households located at least ten kilometers away from the town of Loznica that one can encounter over fifteen cattle heads on average, of different breeds. Due to lower repurchase milk prices, farmers have mostly shut down their production or decreased the number of heads. With the obtained amounts of milk, they started milk processing, i.e. they started producing cheese and skimming cream which they sold from home. There were examples such as these, but this type of processing declined by 60% as a result of the pandemic.

The COVID19 pandemic exerted a great impact on the agricultural production of this region, from the manner of preparing cattle fodder to the cattle products processing industry, primarily dairies and butchersAt the beginning of the pandemic and during the lockdown, general interest in farming declined. The pandemic which started two years ago had a great and mostly negative impact on the economy and various areas of life, including the agricultural production in the Loznica region. The negative impact is mostly reflected on production capacities relating to cattle fodder preparation, as well as on the cattle products processing industry. The most affected industries were the dairy and meat industries. The lack of cattle fodder led to a sudden increase in prices which additionally made cattle production expensive. The biggest increase in prices was related to concentrated cattle fodder, primarily corn and soybeans. A large number of producers resorted to “cheaper” bulky cattle fodder, i.e. grazing, of alfalfa and clover hay and silage Naturally, this resulted in a decrease in production which is confirmed by the results from the table, whereby one can notice the decline in an average amount of milk production per head, year in and year out. Pešić et al (2020) state in their work that it is a well-known fact that milkfat is inversely proportional to the amount of milk, i.e. the bigger the amount of milk, the smaller the percentage of milkfat and vice versa, which is confirmed by the research results. In addition, the amount of milkfat is influenced by the diet as well. Bulky cattle fodder has a positive impact on the percentage of milkfat. Proteins in milk can be corrected by the process of selection (Bogdanović et al, 2012) but based on the results presented in the table one can see that the selection process was impeded, which additional had a negative impact on the percentage of milk protein.

Production results of Simmental breed for the period between 2018 and 2021 on the territory of the Municipality of Loznica

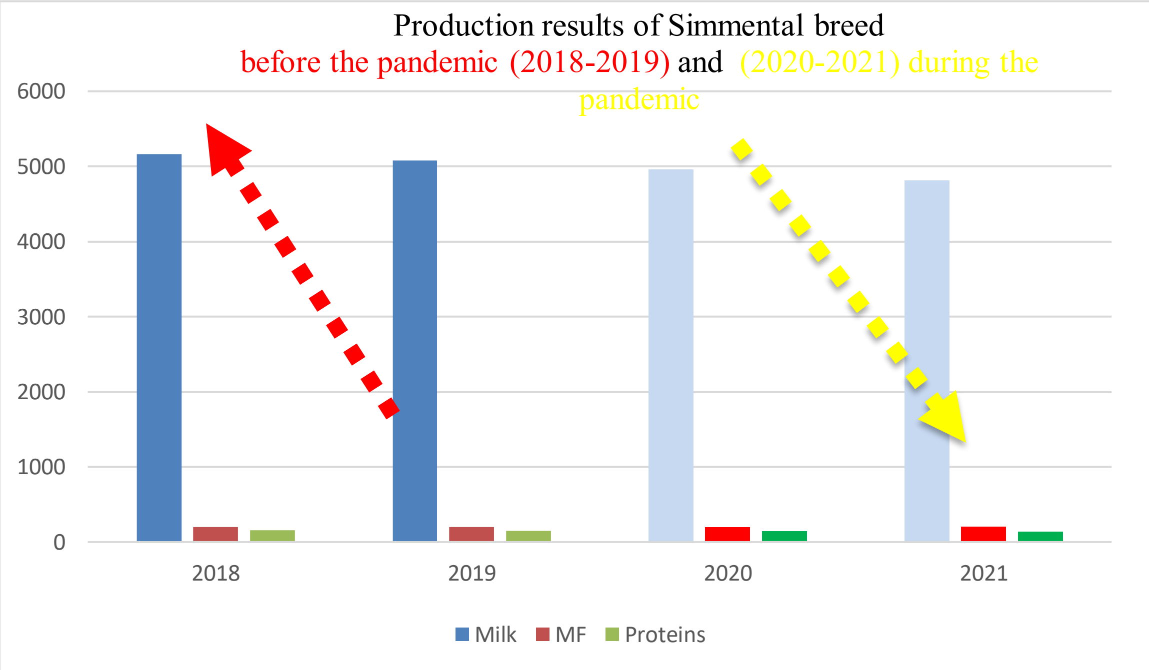
In the Simmental breed production process, the authors monitored the production results of Simmental breed: the total amount of produced milk, the amount of milkfat, and protein. Likewise, the authors have established the deviation from the arithmetic mean and the coefficient of variation within the group. Similar results were obtained by Lazarević et al (2013).

Table 5. Simmental breed production results on the territory of the Municipality of Loznica for the period between 2018 and 2021

Year	Number of cows	Total dairy production	Standard lactation of 305 days									
			$\bar{X}$	sd	CV	INDEX	MM			PROTEINS		
	head	liter	liters	liter	%	%	%	Sd %	Cv %	%	Sd %	Cv %
2018	5045	26 032 200	5160	2.081	5.01	100,00	3,85	0,27	0,067	3,06	0,20	0,044
2019	4500	22 837 500	5075	1.987	4.86	98,35	3,96	0,34	0,104	2,94	0,21	0,047
2020	4365	21 650 400	4960	1.678	4.81	97,77	4,01	0,46	0,020	2,91	0,25	0,072
2021	4250	20 442 500	4810	1.780	4.89	86,97	4,16	0,66	0,391	2,90	0,20	0,043

Source: The Archive of PSSS Loznica

The presented results indicate that the trend in decreasing the number of cattle heads was followed by the trend in a decreased amount of produced milk, year in and year out.The Table shows that the average percentage of milkfat ranged from 3.85 % to 4.16%. The largest value of milkfat was noted in 2021, while the smallest value was noted in 2018. The protein content was the smallest in 2021 (2.90%), and the largest measured amount was in 2018 (3,06%). The presented results are characterized by a small variability between the years because the research was conducted in the same region with cows of the same breed and on a similar diet. This trend of cattle breeding on the territory of Loznica municipality is a consequence of inadequate and non-standardized cattle diet caused by limited activities of farmers as a result of the COVID19 pandemic.



Graph 1. Simmental breed production results before and during the pandemic

Since all countries, including Serbia, take increasingly strong measures to suppress the spread of the COVID19 pandemic (Naled-5, 2020), self-isolation and temporary closure of businesses, green markets, shops, and other enterprises exerted an impact on the habits and practices relating to the behaviour and work of farmers, which was reflected in the manifestation of agricultural potentials of Simmental breed on the territory of Loznica municipality. The results indicate that the organisation of agricultural production was much easier before the pandemic, while during the pandemic due to difficult working conditions the branch of cattle breeding was seriously affected.

Conclusion

Due to the corona virus pandemic, the livestock, milk production and processing sector has been severely shaken. For several months now, there have been great turbulences in cattle breeding, especially in the fattening and marketing of cattle, but also in the production and processing of milk.

The COVID19 pandemic has had an impact on agriculture as much as on any other branch of the economy. According to the official data, there were 886.000 cattle heads in our country in 2020, which represents the historical minimum in the cattle breeding sphere.

The perennial negative trend in the number of Simmental cattle heads on the territory of the Municipality of Loznica has had an impact on the total milk production, as well as on the average milk production per cow. For practical purposes, various zootechnical measures have been conducted to improve the breed composition of cattle. The low number of Simmental cattle heads had a negative effect on milk quotas when it comes to the territory of the Municipality of Loznica in the context of Serbia.

The COVID19 pandemic had a great impact on the agricultural production of this region, from the manner of preparing cattle fodder to the cattle products processing industry, first and foremost dairy and butchers. The export and import of goods were impeded as well, which also had an impact on cattle breeding. At the beginning of the pandemic and during the lockdown, general interest in farming declined. The pandemic which started two years ago had a great mostly negative impact on the economy and various areas of life, including the agricultural production in the Loznica region.

As the economy is in recession and public debt is in full swing growth, space for future incentives in livestock production is limited. Therefore, further institutional reforms are needed to return the economy to sustainable growth, he said jobs and restored livestock production to Pandemic levels.

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Original scientific paper

A review of pheasant hatching production results at the *Ristovača* pheasant farm in the period between 2019 and 2021

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Abstract

The paper presents the results used from the registry records in the pheasant farm "Ristovača" for the period from 2019 to 2021. They show data on the number of incubated eggs, the number of hatched pheasants and the mortality of pheasants that were achieved by stages of production in the observed period. Today, in the hunting grounds of Serbia, there are a large number of production and technical facilities that are used for artificial breeding of pheasant chickens. Growing pheasants in controlled conditions is a necessity and a need to increase the insufficient natural production of pheasants. The health condition of the parent flock, the general condition of the individuals and the condition of the parent pairs affect the quality of the obtained eggs, ie a larger number of fertilized and a smaller number of scrapped eggs. Based on previous research, it can be concluded that egg hatching of 75% represents the limit of profitability in controlled pheasant breeding conditions. In our research, the average egg hatching was 80.38% and ranged from 77.45% to 81.94%. This effect was the result of high egg fertilization by an average of 87.58%. Poor production results and a high degree of scrapped eggs were observed in the period of bad weather conditions and extreme temperatures (13.48%). The success of growing pheasant chickens largely depends on climatic conditions, so it is recommended to grow pheasants in controlled conditions where it is possible to achieve better production results.

Key words: pheasant, breeding, hunting ground, fertilization, feasibility.

Introduction

The pheasant is one of the most numerous and productive species of feathered game in hunting grounds in Serbia and abroad, owing to their great adaptability to different habitats and various changes that occur in our environment. The increase in the number of pheasant game in hunting grounds is hugely impacted by hunters´ interests in attractive pheasant hunting, as well as by the market needs for quality meat of this particular game bird. The first and primary production is the one that aims to provide a sufficient number of pheasants to inhabit the hunting grounds thus increasing the population of pheasants (Gajić 1994, Beuković & Popović, 2014). To what number should the pheasant population be increased is determined by the hunting demand in hunting tourism, i.e. by the possibility of placing pheasants in hunting grounds. The main reasons for decreasing the number of pheasant population in Serbian hunting grounds are mostly attributed to the human factor: significantly reduced hunting areas, deteriorating natural food resources, increasingly mechanical and chemical nature of agricultural production, increased percentage of hunting, etc. (Đorđević et al., 2012; Popović et al., 2008, 2011). For the same reason, in numerous countries, people have developed the system of artificial growth of pheasants and their subsequent release into the hunting grounds as mature birds. An important role in the artificial growth of pheasants is played by the parent flock. The parent flock can be formed with pheasants obtained from other producers or by catching them, although the most frequent manner of forming the parent flock is using one´s own flock raised specially for those purposes. Individual birds set aside for the parent flock are more superior in characteristics to other individuals, without any indication of later external defects, at the age of eight weeks when one can establish the sex without any doubt. The advantages of this manner of breeding are reflected in achieving higher production capacities, systematic work, and planned production. Such flocks are under constant breeding and health control, and their diet is adjusted to the status and level of production (Gajić & Popović, 2010).

When allocating pheasant chicks for the parent flock one should allocate at least 30% of chicks more than the planned number of mature individuals in the parent flock, due to possible losses in the breeding process. To perform a successful selection of the best individuals one needs to keep an accurate record of game breeding, egg-laying, percentage of fertilization and egg hatching, individual marking of pheasant chicks according to families, and take care of further record and classification. Before releasing pheasants to aviaries, it is necessary to disinfect the equipment there, such as feeders, drinking troughs, and standing posts. Subsequently, one needs to carry out the vaccination of pheasants released into aviaries, either individually or within families. The paper aimed to present the production results of the parent flock at the *Ristovača* pheasant farm conditioned by different internal and external conditions.

Material and Methods

The technology of breeding pheasants at the Ristovača pheasant farm

The largest pheasant breeding farm in Serbia and one of the largest in Europe with a production capacity of 220.000 one-day-old pheasants is located at the game breeding farm *Ristovača* in the town of Bač, and it is managed by Lovoturs, a branch of PU „Vojvodinašume“. The pheasantry covers a total area of 34 ha, whereby 29 ha comprise aviaries for pheasant breeding and rewilding. At the pheasantry, the process of production is technologically complete, from aviaries with breeding boxes and accommodation of one´s parent flock, incubators and battery stations, brooders with wire outlets, and rewilding aviaries. Pheasant chicks from five to ten weeks old are the category of pheasants that hunting associations purchase the most to feed and raise them in their shelters and settle them in their hunting grounds, and their delivery period is from mid-June to the end of August. The pheasants which are 3 to 4 months old are mostly purchased by hunting associations without adequate shelters, i.e. there are no adequate housing conditions and their delivery period is from mid-August to mid-October. In the period between mid-October and the end of January, hunting pheasants are delivered to hunting associations that have highly unfavourable habitats for pheasants, as well as to associations that have their particular hunting ranges intended for intensive hunting of pheasants for commercial purposes, and which are released into hunting grounds immediately before the hunt. Depending on the purpose, i.e. on the time that pheasants spend in aviaries, they are divided into winter and summer aviaries. The pheasants from the parent flock are kept in winter aviaries outside the reproductive season, and summer aviaries during the preparation and production season. The floor area in aviaries is 5m^2 to 10 m^2 per individual bird.

Collecting, keeping, and storing eggs. The biological quality of eggs relates to the fertilization of eggs, the manner and length of keeping them, and to the choice of eggs for hatching. Consequently, the organised manner of collecting and storing eggs, as well as keeping them is essential to obtain better production results. It is most desirable that eggs for one batch of offspring be uniform in age. However, in reality, this is almost impossible to achieve and hatching eggs have to be collected in the period of several days. In that case, the issue of storing eggs is also raised, so that they would not lose much of their quality. Laid eggs are collected six times a day. The first round of collecting eggs has to be done early in the morning when birds are fed, and every next round should take place in the range of three hours – in the afternoon. It reduces the risk of eggshells getting dirty, of hens cracking the eggs, or contaminating the eggs to a greater extent. Likewise, collecting eggs is best done with clean hands to prevent microorganisms to affect the egg or the embryo. To reduce the risk of infection, eggs are collected with two fingers by placing them on the two ends of an egg, the top and the bottom. The collected eggs are placed in special facilities with a temperature set between 10°C and 14°C and relative humidity of 60% (Pešić, M. B., 2015). In these facilities, it is allowed to store eggs for up to seven days, because after that period the biological quality of eggs is reduced as well as the percentage of hatching. Any irregularly shaped egg, which is either too small or too big, is culled immediately. As a rule, eggs are not washed to avoid clogging the pores on the eggshell. After collecting, eggs are kept arranged in trays. If they are kept for more than a few days they should be turned more frequently. Under natural conditions, it is difficult to create a good ambiance for fertile eggs. Thus, one needs to resort to an artificial way of warming them during the winter and cooling them during the summer. Fertile eggs are kept in a dark well-ventilated room. When setting eggs into an incubator it is necessary to disinfect them to destroy the pathogens from the surface which could be transferred into the incubator. All eggs collected for the incubator are disinfected with formaldehyde vapour, 30ml of formaldehyde (40% concentration), and 20 g of hyper manganese. The disinfection of the incubator is mandatory, too.

Setting the eggs, the setting programme, ovoscopy, and egg hatching. To rationally implement all phases of chicks´ hatching and breeding, the eggs are set in the incubator according to the previously devised plan. This is how setting batches are formed. The ovoscopy is done six days after setting them on incubator trays. All tray shelves with the same date and with six-day-old eggs are taken out of the incubator and left in a dark room. For the purpose of ovoscopy one uses a lamp with a handle. It is placed on the lower side of the shelf for candling the eggs to establish whether there is an embryo in an egg or not. All unfertilized eggs are safely culled from the tray shelf. The percentage of unfertilized eggs varies in the laying period from 10% to 17%. To achieve good results, it is necessary to secure a good ambiance for this sort of production. The normal temperature in the incubator is 38°C, while the humidity is from 82% to 84% for old types, and 78% to 80% for the new type. The temperature in the room where the incubator is placed should be between 22 °C and

24 °C, while humidity should be 65%. The relative air humidity in the hatcher is between 86 % and 88%. When placing chicks into batteries the air temperature is 35 °C, whereby corrections are due, i.e. the temperature tends to decrease as little pheasant chicks grow (1 degree per day). The ventilation in the room should be continual.

Coming out (hatching) in the hatcher and transfer to batteries

After 21 days the eggs which had previously been in incubators are now transferred to hatchers where they will stay for four days. In the period of four days, they will hatch and dry. For 25 days in the facilities and batteries, the space is heated and microclimatic conditions are being secured for the arrival of chicks which are now dry and capable of further growth and development. The number of hatched pheasants from set eggs depends on the quality of incubation and the number of fertilized eggs. The temperature in the hatcher is 38 °C, while relative air humidity is from 86% to 88%.

Rearing in batteries

Heating the facilities in which batteries are placed to receive the chicks is of utmost importance for the further growth of little pheasants. The temperature in the room with batteries needs to be stable and it has to be 35°C, and after introducing pheasant chicks every day for the upcoming period, the temperature tends to decrease 1°C at a time. In the room, one should have the heaters and fans turned on at the same time to heat the fresh air that enters the batteries before entering the room. The chicks are fed every day by placing fresh food into the troughs at the edges of batteries. The pheasants are fed with ground marble, whether separately or as a part of the food mixture, because this is necessary at this age for the development of their muscular stomach and the improvement of the digestive function. Daily battery hygiene is mandatory.

The procedure with little pheasants in the battery

Before transferring little pheasants to batteries, a very rigorous selection of one-day pheasants is carried out, whereby strong, vital, and healthy chicks are left in the flock, primarily because pheasants are very sensitive in the first days of life and the biggest losses are recorded in that period. The pheasants´ feed needs to be continuous and continual from the first day of their lives even though little pheasants´ feed starts from the second day because they come into this world with large reserves of unabsorbed yolk. When transferring little pheasant chicks into the batteries one needs to take care of not overcrowding the batteries.

Overcrowding of batteries can lead to unnecessary losses which are reflected in suffocation due to overcrowding, pecking (cannibalism), constant harassment, and the lack of space for rest and time for proper digestion. To reduce the number of dead chicks (mortality rate) it is necessary to carry out daily control of chicks in the facility because accidents happen in batteries as a result of walking on a wire net. Likewise, one should control blow-heaters, artificial hen heaters, as well as the amount of food and water.

Brooders

After two weeks in batteries, pheasant chicks are transferred into brooders. They can be either prefabricated or made of bricks with two-part outlets, whereby each outlet has feeders and drinking troughs. The first outlet is covered with a sandy base, while the other, the grassy one, is without an eave. Both are enclosed with a wire fence. Each brooder has a door on the front which is used by workers to communicate when they feed the chicks and control the water flow into the covered part. It is possible to release the chicks from brooders into an aviary by lifting the wire and allowing the chicks to go freely into the part with tall and unmown grass.

Collective aviaries (rewilding)

As the pheasant chicks mature and grow, they need to change their habits and become prepared for rewilding, i.e. for natural habitats. After three to four weeks spend in brooders, pheasants are transferred into a specially enclosed space (aviary) which serves the purpose of rewilding. The aviaries, places for rewilding of pheasants, are fenced with a 1.8m to 2m high wire. The space inside the aviary is planted with different annual crops: corn, hemp, sunflower, etc. Likewise, in this enclosure, some covers serve to protect little pheasants from natural disasters, strong rain, and sun rays. After reaching the age of eight weeks, the chicks are caught and further distributed from those enclosed spaces to the shelters in hunting grounds managed by hunting associations. This phase of breeding (from 3 to 6 weeks of age) is often carried out at the hunting ground. Namely, the majority of hunting associations take little pheasants age 5 to 6 weeks from pheasant farms and they keep them in specially equipped shelters at the hunting ground to help them adapt to the natural environment to the greatest extent possible.

Results and Discussion

The success and profitability of pheasant farms depend on the percentage of fertilized eggs and eggs hatching. In practice, it has been recorded that the number of fertilized eggs and the number of hatched chicks is smaller in the first and last production batches, while the reasons are manifold. One of the reasons is the laying capacity which is uneven in the beginning, the eggs are smaller and often deformed. Likewise, weather conditions such as low temperature, rain, and frequent temperature fluctuations can be the factors. In the last batches, in addition to weather conditions and laying percentage, there are also problems in birds themselves. Roosters are worn out, and on hens´ backs, one can frequently notice injuries from roosters´ sharp claws during mating. A correct and precise record of production results is one of the conditions for proper business decision-making. In Table 1 one can notice that the first egg setting recorded in 2019 was on April 7, while the last egg setting to incubators recorded in 2019 was on June 16. In this period the pheasant farm had eleven iterations whereby there were 196.568 eggs set in incubators, while the number of hatched chicks was 141.380, which is 81,94%.

Table 1. – The results of hatching of pheasant chicks per iteration in 2019

Batch	Date of setting the pheasant eggs	The number of set eggs (piece)	Eggs culled after candling		The number of fertilized eggs	% of hatched eggs	Hatched chicks	
			piece	%			piece	%
1.	07.04.	8820	1317	14,93	7503	85,06	6280	83,7
2.	14.04.	17640	1902	10,78	15738	89,21	12900	81,97
3.	21.04.	21336	2554	11,97	18782	88,02	15900	84,66
4.	28.04.	21336	2363	11,08	18973	88,92	16280	85,81
5.	05.05.	21336	2279	10,68	19057	89,31	15880	83,33
6.	12.05.	21336	2035	9,538	19301	90,46	16150	83,67
7.	19.05.	21336	2461	11,53	18875	84,46	15640	82,86
8.	26.05.	21336	2409	11,29	18927	88,7	15500	81,89
9.	02.06.	17640	2238	12,69	15402	87,31	12470	80,96
10.	09.06.	21336	3529	16,54	17807	83,45	13020	73,12
11.	16.06.	3116	932	29,91	2184	70,08	1360	62,27
Total/average		196.568	24.019	12,22	172.549	87,78	141.380	81,94

Source: internal documents of the Ristovača pheasant farm.

By observing the data per iteration one can notice that the percentage of hatching is the most favourable in the third and fourth iteration and that it was 84,66% and 85,81%. The eggs from these iterations were in incubators from April 21 to May 16, 2019, i.e. from April 28 to May 23, 2019. The largest percentage of fertilized eggs was in the sixth iteration and it was 90,46%, while the worst was in the eleventh iteration and it was 70,08%. The worst result was recorded in the eleventh iteration and it was 62,27%. The reason for decreased hatching and fertilization percentage was a high temperature in mid-June, i.e. the eggs were set on June 16, and the pheasant chicks were hatched on July 11, 2019. Research studies conducted by some authors (Jović, 1960; Jović, 1964; Gajić & Jović, 1969, Gajić 1975) show that with the sex ratio of 1:12 in favour of female pheasants, fertilization was 85%. Nowadays, in Europe as well as in Serbia collective growth is practiced, with 80 to 140 chicks per box and the sex ratio of 1:8-10, which has proved to be more practical. With this manner of breeding, the laying capacity was 5% to 10% smaller, but the percentage of fertilization was between 80% and 95% (Popović & Stanković, 2009).

Egg fertilization in the natural environment can achieve a value of 96%, but due to large losses (up to 85%), the real growth per hen is rather poor (Bojović, 2012).

The results of hatching of pheasant chicks in 2020

By observing the data per iteration one can notice that the percentage of hatching was the best in the second and fifth iteration and that it was 84,23% and 83,59%.

The eggs from those iterations were in the incubator from April 12 to May 7, 2020, i.e. from May 3 to May 28, 2020.

Table 2. The results of hatching of pheasant chicks per iteration in 2020

Batch	The date of setting the pheasant eggs	The number of set eggs (piece)	Eggs culled after candling		The number of fertilized eggs	% of fertilized eggs	Hatched chicks	
			piece	%			piece	%
1.	05.04.	8820	1219	13,82	7601	86,17	6000	78,94
2.	12.04.	17640	2099	11,90	15541	88,10	13090	84,23
3.	19.04.	17640	1825	10,35	15815	89,65	12320	77,90
4.	26.04.	17640	2181	12,36	15459	87,63	10400	67,27
5.	03.05.	17640	2327	13,19	15313	86,8	12800	83,59
6.	10.05.	17640	1623	9,201	16017	90,79	12900	80,54
7.	17.05.	17640	1928	10,93	15712	89,07	12600	80,19
8.	24.05.	17640	2409	13,66	15231	86,34	12586	82,63
9.	31.05.	17640	2417	13,70	15223	86,29	9550	62,73
10.	07.06.	17542	3508	20	14034	80,0	11400	81,23
11.	14.06.	15092	3066	20,32	12026	79,68	8700	72,34
Total/average		182.574	24.602	13,48	157.972	86,52	122.346	77,45

Source: internal documents of the Ristovača pheasant farm.

The largest percentage of fertilized eggs was recorded in the sixth iteration and it was 90,79%, while the worst was in the eleventh iteration and it was 79,68%. The worst result was recorded in the fourth and ninth iterations. In the fourth iteration, the hatching percentage was 67,27%, while in the ninth iteration it was 62,73%.

The results of hatching of pheasant chicks in 2021

By observing the data in Table 3 one can notice that the percentage of hatching per iteration is the best in the fourth and seventh iteration, and it was 84,34% and 86,07%. The eggs in those iterations were in the incubator from April 25 to May 20, 2021, i.e. from May 16 to June 10, 2021. The largest percentage of fertilized eggs was in the third iteration and it was

90,89% and the smallest in the eleventh and it was 82,85%.

The worst result was recorded in the first iteration and it was 67,495. In addition to the first, the second, third and last iterations did not provide satisfactory results which were expected to some extent. According to the research conducted by Ristić (2006), the percentage of fertilization was around 87,25%.

Table 3. The results of hatching of pheasant chicks per iteration in 2021.

Batch	The date of setting the pheasant eggs	The number of set eggs (piece)	Eggs culled after candling		The number of fertilised eggs	% of fertilized eggs	Hatched chicks	
			piece	%			piece	%
1.	04.04.	8820	1367	15,5	7453	84,5	5030	67,49
2.	11.04.	17640	1877	10,64	15673	88,84	11675	74,07
3.	18.04.	17640	1606	9,104	16034	90,89	12630	78,77
4.	25.04.	26460	2651	10,02	23809	89,98	20080	84,34
5.	02.05.	26460	2509	9,482	23951	90,51	20160	84,17
6.	09.05.	17640	1764	10	15876	90	13200	83,14
7.	16.05.	26460	2672	10,10	23788	89,9	20475	86,07
8.	23.05.	26460	3118	11,78	23342	87,62	19040	81,57
9.	30.05.	17640	2101	11,91	15539	88,08	12700	81,73
10.	06.06.	17640	2998	17	14642	83,0	12200	83,32
11.	13.06.	13328	2285	17,14	11043	82,85	8500	76,97
Total/average		216.098	24.948	11,54	191.150	88,45	155.690	81,41

Source: internal documents of the Ristovača pheasant farm.

The results of hatching of pheasant chicks per year

As it has already been noted, the smallest percentage of hatching eggs was in 2020, and the largest in 2019. By monitoring the number of fertilized eggs it was established that the percentage of fertilised eggs was the largest in 2021, but due to a large number of factors (the incubator mode, frequent and heavy rainfall, and frequent temperature fluctuations), the number of fertilized eggs and consequently the percentage of hatched chicks were smaller in comparison to the number of fertilized eggs.

Table 4. The results of hatching per year in the period between 2019 and 2021

Year	The number of set eggs	The number of fertilized eggs	% of fertilized eggs	The number of hatched chicks	% of hatching (relative to the number of set eggs)	% of hatching (relative to the number of fertilized eggs)
2019.	196.568	172.549	87,78	141.380	71,92	81,94
2020.	182.574	157.972	86,52	122.346	67,01	77,45
2021.	216.098	191.150	88,45	155.690	72,04	81,41
Total	595.240	521.671	87,64	419.416	70,46	80,38

Source: internal documents of the Ristovača pheasant farm.

Conclusion

The success of growing pheasant chicks largely depends on climate factors, which are beyond human control. Temperature fluctuations, cold and humid weather can cause losses up to 50%, as well as high temperatures. Bearing in mind that the Ristovača pheasant farm conducts intensive pheasant growth under direct human influence, this manner of growth requires the large engagement of breeders, from building facilities, forming the parent flock, organising technical processes during production, collection, storing and keeping eggs, setting the eggs, all the way to growing the pheasant offspring. The breeding of pheasants at the pheasant farms and releasing their offspring into the hunting grounds has enabled the sustainable number of this type of game.

Forming the parent flock under the conditions of intensive production can have a positive impact on the quality of bred pheasants because the parent flock is under constant health control while the obtained chicks are of good quality.

In recent years an increasing amount of attention is paid to the intensive growth of pheasants at pheasant farms because this manner of growth provides one with a larger and safer number of offspring of this type of game, which has been endangered in recent years due to changes in environmental conditions. Owing to the indicated problems and shortcomings it is necessary to continue with the research so that the current experiences of producers as well as implemented measures could be corrected to achieve better production results per set chick of the parent flock.

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Original scientific paper

Effects of high water temperature on embryonic development and different times of initial nutrition on survival and growth of juvenile rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The aim of this study was to determine the duration of individual stages of embryonic development at high water temperatures, the effect of initial feeding time on survival of rainbow trout to three months of age and growth characteristics of three-month-old rainbow trout. The research was carried out in laboratory conditions for 144 days, and consisted of three parts; I - effects of high water temperature (15.16±0.79°C) on the duration of embryonic development, II - effects of initial feeding time on survival of rainbow trout to three months of age and III - effects of initial feeding time on growth of three-month-old rainbow trout. Embryonic development in stages lasted; 14 days (221 degree day) until the appearance of the eyes, 18 days (280 degree day) until the beginning of the hatch, 24 days (368 degree day) the hatch was completed and 33 days (500 degree day) all the fish swam. In the second part, after swimming, two groups of 300 fish were formed. The initial diet with commercial feed in the G_{12} group started 12 (528 degree day) and in the G_{16} group 16 days (580 degree day) after the outbreak. Mortality during the second period (G_{12} = 5.33% and G_{16} = 3.33%) was not statistically significant ($p > 0.05$). In the third part, it has been determined a statistically significant difference in mean weight, total length and fork length ($p < 0.05$) was found, as a result of different time initial diet. In the second week, a significant difference ($p < 0.05$) was found only for the fork length, and no significant differences were found until the end. No significant difference was found between the observed groups in the condition factor (CF), the feed conversion ratio (FCR), the specific growth rate (SGR) and the thermal unit growth coefficient (TGC).

Key words: embryonic development, survival, initial diet, growth, rainbow trout

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a commercially very important cold-water species that is bred in many countries around the world, practically wherever there are appropriate conditions for it. Rainbow trout has good growth characteristics and a fast production cycle, which is highly dependent on the conditions of the breeding environment and the applied cultivation technology. Since it is a cold-water, salmonid species, its natural habitat are clean, clear and cold waters of the first class of quality. Accordingly, the optimal temperatures for spawning and embryonic development of rainbow trout are 8-10ºC, and for the initial breeding 10-12ºC (Marković and Mitrović - Tutundžić, 2003). Higher water temperature causes faster embryonic development, but also greater losses. Adamek (2006) states that, at a water temperature of 15ºC, the survival of rainbow trout during embryonic development is 75%. Myrick and Cech (2001) and Carter (2005) state that the optimal temperatures for incubation of rainbow trout eggs are 7-10ºC, and water temperatures <5ºC and > 10ºC affect the lower survival of rainbow trout during embryonic development.

Material and Methods

The experiment was carried out in laboratory conditions for 144 days, and it consisted of three segments; I - effects of high water temperature (15.16±0.79ºC) on the duration of embryonic development, II - effects of initial feeding time on survival of rainbow trout to three months of age and III - effects of initial feeding time on growth of three-month-old rainbow trout.

The first part of the experiment: fertilized rainbow trout eggs were placed in embryonic development trays in the aquaculture laboratory of the Faculty of Agriculture in Banja Luka after spawning and fertilization. The fertilized eggs (2,300 eggs / trays, a total of 6,900 eggs) were placed in 3 trays placed in flowing aquariums. The dead eggs was counted and removed from the trays every day, and the eggs was not treated with means for the prevention of fungal infections.

The second part of the experiment: after swimming, 600 fry were divided into two groups (300 fry / group) and three repetitions (100 fry / repetition) per group. Nutrition in the G_{12} began 12 days (528 degree day) after the end of the hatching, and in the G_{16} 16 days (580 degree day) after the end of the hatching. The second diet period lasted 65 days for the G_{12} group and 61 days for the G_{16} group. Average weight (g) ± standard deviation (SD) was determined after 65 days in group G_{12} and after 61 days in group G_{16} (scale Acculab, accuracy 0.1 g).

The third part of the experiment: the same pattern was continued with a smaller number of fish, 180 fish were isolated from a random sample, 90 fish / group with three replicates (30 fish / replicate) and the diet was continued for the next 42 days to determine growth characteristics. Total body length (TL), fork length (FL) and body weight (BW) were measured at baseline and at two-week intervals. Total body length (cm) and fork length (cm) were measured with an ichthyometer (accuracy 0.1 cm) and body weight with an Acculab scale (accuracy 0.1 g). Prior to measurement, individuals were anesthetized using the anesthetic 2-phenoxyethanol (2.5 ml / 10 liters of water). The average weight (g) ± standard deviation (SD) of two-month-old juvenile rainbow trout in G_{12} was 3.04±0.60 at the beginning (70 days old), and in G16 2.72±0.61. Commercial feed with the following composition was used for nutrition: crude protein 50%, crude fat 20%, crude fiber 1%, crude ash 8.5%. Daily dietary norms were determined based on fish weight and water temperature as well as recommended dietary tables of feed producers.

Water temperature and dissolved oxygen content were measured with an oximeter (Oxi 330i / SET 2B20–0011 WTW Germany) and pH with a pH meter (pH 330i / SET 2A20– 1011 WTW Germany). Conversion rate, survival and growth characteristics were determined using the following formulas:

Condition factor (CF): $CF = \left(\frac{BW}{L^3}\right) \times 100$

BW – body weight (g), L – body length (cm).

Feed conversion rate (FCR): $FCR = \frac{F}{G}$

F - feed consumption, G - achieved weight gain.

Specific growth rate (SGR): $\mathrm{SGR} = \left(\frac{(\mathrm{InFBW} - \mathrm{InIBW})}{\mathrm{D}}\right) \times 100$

where: FBW - final body weight (g), IBW - initial body weight (g), ln - natural logarithm, D - number of days.

Survival (%) = = $(N_t/N_0)*100$

N_t and N_0 number of fish at the end and number of fish at the beginning (n)

$$TGC = \frac{\left[FBW^{1/3} - IBW^{1/3}\right]}{\sum\left[T \times D\right]} \times 100$$

FBW – final body weight (g), IBW – initial body weight (g), T – water temperature (°C), D – number of days.

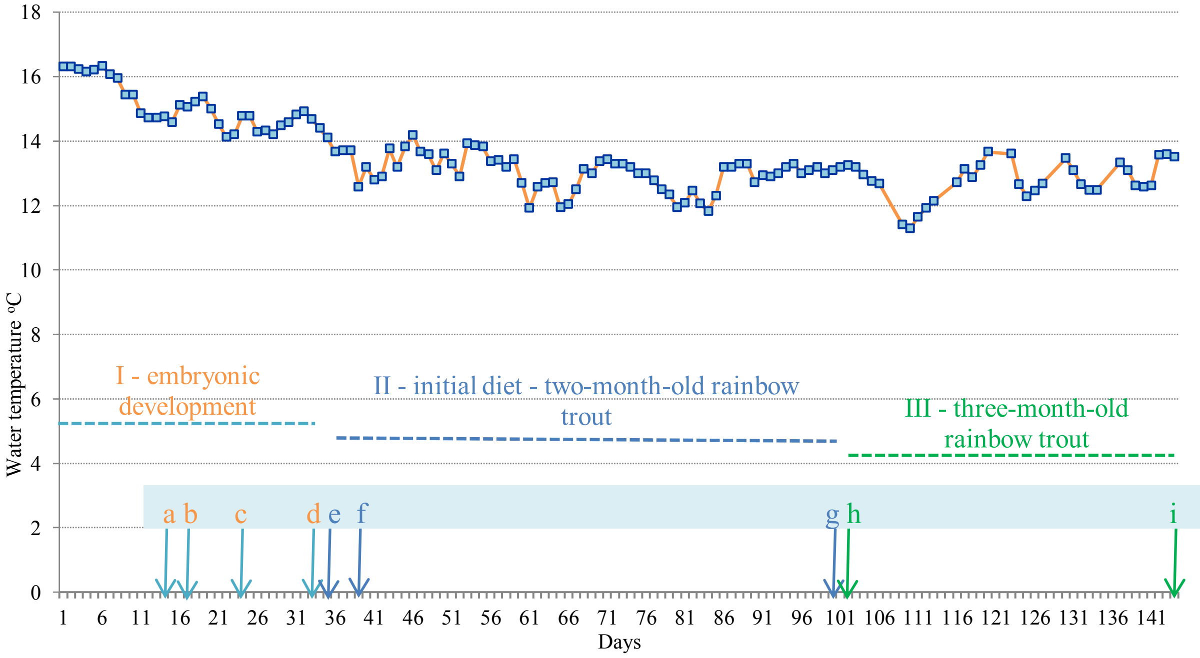
Statistical analysis determined: average value, standard deviation and coefficients of variation, and the significance of differences in the observed characteristics using the t test.

Results and Discussion

During the realization of the experiment, the water temperature was different in some phases. The highest water temperatures were during embryonic development, in the further part of the experiment the temperatures had a decreasing trend.

First part

The water temperature during embryonic development was significantly higher than the desired temperature for incubation of fertilized rainbow trout eggs, which caused more intensive embryonic development (Graph 1), but also higher mortality of fertilized eggs. During embryonic development, the average water temperature (°C) ± standard deviation (SD) was 15.16±0.79 (min. 13.90; max., 16.3°C), with a coefficient of variation of 5.23. Weber et al. (2016) state that on the first day of incubation (after fertilization) at a water temperature of 5°C, survival is lower compared to survival at water temperatures of 10°C and 14°C during incubation.



Graph 1. Water temperature during the experimental period (144 days)

Legend:

a - I – stage of eyes (14 days after fertilization, average degree day±SD; 221±2.97 degree day)
b - I – beginning of hatching (18 days after fertilization, average degree day ±SD; 280±4.24 degree day)
c - I – completed hatching (24 days after fertilization, average degree day ±SD; 368±6.33 degree day)
d - I – all fish swam (33 days after fertilization, average degree day ±SD; 500±7.77 degree day)
e - II – initial diet in G_{12} (12 days from the end of hatching to the two-month-old rainbow trout, 528 degree day)
f - II – initial diet in G_{16} (16 days from the end of hatching to the two-month-old rainbow trout, 580 degree day)
g - II – two-month-old rainbow trout
h - III – start of cultivation of two-month-olds rainbow trout
i - III – completion of cultivation of three-month-olds rainbow trout

The effects of higher water temperature are reflected in faster embryonic development, according to Hinshaw & Thompson (2000) that at a water temperature of 12.77ºC it hatching about three weeks after fertilization, and at 7.22ºC in about seven weeks. Savić et al. (2012) state that at an average water temperature of 10.4ºC, the length of embryonic development of rainbow trout, from fertilization to swimming, was 44-47 days. Hoitsy et al. (2012) state that is required 500 degree day from fertilization to swimming of the rainbow trout, in accordance with the results obtained in this study (Graph 1). Higher water temperatures during embryonic development rainbow trout lead to increased mortality, which was 67.72% in this study. The cause of high mortality, in addition to high temperature, is partly due to the fact that the eggs was not treated with chemical agents to combat fungal infections. The dead eggs were manually removed. Barnes et al. (2000, 2005) recommend the use of chemical agents to control fungal infections during embryonic development of rainbow trout. Savić et al. (2012), Kocaman et al. (2009), Marković & Mitrović - Tutundžić (2003) state that at optimal water temperature and other water quality indicators, mortality during embryonic development of up to 10% is an acceptable loss in this phase of development.

Part two

During the initial feeding and during the first two months, the average water temperature (°C) ± SD was in G_{12} 12.96 ±0.68 (min. 11.10; max., 14.60°C) with a coefficient of variation of 5.28, and in G_{16} 13.01±0.73 (min. 11.20; max., 14.60°C) with a coefficient of variation of 5.59.

After 12 days (from fertilization 528 deegre days) from the end of the hatching, the feeding of rainbow trout began with commercial feed in the G_{12} group, and in G_{16} the feeding began 16 days (from fertilization 580 deegre days) after the end of the hatching. A statistically significant difference ($p < 0.05$) in the average body weight between the observed groups was found after 65 (G_{12}) and 61 days (G_{16}) of the diet. The average body weight±SD (g) in G_{12} was 2.89±0.13, and the coefficient of variation of body weight was 4.37, while in G_{16} the average body weight was 2.65±0.08, and the coefficient of variation of body weight was 3.02. Savić et al. (2012) present data on the achieved average weight of two-month-old rainbow trout from 0.95 to 2.52 g, grown at an average water temperature of 10.5°C. Gunter and Grant (2016) investigated the influence of initial feeding time on rainbow trout growth characteristics, starting with feeding 13, 15, 17, 19, 21 and 25 days after hatching, at an average water temperature of 12.2 °C and found that 21 and 25 days of the initial diet are similar, and rainbow trout achieve the best growth with less variation in size, when the initial diet of commercial feed begins 21 days (462 deegre days) or 25 days (550 deegre days) after the end of the hatching.

The third part

During the cultivation of three-month-old fry rainbow trout, the analyzed water quality indicators were similar in both observed groups (Table 1).

Table 1. Average values±standard deviation (SD) and coefficients of variation of measured water quality indicators in the third part of the experiment

Indicator	G_{12}		G_{16}	
	Mean±SD	CV	Mean±SD	CV
Water temperature (°C)	12.65±0.64	5.07	12.65±0.58	4.62
Dissolved O_2 content (mg/l)	8.43±0.48	5.66	8.56±0.60	7.06
Water saturation O_2 (%)	80.70±3.47	4.30	81.60±5.94	7.28
pH	7.44±0.13	1.70	7.44±0.21	2.84

At the beginning, at the rainbow trout age of 70 days, a statistically significant difference was found in the mean ($p < 0.05$) weight, total length and fork length, which has not been present for 84 days, except for the fork length and on the eighty-fourth day, a statistically significant difference was found (Table 2). By the end, there was no significant difference in the mean weight and body length, although it is evident that fish from the G_{12} group have a higher body weight and length, which can be explained by the earlier start of the diet in that group. With increasing weight, the coefficients of variation trend to increase, which indicates an increase in differences in individual body weight within groups. The situation is similar with the total length and fork length.

Table 2. Body weight and length (average±SD) and coefficients of variation (CV) of three-month-old juvenile rainbow trout (age 70-112 days)

Parameter	Fry age (days)	G_{12}		G_{16}	
		Mean±SD	CV	Mean±SD	CV
Body weight (g)	70	3.04^{a}±0.60	19.81	2.72^{b}±0.61	22.40
	84	4.62^{a}±1.12	24.13	4.35^{a}±1.02	23.31
	98	7.13^{a}±1.78	24.92	6.90^{a}±1.72	24.93
	112	12.01^{a}±3.44	28.68	11.82^{a}±3.16	26.76
Total body length (cm)	70	6.44^{a}±0.41	6.30	6.27^{b}±0.46	7.39
	84	7.31^{a}±0.56	7.64	7.15^{a}±0.60	8.37
	98	8.51^{a}±0.78	9.16	8.34^{a}±0.78	9.36
	112	9.82^{a}±1.07	10.94	9.77^{a}±0.96	9.79
Fork length (cm)	70	6.12^{a}±0.39	6.42	5.94^{b}±0.45	7.61
	84	6.98^{a}±0.53	7.66	6.77^{b}±0.57	8.45
	98	8.11^{a}±0.74	9.13	7.95^{a}±0.76	9.59
	112	9.38^{a}±1.04	11.08	9.35^{a}±0.95	10.18

Values with different superscript in the same row differ significantly ($p < 0.05$).

The analysis of growth characteristics (Table 3) shows that in the G_{16} group, a higher specific growth rate (SGR) and thermal unit growth coefficient (TGC) was achieved, which can be attributed to the effect of compensatory growth rainbow trout in this group, with a slightly higher conversion rate and lower survival. In general, it can be said that the analyzed growth characteristics do not indicate a significant deviation in value, and it can be said that the time of initial exogenous diet was well chosen 12 and 16 days (528 and 580 deegre days) after the end of the hatching. Gunter and Grant (2016) stating that the optimal time of the initial diet, at an average water temperature of 12.2 °C, are 21 and 25 days after the end of the hatching to achieve a good growth of farmed rainbow trout. No significant difference in the analyzed growth characteristics rainbow trout was found during the three-month-old rearing period (Table 3), although higher values of CF, FCR, SGR and TGC were evident in the group where the initial feeding began 16 days after hatching, with survival in the same group being lower compared to the group with the time of the initial feeding 12 days after the end of the hatching in which there was no mortality.

Table 3. Growth characteristics (CF, SGR, TGC) and FCR of three-month-old rainbow trout

Parameter	G_{12}		G_{16}	
	Mean±SD	CV	Mean ±SD	CV
CF	1.35 ± 0.20^{a}	14.89	1.36 ± 0.17^{a}	12.55
FCR	0.60 ± 0.07^{a}	12.19	0.62 ± 0.08^{a}	12.90
SGR	3.27 ± 0.40^{a}	12.19	3.50 ± 0.31^{a}	8.73
TGC	0.158 ± 0.04^{a}	24.46	0.166 ± 0.04^{a}	22.31

The SGR is higher in G_{16}, in individuals with later initial feeding times, according to Gunter and Grant (2016) who indicate a continuous increase in SGR in each subsequent group with later initial feeding.

Conclusion

High water temperatures (≈15 °C) affect the more intensive embryonic development of the rainbow trout embryo, but also the more pronounced mortality. During the rearing of juvenile rainbow trout up to the age of two months, there was no significant difference in mortality, which indicates that different times of the initial diet were well chosen. On the other hand, the effects of the time of the initial diet on the growth characteristics of two-month-olds rainbow trout are reflected in a significant difference in the mean average body weight. Significant differences in body weight, total length and fork length were found at the beginning of the third month of age, after which the difference is lost, indicating compensatory growth, although body weight and body length were not reached. This is indicated by the analyzed parameters of growth characteristics, higher SGR, CF and TGC of rainbow trout from the G_{16} group.

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Original scientific paper

Phenotypic description of the Lipicane horses' population from Bosnia and Herzegovina and Serbia

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Abstract

Lipicane horses play an important role in horse breeding in Bosnia-Herzegovina and Serbia, particularly through the studs Vučijak, Karađorđevo and Kelebija. The breeding goals at these stud farms are different, and have results in differences in the phenotype of the horses. The aim of this study was to present the means and differences in body traits of Lipicane horses between three stud farms. Out of a total of 225 horses (88 stallions and 137 mares) following body measurement were recorder: height at withers, circumference of chest and circumference of cannon bone forelimb. All horses were at least 4 years or older at the time of measuring. Based on morphological measures, chest girth and boniness index were calculated. The results showed that Lipicane horse from these three stud farm are significantly different ($p<0,01$). The biggest horses were in Kelebija, middle in Karađorđevo and the smallest were in Vučijak stud farm. We can conclude that the Lipizzan population at the Vučijak and Karađorđevo stud farms is on average lower than recommendations of LIF, while the horses at the Kelebija stud farm are at the lower limit. Observed by sex, we see that stallions from the stud farm Karađorđevo and Kelebija as well as mares from the stud farm Kelebija are in accordance with by international standards, while mares and stallions from the Vučijak stud farm as well as mares from the Karađorđevo stud farm are below this standard.

Key words: stud farm, Lipicane horse, body measurement, difference

Introduction

The Lipicane horse belongs to the oldest European horse breeds, and it is a result of strict, systematic selection throughout almost 450 years. According to the Lipizzan International Federation (LIF), there are 11 national stud farms of Europe. In addition, in 20 countries from Europe, America, Australia and Africa, private breeding organizations are registered. The total number of Lipicane horses is 11.895 (LIF, 2020). Considering that all private bred Lipicane horses are originating from state-owned nucleus herds which are bred in closed herds on a long term, the genetic diversity of this breed can be considered small (Zechner et al., 2001).

The morphological differences between the horses from different stud farms, depend on the breeding goals, which differ from one country to another, or from the stud farm to stud farm. Zechner et al. (2002) notes that the breeding goals of the stud farms have been different and are partly changing over time. The primary goal of the Austrian stud farm is to provide horses for classical dressage, the Hungarian stud farm has specialized in breeding of top horses for carriage driving, the Slovenian, Slovakian and Croatian stud farms are breeding riding horses, while the Romanian studs providing stallions for improvement of the local farm horse population (Rogic et al., 2018).

The national stud farm Vučijak was founded in 1946s in Prnjavor municipality. The primary goal of establishing the stud was to improve the existing horse population in North Bosnia. According to Praček (1999) breeding goal of founding stud farm Vučijak was to create a horse with smaller body frame suitable for driving and carrying. Soon the breeding of Lipicane horses spread to other parts of Bosnia and Herzegovina, thanks to the stud farm Vučijak and its continuous work directly influenced the breeding quality of private breeders (Stojanović, 2006). The Lipicane horses from the stud farm Vučijak are smaller than Lipicane from other European stud farms (Rogić et al., 2018). In Serbia stud farms (state and private) and individual breeders (over 200 individuals and legal entities) breed Lipicane. Karađorđevo Stud Farm is currently the country's oldest stud farm, with a tradition of breeding Lipicaners for 90 years. This stud played a very important role in the transmission of tradition, methods, knowledge and experience to other studs and individual breeders, primarily setting a good example of organized selection work to improve and preserve this horse breed. With its own reproduction, augmented by partial purchase of breeding mares from the disbanded Fruška Gora and Dobričevo Lipicane stud farms, but also purchases from and exchanges with the Lipica, Đakovo, Lipik and Vučijak stud farms, Karađorđevo Stud Farm formed a consolidated breed. Kelebija Stud Farm also has great importance for the breeding of Lipicane horses in Serbia, as it formed an initial breeding herd from Karađorđevo Stud Farm, individual Lipizzaner breeders and owners and from other state stud farms in the former Yugoslavia (Lipica, Đakovo, Vučjak and Lipik). The goal of Kelebija Stud Farm is to breed Lipicane horses for harness sports and dressage riding.

Zechner et al. (2001) notes that one part of characterization of a breed involves the morphological study of horses. The analysis of morphological measurements of European Lipicane horse populations have been the subject of multiple scientific studies (Zechner et al., 2001; Rastija et al., 2004; Baban et al., 2006, Pallotino et al., 2015, Druml et al., 2016). However, only few studies about conformation traits and body measurements of Lipizzan horses from stud farm Vučijak have been published recently (Važić et al., 2016, Rogić et al., 2018, Rogić et al., 2019). Štrbac and Trivunović (2014) used 566 Lipicane horses from Serbia to determine mean withers heights, chest girths and cannon bone circumferences and analyse the effects of stallion bloodlines, age and sex on the three measured traits.

The measurement of various body dimensions of horses plays a significant role in quality improvement, genetic breeding, health, and soundness. In the management techniques for race and riding horses, understanding the body conformation of horses is a major factor (Matsuura et al., 2021). Cannon bone circumference, chest girth, and withers height are important biometric measures that are useful to describe the horses body conformation correctly. Also, they are useful to evaluate how the conformation might be modified thanks to different breeding purposes such as: racing, jumping, and other equestrian disciplines (Giontella et al., 2020). The goal of this study was to collect and compare the data of Lipicane body measurements from three stud farms: Vučijak, Karađorđevo and Kelebija. The aim of this paper was to present the means and differences for these studs of 3 standard body measurements. According to LIF (2010) ideally, the adult horse should measure withers height between 153 cm and 158 cm. However, the different breeding goals followed by the various studs, as well as specific characteristics and traits of lines and families on the analyzed studs, could be reflected by differences in the general appearance of horses.

Material and Methods

Data were recorded at stud farms Vučijak, Karađorđevo and Kelebija. The numbers of stallions and mares measured at each stud are given in Table 1. A total of 225 individuals (88 stallions and 137 mares) were recorded. As the Lipicane horse is late maturing, only individuals 4 years or older were measured.

Table 1. Number of horses measured

Stud	Mares	Stallions	Total
Vucijak	53	27	80
Karađorđevo	13	14	27
Kelebija	71	47	118
Total	137	88	225

All measurements were taken on the left side of the horse while it was standing on flat ground with all feet on the ground and legs parallel. Circumference measurements were taken using a tape, while the height at withers were taken using a stick. The following traits were recorded: height at withers-HW (distance from the highest point of the withers to the ground), circumference of chest-CC (circumference around the chest behind the front legs) and circumference of cannon bone-CCB forelimb (smallest circumference of cannon bone of the forelimb) (Fig. 1.).

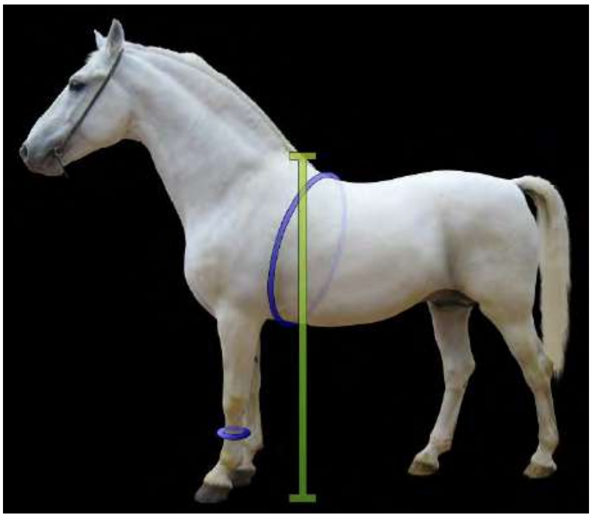

Figure. 1. Characteristic recorded measurements

On the basis of the measures following indices were calculated by Sadek (2006):

1. Boniness index = (CCB/HW) x 100, that relates the thickness of the horse′s bones compared with its height and can reflect the capacity of the horse in all gaits, jumping and in carrying a rider while remaining well balanced, and
2. Chest girth index = (CC/HW) x 100, that expresses the volume of the chest in comparison with the general height of the horse.

Statistical analysis

The obtained results were processed by simple analysis of variance using the statistical program SPSS (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Stud means were estimated for all populations, as well as separately from mares and stallions. The significance of differences between the means of these parameters in accordance to the stud farms and sex were tested using the Duncan test.

Results and Discussion

Then mean values of three standard measurements at the different studs, for whole populations, and for mares and stallions are given in table 2. The largest values for height at withers were found at Kelebija, then Karađorđevo and the smallest values were found at Vučijak. The same results are found for other two measures. For all three measures the highest values are found at Kelebija horses, then Karađođevo, and the smallest values are found at Vučijak horses. The pairwise comparison of means reveals significant differences for recorded measurements. Statistically highly significant difference (p <0.01) was found in the HW between the stud. According CC statistically highly significant difference (p <0.01) was found between Vučijak and other two studs. Furthermore, the difference in the CCB was statistically significant (p <0.05) between Vučijak and Kelebija.

Furthermore, the mean values of recorded measures at the different studs for mares and stallion were showed the similar results. The stallions are larger the mares, and the largest mares and stallions are at Kelebija in compare with Karađorđevo and Vučijak. Statistically highly significant difference (p <0.01) between the stud farms were found for HW and CC, while statistical significant differences was no found for the CCB. Stallions from Vučijak were significantly smaller than stallions from Karađorđevo and Kelebija. Mares for Kelebija were significantly higher than mares from Karađorđevo and Vučijak. The pairwise comparison of means reveals significant differences for recorded traits. Based on these few measurements, it is obvious that horses from Kelebija are on average bigger than horses from other studs. From other side, the horses from Vučijak are on average smaller than horses from Karađorđevo and Kelebija.

Table 2. The means of morphometric measurement of Lipicane horses in accordance to stud farm

	Vučijak	Karađorđevo	Kelebija	P
Whole populations				
No of horses (n)	80	27	118	
HW	149.10^{a}	152.59^{b}	154.98^{c}	<0.01
CC	177.33^{a}	183.93^{b}	186.36^{b}	<0.01
CCB	18.49^{a}	$19.58^{a,b}$	20.17^{b}	<0.05
Stallions				
No of horses (n)	27	14	47	
HW	149.61^{a}	154.71^{b}	156.21^{b}	<0.01
CC	177.19^{a}	185.07^{b}	186.11^{b}	<0.01
CCB	19.43	19.97	21.00	NS
Mares				
No of horses (n)	53	13	71	
HW	148.84^{a}	150.31^{a}	154.17^{b}	<0.01
CC	177.41^{a}	$182.69^{a,b}$	186.52^{b}	<0.01
CCB	18.01	19.27	19.33	NS

NS: non-significant; values marked by letters ($^{a, b, c}$) in one row describe significant differences; height at withers-HW, circumference of chest-CC and circumference of cannon bone-CCB

Zechner et al. (2001) were made morphological descriptions of Lipicane horse populations from seven Europe countries (eight national stud farms): Lipica, Piber, Đakovo, Lipik, Fagaras, Monterotondo, Szilvasvarad and Topol'cianky. When we compare the results for the height at withers, we can conclude that the stallions from Karađorđevo are the smallest in relation to the European population of Lipicane stallions, while the stallions from Kelebija are higher than stallions from Lipica, Monterotondo, Piber and Topol'cianky. Only mares from Piber were not smaller then mares from Karađorđevo. Mares from Kelebija are higher than mares from Beclan, Lipica, Monterotondo, Piber, Szilvasvarad and Topol'cianky.

Table 3. The means of body indices of Lipicane horses in accordance to stud farm

	Vučijak	Karađorđevo	Kelebija	P
Whole populations				
No of horses (n)	80	27	118	
Boniness index	12.42	12.64	13.21	NS
Chest girth index	118.97	120.25	120.55	NS
Stallions				
No of horses (n)	27	14	47	
Boniness index	12.98	12.78	13.57	NS
Chest girth index	118.45	119.14	119.63	NS
Mares				
No of horses (n)	53	13	71	
Boniness index	12.13	12.54	12.82	NS
Chest girth index	119.23	120.98	121.53	NS

NS: non-significant

According the chest circumference stallions from Karađorđevo and Kelebija are higher than stallions from Beclan, Monterotondo and Piber. Mares are higher than mares from Beclan and Fagaras. According the circumference of cannon bone the stallions from Karađorđevo has the smallest values, also mares expect from Lipica and Monterotondo. Stallions from Kelebija has the highest value of CCB together with stallions from Szilvasvarad and Topol'cianky. From the other side, mares from Kelebija has the smallest value of CCB, expect mares from Lipica and Monterotondo.

Mares and stallions from Vučijak are the smallest for all three recorded measures. This is related to the primary goal of establishing the stud Vučijak, which was to improve the existing horse population in North Bosnia, respectively to get the horse with smaller body frame suitable for driving and carrying. The mean values of body indices at the different studs, for whole populations, and for mares and stallions are given in table 3. The highest values for boniness and chest girth index were found at Kelebija, then Karađorđevo, and the smallest values at Vučijak. Results showed that there is no significant differences for indices between studs, respectively, between stallions and mares. In compare with Arabian horses the boniness (12.4) and chest girth indices (114.9) value are smaller than Lipizzan horses (Sadek, 2006).

Conclussions

This work is the first report of differences between Lipicane horses from Bosnia and Herzegovina and Serbia. According results we could conclude that Lipicane horses from Vučijak are the smallest, and from Kelebija are the biggest. Lipicane horses from Karađorđevo are in the middle. If we compare the obtained results with the recommendations of LIF, we can conclude that the Lipicane population at the Vučijak and Karađorđevo stud farms is on average lower than recommended, while the horses at the Kelebija stud farm are at the lower limit. Observed by sex, we see that stallions from the stud farm Karađorđevo and Kelebija as well as mares from the stud farm Kelebija are in accordance with by international standards, while mares and stallions from the Vučijak stud farm as well as mares from the Karađorđevo stud farm are below this standard. However, it is known that all stud farms during their breeding work were aimed at forming an own-specific type of Lipicane horses by which they would be recognizable and which would correspond to the use in accordance with the goals of that stud farm and the area where it is located. Also, the specific characteristics and traits of lines and families on the analyzed studs, could be reflected by differences in the general appearance of horses. In order to be able to discuss in more detail, it is necessary to conduct additional research and measurements of other exterior traits that are important for the morphological characterization of the breed.

Acknowledgments

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Original scientific paper

The occurrence of heavy metals (Pb and Cd) in the kidneys of wild boar (*Sus scrofa*), mangulica and fattening pigs

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Abstract

The heavy metals as lead (Pb) and cadmium (Cd) are possible contaminants of food and feed. For the purpose of this work the samples of kidneys of fatteners, Mangulica pigs and wild boar were analyzed. Fattening pigs live in control conditions up to six months, while Mangulica pigs and wild boar live longer and in uncontrolled conditions. In total 20 samples of kidneys were analyzed (2 wild boars, 6 Mangulica and 12 fattening pigs). In all samples the concentration of lead and cadmium was above the limit of detection. For cadmium the limit of detection (LOD) was 2.32 µg/kg, while limit of quantification was 7.04 µg/kg. For lead LOD was 4.26 µg/kg and LOQ was 12.9 µg/kg The concentration of lead and cadmium in samples of kidneys of fattening pigs was in the allowed range while for the Mangalica pigs and wild boar the concentration of lead in some samples exceeded the permitted levels. The maximum concentration of lead and cadmium was found in samples of kidneys of Mangulica and were 1069 µg/kg and 484 µg/kg respectively. The average concentrations of lead for the Mangulica, wild boar and fattening pigs were 759, 731 and 243 µg/kg respectively. In all samples the concentration of cadmium was in the allowed range, with the average concentration from 337 µg/kg. There is a need for the further research.

Key words: heavy metals, kidneys, mangulica, wild boar

Introduction

Pork is one of the most used meats in the nutrition in the Republic of Serbia. The annual production of pork in Serbia is about 307 thousand of tons per year (Statistical Office of the Republic of Serbia 2018). Except the pork produced from the fattening pigs, the meat and some traditional meat products made from the Mangulica and wild boar can be found on the table. Mangulica are autochthonous breed of pigs and in some characteristics it is very similar to the wild boar. In the developed countries apart from the quantity the quality of meat is of the enormous importance. Liver and kidneys are edible offals which is the main place of accumulation of the majority of unwanted contaminants. While checking the quality of the offals the quality of the meat in general and meat products produced from it is checked. Heavy metals as lead (Pb) and cadmium (Cd) do not have any function in animal or human organism (EFSA 2009; EFSA 2010). However, due to their presence in the environment it can be found as residues in edible tissue or in the bones of animals (Liu, 2003; Kenntner et al. 2007; Bilandžić et al., 2010; Petkovšek et al. 2014; Polovinski-Horvatović et al., 2021). Cadmium or lead can be found in nature due to some nature event as meteor fall, volcanoes activity or forest fires (Hutton, 1983).

However, it can be much more often found in the nature due to the human activity, in the proximity of heavy industry, thermo plants, or as the results of intensive agriculture activities (Krčmar et al. 2017; Mandić-Rajčević et al. 2018). Once when they enter human or animal body, they have very long half-life. There is the legislation for the allowed concentration of heavy metals in edible offals (RS Pravilnik 2011). The allowed concentration for the lead and cadmium in liver is 500 µg/kg, while the allowed concentration for cadmium in kidneys as the main place of accumulation is double and it is 1000 µg/kg. Wild animals have important role in the assessment of the exposure to heavy metals in the environment (Tataruch and Kierdorf 2003). Fattening pigs are usually about six months old at the time of the slaughter. They live in controlled conditions with water and feed provided on the farm. Mangulica is autochthonous breed of pig, extensively produced on small scale, family (backyard) farms. Mangulica matures late and is one of the fattiest pigs. The fattening period is very long up until they reach wanted weight (about 120-130 kg). They are fed with locally produced corn without any premix or controlled feed. The meats of Mangulica are used for the production of some of the traditional meat products as bacon, ham or sausage which are highly valued traditional dry meat products by the consumers (Jokanović et al. 2013).

Wild boar (*Sus scrofa*) are hunting game and are regularly harvested during the hunting season. Meat from wild swine is not very often on the market, if ever. Hunters, their families and friends have more access to the meat of free living game. Wild boars' meat is considered delicacy. However, free living game generally has the higher concentration of heavy metals then the farm animals. The purpose of this work was to compare the average concentration of two heavy metals in the kidneys from three different population of pigs (fatteners, Mangulica and wild boar).

Material and Methods

Sampling

Kidney samples were taken from random fattening pigs on the slaughter line s in December 2020. The fattening pigs were approximately six months old, weighing 100-110 kg and originating from commercial pig farm in Vojvodina. The hogs' diet was based on the locally produced corn and soybean meals and was formulated to meet the animals' nutritional needs. The fattening of the hogs was divided in three phases: the starting, growing and finishing periods. The samples of Mangulica kidneys were taken from two different farms in Vojvodina, mangulice were approximately 18 month old, reared in free range condition. The samples of wild boars' kidneys were taken after the hunting was finished, during the hunting season 2020. Before analyzing all samples of the kidneys were checked and samples with any indication of damage made by shot were excluded from the analysis. Upon homogenization, all the samples were frozen at -20 °C until further analysis.

Analytical methods

Lead and cadmium concentrations were determined after the wet sample digestion in concentrated nitric and hydrochloric acid (3:1, v/v) using a Reacti-ThermTM Heating/Stirring module Thermo Scientific (Rockford, USA). The analysis was performed by using a Perkin-Elmer PinAAcle 900T THGA/FL atomic absorption spectrometer, a longitudinal Zeeman-effect background correction system, equipped with a transversely heated graphite atomizer (THGA) and an AS900 auto sampler. Hollow cathode lamps (HCL) were used for lead and cadmium detection. The analysis was carried out according to the method described by et al. Beuković et al. 2015. For cadmium the limit of detection (LOD) is 2.32 µg/kg, while limit of quantification is 7.04 µg/kg. For lead LOD is 4.26 µg/kg and LOQ is 12.9 µg/kg

Results and Discussion

The results of the occurrence of the two heavy metals (lead (Pb) and cadmium (Cd)) are showed in the tables No 1 and No 2. In total 20 samples were analyzed, 6 from Mangulica, 2 from wild boar and 12 from the fattening pigs. The average lead concentration in all samples was 650 µg/kg, while the maximum of 1069 µg/kg was found in the sample of the kidneys of mangulica. The average concentration of lead in samples of Mangulica and wild boar was above the permitted level. Among the all samples only samples of kidneys of the fattening pigs had the average concentration of lead in the permitted level.

Table 1. The occurrence of lead in the kidneys of wild boar (*Sus scrofa*), Mangulica and fattening pigs (µg/kg, ppb)

	N	Mean	Min	Max	Std. Dev
Mangulica	6	759	404	1069	266
Wild boar	2	731	478	983	358
Fattening pig	12	243	176	310	95
∑	20	650	176	1069	317

All samples of kidneys had the concentration of cadmium in the permitted level; maximum found was 484 µg/kg in the sample of mangulica pigs. Average concentration in Mangulica, wild boar and fattening pigs was 379, 384, 165 µg/kg respectively.

Table 2. The occurrence of cadmium in the kidneys of wild boar (*Sus scrofa*), Mangulica and fattening pigs (µg/kg, ppb)

	N	Mean	Min	Max	Std. Dev
Mangulica	6	379	189	484	104
Wild boar	2	384	341	428	62
Fattening pig	12	165	149	182	23
∑	20	337	149	484	122

It is impossible to completely avoid the contamination of heavy metals in food and feeds and consequently in animals and humans. However, it is possible with the good agricultural practice, knowledge and care for the environment to reduce as much as possible to be in the allowed concentration. In the samples of the kidneys of wild boar, fattening and Mangulica pigs cadmium concentration was in the allowed concentration by the Serbian legislation. However, the concentration of lead was above the legislation limit in the wild boar and Mangulica with the average concentration from 759 and 731 µg/kg respectively. The concentration of lead in samples of fattening pigs was in the allowed range.

The results of lead in kidneys of fattening pigs are in agreement with some previous research done on the edible offals in Serbia (Nikolic et al. 2017) in which was found average concentration from 0.006±0.001 mg/kg. However, sometimes lead in increased concentration can be found as seen in the work of Polovinski-Horvatović et al., 2021 in this work the maximum found value of lead in liver was 0.86 mg/kg. Reasons for the increase are usually inconsequent. However the proven source for the increase of lead in the edible offals (as high as 3.3 mh/kg fresh weight) in fattening pigs in the research from Australia was premix with the lead as impurity (Maclachlan et al. 2015).

The concentration of lead in the kidneys of Mangulica and wild boar are above the legislation. However, the results are expected. Mangulica breed is very similar to the wild boar, fattening period is very long and they live in free range condition. The condition in which Mangulica and wild boar live are without any control, and they eat and drink whatever they can find. The results are similar with the results from the research from Croatia (Bilandžić et al. 2009; Lazarus et al. 2014) in which concluded that meat of free living game as wild boar can be important source of heavy metals if consumed regulary. Moreover in some samples the maximum found concentration was as high as 11.6 mg/kg in kidneys. (Bilandžić et al., 2009). However, the average concentration of lead from 0.016 mg/kg in the samples of Mangulica reared in central Serbia had been much less then in our study (Nikolic et al. 2017).

Conclusion

The results from this research suggest that the occurrence of heavy metals in the wild boar, Mangulica and fattening pigs can pose risk of the increased level of some of the investigated heavy metals. Therefore, there is a need for the further investigation regarding the occurrence, of most of all the lead, in edible tissue of Mangulica and wild boar.

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Original scientific paper

The influence of the saeason on milk urea content in dairy goat farms

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Abstracts

Milk urea content is a useful indicator of protein and energy balanced diet and it is used in systematic control of cows' milk, while in sheep and goat milk it is not determined or controlled. The aim of this study was to determine the urea level in goat milk and examine the influence of season. The observation was made on 10680 samples of milk control records of goats in the period of three years, from year 2019 to 2021. The goats on the three observed farms were reared in a closed system with a balanced diet throughout the whole year. Analyses of raw milk samples were carried out on the FOSS instruments MilcoScan in Laboratory for Milk Quality Control, at the Faculty of Agriculture, Department of Animal Science. According to the season of sampling, milk samples were divided into four groups: group I winter (December – February), group II spring (March – May), group III summer (June – August) and group IV autumn (September – November). Statistical analysis were done using program Statistica 14.0. The average MU content was 39.69 mg/dl (10.40 – 95.40 mg/dl). The study shows statistically highly significant differences in MU content ($P<0.01$) between seasons and also between all observed milk parameters (milk fat, protein, lactose and daily milk yield). The lowest urea content was in spring (37.83 mg/dl), and the highest was in autumn (41.94 mg/dl).

Key words: milk urea, goat milk, milk parameters

Introduction

Milk urea, as a part of the non-protein fraction of nitrogen in milk, represents the final product of protein metabolism in the rumen of ruminants. Toxic ammonia comes into the liver by portal bloodstream and undergoes there the transformation to urea. Urea is then released from the liver in the bloodstream to reach, mainly, the kidneys and is excreted in the urine. However, a minor proportion of urea is excreted with milk or is recycled, via saliva, to reach the rumen. There is a highly positive correlation between blood urea and milk urea (Butler et al., 1996; Broderick and Clayton, 1997; Jonker et al., 1998; Nousianen et al., 2004). Milk urea concentration is a useful indicator of energy and a protein balanced diet in ruminants. Protein is typically the most important and expensive nutrient in the diet of dairy goats which needs to be efficiently utilized. The source of dietary CP and energy fed to the dairy animals significantly influence the utilization of N and energy in the rumen and the flow of nutrients to the small intestine.

Today, the ecological sustainability of livestock is becoming increasingly important. Nitrogen excretion from dairy cows, goats, and sheep can lead to air and groundwater pollution (Santos et al., 2014). Often their diets have a much higher protein content than they need. Therefore, animal production systems, which are important to the food supply worldwide, have to pay greater attention to being sustainable and environmentally friendly. The goats milk quality, chemical composition, and quantity depend on genotype (breed) and external (environmental) influences and include nutrition, seasonal variations in air temperature, lactation stage, animal health and herd management (Jenness, 1980; Park, 1990; Antunac i sur., 2001a, b; Lindmark -Manssonisur., 2003; Guois ur., 2004; Ţanisur., 2006) while Mioč i Pavić (2002) also state the order and duration of lactation, physical development, skin age, litter size and kidding season.

While the determination of milk urea concentration has practical application in monitoring the nutritional and reproductive status of dairy cows, it has not been systematically implemented in the production of goat (and sheep) milk. The reason lies in the fact that the interpretation of results is not fully standardized due to numerous influences such as nutrition, breed, stage of lactation, parity, season, milking time, body weight, production, the chemical composition of goat milk, etc. (Schepers and Meijer, 1998; Giaccone et al., 2007; Abdouli et al., 2008; Goetsch, 2019).

There is very little data on the urea content in goat milk, primarily due to the lower economic importance of goats, i.e. goat milk (compared to cow milk), their seasonal polyestrous cycles, and different ways of breeding and keeping (Goetsch, 2019). According to Brun-Bellut et al. (1991) an acceptable range for the MUL is 28–32 mg/dl, and according to Rapetti et al. (2009), the acceptable range is 23–34 mg/dl. Determination of urea content is closely related to milk production efficiency (Santos et al., 2014), reproductive traits of goats (Godden et al., 2001a; Marenjak et al., 2004; Mellado et al., 2004; Butler, 2005; Biswajit et al. et al., 2011), milk processing properties (Pretto et al., 2013; Bland et al., 2015) and environmental pollution (Santos et al., 2014). A balanced diet is considered to be the basis for efficient goat milk production. According to research, feeding costs account for about 50-70% of direct costs in goat milk production (Grgić, 2013; Kostelić, 2019).

Balanced feeding structure, balancing the actual needs of the individual, the use of alternative and cheaper sources of protein in order to reduce the production cost, at the same time increasing the quantity and quality of produced milk (Santos et al., 2014). Bendelja et al. (2016) found that feeding with different amounts of CP (crude protein) in the meal resulted in increase of MU. Urea serves as a good indicator of the goat's energy and protein supply. It can be assumed that setting the limit value of urea concentration in goat milk would reduce feeding costs due to more rational protein (the most expensive dietary ingredient) intake, and feeding goats according to their actual needs would increase the farm's profit. Many farmers are still convinced that a generous protein supply can increase milk yield and quality. On the contrary, only the feed cost and environmental impact increase. For dairy goats, only few studies investigated the relationship between MU content and nitrogen excretion (Ciszuk and Gebergziabher, 1994; Decandia et al, 2011). The aim of this study was to determine the milk urea level in goat milk and examine the influence of season on MU content.

Material and Methods

Milk samples were collected from three goat farms in the period of three years from 2019 – 2021, as a part of regular milk recording control (ICAR, 2019). There were no changes in the feeding regime throughout the lactation period and the goats on the farms were in closed system with balanced diet throughout the whole year. Milk urea content and milk chemical composition were determined by infrared test method (ISO 9622:2013) by MilcoScan FT in Laboratory for Milk Quality Control, at the Faculty of Agriculture in Novi Sad. The Laboratory is accredited in accordance with the international standard ISO 17025:2017 an ICAR guidelines (2020).

The complete data set consisted of 10680 samples of milk control of goats from three farms. Data included date of recording (season), daily milk yield, milk fat, protein, lactose and milk urea (MU) content. According to the season of sampling milk samples were divided into four groups. Group I – winter (December – February), Group II – spring (March – May), group III – summer (June – August) and group IV – autumn (September – November). The average values and variability of the examined traits, as well as the effect of the season on these traits were studied using the PROC UNIVARIATE and PROC GLM procedures within the Statistica software package (Ver. 14.0 StatSoft Company). Post-hock analyses (Duncan test) was used to determine statistically significant differences between the mean values of different seasons, with significance levels of $P<0.05$ and $P<0.01$.

Results and Discussion

The average results for milk urea content (MU), milk fat, total protein and lactose percentages and daily milk yield (DMY) are presented in Table 1.

Table 1. Means, standard deviations (SD) and coefficient of variation (CV) of analyzed variables

Trait	N	Mean	Min	Max	SD	CV (%)
Fat (%)	10680	3.09	1.01	7.97	0.91	29.33
Protein (%)	10680	3.18	1.88	6.63	0.54	17.09
DMY (kg)	10680	1.58	0.10	6.40	0.89	56.66
Lactose (%)	10680	4.25	3.01	5.19	0.26	6.14
MU (mg/dl)	10680	39.69	10.40	95.40	9.89	24.93

The mean MU concentration was 39.69 mg/dl, with high variability (SD=9.89). Mean MU level was higher than the optimal values, for dairy goats, considered by Brun-Bellut et al. (1991). Rapetti et al. (2009) analyzed bulk goat milk and concluded that about of 75% samples had the higher MU level the level suggested by Brun-Bellut et al. (1991). Sandrucci et al. (2019) reported a similar level of MU (39.70 mg/dl). Todaro et al. (2005), Giaccone et al. (2007) and Bendelja (2018) reported higher mean level of MU (43.70 mg/dl, 43.57 mg/dl, and 41.43 mg/dl respectively), but Bonanno et al., (2008) found a lower level of MU (22.5 mg/dl). Rapetti et al. (2009) and Sanducci et al. (2019) in their researches, also found a wide range of variation MU content (11.9 – 67.5 mg/dl and 11.40 - 90.90 mg/dl). Mean milk fat content (3.09%) was lower than mean protein content (3.18%), Sandrucci et al (2019) in their research found that more than half of about 7000 individual goats milk samples (51.4%) was characterized by milk fat / protein reversion, but they found higher content of milk fat, protein and lactose in goats milk. The mean values for milk fat (3.09%) and protein (3.18%) determined in this study were lower than the average values for goat milk reported by Todaro et al. (2005), Bonanno et al. (2008), Park and Haenlein (2010), and also lactose content (4.25%) was lower the lactose content found Sandrucci et al (2019), Todaro et al. (2005), and Rapetti et al. (2014). Bendelja and Ljoljić (2019) in their research found lower content of protein and lactose, but higher level of milk fat. Average milk production was 1.58 kg, Sanducci et al. (2019) found lower milk yield. The average results of raw milk composition observed by season are presented in Table 2.

Table 2. Average composition of raw milk, observed by season

Season	N	Milk fat (%)	Protein (%)	DMY (kg)	Lactose (%)	MU (mg/dl)
1	1047	3.43^{a}	3.34^{a}	1.31^{a}	4.37^{a}	40.95^{a}
2	3247	3.15^{b}	3.06^{b}	1.76^{b}	4.33^{b}	37.83^{b}
3	3876	2.81^{c}	3.05^{b}	1.58^{c}	4.19^{c}	39.44^{c}
4	2510	3.32^{d}	3.45^{c}	1.44^{d}	4.17^{d}	41.94^{d}

Average within the same column with different superscripts (a,b,c,d) differ significantly (P<0.01)

According to the results in Table 2, we can see a large influence of season on observed milk parameters (fat, proteins, lactose, MU, and DMY). There was a highly statistically difference between all four groups related with season P<0.01.

Our results are in accordance with other research, Žarković et al. (2019) found a statistically significant difference between the content of milk fat, protein, and total solids in goat's milk according to the seasons.

The lowest level of MU was in spring (37.83 mg/dl) and the highest level in autumn (41.94 mg/dl). Giaccone et al. (2007) also determined the highest MU level in winter months in comparison with spring months, and in July found the highest content of MU. Rapetti et al. (2009) had opposite conclusions, they reported that MU level increase after kidding season (January – March) from 34 to 40 mg/dl, and reaching a peak from April to June (44 mg/dl) and decreased until November (37 mg/dl). The other authors found the highest MU content during the summer months (Carlson et al., 1995; Hojman et al., 2004, Konjačić et al., 2010; Bendelja et al., 2011). They explain that the cause of higher milk urea content may be associated with the availability of voluminous feed.

From these data (Table 2), it is evident that during the summer the content of protein and fat was the lowest, and in autumn the lowest lactose content (4.17 %) and milk yield (1.44 kg). Žarković et al. (2019) also found the lowest milk yield in autumn, and the lowest fat and lactose content in summer, and protein content in spring. Milk fat content was the highest in winter, ad protein content in autumn when is also the highest MU content (41.94 mg/dl).

Conclusion

The season is an important factor in the variability of milk urea (MU) content and has a strong influence on other observed milk parameters (milk fat, proteins, lactose, and DMY).

The lowest MU content was during spring (37.83 mg/dl) and summer (39.44 mg/dl) months, and the highest in autumn (41.94 mg/dl). During the whole year the MU content was higher than the recommended optimal values.

The urea content in goat's milk can be used to fine-tune the protein composition of the diet that optimizes the utilization of nitrogen for milk production, in order to improve the efficiency of milk N and reduce the excretion of N urine.

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ICAR – The global standard for livestock data (2020): Section 12 – Guidelines for Milk Analysis

Original scientific paper

The impact of breeding region and lactation on milk yield traits in the of Simmental cattle population of the Republic of Serbia

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Abstract

The objective of this research was to study phenotypic expression and factors that can affect milk yield traits in the population of Simmental cattle breed in the Republic of Serbia. The research was conducted on a set of data that included records on the production and origin of Simmental breed cows displayed both in regional and municipal exhibitions in the territory of the Republic of Serbia in the period from 2004 to 2017. A final data set included records on production and origin of 1176 Simmental breed cows. The animals were raised in the area covering 9 regions of the Republic of Serbia. The research included most important milk yield traits in standard lactation: milk yield, milk fat content, milk fat yield, yield of 4% fat-corrected milk (4%FCM). An average milk yield in studied population accounted for 5.520±919 kg, milk fat content 3.94±0.11%, milk fat yield 218±38 kg, while the yield of 4% fat-corrected milk accounted for 5.474±933 kg. It was determined that region, age and lactation had a very high statistically significant effect on studied traits while the age of cows had no statistical effect.

Key words: milk traits, Simmental breed, breeding region

Introduction

In the Republic of Serbia milk production represents the most important branch of livestock production and it takes place in quite different zootechnical conditions. Milk production is affected by a number of factors which can be divided into genetic and non-genetic ones.

When it comes to breeds which the animals that produce milk belong to Simmental and Holstein Friesian breeds are predominant ones. Simmental breed originated from Switzerland but over time it spread throughout a whole world being known as "Fleckvieh" in Germany and Austria, "Pie Rouge", "Montbeliard" and "Abondance" varieties of this breed in France and "Pezzata Rossa" in Italy. It was created by an intensive selection in pure breed. Apart from that, ameliorative cross breeding with dairy breeds was conducted particularly with Red Holstein breed. Simmental breed is also the most numerous cattle breed in the Republic of Serbia participating with 80 % in total number of cattle (Skalicki et al., 2007).

In Serbia Simmental breed was first imported in the 19th century. The animals were imported from Hungary, Czechoslovakia, Switzerland, Austria and Germany. Development of this breed in Serbia was significantly contributed by an artificial insemination. Two centeres for artificial insemination developed, one in 1952 in Krnjaca and the other in 1957 in Velika Plana. The population of Simmental cattle in Serbia is predominantly created by melting crosses of indigenous breeds of cattle using Simmental bulls or their semen in the second half of the 20th century.

Simmental cattle is mostly raised in central Serbia as a breed of dual production capabilities. Simmental dairy production in our country falls behind an average world dairy production primarily due to bad nutrition and management. According to the 2019 breeding programme (Main breeding program in cattle breeding - Simmental breed. Institute of Animal Husbandry - Zemun, Belgrade, 2019 for Simmental breed an average milk production in standard lactation should reach 6500 kg milk with 4.10% milk fat and 3.60% protein.

Milk yield in Simmental breed in different countries varies depending on quality of a breeding stock, selection programme and breeding conditions. Milk yield in Simmental breed that is mostly imported from Germany and Austria and raised in Serbia is 6000-7000 kg with 4.1% milk fat and 3.6% protein. Some breeding stocks of Simmental breed produce milk yield over 8000 kg. Niksić et al. (2011) report that milk yield ranges between 4400-5500 kg depending on a breeding stock. An average milk yield in Simmental breed of Serbia accounts for 4810 kg milk with 3.9% milk fat and 3.2% protein.

Pantelic et al. (2020) reported that average milk yield in first-calf heifers in 2019 accounted for 4692 kg milk with 3.98% milk fat and 3.18% protein. The yield of milk fat and protein was 187.27 kg and 149.64 kg respectively. The average milk production in Germany in 2016 accounted for 7568 kg with 4.19% milk fat and 3.52% protein (https://www.milcherzeugerverband-bayern.de/) and was significantly higher compared to the production determined in our population of Simmental cattle by above mentioned authors. In

Bulgaria the average milk production of Simmental cows for period from 1999 to 2017 was 5.016±70.81 kg with 4.217±0.024% milk fat in lactation lasting 305 days. In addition, it was determined that farm and year of calving had a significant effect on studied traits (Karamfilov et al., 2019).

Milk yield in dairy cattle of Austria from 1950 to 2010 increased from 3000 kg to 6850 kg. In 2012 milk production accounted for 7000 kg milk (Gruber and Stegfellner, 2015). Petrovic et al. (2009) determined that quantitative traits of milk yield in first-calf heifers were under a strong effect of a breeding region and showed a highly significant deviation from a general average ($P<0.01$). In addition, significant deviation on the yield of milk and milk fat was caused by a year of calving. Pantelic et al. (2021) in their study which included 2589 first-calf heifers of Simmental breed raised on the farms of individual livestock producers in the region of Central Serbia determined that milk yield traits showed a high variability depending on breeding region in which the animals were raised. The aim of the study was to examine the influence of the region of rearing and lactation on the milk yield characteristics of Simmental cows in Republic of Serbia.

Material and Methods

The trial was conducted on a data set that included records on production and origin of cows of Simmental breed exhibited in regional and municipal exhibitions in the territory of the Republic of Serbia in the period from 2004 to 2017. A final set of data contained records on production and origin of 1176 cows of Simmental breed. The animals were raised in the area of 9 regions of the Republic of Serbia. Displayed animals were born in the period from 1992 to 2015 and were exhibited in the age from 2 to 13 years, that is, from 1st to 10th lactation. Production results of animals were taken from the exhibition catalogue and represented production records from herd book and were obtained in a regular procedure of control of productivity.

The research included most important milk yield traits in standard lactation such as follows:

- Milk yield (MY) (kg),
- Milk fat content (MFC) (%),
- Milk fat yield (MFY) (kg),
- Yield of 4% fat-corrected milk (4%FCM) (kg) calculated by formulas:

$4\%FCM = 0.4M + 15F$ where is: M- milk quantity (kg); F- milk fat quantity (kg).

In research the effect of the region and the year of exhibition was studied along with the age of cows in the time of the exhibition and the lactation in which the cows were exhibited for mentioned milk yield traits in standard lactation. The animals were displayed in the exhibitions in 9 regions: 1- Podunavski, 2- Rasinski, 3- Branicevski, 4- Kolubarski, 5- Jablanicki, 6- Beogradski, 7- Nisavski, 8- Sumadijski and 9- Borski. However due to a relatively small number of animals that were exhibited after the third lactation (140 cows), they were classified into a category of cows exhibited in their fourth or higher lactation.

The average values and variability of studied traits were calculated within PROC MEANS procedure of SAS programme package (version 9.4). The effect of factors on analyzed traits of milk yield was investigated within PROC GLM procedure of SAS programme package while an applied fixed model had a following form:

$Y_{ijkl} = \mu + R_i + G_j + S_k + L_l + e_{ijkl}$, where

Y_{ijkl} – is a phenotypic expression of milk yield traits,

μ- population average,

R_i-fixed effect of the region of exhibition (i=1-9),

G_j-fixed effect of the j year of exhibition (j=2004-2017),

S_k-fixed effect of the k age in the moment of exhibiting expressed in years (k=1-14),

L_l- fixed effect of the l lactation (l=1-4),

e_{ijkl}-random error.

Within the PROC GLM procedure of SAS programme package the values of the least square means (LSM) were calculated as well.

Results and Discussion

Phenotypic expression and variability of studied milk yield traits are shown in Table 1.

Table 1. Mean values and variability of milk yield traits

Trait	**N**	$\bar{x}$	σ^2	σ	**Min**	**Max**	**Cv (%)**
Milk yield (kg)	1176	5520	844039	919	3660	9750	17.00
Milk fat content (%)	1176	3.94	0.01	0.11	3.47	4.40	2,.73
Milk fat yield (kg)	1176	218	1438	38	137	374	17.00
Yield of 4% fat corrected milk	1176	5474	871295	933	3523	9167	17.00

On the basis of the records it can be seen in Table 1 that the average milk yield in studied population was 5.520±919 kg, milk fat content 3.94±0.11%, milk fat yield 218±38 kg while the yield of 4% fat-corrected milk was 5.474±933 kg.

Medic et al. (2006), as well as Niksic et al. (2011), determined lower mean milk yield for about 1000 kg while the content of milk fat in their research was lower by 0.04%. Genetic improvement in production capabilities achieved in cows had caused mean values obtained in this research to be far higher than in the research of Medic et al. (2006). This difference in mean production occurred because conducted their research in wider population of cows while our research included only the cows that participated in the exhibitions, i.e. the highest quality cows of the population. Similar values were found in their research Cziszter et al. (2017) in population of Fleckvieh cow in Romania.

The effect of the region of holding exhibition, years of the exhibition, age of animal and lactation on the yield of milk and content of milk fat were analyzed in the research. Table 2 shows the significance of factors included in the analysis.

Table 2. Values of F-test for studied factors

Trait	Region	Year of exhibition	Age	Lactation	R^2
	df1=8	df1=6	df1=10	df1=3	
Milk yield	<0.01**	<0.01**	0.7322 ns	<0.01**	0.22
Milk fat content	<0.01**	<0.01**	0.0799 ns	<0.01 **	0.16
Milk fat yield	<0.01**	<0.01**	0.6892 ns	<0.001 **	0.23
4% fat corrected milk	<0.01**	<0.01**	0.7123 ns	<0.01 **	0.23

**- high statistically significant effect, ns- no statistically significant effect

Table 3 shows the least square means (LSM) for studied traits according to the region of holding exhibition. In Table 3 we can see that region, age and lactation had a very high statistically significant effect on studied traits, while the age of cows had no statistically significant effect. Determination of model ranged from 0.16 for the milk fat content in standard lactation to 0.23 for 4% FCM.

Table 3. LSM values for studied traits according to the region of holding exhibition

	Region								
Trait	Podunavski	Rasinski	Branicevski	Kolubarski	Jablanicki	Beogradski	Nisavski	Sumadijski	Borski
MY (kg)	5814	5982	5802	5098	5434	5946	4452	5804	5586
MFC (%)	3.96	3.97	3.93	3.98	3.93	3.97	3.92	4.03	3.82
MFY (kg)	231	237	228	203	214	236	176	233	214
4%FCM(kg)	5784	5949	5738	5082	5377	5923	4416	5823	5443

MY – milk yield, MFC- milk fat content, MFY- milk fat yield, 4%FCM- yield of 4% fat corrected milk

The highest milk yield as of 5.982 kg was recorded in Rasinski district while the lowest one as of 4.452 kg was recorded in Nisavski district. Milk fat content varied in considerably smaller limits in relation to milk yield where Sumadijski district stands out with highest milk fat content as of 4.03%, while the lowest content of milk fat was recorded in Borski district.

Maximum milk fat yield and 4% fat-corrected milk were recorded in Rasinski district being 237 kg of milk fat and 5.949 kg of 4% fat-corrected milk while the lowest yield was recorded in Nisavski district where it accounted for 176 kg milk fat and 4.416 kg 4% fat-corrected milk.

Differences obtained in milk yeild, milk fat content, milk fat yield and 4% milk fat-corrected milk are the consequence of different conditions of raising which dominate in these districts but also of the application of different semen during artificial insemination, therefore, the impact of different sires on production of their daughters**.**

The same conclusion was reached also by Petrovic et al. (2009) in their research. They determined that milk yield, besides region, is affected by the year of calving as well. Similar research was conducted by Pantelic et al. (2021) who obtained the same conclusion.

Table 4. LSM values for studied traits according to lactation

	Group of lactation			
Trait	1	2	3	4
MY (kg)	5052	5553	5703	5877
MFC (%)	3.92	3.94	3.96	3.97
MFY (kg)	198	219	226	233
4% FCM(kg)	4990	5505	5668	5852

MY – milk yield, MFC- milk fat content, MFY- milk fat yield, 4%FCM- yield of 4% fat corrected milk

Table 6 shows LSM values for studied traits according to lactation. The highest milk yield was realized by the cows in group 4, i.e. by the cows that realized 4 and more lactations their production being 5.877 kg while the lowest milk yield was realized by the first-calf heifers as of 5.052 kg. Milk fat content had the same trend of rise being the lowest in the cows in the first group i.e. first-calf heifers as of 3.92%, while the highest one was obtained by the cows in group 4 as of 3.97%. The highest increase regarding milk yield occurred between the first and the second lactation while later on this increase gradually declined. The yield of milk fat and 4% fat-corrected milk was lowest in the first-calf heifers and accounted for 198 kg milk fat and 4990 kg 4% fat-corrected milk. The highest mean values of these parameters were recorded in the cows in the group 4 being 233 kg milk fat and 5852 kg 4% fat-corrected milk.
Gruber and Stegfellner (2015) in their research concluded the same. They confirmed that the number of lactations has a significant effect on milk yield and that the highest leap in milk production occurs between the first and the second lactation while later on the increase gradually declines. They also reported that the content of milk fat did not change significantly but that the milk yield increased due to the increase of the milk quantity which is the case here as well.

Conclusion

The research conducted confirmed that there are high statistically significant differences in expressing the analyzed traits of milk yield between the regions in which the animals were raised. This difference between the regions with the highest and with the lowest production amounted to even 1530 kg. Such a difference indicates a large heterogeneity of breeding conditions and practices as an applied technology of production. Lactation and year of the exhibition also highly statistically affected variability of analyzed milk yield traits. In the next period it will be necessary to work on optimizing all zootechnical conditions on dairy farms in order to create more favorable conditions for milk production and achieve the results obtained by the cows in other populations of Simmental cattle breed.

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Professional paper

Free traffic in robotic milking of cows through ethological and welfare approach

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Abstract

The cow welfare it is subject to various influences, in both negative and positive ways, such as: social interactions with other cows, human-animal interactions, management systems, nutrient supply, barn design, climate, etc. Two basic behaviours that are important in ethology of animals are eating and resting. The milking was incorporated between those two needs in robotic milking, or it was given to the free will of the animal itself. Robotic milking has gained widespread acceptance, as a way to reduce labour on dairy farms, increase milk production and simultaneously improve dairy cow welfare by allowing cows to choose when to be milked. The free cow traffic is one of the variations of cow traffic strategies, where cows can access feeding and resting areas of the barn with no restriction. The basic concept for such traffic is increase the comfort of cows, and compliance with the five freedoms of animal welfare.

Key words: dairy cows, free traffic, robotic milking, ethology, welfare

Introduction

Robotic milking has gained widespread acceptance, as a way to reduce labour on dairy farms, increase production per cow, and improve the lifestyle of dairy farm families (De Koning, 2010). According to Rodenburg (2017) robotic milking reduces labour demands on dairy farms of all sizes and offers a more flexible lifestyle for farm families milking up to 250 cows. Barkema et al. (2015) stated that the percentage of herds using this technology is highest in Scandinavian countries and the Netherlands. The Automatic Milking Systems (AMS) have the positive effect on udder health and on cow management, behaviour, health, and welfare (Hovinen and Pyoralia, 2011; Jacobs and Siegford, 2012). The AMS also have some of the negative effect on the cows and farmers, for example: the high investments in robots and there servicing (Maculan and Lopes, 2016), increased levels of free fatty acids in milk (Sapru et al., 1997), decreasing of milk fat and protein (Bach et al., 2009) or increasing of subclinical ketosis (Tatone et al., 2017; King et al., 2018). It is crucial to have well-functioning cow traffic with cows voluntarily visiting the milking unit if the farmer want to obtain the advantages that an Automatic Milking Systems can provide.

Cow Welfare

The cow welfare it is subject to various influences, in both negative and positive ways, such as: social interactions with other cows, human-animal interactions, management systems, feeding practices and nutrient supply, barn design, climate, and other environmental conditions (Wiktorsson and Sørensen, 2004). The schematically explanation for environmental influence and indicators for dairy cow welfare were given is Figure 1. (Webster, 2020). Cows in Automatic Milking Systems (AMS) have more freedom to control their daily activities and rhythms and have more opportunities to interact with their environment (Jacobs and Siegford, 2012). Automatic milking systems have the potential to increase milk production by up to 12%, decrease labour by as much as 18%, and simultaneously improve dairy cow welfare by allowing cows to choose when to be milked. However, most AMS are single-stall units, resulting in an isolated milking experience that drastically differs from most conventional parlour systems.

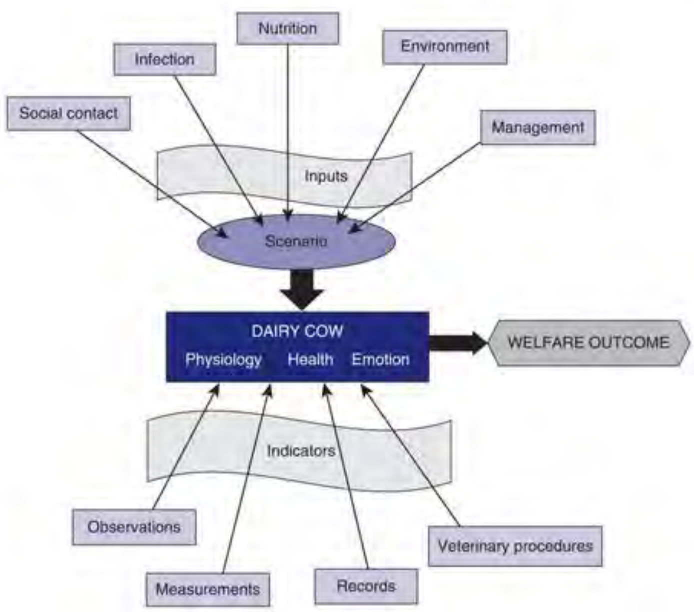

Figure 1. Dairy cow welfare and the environment: inputs and indicators (Webster, 2020)

Cow Traffic

According to Forsberg (2008) the cow traffic refers to the way that the cows can move in the barn in order to perform daily activities such as feeding, lying down and milking. Two basic behaviours that are important in ethology of animals are eating and resting. Knowing that, the milking were incorporated between those two needs of animals in AMS, or it was given to the free will of the animal itself. In herds with AMS the four common variations of cow traffic strategies are used. The first variation is free cow traffic, where cows can access feeding and resting areas of the barn with no restriction. The second variation is guided (forced) cow traffic "milking first", with one-way gates blocking the route from the resting area to the feeding area. Cows leaving the resting area must enter the milking box to be milked if the interval since the last milking makes them eligible or refused if the milking interval is too short. After passing through the milking stall, the cow is released to the feeding area and can return to the resting area through a one-way gate. The third variation, guided (forced) cow traffic with preselection, adds an entry lane where a sort gate directs cows eligible for milking to the commitment pen and ineligible cows to the feeding area. This reduces waiting times for milking and for feed because only cows that are eligible for milking pass through the milking stall. Preselection can also be provided by selection gates in crossovers away from the robot, which open only for cows ineligible for milking. The fourth variation, guided (forced) traffic "feed-first", is a reversal of variation 3 that allows cows access to the manger from the resting area via one-way gates but ensures that they can return to the resting area only through the robotic milking stall or through preselection gates that direct cows ineligible for milking directly to the free-stalls or bedding pack.

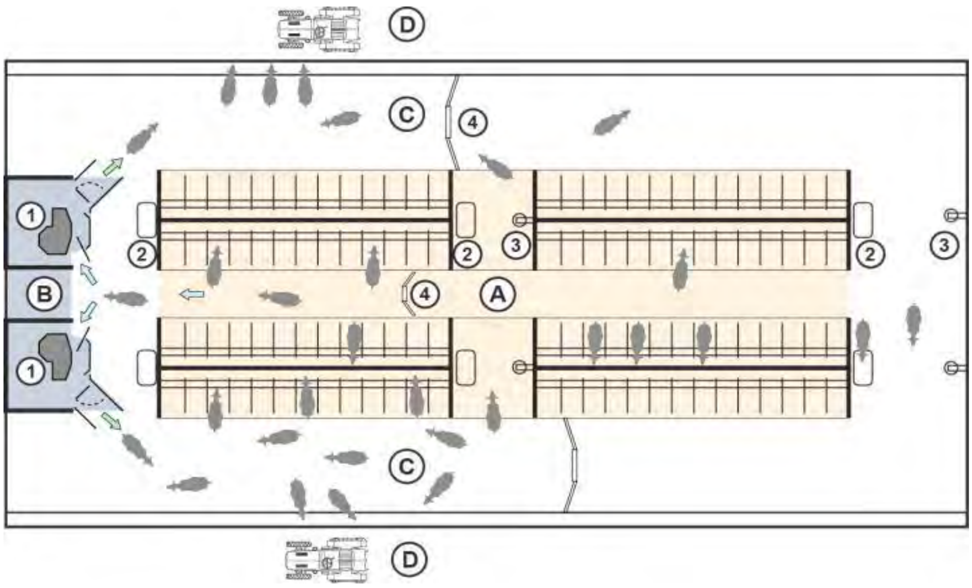

Figure 2. Example of the farm design with free cow traffic with an AMS (Unal et al., 2017) (A-Resting area, B-Milking room, C-Feeding area, D-Feeding Line, 1-AMS, 2-Troughs, 3 Brushes 4-Manure scraper)

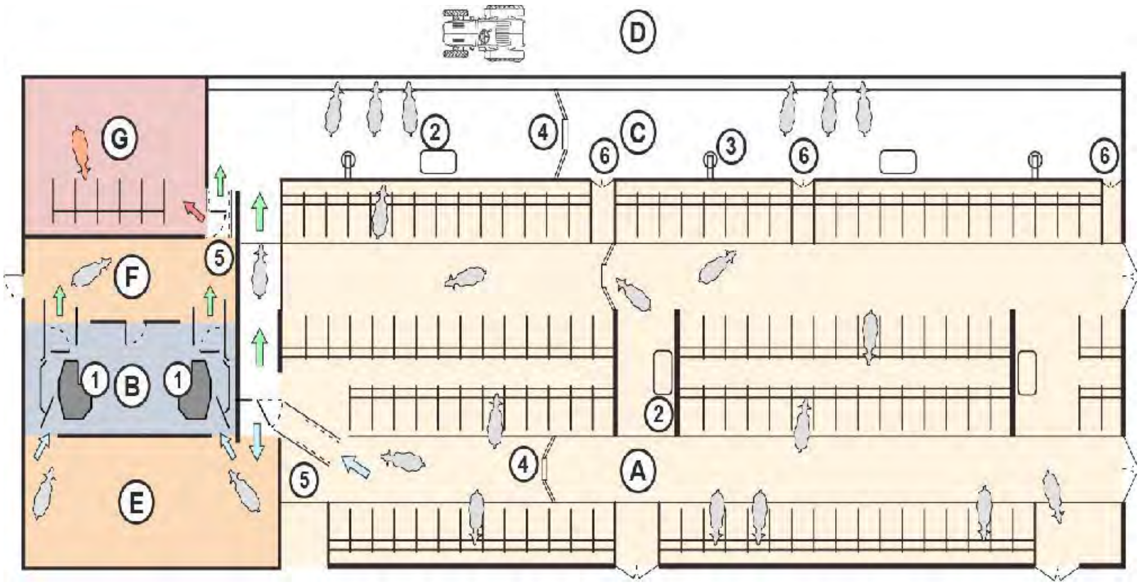

Figure 3. Example of the farm design with guided (forced) cow traffic "milking first" with an AMS (Unal et al., 2017) (A-Resting area, B-Milking room, C-Feeding area, D-Feeding Line, E-Waiting area, F-Exit area, G-Separation area, 1-AMS, 2-Trough, 3-Brush, 4-Manure scraper, 5-Smart gate, 6-One way gate)

The choice of guided versus free traffic can have a substantial effect on both labour efficiency and cow comfort and is an important consideration in the design of automatic milking system (AMS) facilities. Studies show that the number of cows fetched can be decreased by forcing the cow to enter the AMS stall or an associated selection gate in route from the resting area to the feed manger or on her return from the manger to the resting area (Harms et al., 2002; Rodenburg and Wheeler, 2002; Bach et al., 2009).

Free traffic

The free cow traffic is one of the variations of cow traffic strategies, where cows can access feeding and resting areas of the barn with no restriction. The free traffic implies that cows can choose when they want to: eat, drink, rest and be milked. There are no fences or separation gates. The cows can live freely, without restrictions or constraints. During milking, the cows are rewarded with concentrate, with the result that they are happy to be milked.

The basic concept for free traffic is increase the comfort of cows. It also allows compliance with the five principles (five freedoms) of animal welfare, freedom from hunger and thirst, freedom from physical and thermal discomfort, freedom from pain, injury and disease, freedom to display their natural behaviour, freedom from fear and stress (Figure 4).

Figure 4. Five freedoms of animal welfare (European Commission, Euroactiv, https://www.euractiv.com/section/agriculture-food/infographic/animal-welfare-in-the-eu/)

In terms of animal welfare, the von Keyserlingk et al. (2009) emphasized that the unnaturalness of modern housing conditions is one of the greatest sources of public concern and that freedom to express normal behaviour is one of the 5 freedoms that serve as hallmarks of animal welfare (Croney and Anthony, 2011). The free traffic cows enable greater cow comfort and welfare making farming more acceptable in public opinion.

According to Gygax et al. (2007) reported that milking frequency was higher in early lactation with free traffic and higher later in lactation with guided traffic. Harms et al. (2002) reported that the number of meals was higher with free cow traffic than with either guided or guided with preselect traffic. They also reported that forage intake decreased when cows were switched to guided traffic. Cows with free access to forage in the manger spent more time eating and less time standing in free-stalls (Hermans et al., 2003), the lower ranking cows had longer waiting times in the commitment pen compering to dominant cows (Melin et al., 2006).

Table 1. Feeding and milking behaviour of cows with free traffic versus guided traffic systems (Bach et al., 2009)

Item (per cow per day)	**Free Traffic**	**Guided Traffic**	**SE**	**P**
Total milkings (no.)	2.2	2.5	0.04	<0.001
Fetched milkings (no.)	0.5	0.1	0.03	<0.001
Total eating time (min/day)	168	147	8.97	0.19
Daily meals (no./day)	10.1	6.6	0.30	<0.001
Concetrate intake (kg)	2.5	2.5	0.09	0.99
Milk production (kg)	29.8	30.9	1.74	0.32
Milk fat (%)	3.65	3.44	0.078	0.06
Milk protein (%)	3.38	3.31	0.022	0.05

Bach et al. (2009) in their research of comparison of free and guided ("milking first") traffic systems, get results witch illustrate that milking and eating behaviour, were all influenced by the choice of traffic system, but milk production and milk composition were similar (Table 1.).

Ketelaar-de Lauwere et al. (2000) emphasizes a one problem with free cow traffic that cows with the intention to visit the milking unit might withdraw and perform some other activity if the robot is occupied. Such an undesirable behaviour of cows can be avoided according the previous research (Uetake et al., 1997) by placing a waiting area in front of the milking unit where cows will have to remain until they have passed the unit. Research of the Ketelaar-de Lauwere et al. (2000) showed a negative effect of the closed waiting area, because the cows spent less time in the feeding area compared to cows in Free cow traffic without a closed waiting area. According to Markey (2013) free cow traffic without waiting area, resulted in slightly more milking's per robot and day than guided "feeding first" traffic, and in terms of cow performance, except milk conductivity, free cow traffic both with and without waiting area seems to be more favourable than feeding first traffic.

Conclusion

Despite some of the negative aspects of intensive milk production in terms of cow welfare, population growth is obvious and the need for food has increased. Today's market offers modern technology and performance of dairy farms that greatly improve housing conditions and welfare of cows. Some of the solutions are AMS and free traffic of cows. Farmers who choose to use AMS decide for themselves which robot producer and which approach to cow traffic will use.

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Слободан промет у роботској мужи крава кроз етиолошки приступ и добробит

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Сажетак

Добробит крава је подложна различитим утицајима, како негативним тако и позитивним, као што су: друштвене интеракције са другим кравама, интеракције људи и животиња, системи управљања, снабдевање хранљивим материјама, дизајн штале, клима, итд. Два основна понашања која су важна у етологији животиња су једење и одмарање. Мужа је инкорпорирана између те две потребе у роботској мужи, или је дата слободној вољи саме животиње. Роботска мужа је постала широко прихваћена, као начин да се смањи радна снага на фармама млека, повећа производња млека и истовремено побољша добробит млечних крава омогућавајући кравама да бирају када ће бити помужене. Слободни промет крава је једна од варијација стратегија промета крава, где краве могу приступити просторима за храњење и одмор у штали без ограничења. Основни концепт оваквог промета је повећање удобности крава, и поштовање пет слобода добробити животиња.

Кључне ријечи: музне краве, слободан промет, роботска мужа, етологија, добробит

Professional paper

Physiological and ethological aspects of horse feeding

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Abstract

The paper aimed to define frequency of feeding (frequency of rations) "modern" horse, regarding the physiological and ethological aspects of horse. From an evolutionary and physiological point, the horse was built for constant feeding. The rations have to provide an adequate supply of energy and nutrients and largely affect the horses' ethology. The management of feeding and quality of ration affects the horses' health and welfare. Today horses are far less physically active than before. The frequency of rations affects the physiological parameters of digestion and the aetiology of the horse. How to balance the appropriate ration frequency considering the physiological and ethological aspects, and the usage of the horse itself depends primarily on the daily amount and quality of feed required for the horse to remain fit. Usually, adult horses have two rations a day with a 10-hour daily interval and 14-hours nightly interval between rations. The frequency of a horses' ration varies depending on the way of keeping, category, physiological condition and usage. The effect of feeding on the health and welfare of horses is mainly focused on the appropriate intake of energy and nutrients and on the consideration of the aetiology of feeding, limiting concentrates to specific needs, availability of water and hygienic correctness of feed and water. Knowledge of the specific feeding needs of horses in physiology and aetiology should be considered an essential part of preserving the horses' welfare.

Key words: feeding, horse, feeding frequency

Introduction

Looking ago 100 years when horses were used in agriculture, transportation and the army, they were fed four to five times a day. The horses from the ration had to obtain enough energy for their daily work. This fact was applied by horse traders and buyers because horses were healthier at that time and they found a buyer faster. The "modern" horse is faced with completely different conditions (Davidson and Harris 2007). The work is less physically strenuous and horses move considerably less than before (Sakač et al., 2011). The frequency of feeding (number of rations per day) should be harmonized with the usage of horses since it simultaneously affects the physiological parameters of digestion, especially the stomach and the rest of the digestive tract, as well as the aetiology of horses. The impact of feeding on the health and well-being of horses includes in particular an adequate supply of energy and nutrients. The understanding of horse feeding is obtained by considering the physiological behaviour (ethology) of feed intake, especially voluminous feed, limiting the intake of concentrated feed that depends on work, water quality and availability and hygienic feed. Typical stereotypes in horses, such as swallowing air or knitting, indicate a limited sense of well-being, and feeding errors can contribute to the development of stereotypes. According to Ellis (2010), the "biological basis of behaviour" includes physiological mechanisms that direct and control the body (nervous and endocrine systems), which adapt, change due to genetics, but also due to adaptation to breeding that allows interaction, perception and experience within the environment.

According to Benz et al. (2014) in horse breeding, feeding is not only for feeding horses but also for their occupation. Continuous, moderate consumption of voluminous feed enables the maintenance of a healthy digestive system. Voluminous feed plays an important role in feeding horses, not only from a nutritional point of view. Horses have an instinctive need to constantly move and "bite" because of their bent development. Because the horse is constantly eating in the wild, the horse's stomach is constantly producing stomach acid, which can only neutralize saliva. Saliva is excreted only when chewed. If the stomach is at rest for more than four hours, it continuously produces stomach acid which irritates the mucous membranes. The consequences of inappropriate feeding can be a stomach ulcer or colic. Inadequate feed intake is described as an important factor in many horse behavioural disorders. In addition to stereotypes, some health disorders such as gastrointestinal disorders, muscle damage or obesity are caused by nutritional errors that seriously impair the well-being and development of the disease, such as the occurrence of laminitis that accompanies obesity. In most cases, feeding errors occur due to insufficient knowledge of the specific needs of horses, and only in a few cases intentional errors can be assumed. Davidson and Harris (2007) warn of a discrepancy in horse feeding with two large rations a day. These modern practices have advantages but also potential disadvantages for the horse, (nutritional and ethological) which can affect well-being. The growing interest in animal welfare and knowledge of the physiological and ethological needs of horses has led to the development of different types of horse management and housing systems (Marliani et al., 2021). According to Placci et al. (2020) in the equestrian world, two different types of management can be distinguished: traditional management and natural boarding, each of which has its advantages and disadvantages.

The paper aimed to define frequency of feeding (frequency of rations) "modern" horse, regarding the physiological and ethological aspects of horse.

Physiology of horse digestion

According to Ellis (2010), food intake is controlled by multiple energy-related homeostatic signals (such as the hormones ghrelin and leptin) as well as somatosensory (taste, touch, smell) and motivational stimuli across the peripheral and central nervous systems. Free-range horses tend to eat 10-15 different feeds in 24 hours and are fed for about 10-14 hours a day. Voluntary dry matter intake ranges between 1.3-1.7% body weight (BW) for straw, 1.9% BW for hay and 2.6% BW for fresh grass. Chewing one kilogram of fresh matter in an adult horse ranges around 3400 for straw, 2700 for hay and 1800 chewing for chopped fodder with corresponding swallowing times of 45-35-20 minutes/kg (Ellis, 2010). The chewing rhythm (chewing/min) remains mostly constant for each animal, while intake rates (g / min) vary according to moisture content, fracture properties, teeth, and organoleptic perception of feed (Ellis, 2010). Consumption of concentrated food takes about 18 minutes/kg (for oats). Adding chopped fodder for concentrated food by a rate of 20-38% increases the time of food intake by 50-100%. Furthermore, adding oil to animal feed increases feed intake time.

Irregularities in the feeding and/or well-being of horses indicate atypical reaching for food such as geophagy (eating earth or sand), chewing wood and coprophagy. According to Ellis, (2010) for atypical food reach, a ration for horses enriched with structural fibres is suggested. According to Wyss et al. (2016), the distribution of meals up to twelve times in 24 hours significantly contributes to feeding adjusted to the physiology of horse digestion.

Voluminous food in horse feeding

Voluminous feed, whether it is in form of grazing, fresh green mass or hay, should always be available. This method of feeding management is not possible in all breeds and in all horses (overweight). Furthermore, the increased hay intake increases water consumption, which should always be available. The preparation of quality hay is laborious (DLG-verlag, 2003) and is exposed to a high weather risk when drying on the ground which can lead to large changes in the quality itself. The optimal time to cut the grass to produce hay to feed the horses would be from the beginning (about 30% of the grass blooms) to the middle of flowering. During this phase, the metabolic energy level is 7-8 MJ ME / kg dry matter (DM), the crude fibre content of about 27% or more and crude protein below 12% in DM (DLG-verlag, 2003). A healthy horse should be able to get as much energy as possible from the voluminous fodder (hay). Hay quality should be medium to good and consumed in the amount of 2 kg / 100 kg BW. Horses consume hay at a different rate than concentrated feed. For the same amount of feed, horses consume hay four times longer. Horses graze on pasture for 12 to 20 hours. Since grass contains a lot of water (70-90%), more grass must be eaten until as much weight as dry hay is absorbed (water content 10-15%) (Lüem, 2021). Adult horses that eat six to eight hours of voluminous feed meet 80% of the nutrients per day, and 20% should be provided from a concentrated feed. If horses are deprived of a concentrated portion of a ration, the daily intake of voluminous feed is extended to 12-16 hours (Lüem, 2021). The time of intake of voluminous feed can be extended with automatic feeders. Automatic feeders are suitable for horses with low energy requirements or horses on a diet. Intake of voluminous feed is slightly more frequent in daylight, while at night it is slightly less. The frequency of feeding affects the intake of voluminous feed. When horses receive 8 small concentrated feed rations, they consume more voluminous food than if they receive only two concentrated rations. If a horse needs to eat more hay, it is often given small amounts of concentrated feed. According to Benz et al. (2014), the period of consumption of voluminous feed is greatly influenced by the characteristics of the feeder (extends the consumption time by 50%). Furthermore, Clegg et al. (2008) found that feeder characteristics did not affect horse physiology (digestibility, plasma or pulse cortisol concentration).

Concentrated feed in horse feeding

Concentrated food can be offered through 3-4 rations in the amount of 0.3 kg / 100 kg BW per ration. Vegetable oils can be supplemented in the amount of 20-50 ml / 100 kg BW daily after a period of adaptation. Complex foods with increased protein content (about 12%) and soy meal (10-20 g / 100kg / BW) can be used for protein supply. An increased proportion of crude fibre in a ration can be achieved with foods that contain pectin such as beet pulp (0.2 kg / 100 kg BW) (Lüem, 2021). Furthermore, in adult horses, excessive mineralization with calcium (Ca) and phosphorus (P) and vitaminization with vitamins A and D should be avoided. A positive effect on the horses' welfare has an increase in the number of concentrated feed rations and at the same time a decrease in the amount of feed in the ration. The advantage is the glycemic response of the meal and its consequences. The glycemic response changes significantly when the daily amount of feed is given in one, two or more meals (Philippeau et al., 2014). One meal leads to a higher glycemic index and as a result higher insulin secretion (Harris and Geor, 2009). The glycemic response to a morning meal is strongest, no matter how many feed rations are given during the day. This should be considered in horses where the glycemic response plays a role and should be controlled (e.g. endurance). Furthermore, the order of feeding also affects the glycemic response. For horses receiving only one concentrated ration, the glycemic response is higher when hay is given 15 minutes before the concentrated feed, and lower when hay is given 15 minutes after a concentrated feed (Rodiek and Stull, 2007, Lüem, 2021). This is not noticed when horses receive several concentrated feed rations a day. Horses that receive concentrated feed before voluminous often bite the wood. Feeding concentrated feed also affects air swallowing, knitting and other stereotypes in horse behaviour. Stereotypical behaviours such as chewing wood or swallowing air are the result of boredom but can be caused by inflammation, ulcers, or abnormal stomach acidity.

Number of rations per day

According to Davidson and Harris (2007) and Meyer and Coenen (2014), adult horses are typically fed twice daily. During the day they have an interval between rations of 10 hours and at night 14 hours. In addition to the mentioned glycemic index, the capacity of the digestive tract should be taken into account when administering large amounts of concentrate (Harris and Geor, 2009). Large amounts of concentrated food can overcome the small intestine's ability to break down grains and absorb starch. Too much undigested grain reaches the back of the intestine. The consequences of microbial fermentation or non-fermentation can range from diarrhea, colic to laminitis. As a general rule, no more than 500 g of cereal concentrate (> 30% starch) should be given per ration per 100 kg of body weight (Lüem, 2021). For larger quantities, it should be divided into several smaller rations. If the starch content is higher, the recommendations for the amount per ration are even lower as shown in the table below (Table 1).

Table 1. Maximum mass of ration for optimal starch digestion in the small intestine (Lüem, 2021)

Body weight (BW)	< 30%	30 – 40 %	> 40 %
400 kg	2.00 kg	1.20 kg	1.00 kg
450 kg	2.25 kg	1.35 kg	1.125 kg
500 kg	2.50 kg	1.50 kg	1.25 kg
550 kg	2.75 kg	1.65 kg	1.375 kg
600 kg	3.00 kg	1.80 kg	1.50 kg
650 kg	3.25 kg	1.95 kg	1.625 kg
700 kg	3.50 kg	2.10 kg	1.75 kg

Rations with more than 1.1 g of starch per 1 kg of body weight increase the risk of stomach ulcers. Gastric ulcer is also closely associated with malnutrition. In horses that do not have access to feed for 12 hours, the pH in the stomach drops to 2 (Lüem, 2021) creating conditions for stomach ulcers. The lowering of the pH in the stomach is often noticed even after the feeding of concentrate or pure cereals. In horses kept on pasture, the pH in the stomach is between 4 and 6. Increased frequency of rations is also very important if horses have certain diseases, such as hyperkalemic periodic paralysis. An approach with two large rations a day leads to nutritional imbalances and a negative effect on horses' well-being (Davidson and Harris 2007).

Horse management and housing systems

In the equestrian world, two different types of horse breeding management can be distinguished: traditional management and natural boarding. According to Placci et al. (2020) horses kept in natural boarding have the most favourable endocrine framework (determine DHEA (dehydroepiandrosterone) and cortisol concentration through RIA). This research suggests that this management best suits the ethological and physiological needs of the horse. The results of Marliani et al. (2021) related to the ethological barn model, showed that horses spent most of their time searching for food, followed by behaviours at rest and movement. Social behaviours (e.g., allogrooming, olfactory research) were rare, and stereotypical behaviours (e.g., oral and locomotor stereotypes) accounted for 2.74% ± 2.74% of total time. The percentage of time spent searching for food, resting and moving reflects the calculation of activity observed in wild horses that roam freely. However, the rare occurrence of positive social interactions and the presence of some stereotypes could be aspects for improvement. This kind of ethological barn housing could be considered a good alternative to traditional horse management and could offer a better compromise between horse needs and human management goals.

Conclusion

The appropriate frequency of feeding (frequency of rations), taking into account physiological and ethological aspects, depends on the daily amount of feed necessary for a horse to stay in shape. Frequent and constant access to feed either grass or hay is a key point of optimized feeding technology. Adequate rations of concentrate affect the glycemic response, avoid overuse of the digestive tract, and will affect horse behaviour. If a lot of calories are needed and starchy foods are used, the frequency of meals needs to be adjusted.

Presumably not every horse needs four rations as their hard-working ancestors do. The frequency of rations during the day is equally important in optimized horse feeding as the quality of ration. The ethological and physiological needs of horses are largely met in the natural boarding system of horse breeding management. Today in the equestrian world there is a growing interest in animal welfare. Therefore, it is necessary to modify the management and housing systems following the physiological and ethological needs of horses.

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Fiziološki i etološki aspekti hranidbe konja

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Sažetak

Cilj rada bio je prikazati optimalan broj obroka „modernog“ konja tijekom dana uzimajući u obzir fiziološke i etološke aspekte konja. S evolucijske i fiziološke točke, konj je izgrađen za stalno hranjenje. Obroci moraju osigurati odgovarajuću opskrbu energijom i hranjivim tvarima i uvelike utječu na etologiju konja. Upravljanje hranidbom i kvalitetom obroka utječe na zdravlje i dobrobit konja. Danas su konji daleko manje fizički aktivni nego prije. Učestalost obroka utječe na fiziološke parametre probave i etiologiju konja. Kako uravnotežiti odgovarajuću učestalost obroka s obzirom na fiziološke i etološke aspekte, te korištenje samog konja ovisi prvenstveno o dnevnoj količini i kvaliteti hrane koja je potrebna da konj ostane u formi. Odrasli konji obično imaju dva obroka dnevno s 10-satnim dnevnim intervalom i 14-satnim noćnim intervalom između obroka. Učestalost obroka konja varira ovisno o načinu držanja, kategoriji, fiziološkom stanju i korištenju. Učinak hranidbe i kvalitete obroka na zdravlje i dobrobit konja uglavnom je usmjeren na odgovarajući unos energije i hranjivih tvari te na razmatranje etiologije hranjenja, ograničavanje koncentrata na specifične potrebe, dostupnost vode i higijensku ispravnost hrane i vode. Poznavanje specifičnih hranidbenih potreba konja u fiziologiji i etiologiji treba smatrati bitnim dijelom očuvanja dobrobiti konja.

Ključne riječi: hranidba, konji, frekvencija hranjenja

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