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THE ASSESSMENT OF PHYSIOLOGY PARAMETERS OF WILLOW PLANTS AS A CRITERION FOR SELECTION OF PROSPECTIVE CLONES

ABSTRACT: Bioenergy production based on short rotation coppice willow plantations (SRC) is an effective direction both for economic and environment profit. The yield of willow wood can amount to 10-15 tons per hectare of dry biomass per year and the cost of thus obtained energy is lower in comparison with other energy crops. In order to achieve high yield and profitability, the use of special willow clones is necessary. Species most often used in selection for biomass production are shrub type willows: *Salix viminalis*, *Salix dasyclados* and *Salix schwerini*, while the clones tested in this paper were also of tree species *Salix alba*. The productivity and some physiology characteristics of Serbian selection clones of *Salix alba* (Bačka, Volmianka and Drina) and Swedish selection clone Jorr (*Salix viminalis*) were investigated in greenhouses and in field conditions. As the result of testing three clones of *Salix alba* – Bačka, Volmianka and Drina, having special preferences and adaptability to different environmental conditions, these were included in State register of Republic of Belarus in 2013. In our experiment it was also satisfactory that specific properties of willows (intensity of transpiration and photosynthesis, water use efficiency and others), were conserved both in greenhouses and in field conditions. This factor gives opportunity to select prospective clones of willows at an early stage of ontogenesis for further testing.

KEYWORDS: bioenergy, willow, selection, varieties, physiology parameters

INTRODUCTION

Bioenergy production based on short rotation coppice plantations (SRC) is widely introduced in some European countries, USA and Canada [Rodzkin *et al.*, 2012]. Willows have a special place among energy crops due to their high potential in productivity and broad tolerance to environmental factors.

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The average yield of willow wood is 10–15 tons per hectare of dry biomass per year [McElroy 1986], which results in a lower cost of energy obtained from willow wood compared to other crops [Keolian 2005].

The assessments of cost of energy crops production on the basis of total costs and risk assessment were done in Sweden [Rosenqvist and Nilsson 2007], where the cost of energy from willow biomass was 4–5 Euro/GJ, from poplar 5–6 Euro/GJ, from hemp 8–9 Euro/GJ, from reed 6.5–6 Euro/GJ, from silver-grass 7.9–8.5 Euro/GJ and from triticale 6.7–7.1 Euro/GJ. In Poland, the assessment of cost of energy crops has shown that the highest profit was from willow wood [Krasuska 2011]. According to Polish agricultural market, energy crops can be as competitive as cereal crops.

In order to achieve high yield and profitability, the use of special willow clones is necessary. In the former USSR the special breeding program of willow clones selection started in 1960s and 1970s [Skvortsov 1968]. The willow biomass was used for baskets, furniture, as building material and for other purposes [Levitskiy 1965]. As prospective candidates, hybrid varieties of willow Jarvim, Omvim, (*Salix schwerinii*), Chillin-3, (*Salix viminalis* x *Salix chilkooans*), Jikin-7, (*Salix viminalis* x *Salix purpurea*) were selected, and during field testing the highest biomass production was obtained by varieties of *Salix schwerinii*.

Selection of willow for energy purposes started after 1970's. The following species of willow were most actively used for selection: *Salix viminalis*, *Salix dasyclados*, and *Salix schwerinii*. These species were characterized as high productive with a large number of sprouts and fast sprout re-growing. They belong to bush types of willow [Caslin *et al.*, 2012; Tuck *et al.*, 2006]. But along with these characteristics for selection, it is necessary to assess other features. For example, *Salix dasyclados* is characterized by lower requirements regarding oxygen and nutrients supply when comparing to *Salix viminalis*. *Salix purpurea* and *Salix acutifolia* successfully grow under condition of water shortage, as opposed to *Salix nigra* which can resist extra flooding, and so on [Parfenov 1986]. Taking in consideration that willow wood can grow on different types of soil, influence by a number of environmental factors, it is necessary to broad of base of willow species and hybrids for selection [Tahvanainen and Rytönen 1999; McKay 2011; Rodzkin *et al.*, 2013]. In this article, the results of our study of selection of willow *Salix alba* are presented.

MATERIAL AND METHODS

The clones of *Salix alba* were selected at the Institute of Lowland Forestry and Environment in Novi Sad, and clone Jorr (*Salix viminalis*) is Swedish selection. The experiments were carried out in greenhouses in Mitscherlich pots and under field conditions in the International park “Volma” (Republic of Belarus). Soil properties are presented in Table 1. The field experiment was

designed as 25 m² plots in 4 repetitions with 1.40x0.70 m spacing. The following physiological characteristics of willow plants were measured: water use efficiency (WUE), transpiration (E), photosynthesis (A), and stomatal conductance (GS). The results were measured by means of LC pro+ Portable Photosynthesis System (ADC BioScientific Ltd. company).

Transpiration, water deficit, water retained abilities, morphology parameters of willow plants were measured under field conditions by weighing the leaves on the torsion balance [Grodzinski 1973]. The soil of experimental plot was sandy loam (Table 1). The density of willow planting was 16,000 per hectare.

Table 1. *Agrochemical characteristics of soil*

Experimental plot	Soil characteristics				
	Structure	Humus %	P ₂ O ₅ , mg/kg	K ₂ O, mg/kg	pH _{KCl}
Volma	Sandy clay	2.2	76.2	96.9	6.05

RESULTS AND DISCUSSION

Due to its tree form, *Salix alba* was not actively used in the selection for SRC plantations. The reason could be in the risk of insufficient number of sprouts after the first year cutting. Our results showed that the average number of new sprouts after cutting of *Salix alba* varied from 3.5 to 3.9, *Salix viminalis* – 3.1–4.1, and *Salix dasyclados* – 3.9–4.2 (Table 2). These characteristics indicated that clones of *Salix alba* were competitive to other species of willow. The Serbian candidate clones of *Salix alba* were estimated both in greenhouses and under field conditions. The high potential of willow clones selected within *Salix alba* species was also recorded for the conditions of Russia [Parfenov 1986] where yield of hybrids *Salix fragilis* x *Salix alba* was higher than yield of *Salix viminalis* and ranged from 7 to 10 tons of dry biomass per hectare.

One of the problems in selection of perennial crops is the duration of the process, where testing of prospective clones lasts for several years until final selection of the best cultivar. In our experiments was investigated the ability of willow clones to keep their parameters both in greenhouses and under field conditions. The key factor for willow production is the water regime of plants. Willows belong to the group of phreatophytes, having an increased demand for water during vegetation. Therefore, the focus on water regime in willow plants is obligatory in the investigation.



Figure 1. Seedlings of *Salix alba* after cutting at experimental plot “Volma” in 2013.

The results of comparison of Serbian selection of *Salix alba* candidate clones (Drina, Volmianka and Bačka) with widely used Swedish clone Jorr, regarding the intensity of transpiration in greenhouse, is presented in Figure 2.

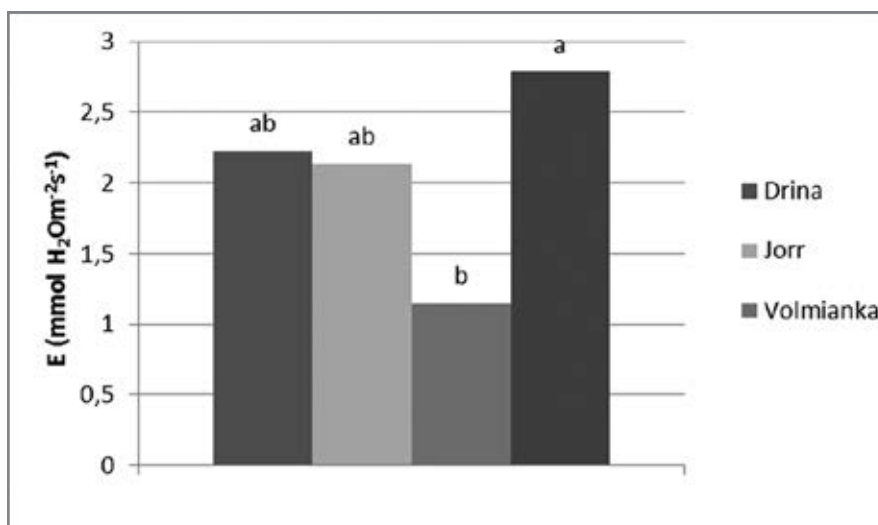


Figure 2. Intensity of transpiration of willow clones in greenhouse.

The highest transpiration was observed for clone Bačka, while the lowest transpiration rate was with clone Volmianka. The transpiration of clone Jorr (*Salix viminalis*) was moderate, when compared with other clones, as presented in Figure 2.

As already mentioned, energy willow plantations can be grown under different environmental conditions, including areas with low water supply, for example post-mining peatlands, degraded peaty soils, etc. Therefore, a very important factor for willow clones is the water use efficiency (WUE). The results of water use efficiency (WUE) in greenhouse experiment are presented in Figure 3.

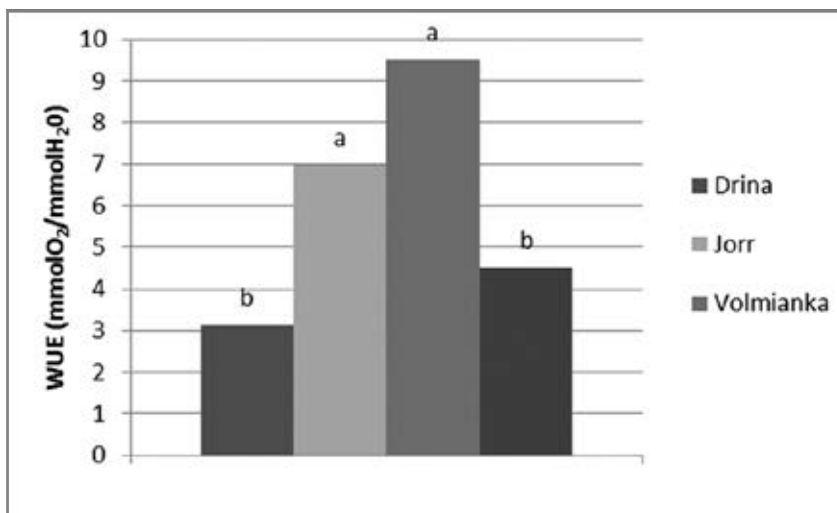


Figure 3. Water use efficiency of willow clones in greenhouse

The best results of WUE were recorded for clone Volmianka (*Salix alba*). As it was identified earlier, this clone had the lowest level of transpiration. Clones of willow tested in greenhouses during early stage of ontogenesis were also tested under field conditions. Results of measurements of intensity of transpiration are presented in Figure 4.

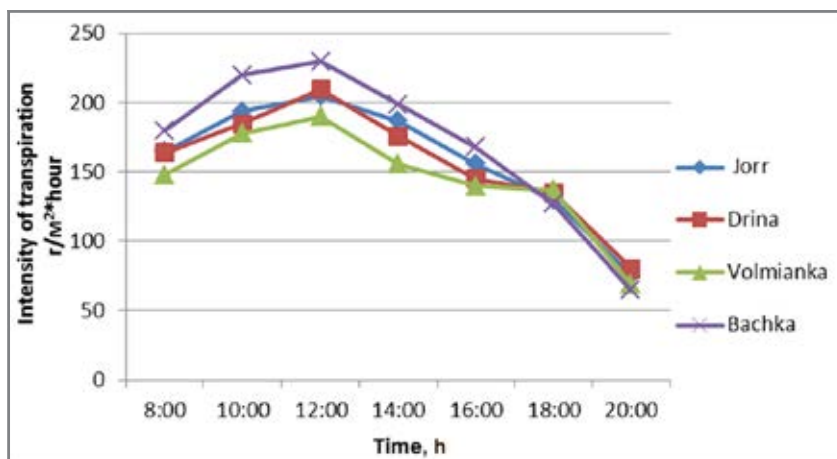


Figure 4. The diurnal dynamics of transpiration of willow clones under field conditions.

Diurnal dynamics of transpiration showed that the lowest level of transpiration, the same as in greenhouse experiment, was observed for clone Volmianka, while the highest transpiration was recorded for clone Bačka. The results of diurnal dynamics of transpiration showed one peak (typically at midday) for all clones. Our results are in accordance with the results of transpiration dynamic presented in other publications [Kostjuchenko 2009].

Water retention ability represents a physiology indicator related to water use efficiency. The results of this parameter are presented in Figure 5.

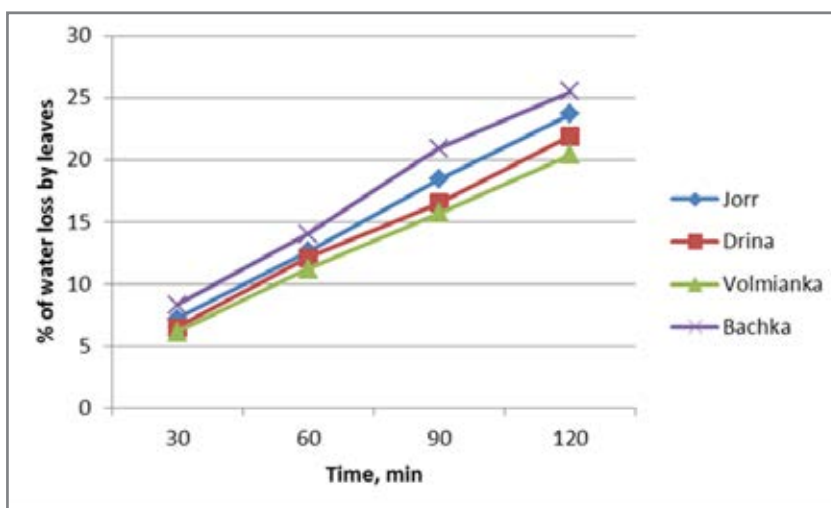


Figure 5. The dynamic of water loss by leaves of willows under field conditions

As presented in Figure 5, the minimal level of water loss was observed for clone Volmianka. It indicates that this clone can economize water and spend it efficiently under field conditions, which was also identified in greenhouse experiment. Plants of willow clone Bačka showed maximal discharging of water both in field and in greenhouse.

Results of water deficit dynamics (Figure 5) correlate with water deficit parameters in willows. Minimal water deficit on average was recorded for clone Volmianka, while maximal was recorded for clone Bačka. These results indicate that clone Volmianka has the best water retention capability and low water deficit parameters, resulting in better drought resistance and tolerance to unfavorable environmental conditions.

Results of morphology characteristics of clones showed higher productivity of *Salix alba* clones compared to clone Jorr (*Salix viminalis*), Table 2.

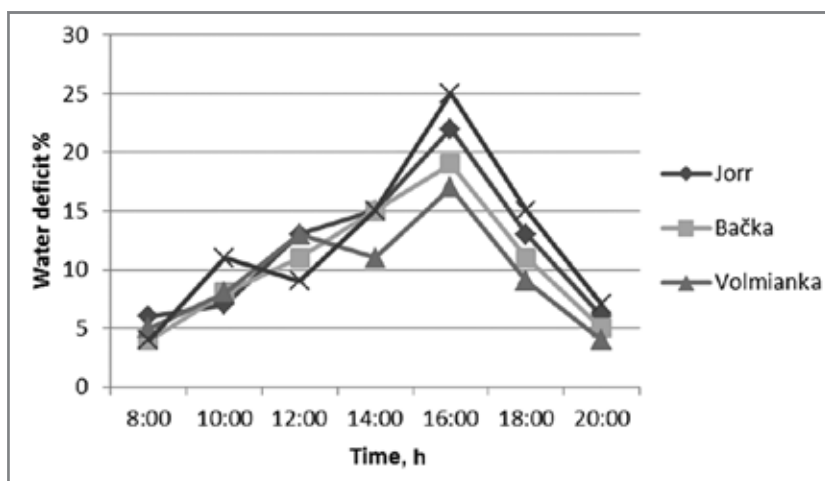


Figure 5. Water deficit dynamics of willow clones

All results of testing of three Serbian varieties of willow selected from *Salix alba*, characterized by intensive growth and sprout re-growing, were included in State register of Republic of Belarus in 2013. Every variety has special preferences and can be adapted to different environmental conditions. Bačka showed higher productivity than other clones during the testing. Volmianka is characterized by special water regime and this clone can be successfully adapted to the areas with low water supply. Drina, as it was identified in our experiments, accumulates heavy metals to biomass less than other clones. This clone can be used for reclamation of areas polluted by heavy metals (e.g. along the roads, near the livestock breeding complexes, etc.) [Rodzkin *et al.*, 2010; Rodzkin and Pronko 2010].

Table 2. Morphology characteristics of willow clones under field conditions

Clone	Year	Parameter		
		Height of plants, cm	Stem diameter mm	Number of sprouts
Jorr	2012	206	12.5	3.6
	2013	401	25.2	-
Bačka	2012	226	14.0	3.5
	2013	428	28.5	-
Volmianka	2012	225	13.6	3.4
	2013	417	26.9	-
Drina	2012	197	13.5	3.4
	2013	387	27.3	-
LSD ₀₅	2012	9.2	0.53	0.28
	2013	14.6	1.12	0.28

CONCLUSIONS

According to prognoses of World Energy Council, energy consumption will increase twofold until 2050. Over 40% of energy demands will be covered by renewable energy resources, including bioenergy with 32%. It is obvious that development of bioenergy production will have the priority, which includes crop remains, energy cultures, animal waste and energy biomass plantations like poplars, willows, black locust and eucalyptus.

Results of our testing of *Salix alba* L. clones (Bačka, Drina and Volmianka) and *Salix viminalis* L. clones (Jarr) showed good adaptability in Belarus. Water regime of investigated clones shows differences in transpiration and water use efficiency, with clone Bačka surpassing other investigated clones, while clone Volmianka showed the highest potential for drought conditions.

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ИСПИТИВАЊЕ ФИЗИОЛОШКИХ ПАРАМЕТАРА ВРБА КАО КРИТЕРИЈУМ ЗА СЕЛЕКЦИЈУ ПЕРСПЕКТИВНИХ КЛОНОВА

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РЕЗИМЕ: Производња биоенергије у засадима са кратким турнусима (SRC) врба ефикасан је правац истраживања, како са економског, тако и са аспекта заштите животне средине. Принос дрвне масе код врбе може достићи 10–15 тона по хектару суве биомасе годишње и трошковима добијене енергије нижим у поређењу са другим енергетским културама. У циљу постизања високог приноса и профитабилности треба користити посебне клонове врбе. Најчешће врсте коришћене у избору за производњу биомасе су украсни типови врбе као што су: *Salix viminalis*, *Salix dasicladus* и *Salix schverini* док су у овом раду испитивани клонови врбе: *Salix alba*. Продуктивност и неке физиолошке карактеристике српске селекције клонова *Salix alba* (Бачка, Волмианка и Дрина) и шведски клон Jorr (*Salix viminalis*) су испитивани у стакленицима и у теренским условима.

Тестирања три клона *Salix alba* – (Бачка, Волмианка и Дрина) који имају посебне склоности и прилагођени су на различите услове животне средине тестирани су такође у условима Белорусије и ти клонови су укључени у државни регистар Републике Белорусије у 2013. У нашим експериментима, са наведеним клоновима, испитивана су следећа својства врбе (интензитет транспирације и фотосинтезе, ефикасност коришћења воде и других показатеља), како у стакленицима, тако и у спољним условима. Овакав приступ даје могућност да се изаберу перспективни клонови врбе у раној фази онтогенезе те се могу користити за даља тестирања.

КЉУЧНЕ РЕЧИ: биоенергија, врба, селекција, сорте, физиолошки параметри

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EFFECTS OF BIOCHAR APPLICATION ON MORPHOLOGICAL TRAITS IN MAIZE AND SOYBEAN

ABSTRACT: This paper analyses the effects of the biochar application morphological traits in maize and soybean under semi-controlled conditions. During the study, the increasing doses of biochar (0%, 0.5%, 1, 3, and 5%) were incorporated in three soil types: Alluvium, Humogley and Chernozem to determine plant height and shoot weight. The experiment was set up as fully randomized design with three repetitions. The plants were grown in pots of 5 l with controlled watering and N fertilization. The research results have shown that there are differences in terms of biochar effects on soils. The greatest effect on plant height and shoot weight was obtained when the biochar was applied to Humogley soil and lower effects were found on the Alluvium soil. The increase in aboveground mass of maize and soybeans was significantly conditioned by adding different doses of biochar. Based on these results, it can be concluded that adding biochar can significantly affect the growth of plants. This is a consequence of the changes it causes in soil, which requires further tests to complement the current findings.

KEYWORDS: biochar, soil, maize, soybean, plant height, shoot biomass

INTRODUCTION

Arable soils are among the largest and most important natural resources of all mankind [Wall and Six 2015]. To protect the arable soil from degradation preventive measures are the most important, such as identification of hazards and detection of appropriate solutions to overcome risks of agricultural intensification. Today, a great effort is made to improve and utilize less productive soils and restore their initial fertility. One of the possible solutions

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for the amending of degraded soils is biochar application [Biederman and Harpole 2013; Lehman *et al.*, 2005]. Biochar is a solid material obtained in the process of carbonization, pyrolysis of biomass, usually of plant origin. The manufacturing process is similar to the process of obtaining charcoal with a difference in the used raw materials. Soil biochar amendment is based on two thousand years old experience, which in recent decades has been renewed because of proven multiple benefits [Chan *et al.*, 2007]. This importance is largely long-term, but also reveals the short-term effects [Mann 2005].

Traditional charcoal production uses carbon dioxide sequestered into woody biomass tissue via the process in which tissue of biological origin is burnt (or charred) in the absence of, or at low levels of oxygen to produce 'biochar' [Preston and Schmidt 2006]. After pyrolysis, approximately 50% of the carbon contained in the original source of biomass can be retained within the biochar. However, recovery rates are highly dependent of the pyrolysis process. Among the many elaborated effects, the most beneficial result of the biochar application could be sequestration of the atmospheric carbon with the consequence to global climate [Laird 2008; Woolf *et al.*, 2010]. Many studies confirmed that soil incorporated with biochars can improve plant growing [Yamato *et al.*, 2006; Steiner *et al.*, 2007]. According to Lehmann *et al.* [2003] biochar incorporation induces soil alkalization which can increase soil nitrification and N levels. Increases in soil pH are likely to affect electrical conductivity (EC), cation exchange capacity (CEC) and increase alkaline metal (Mg^{2+} , Ca^{2+} and K^+) oxides. Likewise, it reduces soluble forms of aluminium, which is suggested as the most significant biochar factor affecting P solubility [DeLuca *et al.*, 2009]. The presence of biochar in the soil can provide a physical niche for growing hyphae and bacteria [Warnock *et al.*, 2007]. Beneficial effects of biochar have been elaborated in studies word wide. However, there is a lack of experimental confirmation of the biochar application in our agricultural science. Researches of biochar use have been mainly conducted on soils under tropical and humid climatic conditions, which are more degraded and have a lack of soil organic carbon [Šeremešić *et al.*, 2014]. Therefore, the aim of this study is to investigate the possibility of biochar application and doses on the contrasting soil types under temperate climatic conditions.

MATERIALS AND METHODS

In order to evaluate the dose-response pattern of biochar application, a pot experiment was set up under semi-controlled conditions of the vegetation shed at the Faculty of Agriculture, University of Novi Sad. The study included maize and soybean growing in three soil types Chernozem (C), Alluvium (A) and Humogley (H) and five application rates of biochar 0, 0.5%, 1%, 3, and 5% in a fully randomized experimental design with three replicates. The biochar doses were equivalent to application rates of 0.0, 12.5, 25, 75, 125 t biochar ha⁻¹ assuming a soil bulk density of 1.25 g cm⁻³ up to a depth of 20 cm. Chemical analyses of biochar used in this study are presented in Table 1. Total carbon content of

74.5% was measured in 30 mg soil sub-samples on an elemental analyzer (CHNS). The obtained values corresponded with those described by Jindo *et al.* [2014].

Chemical soil properties are presented in Table 1. Preparation of the substrate preceded plant growing. Soil was first mixed with the biochar, and then the pots were filled with the substrate in order to ensure similar bulk density of substrate in pots (5 l – 20 cm of diameter; 20 cm of height). Pots were filled 2 weeks before sowing and watered to maintain soil water regimen and establish the stabilization of physical and chemical soil properties.

Table 1. *Chemical soil properties of the investigated soil types*

Soil type	Depth (cm)	pH		CaCO ₃ %	Total C Humus %	Total N %	AL-P ₂ O ₅	AL-K ₂ O
		KCl	H ₂ O				mg/100g soil	
Biochar	-	7.54	8.24	1.6	74.51	0.54	53.8	291.0
Chernozem (C)	0–30	7.21	8.13	1.04	2.75	0.159	23.96	29.08
Alluvium (A)	0–30	7.38	8.26	3.80	1.72	0.148	102.5	14.1
Humogley (H)	0–30	6.98	5.99	0.33	3.66	0.183	29.98	49.71

Soybean Favorit (NS Seme) as short vegetation variety (maturity group 000) and maize line with short vegetation (<1m in height) was used in this experiment. The nitrogen (in the form of NH₄NO₃) fertilization was applied in post-emergence stage to ensure undisturbed plant growth. The moisture of soil substrate was maintained by watering at an optimal level, between 70–80% of the water retention capacity of soil in pot to prevent water stress. Plants were harvested by cutting aboveground biomass for determination of yield and morphological properties. The plant material was dried in an oven at 105 °C for 48 h, after which the absolute dry mass was determined by using the technical scale. The soil substrates were also analyzed in order to determine their chemical properties. The data reported was assessed by analyses of variance (ANOVA). Analysis of variance was used to separate the treatment means when there was a significant difference at the $p < 0.01$ and $p < 0.05$ level. The analyses were conducted using the statistical software package Statistica 12.6. (StatSoft Inc., USA).

RESULTS AND DISSCUSION

In the combined analysis of variance, the effects of soil ($P < 0.0004$) and interaction of soil type and biochar doses ($P < 0.0261$) showed significant F-test for maize plant height (Table 2). The soil accounted for 27.6% of total height variation, whereas the interaction is responsible for 26.89% of total variation and 37.97% variation derives from residual influences. Biochar application has not significantly affected maize height in our study. Contrary to our study, the application of 50 t/ha biochar to acid soil increased height and the fresh weights of the maize aerial part [Rodríguez and Preston 2009]. Study of saline

soil (pH 8.52) in the pot experiment and the application of biochar recorded 17.7 to 25.8% increase in the maize shoot length with the maximum of 78 cm plant⁻¹ 30 days after sowing (Saranya *et al.*, 2011).

Table 2. *Analysis of variance for maize plant height*

Sources of variation	d.f.	s.s.	SS %	m.s.	F	P
Soil (A)	2	2210.0125	27.59	1105.0062	10.171**	0.0004
Biochar (B)	4	342.9792	4.28	85.7448	0.789	0.5407
Interaction (A x B)	8	2153.8208	26.89	269.2276	2.478*	0.0261
Blocks	2	261.8792	3.27	130.9396	1.205	0.3096
Error	28	3042.1208	37.97	108.6472		
Sum	44	8010.8125	100			

d.f. – degrees of freedom, s.s. – total sum of squares, s.s.% – sum of squares relative to total sum, m.s. – mean squares

The analyses of variance for maize shoot biomass reveal that the soil very significantly influenced maize dry biomass ($P < 0.0000^{**}$), while doses of biochar ($P < 0.0114^{*}$) and interaction of soil type and biochar doses ($P < 0.0159^{*}$) showed significant F-test for dry maize biomass (Table 3). The soil accounted for 80.60% of total shoot biomass variation, whereas the residual influences accounted for only 8.39%. It appears that in maize growing different soil types showed higher effect regardless of biochar doses. Some researchers reported no changes in the maize production in the first year after biochar amendment, but a significant increase was observed in the following years Major *et al.* [2010]. According to Yamato *et al.* [2006] maize production was significantly increased after the application of bark charcoal under a fertilized condition in an infertile soil environment. A positive effect of biochar addition on maize dry biomass could be ascribed to higher soil N-retention also observed in Baronti *et al.* [2010]. Although some positive effects were observed, we assume that in our study biochar addition could manifest more beneficial effects to maize growing if added earlier (in the autumn).

Table 3. *Analysis of variance for maize shoot biomass*

Sources of variation	d.f.	s.s.	SS %	m.s.	F	P
Soil (A)	2	1563.3114	80.60	781.6557	134.473**	0.0000
Biochar (B)	4	85.9029	4.43	21.4757	3.695*	0.0114
Interaction (A x B)	8	126.6447	6.53	15.8306	2.723*	0.0159
Blocks	2	0.8770	0.04	0.4385	0.075	0.9274
Error	28	162.7571	8.39	5.8128		
Sum	44	1939.4932	100			

d.f. – degrees of freedom, s.s. – total sum of squares, s.s.% – sum of squares relative to total sum, m.s. – mean squares

Higher maize plant height was observed on Humogley soil, while application of biochar resulted in significantly lower plant height on Alluvium soil. Maize shoot biomass was significantly higher on Humogley soil compared with Chernozem and Alluvium. Obtained results indicate that the ameliorative effect of biochar is largely related with pH increase and N availability to plants. Our results with maize are in accordance with those presented by Zhang *et al.* [2011] who suggested that positive effects of biochar application in field crop production could be also observed in the calcareous soils.

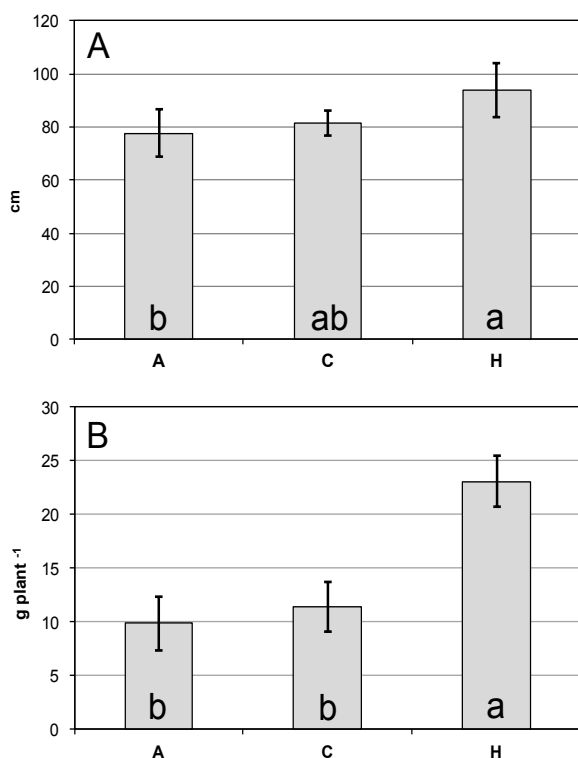


Figure 1. Maize plant height (A) and shoot biomass (B) after biochar addition on Alluvium (A), Chernozem (C) and Humogley (H) (^{abc}Column marked with the different letters within treatments differ significantly at $P \leq 0.05$; Error bars indicate standard deviation)

The analyses of variance for soybean plant height indicate very significant effects of soil type and biochar doses on the plant height ($P < 0.0015^{**}$) and ($P < 0.0001^{**}$), respectively (Table 4). The biochar doses accounted for 40.27% and soil types for 17.66% of total height variation, whereas the interaction (A x B) is responsible for 26.89% of total variation and 37.97% variation derives from residual influences. Soybean height appears to be significantly influenced by biochar doses compared to maize.

Table 4. *Analysis of variance for soybean plant height*

Sources of variation	d.f.	s.s.	SS %	m.s.	F	P
Soil (A)	2	461.5661	17.66	230.7831	7.900**	0.0015
Biochar (B)	4	1052.6328	40.27	263.1582	9.009**	0.0001 9.d
Interaction (A x B)	8	254.4651	9.73	31.8081	1.089	0.3892
Blocks	2	27.2328	1.04	13.6164	0.466	0.6362
Error	28	817.9235	31.29	29.2116		
Sum	44	2613.8203	100			

Soybean shoot biomass was significantly affected by soil type and biochar level ($P < 0.000^{**}$). Biochar doses showed considerable fraction in total variation (42.99%) indicating positive response of soybean to increased amount of biochar application. The error accounted for 25.06% of total shoot biomass variation. It clearly showed positive and higher reaction of soybean to biochar application compared to maize. Also, soil types had less effect to morphological trait manifestation in soybean compared to maize. Sun *et al.* [2012] suggested that biochar incorporation to brown soil might bring potential benefit to soybean production from N retention in soil and enhanced microbial turnover that resulted with P and K feedback. Our results correspond with Yin *et al.* [2012] study on acid black soil where soybean yield increased by 35.97% compared with the control.

Table 4. *Analyses of variance for soybean shoot biomass*

Sources of variation	d.f.	s.s.	SS %	m.s.	F	P
Soil (A)	2	9.1981	27.02	4.5991	15.098**	0.0001
Biochar (B)	4	14.6356	42.99	3.6589	12.012**	0.0000
Interaction (A x B)	8	1.6324	4.79	0.2041	0.670	0.7162
Blocks	2	0.0440	0.13	0.0220	0.072	0.9302
Error	28	8.5291	25.06	0.3046		
Sum	44	34.0393	100			

Significant effects of biochar application on the soybean shoot was observed on Humogley soil compared with soybean height that was observed on Chernozem (Figure 2). Regarding shoot biomass, Humogley significantly influenced its formation compared with Alluvial soil. Obtained result could be explained with better water holding capacity of Humogley.

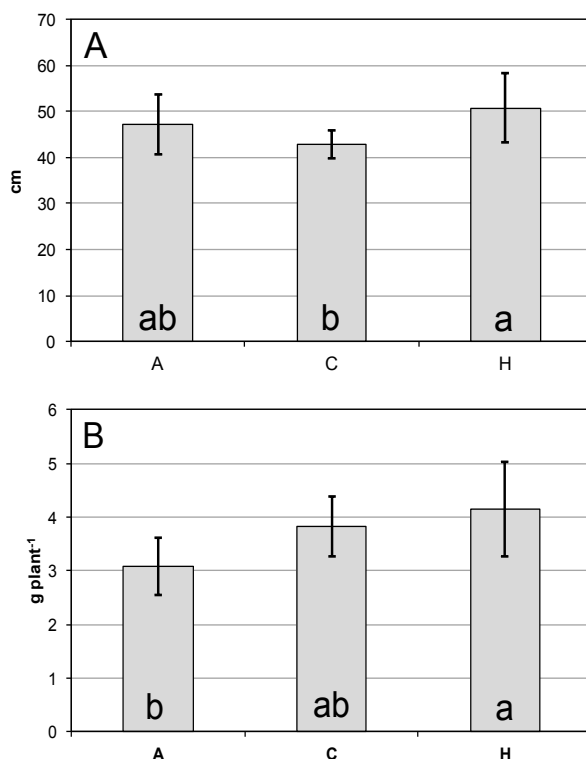


Figure 2. Soybean plant height (A) and shoot biomass (B) after biochar addition on Alluvium (A), Chernozem (C) and Humogley (H) (^{ABC}Column marked with the different letters within treatments differ significantly at $P \leq 0.05$; Error bars indicate standard deviation)

CONCLUSION

Humogley soil showed higher response of the observed traits compared to Chernozem and Alluvium regardless of biochar doses. In maize experiment, different soil types exerted higher influence on the plant height and shoot biomass, while in the soybean experiment biochar application showed significant effects. Our study indicates better response of soybean to biochar application than maize. Based on the obtained results, biochar addition could contribute to crop growing, while additional examinations must be performed to identify doses of biochar corresponding to different soil types.

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ЕФЕКАТ БИОУГЉА НА МОРФОЛОШКА СВОЈСТВА КУКУРУЗА И СОЈЕ

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САЖЕТАК: У раду је испитиван утицај примене биоугља на морфолошка својства кукуруза и соје у полуконтролисаним условима. Примењене су растуће дозе биоугља на три типа земљишта: алувијум, чернозем и хумоглеј и праћена је висина биљака и маса надземног дела. Оглед је постављен по рандомизираним распореду са три понављања у судове запремине 5 литара, а сетва је извршена у месецу мају. Резултати истраживања су показали да постоје разлике у погледу испитиваних земљишта и примењених доза биоугља. Најбољи ефекат је добијен када је биоугаљ примењен на земљиште хумоглеј а најмањи утицај примене биоугља је утврђен на алувијалном земљишту. Пораст надземне масе кукуруза и соје био је у значајној мери условљен додавањем различитих доза биоугља. На основу добијених резултата може се закључити да додавање биоугља може значајно утицати на пораст биљака који је последица промене које он изазива у својствима земљишта, али да је неопходно наставити даља испитивања како би се употпунила досадашња сазнања. Утврђено је да су испитивана својства код соје испољила већу реакцију на примену биоугља у односу на кукуруз.

КЉУЧНЕ РЕЧИ: биоугаљ, земљиште, кукуруз, соја, висина биљака, маса надземног дела

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BARIUM CONCENTRATION IN GRAIN OF *AEGILOPS* AND *TRITICUM* SPECIES

ABSTRACT: The aim of this study was to evaluate the concentration of barium in grain of various *Aegilops* and *Triticum* species with different genomes. The studied species differed significantly with respect to the concentration of barium. The grain of wild diploid *Aegilops speltoides*, the donor of B genome, contained significantly higher Ba concentration than all other analyzed genotypes. Wild and cultivated tetraploid wheats (*Triticum diciccoides*, *Triticum dicoccon*, *Triticum turgidum* and *Triticum durum*) had the lowest Ba concentration in grain. The modern cultivated hexaploid varieties presented substantial variation in grain concentration of barium. The highest Ba concentration (3.42 mg/kg) occurred in Serbian winter wheat variety Panonnia.

KEYWORDS: barium concentration, grain, wheat, genotypes, diploid, tetraploid, hexaploid

INTRODUCTION

Barium (Ba) is a silvery-white alkaline earth metal that occurs naturally in different compounds. Ba is relatively abundant in the earth's crust, with mean values ranging between 265 and 835 µg/g dry weight (DW) depending on the soil type [Lide 2005]. Ba is not a very mobile element in most soil systems due to the formation of water-insoluble salts and their partition into soils and sediments [WHO 2001].

Ba has not been reported as an essential trace element for plants, and it was included in a list of elements that pose a risk to human health and are most com-

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monly found in cases of soil contamination [USEPA 2009; CETESB 2001]. Ba absorption by plant species grown in polluted areas has been observed by Abreu *et al.* [2012]. Pais *et al.* [1998] found that Ba contents of 200 mg/kg could be moderately toxic and that 500 mg/kg could be considered toxic for plants. Therefore, there is an increasing concern regarding Ba in plants, especially in edible plants, because Ba can cause damage in the human body. The ingestion of Ba can result in several human health problems such as: high blood pressure, muscular paralysis, gastrointestinal disturbances, kidney damage, respiratory failure, and, in some cases, even death [Jacobs *et al.*, 2003; Lenntech 2005].

The land is enriched in Ba from the atmosphere with a range of 40–80 g/ha/year. In atmosphere Ba comes from the production of barium compounds, combustion of coal and oil, and in arable land primarily by using mineral fertilizers. Manure contains Ba from 20 to 100 mg/kg, compost from 35 to 100 mg/kg, slaughterhouses meat meal from 4 to 16 mg/kg, sludge wastewater from 110 to 200 mg/kg [Kádár *et al.*, 2012; Kádár 2013], phosphatic fertilizers 200 mg/kg, and the means of calcification 150 to 250 mg/kg [Kabata-Pendias and Pendias 1984].

The effects of Ba on plant growth are in general toxic as small concentrations can retard growth [Robinson *et al.*, 1938]. The various harmful effects of Ba in cereals, such as reduction in germination, root-shoot length, changes in the activity of various enzymes and grain yield, are reported [Debnath and Mukerija 1982; Iqbal and Naz 1989; Suwa *et al.*, 2008].

Ba concentration in the dry matter of plants varies within a wide range from 1 to 200 mg/kg. Körös [1980] reported the plants of the family Myrtaceae characterized by particularly great accumulation of Ba. The legumes are also characterized by larger accumulation of Ba. According to Kádár [2013], Ba concentration in dry matter in monocots, dicots and legume grown on lime chernozem was 23, 41 and 150 mg/kg (respectively), and on serpentine soil 9, 14 and 29 mg/kg (respectively).

The wheat group has evolved through allopolyploidization, namely, through hybridization among species from the plant genera *Aegilops* and *Triticum* followed by genome doubling. Bread wheat (*Triticum aestivum*) is an allohexaploid species ($2n = 6x = 42$, genome BBAAADD) that originated from hybridization events involving three different diploid progenitors: (i) *Triticum urartu*, the donor of the A genome [Dvorak 1976], (ii) a yet undiscovered extant (or extinct) *Aegilops* species closely related to *Aegilops speltoides*, the donor of the B genome, and (iii) *Aegilops tauschii*, the donor of the D genome [Kihara 1944].

This paper focuses on evaluation of barium concentration in whole grain of diploid and tetraploid wheat progenitors and ancestors of common wheat as well as in hexaploid commercial cultivars all grown at the same location for three years.

MATERIAL AND METHODS

Six diploid genotypes with different genome formula BB, AA, and DD, five tetraploids (BBAA) and nine hexaploids (BBAAADD) wheat were used in the experiment. Among the diploid wheats, four were wild and one (*Triticum monococcum* subsp. *monococcum*) was primitive cultivated wheat. Among

the tetraploid wheats used, three genotypes were wild einkorn while two were cultivated. All hexaploids were cultivated cultivars (Table 1).

The wheat genotypes were planted in a randomized complete block design with three replicates at the experiment field of the Institute of Field and Vegetable Crops, Novi Sad (45.2° N, 19.5° E, 80 m elevation), in 2011, 2012 and 2013, on weakly calcareous chernozem. Meteorological data for the experimental years show that the year 2013 was characterized by higher average temperatures as well as precipitation in comparison to years 2011 and 2012 (Table 2).

Field plots of 2.5 m² with 10 rows spaced 10 cm apart were planted with 400 seed per m². In the beginning of October before planting, the experimental area was fertilized with 50 kg N ha⁻¹, and 50 kg P₂O₅ ha⁻¹. The soil at the experimental site was well provided with potassium. The genotypes were planted in mid-October which is the optimal time for winter wheat. There was one top-dressing in early February with 50 kg N ha⁻¹. During the spring the field plots were protected against weeds once without fungicides application. The genotypes were harvested at crop maturity and all hulled genotypes were dehulled by hand. All grain samples selected for this study were visibly intact without any sign of degradation.

Table 1. Genotypes of *Aegilops* and *Triticum* species (after Van Slageren, 1994) examined in the experiments

Species and subspecies	Genome	Name	Source/Origin
<i>Aegilops speltoides</i> ssp. <i>speltoides</i> 1	BB		IPK*
<i>Aegilops speltoides</i> ssp. <i>speltoides</i> 2	BB		IPK
<i>Triticum urartu</i>	AA		IPK
<i>Triticum monococcum</i> ssp. <i>aegilopoides</i>	AA	Wild einkorn	IFVC, SRB**
<i>Triticum monococcum</i> ssp. <i>monococcum</i>	AA	Cultivated einkorn	IFVC, SRB
<i>Aegilops tauschii</i> ssp. <i>tauschii</i>	DD	Goat grass	IPK
<i>Triticum turgidum</i> ssp. <i>dicoccoides</i> (IPK)	BBAA	Wild emmer	IPK
<i>Triticum turgidum</i> ssp. <i>dicoccoides</i> (IFVC)	BBAA	Wild emmer	IFVC, SRB
<i>Triticum turgidum</i> ssp. <i>dicoccon</i>	BBAA	Cultivated emmer	IFVC, SRB
<i>Triticum turgidum</i> ssp. <i>turgidum</i>	BBAA	Rivet wheat	IPK
<i>Triticum turgidum</i> ssp. <i>durum</i> (cv. Durumko)	BBAA	Durum wheat	IFVC, SRB
<i>Triticum aestivum</i> ssp. <i>spelta</i> (cv. Nirvana)	BBAADD	Spelt wheat	IFVC, SRB
<i>Triticum aestivum</i> (cv. Panonnia)	BBAADD	Common wheat	IFVC, SRB
<i>Triticum aestivum</i> (cv. Bankut 1205)	BBAADD	Common wheat	HUN
<i>Triticum aestivum</i> (cv. Bezostaja 1)	BBAADD	Common wheat	RUS
<i>Triticum aestivum</i> (cv. Siete Cerros)	BBAADD	Common wheat	MEX
<i>Triticum aestivum</i> (cv. Florida)	BBAADD	Common wheat	USA
<i>Triticum aestivum</i> (cv. Renan)	BBAADD	Common wheat	FRA
<i>Triticum aestivum</i> (cv. Condor)	BBAADD	Common wheat	AUT
<i>Triticum aestivum</i> (cv. Bolal)	BBAADD	Common wheat	TUR

* IPK – Genebank Gatersleben of the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany; ** IFVC, SRB – Institute of Field and Vegetable Crops, Novi Sad, Serbia; HUN – Hungary; RUS – Russia; MEX – Mexico; USA – United State of America; FRA – France; AUT – Australia; TUR – Turkey.

Table 2. *Monthly mean temperature and precipitation during three years*

Month	Mean air temperature (°C)			Precipitation (mm/m ²)		
	2010/11.	2011/12.	2012/13.	2010/11.	2011/12.	2012/13.
October	9.2	10.7	13.7	67	35	48
November	9.5	2.8	10.1	46	2	36
December	0.8	4.3	0.7	64	50	55
January	0.2	2.0	2.8	25	43	60
February	-0.3	-5.0	4.3	37	67	50
March	5.8	8.3	6.0	26	4	68
April	13.2	13.0	13.5	23	83	30
May	16.7	17.2	18.2	63	51	118
June	21.0	22.6	20.2	37	34	126
Average/Sum	8.5	8.4	9.9	388	366	591
Average yield*	4.88	4.48	5.50			

*average wheat yield in the province of Vojvodina

Milling was carried out using a Perten Laboratory Mill 3100 to produce wholemeal. After digestion of grain wholemeal in a mixture of HNO₃ (65%) and H₂O₂ (30%) total concentrations of Ba were determined by an ICP-OES (Varian Vista-Pro) in Research Institut for Soil Science and Agricultural Chemistry, Budapest. All statistical analyses were done with program XLSTAT-Pro (demo version, Version 3.02.2009).

RESULTS AND DISCUSSION

Like other similar alkaline earth metals barium is poorly mobile in plants. Kastori *et al.* [2007] emphasized the low index of Ba translocation from vegetative organs to grain in wheat. Barium mostly accumulates in the vegetative parts of the plants, in older leaves and stem, and to a much lesser extent in the generative organs (grain). This is confirmed by the results of Kádár and Szemes [1994] in triticale, where Ba concentration in grain was 1.7 mg/kg while in the stem it was 31 mg/kg.

The analyzed *Aegilops* and *Triticum* species differed significantly with respect to the concentration of barium (Tab. 3). The grain of both genotypes of *Aegilops speltoides* with B genome contained significantly higher concentration of barium (9.02 mg/kg and 10.19 mg/kg respectively) than the grain of all others species including related species (*Aegilops tauschii*). This is an indication that species which belong to the same genera can be quite genetically different. Among the diploid wheats the lowest Ba concentration occurred in the wild emmer (*Triticum monococcum subsp. aegilopoides*) (Tab. 3). In the set of tetraploids, wild emmer wheat (from IFVC) contained significantly higher Ba concentration than all other tetraploids including the modern cultivated durum

variety. The primitive cultivated emmer (*Triticum turgidum* subsp. *dicoccon*) had the lowest Ba concentration when compared to all analyzed wheat genotypes (Tab. 3).

Table 3. Concentration of barium (mg/kg, DW) in the whole grain of *Aegilops* and *Triticum* species

Species and subspecies	Year			Average
	2011	2012	2013	
<i>Aegilops speltoides</i> ssp. <i>speltoides</i> 1	6.75	8.38	11.93	9.02 ^b
<i>Aegilops speltoides</i> ssp. <i>speltoides</i> 2	9.67	8.98	11.93	10.19 ^a
<i>Triticum urartu</i>	1.94	2.38	2.23	2.18 ^{efg}
<i>Triticum monococcum</i> ssp. <i>aegilopoides</i>	2.29	1.92	1.55	1.92 ^{efgh}
<i>Triticum monococcum</i> ssp. <i>monococcum</i>	2.28	2.17	2.07	2.17 ^{efg}
<i>Aegilops tauschii</i> ssp. <i>Tauschii</i>	1.92	2.04	2.15	2.04 ^{efg}
<i>Triticum turgidum</i> ssp. <i>dicoccoides</i> (IPK)	1.62	1.65	1.62	1.63 ^{fgh}
<i>Triticum turgidum</i> ssp. <i>dicoccoides</i> (IFVC)	2.12	2.14	2.16	2.14 ^{efg}
<i>Triticum turgidum</i> ssp. <i>dicoccon</i>	0.86	0.98	1.10	0.98 ^h
<i>Triticum turgidum</i> ssp. <i>turgidum</i>	2.52	0.99	1.46	1.66 ^{fgh}
<i>Triticum turgidum</i> ssp. <i>durum</i> (cv. Durumko)	2.02	1.01	1.32	1.45 ^{gh}
<i>Triticum aestivum</i> subsp. <i>spelta</i> (cv. Nirvana)	1.70	1.61	1.51	1.61 ^{gh}
<i>Triticum aestivum</i> (cv. Panonnia)	3.63	2.89	3.74	3.42 ^c
<i>Triticum aestivum</i> (cv. Bankut 1205)	2.65	2.73	3.22	2.87 ^{cde}
<i>Triticum aestivum</i> (cv. Bezostaja 1)	1.71	0.87	1.55	1.38 ^{gh}
<i>Triticum aestivum</i> (cv. Siete Cerros)	4.08	2.45	3.38	3.30 ^{cd}
<i>Triticum aestivum</i> (cv. Florida)	3.34	1.25	3.30	2.63 ^{cdef}
<i>Triticum aestivum</i> (cv. Renan)	3.20	0.87	2.54	2.20 ^{efg}
<i>Triticum aestivum</i> (cv. Condor)	2.22	0.93	2.01	1.72 ^{fgh}
<i>Triticum aestivum</i> (cv. Bolal)	2.92	1.92	2.28	2.37 ^{defg}
Average	2.97 ^a	2.41 ^a	3.15 ^b	

Different letters indicate significant difference at $P < 0.05$ level.

The modern cultivated hexaploid varieties presented substantial variation in grain concentration of barium. The highest Ba concentration (3.42 mg/kg) occurred in Serbian winter wheat variety Panonnia, which is still being produced in Serbia, Bulgaria and Czech Republic. Contrary the well known variety Bezostaja 1 had 2.5 times lower concentration of barium in grain (Tab. 3). Among hexaploid wheats, cultivated spelt variety (cv. Nirvana) had the second lowest level of Ba concentration in grain. The existence of a large variation in concentration of barium in grain of modern cultivated wheat varieties indicates that the concentrations of Ba in hexaploid wheats are genetically controlled. The similar results in tetraploids for Zn concentration reported Cakmak *et al.* [2000].

It is quite clear that two accessions of *Aegilops speltoides* with B genome had exceptionally large concentration of barium in grain, many times larger than all others analyzed genotypes. The main reason for this is that there is a large genetic distance between these genotypes and all other genotypes. Second, high grain Ba concentration in the wild *Aegilops* species is partly related to the lowest grain weights, indicating a role of “concentration effects”. The species from other two diploids with A and D genomes did not differ significantly in barium concentration in the grain (Tab. 4).

Table 4. Concentration of barium (mg/kg DW) in the species with five different genomes

Genome	Year			Average
	2011	2012	2013	
BB	8.21	8.68	11.93	9.61 ^a
AA	2.17	2.16	1.95	2.09 ^{bc}
DD	1.92	2.04	2.15	2.04 ^{bc}
BBAA	1.83	1.35	1.53	1.57 ^c
BBAADD	2.83	1.72	2.61	2.39 ^b
Average	3.39 ^a	3.19 ^a	4.04 ^b	

Different letters indicate significant difference at $P < 0.05$ level.

Table 5. Analysis of variance of concentration of Ba concentration in the whole grain of *Aegilops* and *Triticum* species

Source of variation	df	SS	MS	F value
Genotyp (G)	19	986.32	51.91**	147.02
Year (Y)	2	18.14	9.07**	25.68
G *Y	38	73.67	1.94**	5.49
Pooled error	120	42.37	0.35	

In average, the tetraploids (wild and cultivated emmer) with BA genome had the least concentration of barium comparing to all diploid and hexaploid analyzed genotypes (Tab. 4). It was unexpected that diploids with A and B genome did not significantly differ from cultivated hexaploid modern varieties (Tab. 4). It confirms that plumper grain which occurred in modern hexaploid varieties does not necessarily contain a smaller concentration of barium. This finding is also consistent with studies on various trace elements concentrations of McDonald *et al.* [2008] and Zhao *et al.* [2009].

It is quite obvious that concentration of barium in grain of various wheats is influenced by genotype to a great extent. Results of ANOVA (Tab. 5) show that conditions over the year also had significant effect to Ba concentration in grain, which can be seen in Table 4 as well. The concentration of Ba in the grain of tested wheat genotypes was significantly lower in dry years (2011 and 2012) than in humid year 2013 (Tab. 4).

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КОНЦЕНТРАЦИЈА БАРИЈУМА У ЗРНУ ВРСТА *AEGILOPS* И *TRITICUM*

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РЕЗИМЕ: Циљ овог истраживања био је да се процене концентрације баријума у зрну различитих генотипова *Aegilops* и *Triticum* врста. Испитиване врсте су се значајно разликовале у односу на концентрацију баријума. Зрно дивљег диплоида *Aegilops speltoides*, донатор Б генома садржи знатно већу концентрацију баријума него сви остали испитивани генотипови. Дивља и питома тетраплоидна пшеница (*Triticum dicoccoides*, *Triticum dicoccon*, *Triticum turgidum* и *Triticum durum*) имала је најнижу концентрацију баријума у зрну. Модерне гајене хексаплоидне сорте показале су значајне варијације у концентрацији баријума у зрну. Највеће концентрације (3,42 mg/kg) установљене су код „Паноније”, озиме сорте пшенице произведене у Србији.

КЉУЧНЕ РЕЧИ: концентрација баријума, зрна, пшеница, генотип, диплоиди, тетраплоиди, хексаплоиди

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STRUCTURAL PROPERTIES OF MAIZE HYBRIDS ESTABLISHED BY INFRARED SPECTRA

ABSTRACT: This paper discusses the application of the infrared (IR) spectroscopy method for determination of structural properties of maize hybrid grains. The IR spectrum of maize grain has been registered in the following hybrids: ZP 341, ZP 434 and ZP 505. The existence of spectral bands varying in both number and intensity, as well as their shape, frequency and kinetics have been determined. They have been determined by valence oscillations and deformation oscillations of the following organic compounds: alkanes, alkenes, alkynes, amides, alcohols, ethers, carboxylic acids, esters and aldehydes and ketones, characteristic for biogenic compounds such as carbohydrates, proteins and lipids. In this way, possible changes in the grain structure of observed maize hybrids could be detected.

KEYWORDS: Maize hybrid, grain, structural properties of molecules, infrared spectra, spectral bands

INTRODUCTION

At present, contemporary methods of spectroscopy and biotechnology provide essential progress in diagnostics of a state of organs and vital functions of the whole plant at the molecular level. Vibrational spectroscopy (infrared and Raman) is an unavoidable method in the analysis of spectra originating

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from molecular vibrations, which provide numerous data on structures of observed systems [Krimm and Bandekar 1986; Vasilev 2007; Tarasevich 2012; Sverdlov 1970].

Our previous scientific papers [Radenović *et al.*, 1994a; 1994b; 1995; 1998) described changes in the molecular structure of carotenoids in grain of various maize hybrids and inbred lines and showed that these molecules can be used as molecular markers in evaluation of agronomic values of maize inbred lines and hybrids.

The IR spectroscopy method was applied in the present study to diagnose the state of grain of observed hybrids. It is well known that IR spectroscopy provides the analysis of molecular composition and structure by registering the intensity of oscillations and deformations of molecular bonds [Vasilev 2007; Tarasevich 2012].

The aim of this study was to develop methods for registration of the IR spectrum of grain of observed maize hybrids and to identify structural differences in its biogenic compounds.

MATERIAL AND METHODS

Plant material – The following three hybrids of high quality and with high yields were studied: ZP 341, ZP 434 and ZP 505. These hybrids were developed at the Maize Research Institute, Zemun Polje, Belgrade, Serbia. The stated maize hybrids have been released not only in Serbia, but also in Russia and another three European countries. These hybrids are annually sown on more than one million hectares.

Methods – Overall studies of high yielding and high quality maize hybrids encompass several sets of experiments in which new and standard methods and procedures were applied.

1. Infrared spectroscopy of maize hybrid grain

Measurements of infrared spectrum were done by the IR Furie spectrometer (Shimadzu IR – Prestige 21) in the range of 400–4,000 cm^{-1} . Spectrophotometers used in infrared spectral region, in principle, do not differ from those used in the visible and ultraviolet spectral region. The specifics of the behaviour of IR radiation, particularly with regard to the middle and far spectral region, still impose some differences, first of all, the principles of vibrational spectroscopy, the nature of the materials, sources of IR radiation, the application of thermal detectors, etc.

Shimadzu IR –Prestige 21 is based on the principle of interferometers. Namely, it does not give the spectrum itself, but an interferogram, which is additionally processed by computers and transformed into a common shape of a spectrum – it is called Fourier transformation and therefore this type of spectroscopy is called Fourier transform spectroscopy (FTS). These devices

are particularly suitable for use in the far IR regions and are characterised by high power of breakdown. In order to register the IR spectrum of observed maize hybrids, grain was homogenised and packed as a tablet with the addition of potassium bromide (KBr).

2. Chemical composition of grain of maize hybrids

Methods used to determine the chemical composition of grain of observed maize hybrids are generally accepted and standardised and described in detail in papers written by Radosavljević *et al.*, 2000; White and Johnson 2003; Radenović *et al.*, 2009, 2010.

3. Functional dependence of maize hybrid yields in different locations in Serbia

Studies of functional dependence of high yielding and high quality maize hybrids (ZP 341, ZP 434 and ZP 505) were performed in many locations in Serbia with the application of the standard cropping practices [Videnović *et al.*, 2011].

4. A broad overview of breeding, seed production and technological traits of maize hybrids

Since the hybrids are high yielding and recently developed a broader overview of relevant breeding, seed production and technological traits, properties and parameters obtained by the application of standard ranking methods are presented.

RESULTS

1. Infrared spectroscopy of maize hybrid grain

Grains of the observed maize hybrids (ZP 341, ZP 434 and ZP 505) were homogenised and pressed into the tablet form with the addition of KBr and thus prepared for the measurement of the IR spectrum, Figures 1–3.

The observed IR spectrum was characterised by spectral bands. There was about 20–23 bands in the wavenumber range of 400 to 4,000 cm⁻¹. Spectral bands were differently pronounced, of uneven intensity, with special kinetics and various width in their base. There were 3–5 distinctly pronounced bands, 2–3 bands were moderate, and 4–5 were very low intensity bands. There were several spectral bands that could not be separated, or that indicated the unstable state of the system.

1.1. Infrared spectroscopy of the maize hybrid ZP 341

Figure 1 shows the IR spectrum of the maize hybrid ZP 341 grain. There are three very prominent spectral bands at 3,400, 1,000 and 2,900 cm^{-1} . Moreover, spectral bands at 1,650, 1,175, 2,850 and 1,145 cm^{-1} are also distinctively observable. A detailed survey shows weakly pronounced spectral bands at 3,780, 2,300, 1,550, 1,145, 1,100, 925, 825, 775, 700 and 600 cm^{-1} . There is an indication of an unstable state of the system in the wavenumber range of 400 to 4,000 cm^{-1} : at 3,000, 1,700 and 700 cm^{-1} .

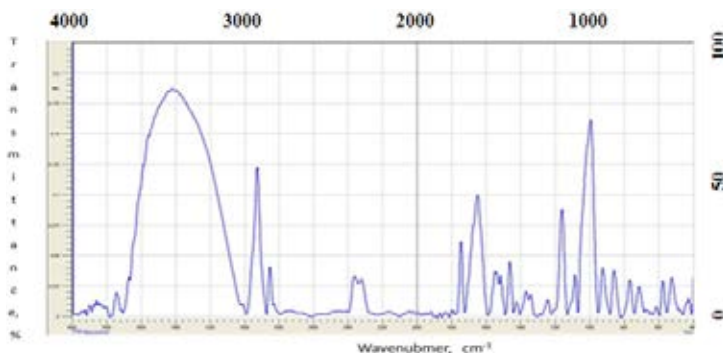


Figure 1. *Infrared spectrum of the maize hybrid ZP 341 grain*

1.2. Infrared spectroscopy of the maize hybrid ZP 434

Figure 2 shows the IR spectrum of the maize hybrid ZP 434 grain. There are four very outstanding spectral bands at 3,400, 1,000, 1,700 and 2,900 cm^{-1} . Furthermore, spectral bands at 2,825, 1,775 and 1,185 cm^{-1} are also particularly observable. Weakly pronounced spectral bands are observable at 3,750, 1,500, 1,225, 1,100, 975, 900, 800, 700 and 600 cm^{-1} . There is also an indication of an unstable state of the system at 3,700, 2,300, 1,800, 1,400 and 550 cm^{-1} .

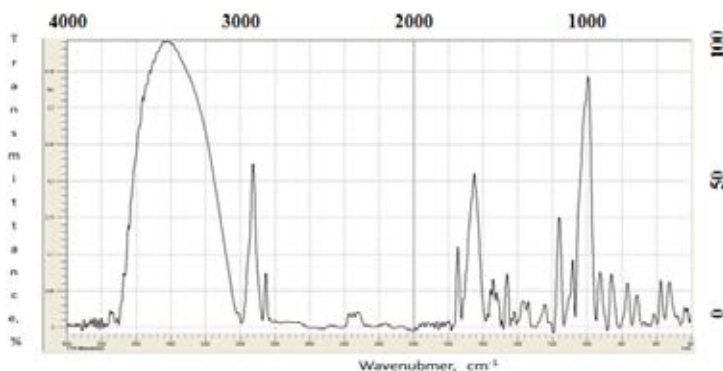


Figure 2. *Infrared spectrum of the maize hybrid ZP 434 grain*

1.3. Infrared spectroscopy of the maize hybrid ZP 505

Figure 3 shows the IR spectrum of the maize hybrid ZP 505 grain. There are eight very significantly expressed spectral bands at 3,400, 2,900, 1,750, 1,000, 2,850, 1,700, 1,450 and 1,150 cm^{-1} . Besides, spectral bands at 3,750, 3,025, 2,350, 1,550, 1,300, 1,100, 900, 775, 700, 575 and 500 cm^{-1} are weakly pronounced. There is an indication of instability of the system at 3,750, 1,900, 1,800, 1,460 and 1,430 cm^{-1} .

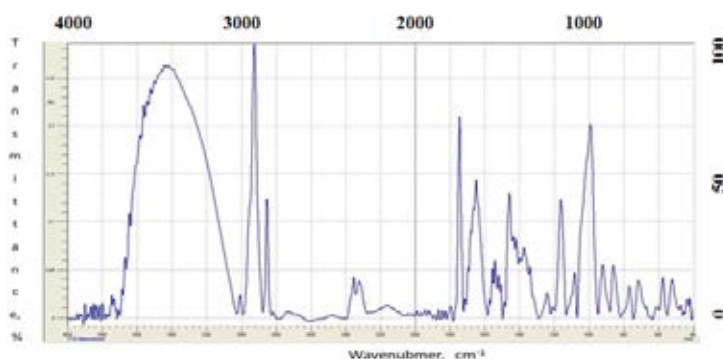


Figure 3. Infrared spectrum of the maize hybrid ZP 505 grain

2. Chemical composition of grains of studied maize hybrids

Results of overall studies of the chemical composition of the studied maize hybrids are presented in Table 1.

Table 1. Results of the analyses of the chemical composition of maize hybrid grains

Chemical composition of grains of maize hybrids	Range of the chemical composition in the literature*	Average chemical composition in the literature*	Average chemical composition of maize hybrids		
			ZP 341	ZP 434	ZP 505
Moisture (%)	7-23	16	11.96	11.56	11.14
Starch (%)	61-78	71.7	70.40	72.04	73.38
Proteins (%)	6-12	9.5	9.75	10.15	9.88
Lipids (oil) (%)	1-5.7	4.3	6.28	6.02	6.38
Ash (%)	1.1-3.9	1.4	1.34	1.40	1.31

* Source: [White and Johnson 2003]

3. Functional dependence of yields of observed maize hybrids in various locations in Serbia

High yielding and high quality maize hybrids: ZP 341, ZP 434 and ZP 505 are primarily intended for cultivation in European maize growing regions. Results of the yields of the stated maize hybrids are presented in Table 2.

Table 2. *Maize hybrid yields (t ha⁻¹) in several different locations in Serbia in the period 2008–2011*

Hybrid	Year				Average	
	2008	2009	2010	2011	t ha ⁻¹	%
ZP 341	7.299	9.318	8.389	7.626	8.158	100.0
ZP 434	7.432	9.522	8.393	7.788	8.284	101.6
ZP 505	7.580	9.706	8.752	7.918	8.489	104.1
Mean	t ha ⁻¹	7.437	9.515	8.511	7.777	8.310
	%	100.0	127.9	114.4	104.6	111.7

Genetic potential of the yield of maize hybrids ZP 341, ZP 434 and ZP 505 was observed in 38, 35, 41 and 37 locations in Serbia in 2008, 2009, 2010 and 2011, respectively. Common cropping practices required for maize hybrid cultivation were applied in trials. Irrigation was not applied. Results of the yields (Table 2) show that there are differences in yields among hybrids, but they are not significant. If the average yield of the hybrid ZP 341 is considered 100%, then the yields of hybrids ZP 434 and ZP 505 are higher by 1.6% (0.126 t/ha⁻¹) and 4.1% (0.331 t/ha⁻¹), respectively. These data point out to the fact that genetic potential of these hybrids is very similar and that only the hybrid ZP 505 has a certain advantages that is actually a result of its somewhat longer growing season. However, yields obtained over years differ much more. Hence, if the lowest yield recorded in 2008 is considered 100%, then yields were much higher in remaining years: 4.6% or 0.340 t/ha⁻¹ in 2011, 14.4% or 1.074 t/ha⁻¹ in 2010 and 27.9% or 2.078 t/ha⁻¹ in 2009. These data unambiguously show to which extent climatic characteristics over years affected the maize yield.

4. *A broad overview of breeding, seed production and technological traits of studied maize hybrids*

Results of these analyses are presented in Tables 3a and 3b.

Table 3a. *Agronomic traits of studied maize hybrids*

Agronomic trait	ZP 341	ZP 434	ZP 505
Type of hybrid	TC	SC	SC
FAO maturity group	300	400	500
Plant height (cm)	210	220	230
Ear height (cm)	100	105	110
1000-kernel weight (g)	350	350	400
Type of kernel	dent	dent	dent
Sowing density (000 plants ha ⁻¹)	70	70	60-65
Leaf position	erect	erect	erect
Resistance to drought	good	good	good
Resistance to diseases	good	good	good
Appearance of leaves at harvest	stay green	stay green	stay green
Growing region (altitude, m)	up to 600	up to 600	up to 500
Silage yield (t/ha ⁻¹)	50	50	60

Table 3b. *Morphological traits of ears of studied maize hybrids*

Morphological trait	ZP 341	ZP 434	ZP 505
Grain moisture	11.96	11.56	11.14
Ear length (cm)	21.53	21.53	23.05
Ear weight (g)	281.43	296.62	309.13
Number of kernel rows per ear	14.6	14.7	15.7
Number of kernels per row	604.1	599.5	706.2
Embryo weight (g)	41.31	43.07	45.18
Kernel weight (g)	240.33	253.56	263.95

DISCUSSION

As already stated, different spectral bands varying in the number, intensity, shape, frequency and kinetics were established in IR spectra of maize hybrids (Figures 1–3). These bands occurred in the wavenumber range of 400 to 4,000 cm^{-1} . The intensity of spectral bands was designated as a transmittance (%) and it ranged from 0 to 100. Spectral bands were determined by valence oscillations and deformation oscillations of numerous functional groups within biogenic organic molecules, starch, proteins and lipids (Table 1). Essentially, this procedure resulted in possible changes in grain structure of studied maize hybrids.

Based on previously stated, the following two questions arise. First, how to acquire information on the individual biogenic organic molecules by valence oscillations and deformations of functional groups that result in appearance of the spectral bands?

And second, are there any differences in totality of IR spectra of grains of observed maize hybrids ZP 341, ZP 434 and ZP 505? If such differences exist, than it can be concluded that various structural properties of grains of studied maize hybrids exist.

The answers to these questions can be, to a great extent, found in Table 4.

The overall observation of the columns in the Table 4, especially those related to intensities of spectral bands, wavenumber values at which the bands occurred, wavenumber range taken over from the references [Vollhardt and Schore 1996], then the observation of the biogenic organic molecules with valence vibrations of functional groups, the information on structural properties of molecules in grains of studied maize hybrids were gathered. Organic molecules from maize hybrids grain were compared with molecules of organic compounds [Vollhardt and Schore 1996], which provided their identification.

When the same parameters for the studied maize hybrids are compared (Table 4), it can be concluded that the structural properties of the hybrids ZP 341 and ZP 434 were similar, while those in the hybrid ZP 505 differed to a greater extent.

Table 4. *Properties of IR spectra caused by valence vibrations of biogenic organic molecules of grains of studied maize hybrids*

Maize hybrid	Intensity of five most pronounced spectral bands, %	Wavenumber, cm ⁻¹	Biogenic organic molecule*
ZP 341	87.5	3,400 3,200–3,650* 3,250–3,500*	alcohols amides
	70.5	1,000 1,000–1,260*	alcohols ethers
	56.0	2,950 2,840–3,000* 2,500–3,300*	alkanes carboxylic acid
	44.0	1,650 1,620–1,680* 1,690–1,750*	alkenes aldehydes ketones
	38.0	1,150 1,000–1,260*	alcohols ethers
ZP 434	100	3,410 3,200–3,650* 3,250–3,500*	alcohols amides
	88.5	1,000 1,000–1,260*	alcohols ethers
	60.5	2,925 2,840–3,000* 2,500–3,300*	alkanes carboxylic acid
	50.0	1,625 1,620–1,680* 1690–1750*	alkenes, aldehydes, ketones
	39.0	1,175 1,000–1,260*	alcohols ethers
ZP 505	93.0	3,410 3,200–3,650* 3,250–3,500*	alcohols amides
	100	2,975 2,840–3,000* 2,500–3,300*	alkanes carboxylic acid
	73.0	1,775 1,735–1,750* 1,690–1,750* 1,710–1,760*	esters aldehydes, ketones carboxylic acid
	67.0	1,000 1,000–1,260*	alcohols ethers
	49.5	1,650 1,690–1,750* 1,620–1,680*	aldehydes, ketones alkenes

*Source: [Vollhardt and Schore 1996]

CONCLUSION

The IR spectrum was for the first time registered in grain of the maize hybrids ZP 341, ZP 434 and ZP 505 by the application of the IR spectroscopy method. According to obtained results the following can be concluded:

- IR spectrum of maize hybrid grain is characterised by 20–23 spectral bands that can occur in the wavenumber range of 400–4,000 cm^{-1} ;
- Spectral bands can be differently pronounced, can be of uneven intensity (transmittance, %), particular kinetics and various width at the base;
- Five spectral bands for each studied maize hybrid were analysed and the following data were gathered: intensity (%), value of the experimentally established wavenumber wavenumber range; literature data were also analysed; possibly present organic molecules;
- The following biogenic organic molecules: alcohols, amines, ethers, alkanes, carboxylic acids, alkenes, aldehydes, ketones and esters were registered (by their functional groups) in the IR spectrum of grain of maize hybrids;
- It can be concluded that the structural properties of the hybrids ZP 341 and ZP 434 are similar, while those in the hybrid ZP 505 differed to a greater extent.

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СТРУКТУРНЕ КАРАКТЕРИСТИКЕ ХИБРИДА КУКУРУЗА ПОКАЗАНЕ ИНФРАЦРВЕНИМ СПЕКТРИМА

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РЕЗИМЕ: У овом раду разматра се примена методе инфрацрвене спектроскопије на зрну хибрида кукуруза ради утврђивања његових могућих структурних карактеристика. Извршено је регистровање инфрацрвеног спектра зрна хибрида кукуруза: ЗП 341, ЗП 434 и ЗП 505. Показано је постојање различитих спектралних трака, како по броју и интензитету, тако и по њиховом облику, фреквенцији и кинетици. До њих се долази валентним осцилацијама и деформацијама функционалних група: алкана, алкена, алкина, амида, алкохола, етра, карбоксилне киселине, естра, алдехида и кетона, које су карактеристичне за биогена једињења угљоводоника, протеина и липида. На овај начин се долази до могућих разлика у структури зрна испитиваних хибрида кукуруза.

КЉУЧНЕ РЕЧИ: хибрид кукуруза, зрно, структурне карактеристике молекула, инфрацрвени спектри, спектралне траке

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DEGRADATION OF LINURON IN SOIL BY TWO FUNGAL STRAINS

ABSTRACT: Two fungal strains were applied to soil polluted with herbicide in order to determine their degradation potential. Three experimental setups were used. In the first setup, the soil in pots was contaminated by linuron in final concentration of 1 ppm. Suspensions of *Phanerochaete chrysosporium* and *Trichoderma asperellum* were applied separately or in combination. Tomato plantlets were transplanted and chlorophyll content in their leaves was determined at two time points during plant growth. In the second setup in pots, the final concentration of linuron was lower, 0.45 ppm. In the third setup 0.1 ppm of linuron was applied in the field plot. Plantlets of lettuce were transplanted and chlorophyll content was measured as indicator of plant stress. The content of linuron in soil was determined by HPLC. The applied fungal strains significantly reduced toxic effect of 0.45 ppm linuron on plants, which was not the case for 1 ppm linuron. Both fungi, applied separately or in combination, were effective in decreasing the linuron content in the soil. However, in field conditions the combination of both fungi was the most effective.

KEYWORDS: *Trichoderma asperellum*; *Phanerochaete chrysosporium*; bioremediation, herbicide, linuron

INTRODUCTION

The demand for food supply increases constantly throughout the world due to the increase of human population. It is predicted that by the year 2050, agricultural production may need to be increased by 60–110%, which can only be achieved through targeted increase in crop yield [Ray *et al.*, 2013].

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Intensive agriculture is highly dependent on the use of chemical pesticides to control plant pathogens. However, these methods are time-consuming and environmentally harmful. As chemicals build up in soil they become toxic to microorganisms and plants, therefore the cleaning of the soil using the remediation is of the primary importance [Verma *et al.*, 2014]. Among different remediation technologies biological methods are very promising as they are easy to operate, do not produce secondary pollution and show higher efficiency in cleaning the soil [Beškoski *et al.*, 2011]. The use of microbes for pesticide removal/degradation from the agricultural soils is widely known, mostly through the enzymatic degradation [Vidali 2001].

Various microorganisms are used for bioremediation purposes, although indigenous species are the ones with best remedial potential.

Among different microorganisms, *Phanerochaete* spp. and *Trichoderma* spp. are recognized as fungi capable for degradation of organic pollutants, such as PAH and POPs. *Phanerochaete chrysosporium*, a basidiomycetous filamentous fungus, is proven to be effective in biodegradation of these compounds. *P. chrysosporium* is a white rot fungus which has a highly efficient lignin degrading enzyme system. With this enzyme system the fungus can also break down different xenobiotic pollutants. In these types of degradation processes, the lignin peroxidase and the manganese peroxidase have a great significance [Vágvölgyi *et al.*, 2014].

Beside these enzyme systems, laccases also oxidize various organic and inorganic compounds such as diphenols, polyphenols, substituted phenols, diamines and aromatic amines with concomitant reduction of molecular oxygen to water [Körmöczy *et al.*, 2013a].

Trichoderma spp. is a widely present cosmopolitan soil borne fungi [Kredics 2014]. Strains of *Trichoderma* spp. exert a number of different capabilities as they are genetically quite diverse [Harman *et al.*, 2004a]. Some of the strains are recognized as promising biocontrol agents, as well as plant growth promoters [Harman *et al.*, 2004b; Kormoczi *et al.*, 2013b]. Moreover, they are known for their potential in bio- and phytobioremediation of toxic compounds, such as pesticides and heavy metals [Woo *et al.*, 2014; Jovičić Petrović 2014]. Like *Phanerochaete* spp., *Trichoderma* spp. is also able to produce laccase enzymes. Strains known for laccase production are *T. atroviride*, *T. harzianum* and *T. assperellum* [Körmöczy *et al.*, 2013a].

Linuron (IUPAC: 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) is a nonselective herbicide used worldwide for the control of grasses and broadleaf weeds in the cultivation of a variety of crop plants, particularly vegetables and cereals. It is absorbed by plant roots and transported passively, via the xylem, to leaves where it inhibits photosynthesis by disrupting Photosystem II (USEPA 1984). Linuron is moderately persistent in soils. In aerobic conditions in the lab its half-life was 75 days, while under field conditions it was 230 days (Pest Management Regulatory Agency 2002). It is known to enter surface waters in agricultural runoff, and its residues have been detected in surface waters, drinking water and foodstuff [USEPA 1995; PMRA 2012].

Linuron is highly toxic to non-target aquatic organisms such as fish and shellfish, while in mammals it disrupts male reproductive function acting as an antiandrogen.

In this work we investigated the remediation potential of *T. asperellum* and *P. chrysosporium* to linuron in soil.

MATERIAL AND METHODS

Examinations of herbicide degradation in soil consisted of three separate experiments. Herbicide linuron was applied in each experiment. Solution was prepared by dissolving of commercial herbicide Afalon in tap water, in order to prepare three different final concentrations of linuron: 0.1 ppm, 0.45 ppm and 1 ppm, in three experimental conditions as it will be explained further below. The treatments in all experiments described below were split in two groups, one with and the other without herbicide applied in soil where plantlets of tomato or lettuce were transplanted. Both groups were split into the following subtreatments differing in the application of fungal suspensions: control, only *Trichoderma*, only *Phanerocheate* and both *Phanerocheate* and *Trichoderma* application.

Experimental conditions 1

Soil mixture was prepared (1:1:5 V ratio of soil:sand:peat) and half of it was sprayed with linuron solution to achieve final concentration in soil of 1 ppm. In the growth chamber experiment 635 g of soil mixture was weighted in transparent Plexiglas pots (depth of 24 cm), covered with aluminum foil. Plants were watered up to 75 % of maximum soil mixture water capacity. Lighting was provided with fluorescent bulbs in 14 h day / 10 h night light regime.

Two days after herbicide application, 5 ml of *P. chrysosporium* suspension (5.84×10^7 CFU/ml) was applied in each pot of *Phanerocheate* subtreatment group. One week after herbicide application, tomato plantlets were transplanted and 5 ml of *T. asperellum* suspension (32.5×10^6 CFU/ml) was applied to pots in only *Trichoderma* or *Phanerocheate* and *Trichoderma* subtreatment group. Non-destructive measurements of plants were done at two time points: two days after transplantation (I) and nine days after transplantation (II). Each treatment was done in three pots, with two plants per pot.

Experimental conditions 2

In order to be comparable with the first experimental setup, the final concentration of linuron in field experiments was calculated per soil weight also, assuming the area of experimental plot and soil depth of 15 cm. So, a half of the field plot was treated with linuron in final concentration of 0.45 ppm.

After seven days, 5 ml of both fungal suspensions were applied according to the described experimental design (*P. chrysosporium* suspension = $0,194 \times 10^7$ CFU/ml, *T. asperellum* suspension = $0,145 \times 10^6$ CFU/ml), followed by transplantation of tomato plantlets five days later. Non-destructive measurements of plants were done one month after fungal suspensions were added. Each treatment was done in plants with four pots (replicates).

Experimental conditions 3

A half of the field plot in this experiment was treated with herbicide linuron in the final concentration of 0.1 ppm in 15 cm soil layer, which is a recommended concentration in common agricultural practice. Both fungal suspensions were applied according to the described experimental design. Two days after herbicide application, 5 ml of the *P. chrysosporium* suspension (1×10^7 CFU/ml) was applied to the future soil transplanting spot. One week after herbicide application, plantlets of lettuce were transplanted and 5 ml of the *T. asperellum* suspension (1×10^6 CFU/ml) was applied. Non-destructive measurements of plants were done one month later.

The content of linuron in the soil was determined at two time points with two weeks interval (two and four weeks after transplantation). Each treatment was done in 10 plants (replicates).

Fungal suspension

Strains *Trichoderma asperellum* SZMC 20866 and *Phanerochaete chrysosporium* 78 SZMC 20961 were from the Szeged Microbiological Collection (SZMC), Department of Microbiology. Fungal isolates were maintained on Potato Dextrose Agar (PDA) medium at 4 °C.

Prior to preparation of fungal suspensions, *T. asperellum* isolate was pre-incubated at 25 °C in the dark, and *P. chrysosporium* isolates were preincubated at 28 °C in the light. Suspensions were prepared as follows: pure culture of *T. asperellum* isolate was grabbed from a Petri dish, resuspended in 100 ml of tap water, and shaken for 2 h on 50 rpm. Pure culture of *P. chrysosporium* isolate was grabbed from 10 Petri dishes, resuspended in 100 ml of tap water, and shaken for 2 h on 50 rpm.

Determination of linuron in soil

Soil samples from pot experiments were taken in 3 replicates and in field experiment in 5–10 replicates. Linuron was determined by HPLC (Agilent 1220 Infinity LC). The column used was a stainless steel Phenomenex Synergy 2.5 µm Fusion RP 100 A (50 mm x 2.1 mm I.D.). The chromatographic conditions were as follows: eluent, methanol-water (65:35, v/v); flowrate, 0.4 ml/min; injection volume, 10 µl; wavelength, 254 nm. Column temperature was ambient.

To obtain a solution of extractable linuron, 0.5 g of soil was shaken with 5 ml of water for 24 h. The suspension was then centrifuged at 14,000 rpm for 30 min and the aqueous extract was separated [Sánchez-Martín *et al.*, 1996].

The response of the detector as referred to peak areas was linear in the range assayed (0.1–1.2 µg/ml) and least squares linear regression analysis of the data provided an excellent correlation ($R^2=0.999$). LOD was 0.03 µg/ml or 0.3 µg/g of soil.

Non-destructive measurements

Chlorophyll content of the leaves was measured nondestructively with SPAD 502 + chlorophyllmeter (Konika Minolta Sensing Inc, Japan). Soil water content and temperature were measured with WET sensor with HH2 (Delta T).

RESULTS AND DISSCUSSION

Experiment 1

Data on soil water content (WET= $30 \pm 2\%$) and soil temperature ($T= 25 \pm 0.2$ °C) measured around each tomato plant used for measurements, indicate that plant growth conditions were uniform and optimal. Chlorophyll content of the leaves (SPAD, rel. units) in plants that were treated with 1 ppm did not decrease in comparison with control plants measured two days after plant transplantation. However, chlorophyll content decreased in plants treated with herbicide nine days after transplantation regardless on the fungal treatment (Table 1). One week after the herbicide application the plants wilted. However, the content of linuron in the soil after plant harvest was lower in all fungal treatments (Table 2).

Table 1. *Parameters of chlorophyll content measured in situ (SPAD) as influenced by herbicide and different fungal treatments in growth chamber experiment. I – refers to the first measurement performed two days after transplantation; II – refers to the second measurement performed nine days after transplantation.*

Treatment	SPAD (rel.units) ±s.d.	Treatment	SPAD (rel.units) ±s.d.
Linuron	I 33.1±0.75 II 30.83±1.86	Control	I 33±0.46 II 37.93±0.64
Linuron and <i>Trichoderma</i> suspension	I 34.17±1.55 II 30.07±2.29	<i>Trichoderma</i> suspension	I 33±0.46 II 37.93±0.64
Linuron, <i>Phanerochaete</i> and <i>Trichoderma</i> suspension	I 33.63±0.57 II 29.83±1.8	<i>Phanerochaete</i> and <i>Trichoderma</i> suspension	I 33.2±0.31 II 39.07±2.21
Linuron and <i>Phanerochaete</i> suspension	I 32.9±0 II 29.2±1.27		

Table 2. The percentage of linuron content in soil related to the value at the beginning of the experiment, as influenced by different fungal treatments in growth chamber experiment.

Treatment	Linuron [%]
Linuron	90
Linuron and <i>Trichoderma</i> suspension	68
Linuron, <i>Phanerochaete</i> and <i>Trichoderma</i> suspension	74
Linuron and <i>Phanerochaete</i> suspension	75

Experiment 2

Data on soil water content (WET= $17.7 \pm 2.3\%$) and soil temperature ($T= 33.8 \pm 1.0\text{ }^{\circ}\text{C}$) measured in the field around each tomato plant used for measurements, indicate that plant growth conditions were uniform and optimal.

Parameters of plant vitality and chlorophyll content in leaves indicate that the application of *P. chrysosporium* fungal strain, alone or in combination with *T. asperellum* fungal strain significantly reduced negative effect of 0.45 ppm linuron treatment of tomato plants (Table 3).

Table 3. Parameters of chlorophyll content measured in situ (SPAD) as influenced by herbicide and different fungal treatments in field conditions.

Treatment	SPAD (rel.units) \pm s.d.	Treatment	SPAD (rel.units) \pm s.d.
Linuron	48 \pm 12	Control	106 \pm 5
Linuron and <i>Trichoderma</i> suspension	54 \pm 13	<i>Trichoderma</i> suspension	106 \pm 8
Linuron, <i>Phanerochaete</i> and <i>Trichoderma</i> suspension	84 \pm 14	<i>Phanerochaete</i> and <i>Trichoderma</i> suspension	104 \pm 10
Linuron and <i>Phanerochaete</i> suspension	79 \pm 17		

The content of linuron in the soil after plant harvest was the lowest after the application of both fungal suspensions (Table 4).

Table 4. The percentage of linuron content in soil related to the value at the beginning of the experiment, as influenced by different fungal treatments in field conditions

Treatment	Linuron %
Linuron	62.5
Linuron and <i>Trichoderma</i> suspension	70
Linuron, <i>Phanerochaete</i> and <i>Trichoderma</i> suspension	46.5
Linuron and <i>Phanerochaete</i> suspension	63

Experiment 3

Data on soil water content (WET= $23 \pm 2\%$) and soil temperature ($T= 17 \pm 1\text{ }^{\circ}\text{C}$) measured in the field around each lettuce plant used for measurements, indicate that plant growth conditions were uniform and optimal. Chlorophyll content of the leaves (SPAD, rel. units) treated with 0.1 ppm linuron did not differ significantly from untreated plants. Basically, there was no difference among the treatments (Table 5). The decrease of linuron content in the soil was the highest when both fungi were applied at both measurement time points (Table 6).

Table 5. Parameters of chlorophyll content measured in situ (SPAD) as influenced by different fungal treatments in greenhouse conditions in lettuce plants. The concentration of applied Linuron was 0.1 ppm.

Treatment	SPAD (rel.units) \pm s.d.	Treatment	SPAD (rel.units) \pm s.d.
Linuron	19.9 ± 2.2	Control	19.3 ± 2.1
Linuron and <i>Trichoderma</i> suspension	19.7 ± 2.3	<i>Trichoderma</i> suspension	22.4 ± 1
Linuron, <i>Phanerochaete</i> and <i>Trichoderma</i> suspension	20 ± 2.5	<i>Phanerochaete</i> and <i>Trichoderma</i> suspension	19.9 ± 3.4
Linuron and <i>Phanerochaete</i> suspension	19.7 ± 3.1		

Table 6. The percentage of linuron content in soil related to the value at the beginning of the experiment, as influenced by different fungal treatments in field conditions

Treatment	Linuron % 2 weeks after plant transplantation	Linuron % 4 weeks after plant transplantation
Linuron	83	42
Linuron and <i>Trichoderma</i> suspension	69	59
Linuron, <i>Phanerochaete</i> and <i>Trichoderma</i> suspension	45	0
Linuron and <i>Phanerochaete</i> suspension	87	27

Investigations of the possibilities of pesticide microbial degradation are of great importance in the field of environmental protection. Species belonging to *Phanerochaete* and *Trichoderma* genus are known for their application in biotechnology, due to their production of lignin peroxidase, manganese peroxidase enzymes [Vágvölgyi *et al.*, 2014], as well as laccases [Da Silva Coelho-Moreira *et al.*, 2013; Körmöczi *et al.*, 2013]. Vágvölgyi *et al.* [2014] showed that *P. chrysosporium* strains exert good degradation potential of herbicides, parabens and phenol derivatives. Also, it is well known that *P. chrysosporium* possesses a great ability to degrade isoproturon, atrazine, propanil, bentazon, and diuron [Da Silva Coelho-Moreira *et al.*, 2013].

CONCLUSION

According to our results, the applied fungal strains significantly reduced toxic effect of 0.45 ppm linuron in plants, which was not the case for 1 ppm linuron. Both fungi, applied separately or in combination, were effective in decreasing the linuron content in the soil. However, in field conditions the combination of both fungi was the most effective. Investigations should be continued in order to determine which metabolites are produced due to microbial degradation of linuron as they could be more toxic than herbicide itself.

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ПРИМЕНА ДВА РАЗЛИЧИТА СОЈА ГЉИВИЦА У ДЕГРАДАЦИЈИ ЛИНУРОНА У ЗЕМЉИШТУ

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РЕЗИМЕ: Да би се испитао потенцијал за деградацију хербицида, два соја гљивица су примењена на земљиште загађено линуроном у оквиру три експериментална система. У првом експерименту са судовима земљиште је загађено линуроном у коначној концентрацији од 1 ppm. Суспензије спора *Phanerocheate*

chrysosporium и *Trichoderma asperellum* су примењене појединачно или у комбинацији. Након расађивања на биљкама парадајза је мерен садржај хлорофила два пута у току вегетације. У другом експерименталном систему примењена је нижа концентрација линурона, у коначној концентрацији од 0,45 ppm. У трећем експерименту примењена је коначна концентрација линурона од 0,1 ppm на експериментаној парцели у пољу. Након расађивања на биљкама салате је мерен садржај хлорофила као показатељ стања стреса. Концентрација линурона у земљишту је одређивана HPLC методом. Примењени сојеви гљивица су значајно смањили токсичне ефекте 0,45 ppm линурона на биљке, што није био случај при концентрацији од 1 ppm линурона. Оба соја гљивица, примењена појединачно или у комбинацији, била су ефикасна у смањивању садржаја линурона у земљишту. Међутим, у условима огледа у пољу најефикаснија је била примена комбинације оба соја.

КЉУЧНЕ РЕЧИ: *Trichoderma asperellum*, *Phanerochaete chrysosporium*, биоремедијација, хербицид, линурон

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PHYLLOSHERE MYCOBIOTA OF *EUCALYPTUS* *CAMALDULENSIS* Dehnh. AND *E. GLOBULUS* Labill.

ABSTRACT: Diversity of phyllosphere microfungi of two *Eucalypti* species (*E. camaldulensis* and *E. globulus*) was investigated using moist chamber method. A total of 19 different taxa of phyllosphere microfungal community were identified in leaves and seed bearing capsules. *Aspergillus niger* was the most frequent isolate in both investigated *Eucalyptus* species along with *Alternaria alternata* and *Penicillium* spp. Saprotrophic species occurred more frequently in *Eucalypti* phyllosphere compared to plant pathogens. Epiphytes were quantitatively prevalent. Microscopic analyses of *E. globulus* phylloplane microfungal community revealed potential inhibitory effect of *Trichoderma viride* against *Eucalypti* pathogenic species *Seimatosporium eucalypti* which formed aberrant, collapsed conidia. The study of phyllosphere mycobiota is of significant importance, considering that numerous leaf inhabiting fungi are in complex interactions with each other and their host plant.

KEYWORDS: epiphytes, *Eucalypti*, fungi, moist chamber, phyllosphere, saprobes

INTRODUCTION

The genus *Eucalyptus* L'Heritier (Myrtaceae) includes over 700 species, mostly tall, forest and woodland trees and shrubs, most of them endemic to Australia [Williams and Brooker 1997]. In the last 200 years, *Eucalypti* have become economically very important plants and one of the most widely planted forest species in the world. Outbreaks of plant diseases in both plantations and native forests have influenced the scientific research of *Eucalypti* in the recent years [Keane 2000].

Almost all vegetative organs are colonized by various microorganisms, including viruses, bacteria, protozoans and fungi forming biofilms together with biopolymer and nutrients composed matrix. Biofilms are usually 10 µm

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thick with its role in host protection against stress factors, excluding the influence on metabolic and genetic changes [Morris *et al.*, 1997]. Microfungi are probably the most important group to investigate since they are well known plant pathogens or colonizers of their tissues. Phyllosphere is a region of biological activity which covers the leaf surface and its interior [Caroll *et al.*, 1977]. Epiphytes grow on the surface of vegetative organs, usually termed as phylloplane, and are exposed to outer environmental factors, while endophytes colonize interior tissues and are in close contact with the inner microenvironment [Kharvar *et al.*, 2009]. Epiphytes colonization depends on biofilm layer thickness. Qualitative and quantitative phyllosphere community characteristics are influenced by nutrients availability, leaf maturity and morphology, as well as the presence of inhibitors [Kinkel 1997]. Phyllosphere fungi have a significant role as biosensors in terrestrial ecosystems through the processes of organic matter decomposition and mineral solubilization, which lead to more increased resistance to pathogens and toxic elements [Dix and Webster 1995]. Both epiphytes and endophytes can cause disease symptoms, while certain endophytes may enhance the tolerance capacity of the plant to survive under stressed conditions [Redman *et al.*, 2002]. Biological significance of phyllosphere fungi has been emphasized and investigated by numerous authors. Therefore, it is of special importance to study microfungal leaf community, especially on economically important plants, such as *Eucalypti*.

The aim of this study was to investigate phyllosphere microfungal community of two economically important *Eucalypti*: *Eucalyptus camaldulensis* Dehnh. and *E. globulus* Labill. collected from different countries.

MATERIALS AND METHODS

Sampling sites

Plant samples were collected from different localities. *E. camaldulensis* leaves and seed bearing capsules were obtained from plantations (Turkey), park trees (Egypt) and the green house of the Institute of Botany and Botanical Garden “Jevremovac” (Faculty of Biology, University of Belgrade, Serbia). Investigated samples of *E. globulus* leaves were collected from park trees (Herceg Novi, Montenegro).

Sampling

Leaf and fruit samples were obtained from 10 randomly chosen *Eucalypti* specimens at every sampling site. Sampling took place in spring 2007. All leaves and fruits were collected directly from living plants and placed in sterile polyethylene bags for transport.

Isolation

Plant samples were placed in Petri dish moist chambers, according to Keyworth [1951], in aseptic conditions and then transferred into thermostat at 25 °C. During the incubation period, microfungi were isolated successively. To obtain pure cultures, all primary isolates were inoculated via single conidial transfer to Malt Extract Agar (MEA) and Czapek Dox agar (CzA).

Identification

During the incubation period, microfungal colonies formed on leaf or fruit surface were initially examined with binocular microscope (Stemi DV4, Carl Zeiss) and photographed.

Mycelium samples were taken from the colony grown on plant leaf or seed-bearing capsule surface, dyed and fixed with glycerol or Lactophenol Cotton Blue. Likewise, plant samples were dissected using a sterile scalpel and dyed with fuchsine acid. All samples were observed under a light microscope (Zeiss Axio Imager M.1, with AxioVision Release 4.6 software).

Identification of fungi was based on the macroscopic features of the colonies growing on MEA and CzA and the micromorphology of the reproductive structures, using identification keys by Raper and Fennell [1965], Pitt [1979], Ellis [1971], Ellis and Ellis [1997], Watanabe [2002] and Samson *et al.* [2004].

RESULTS AND DISCUSSION

A total of 19 different taxa of phyllosphere microfungal community were identified from *Eucalyptus camaldulensis* and *E. globulus* leaves and fruits (Table 1). A total of 16 identified taxa were isolated from *E. camaldulensis* plant parts, including 4 taxa isolated from samples collected in Egypt, 7 from Turkey and 8 from Serbia. On the contrary, only 6 taxa were isolated from *E. globulus* plant parts. The most frequent isolate on both investigated *Eucalypti* was *Aspergillus niger*. *Alternaria alternata* and *Penicillium* spp. were also frequent isolates.

Aspergillus and *Penicillium* species were most frequently isolated from both tested *Eucalypti* plant parts. *A. niger* colonies were documented on leaves of both *Eucalypti*, collected in Turkey and Egypt as well as on fruits of *E. camaldulensis* collected in Egypt. Leaf and fruit surfaces were covered with felt of black conidial heads (Fig. 1a,b). Fruits of *E. camaldulensis* were completely overgrown with *A. flavus* conidial heads (Fig. 1b). *Penicillium* sp. was common isolate from *E. camaldulensis* plant parts covered with dark blue-green colonies. In some cases, conidiophores covering the leaf were rising from stomata-interior (Fig. 2d). Blue-green colonies observed on *E. camaldulensis* samples from Egypt and Serbia, were identified as *P. aurantiogriseum* and *P. citrinum*, respectively.

Table 1. *Phyllosphere microfungi of Eucalyptus globulus and E. camaldulensis*

Isolated taxa	<i>E. camaldulensis</i>			<i>E. globulus</i>
	Serbia	Turkey	Egypt	Montenegro
<i>Acremonium strictum</i> W. Gams	+			
<i>Alternaria alternata</i> (Fr.) Keissl.	+	+		
<i>Aspergillus</i> sp. P. Micheli		+		
<i>Aspergillus flavus</i> Link			+	
<i>Aspergillus niger</i> Tiegh		+	+	+
<i>Aspergillus versicolor</i> (Vuill.) Tirab.		+		
<i>Botrytis cinerea</i> Pers.	+			
<i>Fusarium moniliforme</i> J. Sheld.		+		
<i>Penicillium</i> sp. Link	+	+		+
<i>Penicillium citrinum</i> Thom	+			
<i>Penicillium aurantiogriseum</i> Dierckx			+	
<i>Periconia minutissima</i> Corda				+
<i>Pestalotiospsis</i> sp. Steyaert	+			+
<i>Phomopsis</i> sp. Sacc. & Roum.		+		
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.			+	
<i>Seimatosporium eucalypti</i> (McAlpine) H.J. Swart				+
<i>Stachybotrys atra</i> Corda	+			
<i>Trichoderma viride</i> Pers.				+
<i>Zygosporium masonii</i> S. Hughes	+			
Undefined taxon (Dematiaceae)	+			
Total	9	7	4	6

Trichoderma viride was frequently isolated from *E. globulus* leaves where it formed characteristic, dense, green cover (Fig. 1a). *Seimatosporium eucalypti* picnidia with extruded black cirri were observed on the same leaf samples of *E. globulus* (Fig. 1c). *Alternaria alternata* formed typical branched conidial chains on the leaf surface (Fig. 2e), while *Botrytis cinerea* (gray mold) was isolated from leaves covered with characteristic red gum lerp psyllid honeydew crystalline shells. It is well known that *B. cinerea* is one of the most significant pathogens of *Eucalyptus globulus* Labill, the least resistant species [Zaldúa and Sanfuentes 2010]. *Fusarium moniliforme* was isolated from leaves of *E. camaldulensis* from Turkey where symptoms included presence of white sporodochia.

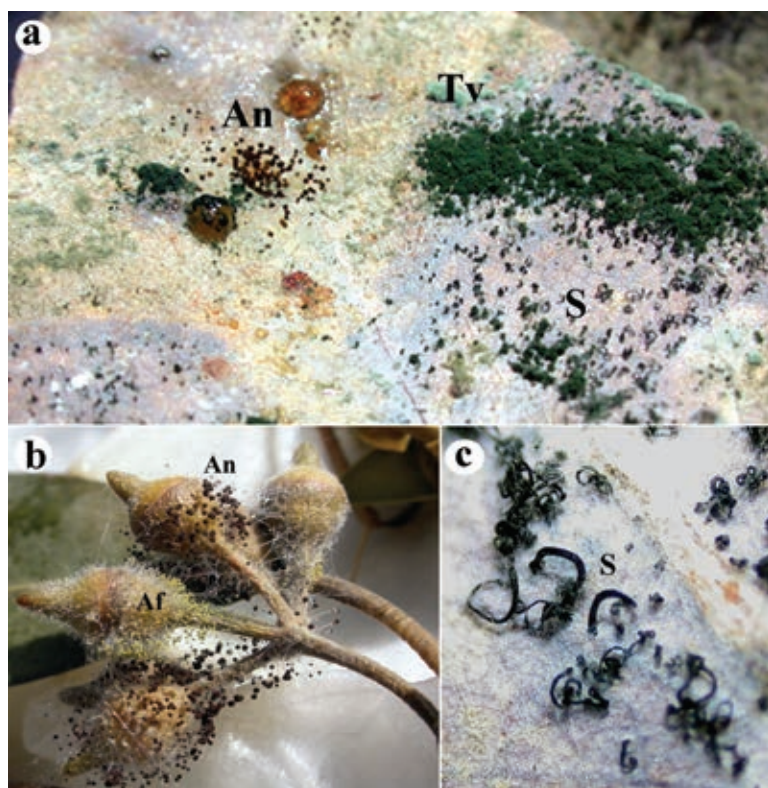


Figure 1. a) Fungal phylloplane community of *Eucalyptus globulus*;
 b) Seed bearing capsules of *E. camaldulensis* overgrown with conidial heads
 c) Picnidia with prominent cirri; *Aspergillus niger* (An), *A. flavus* (Af),
Seimatosporium eucalypti (S) and *Trichoderma viride* (Tv) [Photo D. Lakušić].

Stachybotrys atra was seldomly isolated from *E. camaldulensis* leaves, appearing as black conidial slime heads. *Phomopsis* sp. was rarely isolated from the leaves of *E. camaldulensis*, covered with small, dark-colored picnidia. *Periconia minutissima* was observed in only one sample of *E. globulus* leaves, which were partially covered with long melanized conidiophores (Fig. 2c). *Rhizopus stolonifer* was once documented on *E. camaldulensis* leaf surface overgrown with grey mycelial felt bearing numerous black sporangia. Acervulae containing characteristic conidia with apical setulae of *Pestalotiopsis* sp. were documented on *E. camaldulensis* leaves from Serbia (Fig. 2b). In several trichomes of *E. camaldulensis* leaves, dark-pigmented hyphae belonging to unidentified species of the family Dematiaceae (Fig. 2a) were detected.

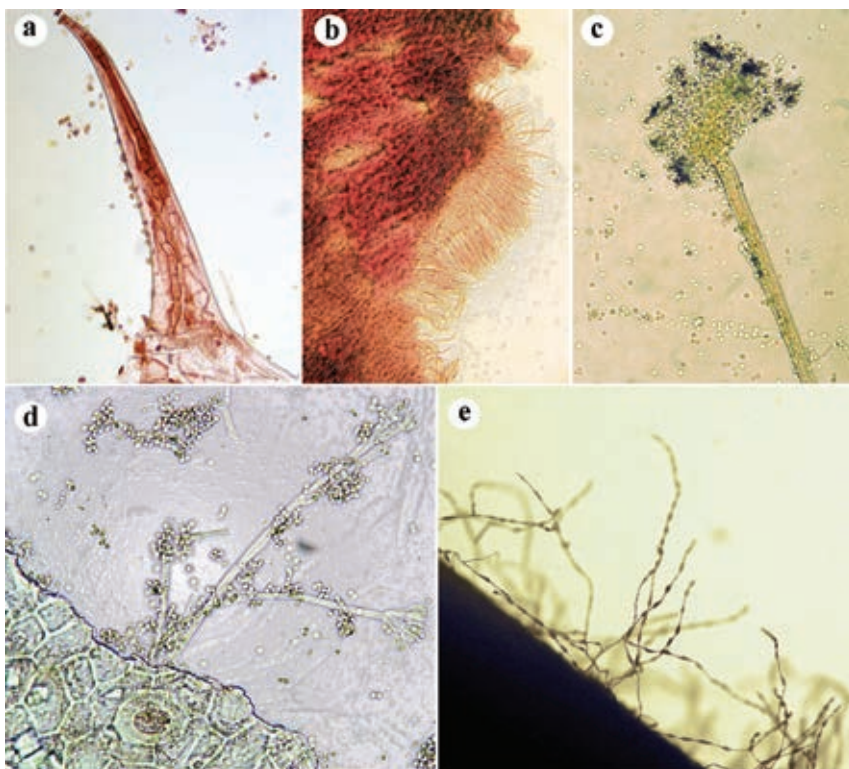


Figure 2. a) Pigmented mycelium of the unidentified taxon from Dematiaceae family inside the leaf trichome of *Eucalyptus camaldulensis*; b) Acervulae of *Pestalotiopsis* sp. on the leaf surface of *E. globulus*; c) Melanised conidiophores of *Periconia minutissima* isolated from the leaf of *E. globulus*; d) Conidiophores of *Penicillium* sp. emerging from stommata-interior of *E. camaldulensis* leaf; e) Conidial chains of *Alternaria alternata* observed on the leaf surface of *E. camaldulensis*

There are several studies related to fungal diversity of *Eucalypti* phyllosphere. Upadhyay *et al.* [1977] reported some species of *Alternaria*, *Fusarium*, *Penicillium* and *Pestalotiopsis* as moderately frequent isolates from *E. globulus* leaves, while *A. niger* and *A. flavus* were least frequent, based on investigated population density. *A. alternata*, *Penicillium cristata* and *Aspergillus* spp. are common isolates from both interior and exterior of the *Eucalyptus citridora* phyllosphere [Kharvar *et al.*, 2010]. In study presented here, isolated phyllosphere fungi of tested *Eucalypti* species belonged to two different ecological groups: epiphytes and endophytes, and most of isolated species belonged to epiphytic saprobes. The most frequently isolated fungi were: *Aspergillus* spp., *Penicillium* spp. and *A. alternata*. Endophytes include species of genera *Pestalotiopsis*, *Phomopsis* and *Seimatosporium*. They are frequently found in phyllosphere of different *Eucalypti*, many of them living as plant pathogens [Sankaran *et al.*, 1995]. Quantitative prevalence of epiphytes is expected, since many

pathogenic endophytes are host specific, while phylloplane fungal community of *Eucalypti* is similar to other plant species [Cabral 1985]. It should be noted that *Penicillium*, *Alternaria* and *Fusarium* genera include both saprobe species and plant pathogen endophytes [Sánchez Márquez *et al.*, 2011; De Aldana *et al.*, 2013].

Saprobe micromycetes (most notably *Aspergillus*, *Penicillium* and *Trichoderma* species) occurred more frequently in *Eucalypti* phyllosphere compared to reported plant pathogens (such as *Alternaria*, *Botrytis*, *Fusarium*, *Pestalotiopsis* and *Phomopsis* species). This could be attributed to saprobes faster growth and greater competition for nutrients against plant pathogens [Upadhyay *et al.*, 1977]. Furthermore, *Aspergillus* and *Penicillium* species are important mycotoxin producers [Samson *et al.*, 2010] which could exhibit negative effect on the growth of other fungi. Additionally, potential biotic interaction was observed on the leaf surface of *E. globulus*, with visible colonies of *A. niger*, *T. viride* and *Seimatosporium eucalypti*. In this case, the isolated plant pathogen *Seimatosporium eucalypti* was determined despite observed aberrant, collapsed conidia. Atypical conidiogenesis is due to strong antifungal inhibitory effect of *Trichoderma* species documented in close proximity of *Seimatosporium* sp. [Zimowska 2004]. It is important to emphasize that many phyllosphere fungi produce a wide range of antimicrobials and some authors have demonstrated antagonistic activity of certain fungal species against others [Naik *et al.*, 2009]. This demonstrates that phyllosphere microfungi exhibit highly complex interactions, including natural systems of phytopathogen control [Ljaljević Grbić 2006].

A large number of isolated epiphytic saprobes, like in this study, suggests that external microenvironment of the leaf surface induces the fungal growth while its internal microenvironment restricts fungal population to some extent [Kharvar *et al.*, 2010]. This is supported by the fact that *Eucalypti* leaf tissues often contain essential oils which are proven to have antimicrobial properties [Safaei-Ghomi and Ahd 2010; Katooli *et al.*, 2011; Bachir and Benali, 2012]. It is argued that plant trichomes could capture and retain airborne particles, including fungal spores [Calo *et al.*, 2006]. This is confirmed with observed growth of the species from family Dematiaceae where visible pigmented mycelium was inside the leaf trichome.

CONCLUSIONS

Phyllosphere microfungal community of *Eucalyptus camaldulensis* and *E. globulus* is diverse, with domination of saprobe epiphytes such as *Aspergillus* and *Penicillium* species. It is evident that isolated species are in complex interactions with each other and their host plant. Since both epiphytes and endophytes can cause various disease symptoms, study of phyllosphere fungal community is of great importance, especially on economically important plants.

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МИКОБИОТА ФИЛОСФЕРЕ *EUCALYPTUS CAMALDULENSIS* Dehnh. И *E. GLOBULUS* Labill.

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РЕЗИМЕ: У раду је испитивана микобиота филосфере две врсте рода *Eucalyptus* (*E. camaldulensis* и *E. globulus*) применом методе влажне коморе. Идентификовано је 19 таксона који улазе у састав заједнице микрогљива површине листова и чаура. *Aspergillus niger*, *Alternaria alternata* и *Penicillium* spp. су најчешће изоловане

микрогљиве са обе испитиване биљне врсте. Сапробне микромицете су чешће документоване у поређењу са фитопатогенима. Такође, уочена је доминација епифитских врста. Микроскопска анализа листа *E. globulus* је показала присуство плодноносних тела *Seimatosporium eucalypti* са конидијама измењене морфологије, највероватније услед инхибиторног ефекта *Trichoderma viride*. Испитивање микобиоте филосфере је од великог значаја, с обзиром да бројне гљиве, колонизатори листова, формирају комплексне биотичке интеракције.

КЉУЧНЕ РЕЧИ: влажна комора, гљиве, епифите, *Eucalypti*, сапроби, фило-сфера

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SCREENING OF AZOTOBACTER ISOLATES FOR PGP PROPERTIES AND ANTIFUNGAL ACTIVITY

ABSTRACT: Among 50 bacterial isolates obtained from maize rhizosphere, 13 isolates belonged to the genus *Azotobacter*. Isolates were biochemically characterized and estimated for pH and halo tolerance ability and antibiotic resistance. According to characterization, the six representative isolates were selected and further screened *in vitro* for plant growth promoting properties: production of indole-3-acetic acid (IAA), siderophores, hydrogen cyanide (HCN), exopolysaccharides, phosphate solubilization and antifungal activity (vs. *Helminthosporium* sp., *Macrophomina* sp., *Fusarium* sp.). Beside HCN production, PGP properties were detected for all isolates except *Azt*₇. All isolates produced IAA in the medium without L-tryptophan and the amount of produced IAA increased with concentration of precursor in medium. The highest amount of IAA was produced by isolates *Azt*₄ (37.69 and 45.86 µg ml⁻¹) and *Azt*₅ (29.44 and 50.38 µg ml⁻¹) in the medium with addition of L-tryptophan (2.5 and 5 mM). The isolates showed the highest antifungal activity against *Helminthosporium* sp. and the smallest antagonistic effect on *Macrophomina* sp. Radial Growth Inhibition (RGI) obtained by the confrontation of isolates with tested phytopathogenic fungi, ranged from 10 to 48%.

KEY WORDS: antifungal activity, *Azotobacter*, IAA, maize, PGP properties, rhizosphere

INTRODUCTION

Maize (*Zea mays* L.) is one of the three world's most widely grown crop with an annual global production of 1 billion t in 2013 [available at FAOSTAT]. In Serbia, maize is grown on about 1.2–1.4 million ha, with a total grain production between 4 and 7 million t per year [Jocković *et al.*, 2010]. Besides genetic potential, achieving higher yields also demands appropriate fertilization.

Recently, plant growth promoting rhizobacteria (PGPR) have been used to enhance crop yield and improve agricultural sustainability. PGPR are directly involved in increased uptake of nitrogen through biological nitrogen fixation,

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synthesis of phytohormones, solubilization of minerals such as phosphorus and production of siderophores that chelate iron and make it available to the plant root [Ahemad and Kibret 2014].

The beneficial effect of *Azotobacter*, applied alone or in mixture with other PGPR strains, on vegetative growth and yield of maize was reported by numerous authors [Biari *et al.*, 2008; Gholami *et al.*, 2009; Jarak *et al.*, 2012]. Yield increase by *Azotobacter* inoculation is a result of nitrogen fixation, as well as the production of growth regulators, antibacterial and antifungal compounds [Mrkovački and Milić 2001; Wani *et al.*, 2013].

Efficiency of microbial preparations can be increased by using the best combination of beneficial microorganisms and it requires a clear definition of useful and necessary properties of a microorganism selected for specific environmental conditions and certain plants. Therefore, the aim of this study was to perform characterization of *Azotobacter* isolates from maize rhizosphere and selection of strains with potential environmental and plant growth properties, as well as antagonistic activities against phytopathogenic fungi, for the purpose of further field application.

MATERIAL AND METHODS

Isolation and characterization of Azotobacter strains

The rhizosphere soil samples were collected from the one-month-old maize plants (hybrid NS6010) grown on calcareous chernozem soil at Rimski Šančevi experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. Chemical soil properties of the experimental field were: pH (in H₂O) – 8.42, nitrogen percentage – 0.152%, calcium carbonate percentage – 5.04%, humus content – 2.05%, available P and K contents – 12.8 and 17.3 mg 100 g⁻¹ soil. The soil paste-plate method [Becking 1981] was used as isolation strategy for *Azotobacter*. The isolates were characterized by their morphological and biochemical characteristics using standard methods [Jarak and Đurić 2004].

The isolates were grown on N free medium adjusted with 1M HCl or 1M NaOH for pH tolerance (5.5 and 9.0) and supplemented with 3% and 7% NaCl for salt tolerance. Determination of the direct impact of antibiotics was performed by the diffusion method using different concentrations of antibiotics (μg ml⁻¹): ampicillin (10, 25), neomycin (10, 30), erythromycin (5, 15), streptomycin (10, 300), chloramphenicol (10), and kanamycin (30).

In vitro screening for plant growth promoting properties

Phosphate solubilization. The ability of isolates to dissolve sparingly soluble inorganic phosphate was determined by spot inoculations on PVK (Pikovskaya medium) [Pikovskaya 1948] and NBRIP (National Botanical Research Institute's phosphate growth medium) [Nautiyal 1999] with 0.5% TCP (Ca₃(PO₄)₂).

Siderophores production. Bacterial ability to produce siderophores was assayed on a chrom-azuroil S (CAS) medium by protocol of Milagres *et al.* [1999].

Exopolysaccharides (EPS) production. In order to test the production of EPS, isolates were grown on the appropriate media supplemented with 0.02% Calcofluor color (Calcofluor White M2R, Sigma) [Reed *et al.*, 1991].

Hydrocyanic acid (HCN) production. HCN production was tested on HCN induction medium supplemented with glycine (4.4 g l⁻¹) [Ayyadurai *et al.*, 2007].

Indole acetic acid (IAA) production. For quantitative analysis of IAA production, a 100 µl 24h-old bacterial suspension was inoculated in liquid N-free medium, supplemented without and with 2.5 and 5 mM of L-tryptophan (as precursor of IAA) and incubated for 48h at standard temperature. Salkowski reagent was mixed with the supernatant (2:1 v/v) and intensity of the developed color was measured at 530 nm [Glickman and Dessaux 1995].

Antifungal activity assay. The ability of the isolates to inhibit the growth of the phytopathogenic fungi (*Helminthosporium sp.*, *Macrophomina sp.* and *Fusarium sp.*) was determined by the method of dual culture [Rodriguez *et al.*, 2000]. Radial Growth Inhibition (RGI) was calculated according to formula: $RGI (\%) = [(r_1 - r_2) / r_1] \times 100$; where: r_1 = radius/growth of mycelium in the control and r_2 = radius/growth of mycelium confronted with a bacterial isolate.

Statistical Analysis. The statistical variation in IAA production by *Azotobacter* isolates and antifungal activity were analyzed using the analysis of variance (ANOVA), followed by mean separation according to Duncan's Multiple Range test ($p < 0.05$).

RESULTS AND DISCUSSION

Among 50 bacterial isolates obtained from maize rhizosphere, according to morphological and biochemical characterization, 13 representative isolates were grouped into genus *Azotobacter* as described in Bergey's Manual of Determinative Bacteriology [Holt *et al.*, 1994]. General properties of test isolates were presented in Table 1. All the isolates were gram-negative and able to use the examined carbohydrate and citrate as carbon sources.

Table 1. Screening of isolates for morphological and biochemical characteristics

Morphology	C utilization	Isolates %	Test reaction	Isolates %
<i>Colony</i> Slimy, glistening, brown to yellow-green on aging, medium to large-size <i>Cell</i> Gr-ve rod-shaped to coccoid cells	Glucose	69	Citratase	84
	Galactose	100	Gelatinase	92
	Sucrose	69	Amilase	0
	Fructose	100	Catalase	100
	Lactose	92	Urease	69
	Mannitol	69	Nitrate reduction	61

Soil properties have a strong impact on a range of processes that influence crop yield, including microbial activity. Bacteria investigated in our study expressed good potential for adaptation. Four isolates had optimal growth on the medium with pH 5.5. All isolates were tolerant to concentration of 3% NaCl, while on the medium with the addition of 7% NaCl growth was not recorded. Resistance to antibiotics depended on the tested isolates, types and concentrations of antibiotics. Neomycin and streptomycin had the largest inhibitory effect, while the most tolerant isolates were *Azt₁*, *Azt₅* and *Azt₇* (Table 2).

Table 2. Screening of isolates for pH and halo tolerance and antibiotic resistance

Isolates	pH and halo tolerance				Antibiotics ($\mu\text{g ml}^{-1}$)									
	pH		NaCl (%)		Amp		Ery		Neo		Str		Kan	Chl
	5.5	9.0	3	7	10	25	5	15	10	30	10	300	30	30
<i>Azt₁</i>	-	-	+	-	r	r	r	s	s	s	s	s	r	r
<i>Azt₂</i>	+	-	+	-	s	s	s	s	r	s	s	s	s	s
<i>Azt₃</i>	-	-	+	-	s	s	s	s	s	s	s	s	s	s
<i>Azt₄</i>	+	-	+	-	s	s	s	s	s	s	s	s	s	s
<i>Azt₅</i>	+	-	+	-	r	r	s	s	s	s	s	s	s	r
<i>Azt₆</i>	-	-	+	-	s	s	s	s	s	s	s	s	s	s
<i>Azt₇</i>	-	-	+	-	r	s	r	s	s	s	s	s	s	r
<i>Azt₈</i>	-	-	+	-	s	s	s	s	s	s	s	s	s	s
<i>Azt₉</i>	-	-	+	-	s	s	s	s	s	s	s	s	s	s
<i>Azt₁₀</i>	+	-	+	-	s	s	s	s	s	s	s	s	s	s
<i>Azt₁₁</i>	-	-	+	-	s	s	s	s	s	s	s	s	s	s
<i>Azt₁₂</i>	-	-	+	-	s	s	s	s	s	s	s	s	s	s
<i>Azt₁₃</i>	-	-	+	-	s	s	s	s	s	s	s	s	s	s

pH and halo tolerance: (+) growth; (-) growth absent; Antibiotic resistance: (s) sensitive; (r) resistant

According to environmental properties, the six representative isolates were selected for further screening on the basis of PGP properties and anti-fungal activity (Table 3). Poor solubility of TCP on PVK and NBRIP has been determined, with the width of the solubilization zone between 1 and 4 mm, whereas a larger solubilising zone, from 4 to 7 mm, was measured only for isolate *Azt₇* on the NBRIP medium. The ability of bacteria to produce siderophores was detected in all isolates except *Azt₇*. Larger orange zone, from 5 to 15 mm, was measured for isolates *Azt₅* and *Azt₁₀*, while the zone of color change for other isolates was between 1 and 5 mm. All isolates except *Azt₇* produced exopolysaccharides, while production of HCN was detected in isolates *Azt₅* and *Azt₁₀*.

Diverse PGPR produce IAA and other metabolically active substances, which lead to an increase in root length, height of above ground plant parts and yield. Bacteria use this phytohormone to interact with plants as part of their colonization strategy, including phytostimulation and circumvention of basal plant defense mechanisms [Spaepen *et al.*, 2007]. In this study, the quantity of produced IAA depended on the applied concentration of L-tryptophan and tested isolates. All isolates produced IAA in the medium without L-tryptophan and the amount of produced IAA increased with concentration of precursor in medium. Isolate *Azt10* was the best IAA producer in the medium without precursor (26.16 $\mu\text{g ml}^{-1}$), while isolates *Azt4* (37.69 and 45.86 $\mu\text{g ml}^{-1}$) and *Azt5* (29.44 and 50.38 $\mu\text{g ml}^{-1}$) produced the largest amounts in medium supplemented with 2.5 and 5 mM L-tryptophan. This is in accordance with the investigation of Govedarica *et al.* [1993]. In their study, *Azotobacter* strains isolated from chernozem soil had the ability to produce auxins, gibberelins and phenols and thus increase plant length, mass and nitrogen content of tomato plants. Similarly, variability within the same PGPR properties in different isolates was recorded by Mahalakshmi and Reetha [2009], while Suresh *et al.* [2010] concluded that the most isolates from maize rhizosphere possessed PGPR characteristics, and therefore should be used as potential biofertilizers.

Table 3. Screening of isolates for PGP properties and antifungal activity

Isolates	P-sol		Siderophore	HCN	EPS	IAA production ($\mu\text{g ml}^{-1}$)			Radial Growth Inhibition (%)		
	PVK	NBRIP				mM L-tryptophan			Macropho- mina	Helminho- sporium	Fusarium
						0	2.5	5			
<i>Azt1</i>	+	+	+	-	+	4.57 ^d	28.71 ^c	36.20 ^c	19.61 ^a	46.76 ^{ab}	38.43 ^{ab}
<i>Azt2</i>	+	+	+	-	+	2.96 ^e	23.73 ^f	24.38 ^f	18.03 ^a	42.28 ^{ab}	39.21 ^a
<i>Azt4</i>	+	+	+	-	+	7.26 ^b	37.69 ^a	45.86 ^b	10.59 ^a	29.36 ^b	35.29 ^{bc}
<i>Azt5</i>	+	+	++	+	+	5.26 ^c	29.44 ^b	50.38 ^a	10.98 ^a	40.79 ^b	27.06 ^{bc}
<i>Azt7</i>	+	++	-	-	-	4.13 ^d	24.66 ^e	28.79 ^e	21.96 ^a	48.25 ^a	34.50 ^{bc}
<i>Azt10</i>	+	+	++	+	+	26.16 ^a	27.09 ^d	30.83 ^d	18.82 ^a	42.79 ^{ab}	30.58 ^c

P-sol: (+) 1-4 mm of halo diameter; (++) 4-7 mm of halo diameter; siderophore: (-) no color change (+) 1-5 mm wide of orange zone; (++) 5-15 mm wide of orange zone; HCN, EPS: (+) positive reaction; (-) negative reaction; IAA: The different letter above the number indicates a significant difference at $P < 0.05$

Azotobacter isolates showed the highest antifungal activity against *Helminthosporium* sp. and the smallest antagonistic effect on *Macrophomina* sp. RGI, obtained by confrontation of isolates with tested pathogens, ranged from 10 to 48%. The largest decrease in growth of *Macrophomina* sp. and *Helminthosporium* sp. was obtained by the confrontation with isolates *Azt1* (19.61%

and 46.76%) and *Azt*₇ (21.96% and 48.25%). The highest antifungal activity against *Fusarium* sp. was registered through confrontation with the isolates *Azt*₁ and *Azt*₂ (38.43% and 39.21%). Other isolates had equally good antagonistic effect against the tested pathogens. Similar findings about fungal growth inhibition and possible application of *Azotobacter* isolates as biocontrol agents were obtained in numerous studies. Subba Rao [2001] proved that isolates of *Azotobacter chroococcum* produced an antibiotic which inhibited the growth of several pathogenic fungi. Investigating the effect of *Azotobacter* isolates against *Apergillus flavus*, *Cercospora* sp., and *Fusarium oxysporum*, Ponmurugan *et al.* [2012] determined the larger inhibition zone at a higher suspension of culture.

CONCLUSION

This study confirmed the occurrence of *Azotobacter* sp. in maize rhizosphere. Results are of practical importance because we demonstrated that the tested isolates produce a considerable amount of IAA, and have a good antifungal activity, partly due to production of siderophores and HCN. Further studies on the performance of these isolates in soil-plant system are needed to establish which traits of selected isolates are useful and necessary for certain environmental conditions and different hybrids.

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ИСПИТИВАЊЕ PGP СВОЈСТАВА И АНТИФУНГАЛНЕ АКТИВНОСТИ ИЗОЛАТА АЗОТОБАКТЕРА

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РЕЗИМЕ: Међу 50 изолата бактерија из ризосфере кукуруза, 13 изолата припадали су роду *Azotobacter*. Изолати су биохемијски карактерисани и испитана је толерантност према реакцији средине, концентрацији соли и резистентност на антибиотике. Након карактеризације, одабрано је шест репрезентативних изолата за даља испитивања PGP својстава: продукције индол-3-сирћетне киселине (IAA), сидерофора, цијановодоничне киселине, егзополисахарида, фосфосолубилизације и антифунгалне активности (према *Helminthosporium* sp., *Macrophomina* sp., *Fusarium* sp.). Осим продукције HCN, PGP својства утврђена су за све изолате осим *Azt*₇. Највећу количину IAA продуковали су изолати *Azt*₄ (37,69 и 45,86 µg ml⁻¹) и *Azt*₅ (29,44 и 50,38 µg ml⁻¹) у подлози са додатком L-tryptophan-a (2,5 и 5 mM). Изолати су испољили највећу антифунгалну активност према *Helminthosporium* sp., а најмањи антагонистички ефекат према *Macrophomina* sp. Проценат инхибиције раста (RGI) добијен суочавањем изолата са испитиваним фитопатогеним гљивама кретао се од 10 до 48%.

КЉУЧНЕ РЕЧИ: антифунгална активност, *Azotobacter*, IAA, кукуруз, PGP својства, ризосфера

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ON THE NEW AND RARE LEPIDOPTERA IN SERBIAN FAUNA

ABSTRACT: New distributional records are given for eight Lepidoptera species in Serbia. *Ethmia lugubris* (Staudinger, 1879) (fam. Ethmiidae) is reported in Serbia for the first time. Larval mines of *Stigmella centifoliella* (Zeller, 1848) (fam. Nepticulidae), photos of *Hypercalia citrinalis* (Scopoli, 1763) (fam. Amphisbatidae) and *Cryphia amasina* (Draudt, 1931) (fam. Noctuidae), as well as male genitalia of *Leptidea reali* Reissinger (1988) – *Leptidea juvernica* Williams, 1946 complex (fam. Pieridae) and *Cryphia amasina* are presented. UTM 10 x 10 km distribution map for three species is provided.

KEYWORDS: Lepidoptera, Republic of Serbia, fauna

INTRODUCTION

The latest comprehensive checklist of Serbian Lepidoptera [Zečević 1996] revealed, among other things, that many families, genera and species had not been sufficiently investigated in the past, as well as that many species had not been found again for even few decades. The knowledge of the species is still insufficient: 1,355 species [Zečević 1996] compared to 4,104 species in Romania [Rákossy *et al.*, 2003], or 3,598 species in Slovakia [Pastoralis *et al.*, 2013], etc. As a result of a recent field research carried out predominantly in eastern Serbia, some interesting data for eight Lepidoptera species have been found. These faunistic data are presented in this paper. Among the recorded species, one Microlepidoptera species has not been known in Serbian fauna so far.

The region of eastern Serbia, towards Bulgarian border, phytogeographically belongs to Moesian phytogeographic province of the Balkan floristical subregion. Due to lack of exploration in the first place, we decided to examine the composition of the fauna Lepidoptera in the gorge of Jelašnička Klisura and Suva Planina Mountain. The results would present the zone of Suva Planina Mt. and Vidlič Mt. influence in the east and Tresibaba Mt. in the north. Rich faunistic material has been collected in the last few years.

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MATERIAL AND METHODS

Specimens were collected with butterfly net, light trap and by collecting mined leaves. The coordinates at which the Lepidoptera were caught were determined using “Garmin e-Trex Vista” GPS device. The photos of specimens *in situ* were taken using Nikon Camera with “AF-S Micro-NIKKOR Lens”.

Fieldwork in protected areas was done on the basis of permits provided by the Ministry of Environment, Mining and Spatial Planning, Republic of Serbia, No. 353-01-1559-2011-03, dated 8 June 2011; No. 353-01-1070/2012-03, dated 12 June 2012.; No. 353-01-916/2014-08, dated 29 May 2014. and No. 353-01-356/2015-17, dated 27 April 2015.

After the preparations, the determination of the specimens was done by the wing-patterns and in some cases by examination of the male genitalia. The preparations were carried out following the well known standard procedure: maceration by boiling in potash, dissecting and cleaning, clearing in xylolum and mounting in Canada balsam. The photos of genital parameters were taken using the “Leica DM 1000” microscope with the “Camera Leica DEC 290”. All the material (specimens and genitalia slides) are deposited in the author’s collection.

The taxonomic order identified according to Nieukerkenn *et al.* [2011], and the nomenclature was done according to Karsholt & Razowski [1996] and Van Swaay *et al.* [2010].

RESULTS AND DISCUSSION

1. *Stigmella centifoliella* (Zeller, 1848) (fam. Nepticulidae)

Material examined: Beograd, Avala Mt., 1 June 2015. Jakšić P. leg. (Fig. 1). Determination was done according to Lödl & Gaal-Haszler [2010]. During the work on medicinal plant research, 152 species of leaf miners from order Lepidoptera in Serbia have been proved [Dimić *et al.*, 2000]. These data include species *S. centifoliella*, but without precise localities. The larvae feed on *Rosa* spp.

In Bulgaria, this species is reported from Eastern Rhodopes [Buszko & Beshkov 2004]; in Croatia from Jastrebarsko, Gonjeva [Matošević *et al.*, 2009].

Ova laid usually underside of leaf mine: June–July, September–October.

2. *Ethmia lugubris* (Staudinger, 1879) (fam. Ethmiidae)

Material examined: Eastern Serbia, the town of Žagubica, Jagnjilo, 350 m; 20 May 2015, 2 ♂♂, Jakšić P. leg.

This is a new species in the fauna of Serbia.

In Bulgaria, species is reported from Rila Mt., Rhodope Mts. and Stara Planina Mt. [Ganev 1983].



Figure 1. Mine of *S. centifoliella* on *Rosa canina*, Beograd, Avala Mt., 1 June 2015, Jakšić P. leg. [Foto: P. Jakšić]

3. *Hypercallia citrinalis* (Scopoli, 1763) (fam. Amphisbatidae)

According to Karsholt & Razowski [1996] there are 26 Amphisbatidae species in Europe and 7 species in former Yugoslavia. Species *Hypercallia citrinalis* [Scopoli 1763] is a member of family Amphisbatidae. Fazekas [2002], Vallhonrat *et al.* [2004], Pastoralis [2010], Rakosy & Wieser [2010] and Pastoralis & Szeőne [2011] are of the same opinion. Following Lesar & Habeler [2005], Bond & O'Connor [2012] and Šumpich *et al.* [2013] this species is a member of family Elachistidae. Finally, by Klepikov [2004], Malkiewicz [2005] and Koçak & Kemal [2009] this species is a member of family Oecophoridae.

Representatives of family Amphisbatidae in former Yugoslavia have not been sufficiently examined yet. The first literary data was given by Scopoli (1763), who described *H. citrinalis* for Slovenia. Rebel [1910] described *Pseudatemelia aeneella* Rebel, 1910 for Hercegovina: Prenj Mt. and Bosnia: Trebević Mt., as well as for Slovenia: Nanos and Gradische Mt.; Rebel und Zerny [1931] recorded *Anchinia daphnella* (Denis und Schiffermüller 1775) and *A. laureolella* Herrich-Schäffer, 1854 for Macedonia (Bulgaria – Greece): Alibotuš Mt., as well as Lesar and Habeler [2005] for Slovenia. Rebel (1904) reported *Pseudatemelia flavifrontella* (Denis und Schiffermüller, 1775) for Bosnia and Hercegovina: Bjelašnica Mt.; Klimesch (1968) specified *P. josephinae* (Toll, 1956) for Macedonia: Šar-planina Mt., Crni vrh, as well as *Fuchsia luteella* (Heinemann, 1870) for Macedonia: Drenovo – Kavadarci.

When it comes to *H. citrinalis* (Scopoli, 1763) species, there is only a few information for former Yugoslavia. Following literature data, there could be specified: Rebel (1904) for Bosnia and Herzegovina (Sarajevo, Džile and Vlasenica); Rebel und Zerny (1931) for Albania (Kula e Lumes) and Serbia (Žljeb Mt.); Lesar & Habeler (2005) for Slovenia (Polzela and Slatina pri Polzeli).

Lemon Flat-body [*Hypercallia citrinalis* (Scopoli, 1763)] is an interesting species. It was recorded in 12 May 2015 in the gorge of Jelašnička Klisura,

310 m above sea level, EN89, N 43°17'55'', E 22°03'42'' (Fig. 2). This new site near the city of Niš connects two already known distant localities: Žljeb Mt. (Western Serbia) and Borovets in Bulgaria [Soffner 1967].



Figure 2. *H. citrinalis*, Serbia, Niš, the gorge of Jelašnička Klisura, 12 May 2015 [Photo: P. Jakšić]

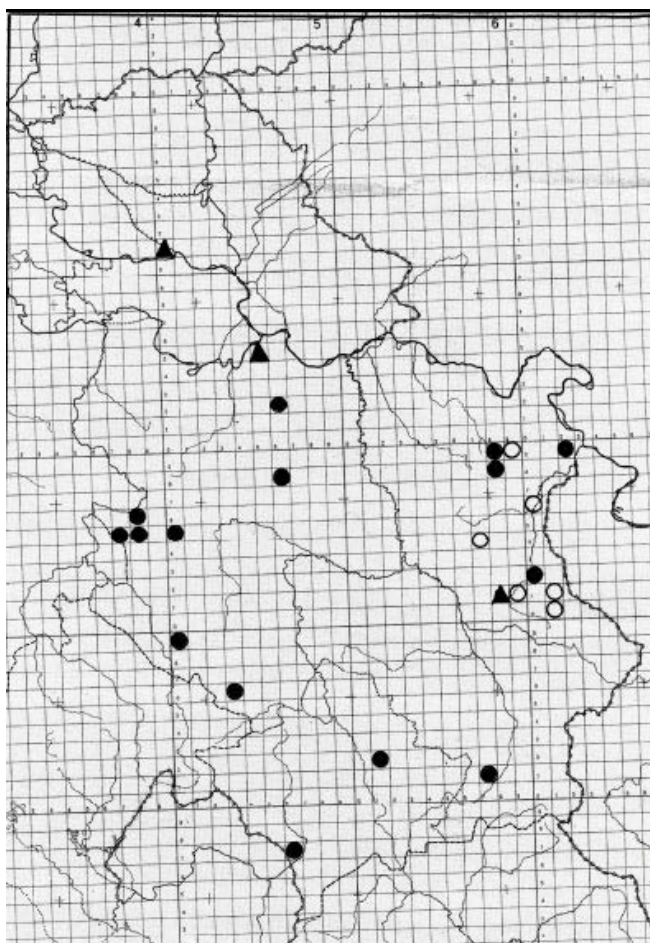
It was found in a developed and well preserved habitat determined as: 6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) (*important orchid sites) (E1.21, E1.22, E1.55). Caterpillars are fed with common milkwort (*Polygala vulgaris*) and chalk milkwort (*P. calcarea*). Petrović [1882] reported *P. vulgaris* for this area.

4. *Leptidea reali* Reissinger (1989) – *Leptidea juvernica* Williams, 1946 complex (fam. Pieridae)

Material examined: Bor, Deljboke, 500 m, 11 August 1994, Jakšić P. leg. (prep. no. SR-2048 and SR-2125); Bor, Savača, 500 m, 9 July 1993, Jakšić P. leg. (prep. no. SR-2123); the Rača River canyon, 16 August 2006., Dodok I. leg. (prep. no. SR-2328); Kosmaj Mt., 24 April 2006, Đurić M. leg. (prep. no. SR-2306); Paštrik Mt., Gorožup, 700 m, 2 July 1996., Jakšić P. leg. (prep. no. SR-2102); the town of Knjaževac, Papratna, 200–300 m; Prijepolje, the Mileševka River, 685 m, 7 June 2015, Jakšić P. leg. (prep. no. SR-2683); Priština, Grmija Mt., 700 m, 3 July 1978, Jakšić P. leg. (prep. no. SR-5740); Negotin, 14 March 1998, Grozdanović A. leg. (prep. no. SR-2301); Rudnik Mt., 600 m, 28 April 1985, Jakšić P. leg. (prep. no. SR-1786); Tara Mt., the Beli Rzav River, 12 August 2006, Dodok I. leg. (prep. no. SR-2330); Tara Mt., Kaluđerske Bare, 11 August 2006, Dodok I. leg. (prep. no. SR-2337); Tutin, Crkvine, the Smolučka Reka River,

23 June 2006, Jakšić P. leg. (prep. no. SR-2314); Vranje, Goč Mt., 18 May 1997, Stošić G. leg. (prep. no. SR-2179); the town of Užice, the gorge of the Đetinja River, 23 April 2006, Dodok I. leg. (prep. no. SR-2336); Zlatibor Mt., Mokra Gora, 22 April 2006, Dodok I. leg. (prep. no. SR-2338) (Map 1, Fig. 3).

L. reali, described previously by Réal as a new species *L. lorkovici*, then as *L. reali* Reissinger [1989], from the eastern Pyrenees, is distinctly different from *L. sinapis* by greatly prolonged ductus bursae, aedoeagus and saccus alone (Fig. 3). Recently, Dincă *et al* [2011] have shown that inside of *Leptidea reali* exist another species – *L. juvernica* Williams, 1946 *L. reali* and *L. juvernica*, differentiated by at least 11 chromosomal fusions/fissions. Recent studies have shown that population in the Balkan Peninsula which used to be known as *L. reali* is now referred to as the new cryptic species *L. juvernica* which occurs in temperate Europe and Asia.



Map 1. The UTM 10x10 km distribution map of the localities of analyzed species:
 ● – *L. reali* – *L. juvernica* complex, ▲ – *C. amasina*, ○ – *K. climene*.



Figure 3. Male genitalia of *L. reali* – *L. juvernica*: Serbia, Bor, Deljboke, 500 m, 11 August 1994, Jakšić P. leg.

5. *Kirinia climene* (Esper, 1783) (fam. Nymphalidae)

Material examined: Tresibaba Mt., 787 m, 23 June 2014, Jakšić P. leg. (Fig. 4; Map. 1). In Tresibaba Mt. this species was found in plant association *Quercetum cerris moesiicum* s. lat. A small colony of about 10 specimens was noticed flying in treetop. In Rtanj Mt. it was treetop of *Sorbus aria* (L) Crantz, and in Tresibaba Mt. it was treetop of *Quercus cerris* L. The localities where this species was recorded are detached and distant from each other. For this reason, there is the question of communication within and between these populations.

Extremely rare species in Serbia are reported from Stol Mt. [Zečević 2002], Rtanj Mt. [Jakšić *et al.*, 2008] as well as from Svrljiške Planine Mt.: Južni izvor, 556, Stara Planina Mt.: the village of Rudinje, 836 m and Zaječar: Beli Breg, 170 m [Đurić & Popović 2011].

In Bulgaria, they are reported only on three localities: Stara Planina Mt.: Gorna Kremna and Sliven [Abadjiev 2001], as well as in Vlahina Mt., the village of Debočica, 1,000 m [Domozetski 2012].



Figure 4. *K. climene*, Tresibaba Mt., 787 m, 23. 6. 2014. [Photo: P. Jakšić]

6. *Erebia alberganus* (Prunner, 1798) (fam. Nymphalidae)

Material examined: *E. alberganus* found at two localities – Stara Planina Mt., Dojkinci, 700 m, 14 June 2015 and the town of Pirot, Crni Vrh Mt., 790 m, 22 June 2015. Both localities are meadows in beech forests. This is the species with large populations.

Parker & Jakšić [1996] reported this species from several localities in Stara Planina Mt.: beside the Toplodolska River, 800–1,000 m; beside the Rakitska River, 1,350 m and beside the Ilijina River, 1,400 m. Van Swaay *et al.* [2007] reported *E. alberganus* from Stara Planina Mt.: Babin Zub, 1,750 m.

7. *Diacrisia sannio* (Linnaeus, 1758) (fam. Erebidae)

Material examined: Fruška Gora Mt., Grabovo, 180 m, 1 ♂, 25 July 2008, Jakšić P. leg.; Fruška Gora Mt., Beočin, 125 m, 1 ♀, 18 July 2008, Jakšić P. leg.

This is an already well-known species of the fauna of Serbia from several localities: Velika Ada Ciganlija; Beograd, Borčanski Kanal; Beograd, Pionirski Grad; Kraljevo; Grocka; Divčibare Mt. (Andus, 1984). Zečević (2002) reported this species from eastern Serbia, Timočka Krajina; also, in Vojvodina, Rotschild (1909–1917) from Deliblatska Peščara (Deliblato sands) and Kereši and Almaši [2009] from Novi Sad: Rimski Šančevi. However, there were no data for this species in Fruška Gora Mt. [Stojanović 2012], these are the first data.

The polyphage larvae feed on different plants: *Galium*, *Plantago*, *Taraxacum*, *Epilobium*, *Urtica*, *Hieracium*, *Rumex*, etc.

8. *Cryphia amasina* (Draudt, 1931) (fam. Noctuidae, Bryophilinae)

Material examined: Beograd, Zvezdara, 185 m, 3 ♂♂, 27 July 2015, Jakšić P. leg. (Figure 5, Map 1). This is the third finding of this species in Serbia.



Figure 5. *C. amasina*, left: adult male, Beograd, Zvezdara; right male genitalia slide SR-2692, x44.

As a new species in the fauna of Serbia Stojanović (2005) found this species in Fruška Gora Mt.: Ledinci-Stokuća, 360 m., 1 ♂, 28 July 2001 and 1 ♂, 18 July 2004. Recently, Beshkov [2015] found *C. amasina* in eastern Serbia, the region of Niš, Koritnjak above Niška Banja, 560 m.

CONCLUSION

Representatives of the following families have been recorded: Nepticulidae, Ethmiidae, Amphisbatidae, Pieridae, Nymphalidae, Noctuidae and Erebidae. Obtained results contribute to the more complete knowledge of the distribution of certain Lepidoptera species in Serbia. These results support the fact that the highland and mountainous regions of the Republic of Serbia, apart from Carpathes, Rhodopes and Stara Planina, as parts of Balkan Peninsula, are one of six European centers of Lepidoptera diversity.

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О НОВИМ И РЕТКИМ ВРСТАМА ЛЕПТИРА (LEPIDOPTERA) У ФАУНИ СРБИЈЕ

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РЕЗИМЕ: Србија са 1.355 до сада регистрованих врста реда Lepidoptera још увек знатно заостаје у познавању фауне ове групе инсеката. У овом прилогу приказани су нови подаци о распрострањењу осам врста Lepidoptera. Врста *Ethmia lugubris* (Staudinger, 1879) (fam. Ethmiidae) утврђена је у околини Жагубице (Јагњило) као нова у фауни Србије. У околини Београда (Авала) приказан је проналазак лисног минера врсте *Stigmella centifoliella* (Zeller, 1848) (fam. Nepticulidae). Изложен је снимак врсте *Hypercalia citrinalis* (Scopoli, 1763) (fam. Amphibatidae) која је утврђена у Јелашничкој клисури. Наведен је и налаз *Diacrisia sannio* (Linnaeus, 1758) (fam. Erebidae) сакупљене на Фрушкој гори (Грабово и Беоцин). Приложени су и снимци гениталне арматуре мужјака врста *Leptidea reali* Reissinger (1989) – *Leptidea juvernica* Williams, 1946 комплекс (fam. Pieridae), утврђене на 15 локалитета у Србији, као и *Cryphia amasina* (Draudt, 1931) (fam. Noctuidae, Bryophilinae) утврђене у Београду (Звездара). Урађена је и УТМ 10x10 km карта распрострањења три врсте у Србији.

КЉУЧНЕ РЕЧИ: лептири (Lepidoptera), Република Србија, фауна

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THE RATIO OF DOMESTIC AND WILD ANIMALS AT NEOLITHIC SITES IN VOJVODINA (SERBIA)**

ABSTRACT: Based on the results of the vertebrate fauna research from 10 Neolithic archaeological sites in Vojvodina (Serbia), two of which belong to **Kőrös culture**, 7 to **Starčevo culture**, and one to **Vinča culture**, the proportional contribution of domestic and wild animals was analysed. These sites were approximately dated between 6000 and 3200 BC. The smallest proportion of domestic animals was recorded at the sites of **Golokut-Vizić** and **Nosa Biserna Obala**, while the biggest one at the sites of **Prosine-Pećinci**, **Zlatara-Ruma** and **Kudoš-Šašinci**. A small proportion of domestic animals at Nosa Biserna Obala shows that the animal husbandry was only just at the beginning, and a high proportion of wild animals testifies about the importance of hunting in economy. These are the characteristics of settlements of Kőrös culture, where goats and sheep dominate among domestic animals. Low proportion of domestic and high proportion of wild animals were recorded at the site of Golokut which, like most of the described sites in this paper, belongs to the Middle Neolithic; this is not characteristic for Starčevo culture and it testifies that hunting was much more important than animal husbandry. What is characteristic for settlements of Starčevo culture is the domination of oxen in the total vertebrate fauna and among domestic animals. At the site of Donja Branjevina-Deronje, the settlement which belongs to Starčevo culture as well, goats and sheep have the biggest proportional contribution. The only analysed settlement in this paper which belongs to the Early Neolithic (Vinča culture) is Gomolava – Hrtkovci where domestic animals dominate, oxen being the most numerous ones.

KEYWORDS: Neolithic, Vojvodina, domestic animals, wild animals

INTRODUCTION

In the territory of Vojvodina there are dozens of archaeological settlements from different periods. Archaeological researches have been conducted there

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over the last eighty years during which an immense sample has been collected, predominantly consisting of bones of vertebrates (Vertebrata), seashells and snail shells (Mollusca). The paper shows data from 10 Neolithic sites, dated between 6000 and 3200 BC [Cerović *et al.*, 1997]. These sites belong to different cultures, Kőrös being the oldest one. The sites of **Nosa Biserna Obala** and **Ludaš Budžak** were discovered in northern Vojvodina. The first site was excavated in 1957 [Bököny 1974], while archaeological digs at other sites were done in 1965 [Bököny 1974]. The next cultural layer is **Starčevo** and it belongs to Early and Middle Neolithic. It is widespread in Vojvodina and the majority of Neolithic sites belong to this culture. Archaeozoological researches for this period were done at the following sites: **Donja Branjevina** near Deronje, excavated in 1987 [Blažić 1992a], **Golokut** near Vizić, excavated in 1973 and 1976 [Blažić 1984], **Starčevo**, where vertebrate bones were first collected in 1932, and where the researches continued in the period between 1969 and 1970 [Clason 1980]. There are also 4 Neolithic sites along the highway through Srem that belong to Starčevo culture: **Malo Kupalovo**-Krnješevci, **Prosine**-Pećinci, **Zlatica**-Ruma and **Kudoš**-Šašinci [Blažić, 1992b]. The earliest cultural layer is **Vinča culture** and it belongs to the end of the Neolithic. Multilayered archaeological site **Gomolava**-Hrtkovci belongs to this period as well. This site, the end of the Neolithic and the beginning of the Eneolithic, is dated between 3800 and 3400 BC [Petrović 1984]. Systematic collecting of osteological material at Gomolava started in 1971 [Clason 1979].

The main goal of archaeozoological researches is to classify remains of animals that were present in human communities, as well as to give the insight into the ratio of wild and domesticated animals, analyse the usage of animals and monitor the domestication process.

Domesticating and animal husbandry, as a mass phenomenon, are present at all Neolithic sites in Europe, and date back to circa 5000 years BC. The characteristic of the Neolithic domestic fauna is the presence of five breeding species: *Bos taurus* – ox, *Ovis aries* – sheep, *Capra hircus* – goat, *Sus scrofa domestica* – pig, and *Canis familiaris* – dog. The process of domestication and animal husbandry did not have the same direction and intensity in all Neolithic settlements. The differences identified during archaeological excavations are the result of various conditions, primarily ecological. These changes are related to the knowledge of domestication process and breeding in different cultures [Blažić 2005].

MATERIAL AND METHODS

The presence of domestic and wild animals is shown according to the vertebrate (Vertebrata) fauna research from 10 archaeological sites in Vojvodina from different Neolithic cultural periods [Radmanović *et al.*, 2014]. Osteological material comes from settlements and necropolises. Determination was done by using the key Schmid [1972] and comparative osteological collections.

RESULTS AND DISSCUTIONS

The Neolithic Age – the New Stone Age – is characterised by the appearance of farming and livestock breeding, and by the intensification of the process of domestication. During the Neolithic, the diet was based on large game hunt. The process of domestication and the type of economy are characterised by specialised animal husbandry and hunting. Breeding of domestic animals and animal husbandry in early period were based on breeding goats and sheep. During the Middle Neolithic, and especially at its end, there is a change in animal husbandry, when oxen became the dominant domestic species. The role of hunting in the diet is reduced, and the most important game species are deer, wild boar and roe deer.

Table 1. *Proportional contribution of domestic and wild animals (vertebrates) at Neolithic sites in Vojvodina*

Culture	Site	Author	% of domestic animals	% of wild animals
Kőrös	Nosa Biserna Obala	Bőköny 1974	37.29	62.71
	Ludaš Budžak	Ibid.	79.08	20.91
Starčevo	Donja Branjevina	Blažić 1992a	66.36	33.63
	Starčevo	Clason 1980	75.11	24.88
	Malo Kuvalovo	Blažić 1992b	66.53	33.47
	Prosine	Ibid.	81.20	18.70
	Zlatara	Ibid.	81.10	18.90
	Kudoš	Ibid.	83.30	16.70
	Golokut	Blažić 1984	35.21	64.79
Vinča	Gomolava	Clason 1979	62.80	37.20

Note: Kudoš – fauna consists of vertebrates and *Mollusca*; Gomolava – proportional contribution was calculated according to bone fragments that were determined up to the level of species

After analysing Table 1, it can be concluded that the smallest proportion of domestic animals is at sites of **Golokut** (35.21%) [Blažić 1984] and **Nosa Biserna Obala** (37.29%) [Bőköny 1974], while the biggest one is at sites **Prosine**, **Zlatara** and **Kudoš** [Blažić 1992b]. A small proportion of domestic animals at **Nosa Biserna Obala** shows that the animal husbandry was just at the beginning, and a high proportion of wild animals testifies about the importance of hunting in economy. These are the characteristics of Kőrös culture settlements, where goats and sheep dominate among domestic animals (Table 2).

Although it belongs to Kőrös culture, the situation at the site of **Ludaš Budžak** is very different. There, domestic animals dominate with 79.08% when compared to the wild ones [Bőköny 1974] (Table 1). The dominant place here, both in the total vertebrate fauna and among domestic animals, occupy sheep and goats (Table 2).

Table 2. *Proportional contribution of domesticated animals at the Neolithic sites in Vojvodina*

Species	Nosa-Biserna obala		Ludaš-Budžak		Donja Branjevina		Starčevo		Malo Kuvallovo		Prošine		Zlatara		Kudoš		Golokut		Gomolava	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
<i>Bos taurus</i>	15.25	40.91	10.38	13.13	15.32	23.08	52.32	69.65	33.33		75.00		30.83		72.20		22.16	62.95	36.94	58.82
<i>Ovis/Capra</i>	22.03	59.09	68.12	86.13	49.52	74.63	19.29	25.69	16.60				20.27		11.10		10.06	28.57	7.81	12.43
<i>Ovis aries</i>							0.20	0.27									0.51	1.45	0.07	0.12
<i>Capra hircus</i>							0.02	0.03					0.27				0.17	0.48	0.11	0.18
<i>Sus scrofa domestica</i>			0.29	0.37	0.98	1.48	2.73	3.63	8.30		6.20		2.77				1.96	5.57	16.33	26.00
<i>Canis familiaris</i>			0.29	0.37	0.53	0.80	0.53	0.71					26.94				0.34	0.97	1.54	2.45

1. % in the total sample

2. % in the contribution of domesticated animals

The proportional contribution of domestic and wild animals at these two sites of the Kőrös culture in Vojvodina is different when compared to the sites of Gyálarét and Rösztke-Lúdvár in Hungary, which also belong to this culture [Bököny 1974], but the above mentioned dominance of goats and sheep was also revealed in this analysis.

It has already been stated that the low percentage of domestic animals was recorded at the site of **Golokut** [Blažić 1984] which, like most sites described in this paper, belongs to the middle Neolithic. This ratio of domestic and wild animals is not characteristic for Starčevo culture, and it proves that hunting played a more important role than animal husbandry, which is related to the fact that Golokut was located at higher altitude in woody terrain of Fruška Gora Mt. What is characteristic for settlements of Starčevo culture is the domination of oxen in the total vertebrate fauna and among domestic animals, which is also true for this Neolithic site (Table 2).

At the sites of **Donja Branjevina** [Blažić 1992a], Starčevo [Clason 1980], **Malo Kvalovo**, **Prosine**, **Zlatara** and **Kudoš** [Blažić 1992b], a high proportion of domesticated species can be observed, with the domination of oxen, excluding the first mentioned site (Tables 1 and 2). At the site of Donja Branjevina, goats and sheep have the biggest proportional contribution.

Concerning the settlements of Starčevo culture, when compared to Divostin [Bököny 1988], Anzabegovo [Bököny 1976] and Sitagroi [Bököny 1986], the sites in Vojvodina have lower proportional contribution of domestic animals and higher contribution of wild ones. The high percentage of domestic animals at these three sites points to a developed animal husbandry. However, after comparing the sites of Lepenski Vir [Bökönyi 1969] and Padina [Clason 1980], which also belong to Starčevo culture, and given the isolation of these two sites and the fact that the population lived mainly from hunting and fishing, there is a far greater proportional contribution of domestic animals, and much smaller contribution of the wild species recorded in Vojvodina. Furthermore, it can be stated that at the sites of Starčevo, Prosine, Kudoš and Golokut, when compared to Divostin, Anzabegovo and Sitagroi, there is a greater proportional contribution of oxen as the dominant species in the total vertebrate fauna. At the sites of Anzabegovo and Sitagroi, the most numerous domestic species are sheep and goats, which did not have their wild ancestors in these areas, so we can assume that they originate from the South or South-East [Lazić 1988].

The only settlement analysed in this research which belongs to the Early Neolithic (Vinča culture) is **Gomolava**, where domestic animals are dominant, with oxen as the dominant species. Pigs – *Sus scrofa domestica*, sparsely present at other sites, are at the second place. In comparison to Vinča layer at the sites of Divostin [Bököny 1988], Anzabegovo [Bököny 1976], and Sitagroi [Bököny 1986], and early layers at the sites of Obre I and Obre II [Bököny 1977], Gomolava site has far greater proportional contribution of wild animals. Presence of wildlife at this site in the proportion of 37.20% corresponds to the results from the site of Crkvine in Kolubara basin [Blažić and Radmanović 2011]. On the other hand, it is lower when compared to Petnica (47.18%) [Greenfield 1986]. There is a difference in the proportional distribution of oxen, sheep and

goats between Gomolava and the above mentioned sites in the Balkan Peninsula, but the dominance of oxen is noticeable. The increase in proportional contribution of oxen can be easily tracked back from the oldest to the youngest layer of Anzabegovo settlement, which confirms that the breeding of oxen gradually became more important during the Neolithic period, even in those settlements where the bases of animal husbandry were sheep and goats. Domination of oxen, ranging between 43.68% and 72.28% among domesticated mammals at the Neolithic sites in the territory of Romania, is mentioned by Stanc *et al.* [2010]. Domination of oxen at the sites from this period shows the developed animal husbandry.

Bököny, 1974 gives the outline of the fauna of the Neolithic sites in the territory of Hungary, of various cultures, especially Tisa culture. The analysis of the presence of domestic animals showed a range from 25.17% at the site of Dėványa-Sártó (Tisa culture) to 91.55% (Tiszavasvári-Keresztfal) which is far wider range when compared to the sites in Vojvodina. With the exception of two mentioned sites of the Kőrös culture, proportional contribution of domestic and wild animals at the other 8 sites in the territory of Hungary is close to the values recorded in Vojvodina. Both sites in Vojvodina and those in Hungary, from the Middle and Late Neolithic, show the already mentioned dominance of oxen, the proportional contribution of which displays similar values in most cases.

CONCLUSIONS

The paper shows data from 10 Neolithic sites in Vojvodina (Serbia), dated between 6000 and 3200 BC. Two sites (**Nosa Biserna Obala** and **Ludaš Budžak**) belong to the Early Neolithic (Kőrös culture); seven sites: **Donja Branjevina**-Deronje, **Starčevo**, **Malo Kvalovo**-Krnješevci, **Prosine**-Pećinci, **Zlatara**-Ruma, **Kudoš**-Šašinci and **Golokut**-Vizić belong to the next layer – **Starčevo culture**, belonging to the Early and Middle Neolithic, while the site of **Gomolava** belongs to the youngest layer – **Vinča culture**, which covers the end of the Neolithic Age.

The smallest proportion of domestic animals was recorded at the sites of **Golokut** and **Nosa Biserna Obala**, while the biggest one at the sites of **Prosine**, **Zlatara** and **Kudoš**.

A small proportion of domestic animals at Nosa Biserna Obala shows that the animal husbandry was just at the beginning, and a high proportion of wild animals testifies about the importance of hunt in economy. Sheep and goats dominate among domestic animals at this site, which is a characteristic for Kőrös culture.

A small proportion of domestic, and a great proportion of wild animals registered at the site of Golokut is not characteristic for Starčevo culture. However, what is the characteristic for Starčevo culture settlements is the domination of oxen in the total vertebrate fauna and among domestic animals.

At the site of **Donja Branjevina**, goats and sheep have the biggest proportional contribution.

Gomolava is the only analysed settlement in this paper that belongs to the Early Neolithic (Vinča culture), where domestic animals are dominant, with oxen as the dominant species.

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ОДНОС ДОМАЋИХ И ДИВЉИХ ЖИВОТИЊА НА НЕОЛИТСКИМ ЛОКАЛИТЕТИМА У ВОЈВОДИНИ (СРБИЈА)**

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РЕЗИМЕ: На основу резултата фаунистичких истраживања кичмењака (Vertebrata) са 10 неолитских археолошких локалитета у Војводини (Србија) од којих два припадају **Керешкој**, седам **Старчевачкој**, а један **Винчанској** култури, анализирана је заступљеност домаћих и дивљих животиња. Датовање ових налазишта процењује се на период 6000–3200. године п. н. е. Најмањи проценат домаћих животиња забележен је на локалитетима **Голокут-Визић** и **Носа Бисерна** обала, док је највећи на налазиштима **Просине-Пећинци**, **Златара-Рума** и **Кудош-Шашинци**. Низак проценат домаћих животиња на Носи Бисерна обала говори да је гајење било у зачетку, а висок проценат дивљих сведочи о важној улози лова у економији. Ово су карактеристике насеља керешке културе којима се прикључује и чињеница да међу домаћим животињама доминирају овца и коза. Низак проценат домаћих, а висок проценат дивљих животиња забележен на налазишту Голокут који, као и већина описаних локалитета у овом раду, припада средњем неолиту, није карактеристичан за Старчевачку културу, а сведочи о томе да је лов имао много већи значај од гајења животиња. Оно што јесте карактеристика насеља Старчевачке културе је доминација говеда и у укупној фауни кичмењака и међу домаћим животињама. На **Доњој Брањевини-Дероње**, локалитету који такође припада Старчевачкој култури, највећу процентуалну заступљеност показују овца и коза. Једини анализиран локалитет у овом раду који припада млађем неолиту (Винчанска култура) је **Гомолава-Хртковци** на којој доминирају домаће животиње, а међу њима говече.

КЉУЧНЕ РЕЧИ: неолит, Војводина, домаће животиње, дивље животиње

**** НАПОМЕНА:**

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INSTRUCTION TO AUTHORS

(www.maticasrpska.org.rs)

This version of Instruction to Authors is valid starting from the year 2012 and the volume number 122

1. General remarks

1.1. ***Matica Srpska Journal for Natural Sciences*** (short title: *Matica Srpska J. Nat. Sci.*) publishes manuscripts and review articles as well as brief communications from all scientific fields as referred to in the title of the journal. Review articles are published only when solicited by the editorial board of the journal. Manuscripts that have already been published *in extenso* or in parts or have been submitted for publication to other journal will not be accepted. The journal is issued twice a year.

1.2. The manuscripts should be written in correct English language regarding the grammar and style. The manuscripts should be submitted electronically as a separate file to **vnikolic@maticasrpska.org.rs** and enclosed with the author's written consent for the publishing of the manuscript.

1.3. Upon the reception of the manuscript, the author shall be assigned with a manuscript code, which has to be referred to in any further correspondence. The authors will be notified about the manuscript reception within seven days and about the reviewers' opinion within two months from submission. All submitted manuscripts are reviewed and proofread.

2. Planning and preparing of the manuscript

2.1. Type the manuscripts electronically on A4 (21 x 29.5 cm) format with 2.5 cm margins, first line indent, and 1.5 line spacing. When writing the text, the authors should use *Times New Roman* size 12 font and when writing the abstract, key words, summary, and footnotes use font size 10.

2.2. First name, middle initial and last name should be given for all authors of the manuscript and their institutional affiliations, institution name, and mailing address. In complex organizations, a full hierarchy should be mentioned (e.g. University of Novi Sad, Faculty of Sciences – Department of Biology and Ecology). The institution of employment of each author should be stated below the author's name. The position and academic degrees should not be cited. If there is more than one author, indicate separately institutional affiliation for each of the authors. Put the name and mailing address (postal or e-mail address) of the author responsible for correspondence at the bottom of the first page. If there is more than one author, write the address of only one author, usually the first one.

2.3. Structure the text of the original articles into Abstract, Key Words, Introduction, Material or Methods, or Material and Methods, Results or Results

and Discussion, Discussion, Conclusion, References, Summary and Key Words in Serbian language, and Acknowledgement (if there is one). Original articles should not be longer than 10 pages, including the references, tables, legends, and figures.

2.4. Titles should be informative and not longer than 10 words. It is in the best interest of the authors and the journal to use words in titles suitable for indexing and electronic searching of the article.

2.5. The authors should submit the title of the article with last name and the initials of the first author.

(if the article has more than one author, *et al.* should be used for other authors) and running title of not more than five words.

2.6. List up to 10 key words using words and phrases that describe the content of the article in the best way and that allow indexing and electronic searching of the paper. List the key words alphabetically and divided by commas.

2.7. The Abstract in English language and Summary in Serbian language should be a short and informative presentation of the article. Depending on the length of the article, the Abstract may have from 100 to 250 words. Summary written in Serbian language can be 1/10 length of the article and should contain the title of the article, first, middle initial, and last names of the authors, authors' institutional affiliation and address, and key words.

2.8. Write the information about financial support, advices, and other forms of assistance, if necessary, at the end of the article under the Acknowledgement. Financial support acknowledgement should contain the name and the number of the project, i.e. the name of the program from which the article originated, and the name of the institution that provided the financial support. In case of other forms of assistance the author should submit the first name, middle initial, last name, institutional affiliation, and the address of the person providing the assistance or the full name and the address of the assisting institution.

3. Structure the Review articles in Abstract, Key Words, Text of the manuscript, Conclusion, and References; submit Summary and Key Words in Serbian language. Review articles should not be longer than 12 pages, including references, tables, legends, and figures.

4. Write brief communication according to the instructions for original articles but not be longer than five pages.

5. References

5.1. List the References alphabetically. Examples:

- (a) Articles from journals: Last name CD, Last name CD (2009): Title of the article. Title of the journal (abbreviated form) 135: 122-129.
- (b) Chapters in the book: Last name ED, Last name AS, Last name IP (2011): Title of the pertinent part from the book. In: Last name CA, last name IF (eds.), Title of the book, Vol.4, Publisher, City
- (c) Books: Last name VG, Last name CS (2009): Title of the cited book. Publisher, City

- (d) Dissertations: Last name VA (2009): Title of the thesis. Doctoral dissertation, University, City
 - (e) Unpublished articles: designation “in press” should be used only for papers accepted for publishing. Unpublished articles should be cited in the same way as published articles except that instead of journal volume and page numbers should write “in press” information.
 - (f) Articles reported at scientific meetings and published *in extenso* or in a summary form: Last name FR (2011): Proceedings, Name of the meeting, Meeting organizers, Venue, Country, 24-29
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- 5.2. References in the text should include author’s last name and the year of publishing. When there are two authors both should be cited, but in case of three or more authors, cite the first author only and follow with et al.
- 5.3. If two or more articles of the same author or authors published in the same year are cited, designate the publishing years with letters a, b, c, etc., both in text and reference list.
- 5.4. The names of the periodicals should be abbreviated according the instructions in the *Bibliographic Guide for Authors and Editors* (BIOSIS, Chemical Abstracts Service, and Engineering Index, Inc.).
- 5.5. Do not translate references to the language of the article. Write the names of cited national periodicals in their original, shortened form. For example, for the reference in Serbian language, put (Sr) at the end of the reference.
6. Units, names, abbreviations, and formulas
- 6.1. SI units of measurement (Système international d’unités) should be used but when necessary use other officially accepted units.
- 6.2. Write the names of living organisms using *Italics* font style.
- 6.3. Abbreviated form of a term should be put into parenthesis after the full name of the term first time it appears in the text.
- 6.4. Chemical formulas and complex equations should be drawn and prepared for photographic reproduction.
7. Figures
- 7.1. Authors may use black-and-white photographs and good quality drawings.
- 7.2. A caption with the explanation should be put below each figure.
8. Tables
- 8.1. Type tables on separate sheet of papers and enclosed them at the end of the manuscript.
- 8.2. Number the tables using Arabic numerals.
- 8.3. Above each table, write a capture with table explanation.
- 8.4. On the left margin, indicate the place of the tables in the text.

9. Electronic copy of the article

9.1. After the acceptance of the article, send a CD with final version of the manuscript and a printed copy to facilitate technical processing of the text. Articles should be written in Microsoft Word format and sent to the Editorial office of the *Matica Srpska Journal for Natural Sciences*, 1 Matica Srpska Street, 21000 Novi Sad (Uredništvo Zbornika Matice srpske za prirodne nauke, Matice srpske 1, 21000 Novi Sad).

9.2. Before printing, the manuscripts shall be sent to the authors for the approval of final version. Corrections of the text prepared for printing should be restricted to misspelling and printing errors as much as possible. For major changes of the text, a fee will be charged. Corrected manuscript should be returned to the Editorial office as soon as possible.

УПУТСТВО АУТОРИМА (www.maticasrpska.org.rs)

1. Опште напомене

1.1 Зборник Матице српске за природне науке / *Matica Srpska Journal for Natural Sciences* (скраћени наслов: *Matica Srpska J. Nat. Sci.*) објављује оригиналне научне радове и прегледне чланке као и кратка саопштења из свих области које обухвата назив часописа. Прегледни радови се објављују само на позив редакције. Радови који су већ објављени у целости или у деловима или су понуђени другом часопису не могу бити прихваћени. Часопис објављује два броја годишње.

1.2. Прихватају се рукописи писани на енглеском језику. Језик мора бити исправан у погледу граматике и стила. Рукопис се доставља електронском поштом као посебан докуменат на адресу: vnikolic@maticasrpska.org.rs, уз обавезну потписану изјаву аутора у вези са пријавом рада за штампу.

1.3. По примању рукописа, аутор ће добити шифру свог рада, коју треба увек наводити у даљој преписци. Уредништво ће обавестити аутора о приспећу рукописа у року од седам дана, а о мишљењу рецензената у року од два месеца од пријема. Сваки рад се рецензира и лекторише.

2. Припрема рукописа

2.1. Текст рада пише се електронски на страни А4 (21x29,5 cm), с маргинама од 2,5 cm, увлачењем првог реда новог пасуса, и размаком међу редовима 1,5. Текст треба писати у фонту *Times New Roman* словима величине 12 а сажетак, кључне речи, резиме и подножне напомене словима величине 10 pt.

2.2. Наводе се име, средње слово и презиме свих аутора рада као и назив установе (без скраћеница) у којој су аутори запослени, заједно са пуном поштанском адресом. У сложеним организацијама наводи се укупна хијерархија (на пример: Универзитет у Новом Саду, Природноматематички факултет – Департман за биологију и екологију). Место запослења наводи се непосредно испод имена аутора. Функције и звања аутора се не наводе. Ако је аутора више, мора се, посебним ознакама, назначити из које од наведених установа потиче сваки од наведених аутора. Контакт адреса аутора (поштанска или електронска) даје се у напомени при дну прве странице чланка. Ако је аутора више, даје се само адреса једног, обично првог аутора.

2.3. Рукопис оригиналног научног рада треба поделити на: Сажетак, Кључне речи, Увод, Материјал или Метод или Материјал и метод, Резултати или Резултати и дискусија, Дискусија, Закључак, Литература, Сажетак и Кључне речи на српском језику и Захвалност (уколико за то постоји

потреба). Оригинални научни radови не смеју бити дужи од 10 страна, укључујући литературу, табеле, легенде и слике.

2.4. Наслов рада треба да буде информативан, али не дужи од десет речи. У интересу је часописа и аутора да се користе речи прикладне за индексирање и претраживање.

2.5. Аутори треба да доставе и текући наслов који треба да садржи презиме и иницијале првог аутора (ако је аутора више, преостали се означавају са “et al.”) и наслов рада у скраћеном облику, не више од пет речи.

2.6. За кључне речи треба користити термине или фразе које најбоље описују садржај чланка за потребе индексирања и претраживања. Број кључних речи не може бити већи од 10. Треба их навести абecedним редом и одвојити зарезима.

2.7. Апстракт на енглеском и резиме на српском треба да представљају кратак информативни приказ чланка. Апстракт у зависности од дужине чланка треба да има од 100 до 250 речи. Резиме на српском језику може бити до 1/10 дужине чланка и треба да садржи наслов рада, имена аутора, средње слово и презимена, назив и место у којима су аутори запослени и кључне речи.

2.8. Податке о финансијској помоћи, саветима и другим врстама помоћи, уколико за то постоји потреба, треба навести на крају рада, под насловом Захвалност. У захвалници за финансијску помоћ треба навести назив и број пројекта, односно назив програма у оквиру којег је чланак настао, као и назив институције која је финансирала пројекат или програм. У случају других видова помоћи треба навести име, средње слово и презиме, установу и седиште лица које је пружало помоћ, а ако је помоћ пружала установа пун назив и адресу.

3. Прегледни рад треба да садржи: Апстракт, Кључне речи, Закључак, Литературу, као и Резиме и Кључне речи на српском. Прегледни radови не смеју бити дужи од 12 страна, укључујући литературу, табеле, легенде и слике.

4. Кратко саопштење се пише по упутствима за оригиналан научни рад, али не сме да буде дуже од 5 страна.

5. Литература

5.1. Литературне наводе треба сложити абecedним редом на следећи начин:

- (а) Чланци из часописа: Презиме CD, Презиме SP (2009): Назив рада. Име часописа (скраћени облик) 135: 122-129.
- (б) Поглавља у књизи: Презиме ED, Презиме AS, Презиме, IP (2011): Наслов цитираног дела у књизи. In: Презиме CA, Презиме IF (eds.), Назив књиге, Вол. 4, Издавач, Град, 224-256.
- (в) Књиге: Презиме VG, Презиме CS (2009): Наслов цитиране књиге. Издавач, Град.

- (г) Дисертације: Презиме VA (2009): Назив тезе. Докторска дисертација, Универзитет, Град.
- (д) Необјављени радови: Навод „у штампи” треба да се односи само на радове прихваћене за штампу. Необјављени радови: цитирати као да се ради о објављеном раду осим што се уместо волумена часописа и броја страна наводи „у штампи”.
- (ђ) Радови саопштени на научним скуповима штампани у целини или у изводу: Презиме FR. (2011): Зборник, Назив скупа, Организатор скупа, Место одржавања, Држава, 24-29.
- (е) Електронски извори:

World Wide Web Sites and Other Electronic Sources

Author last name, Author initial. (Date of publication or revision). Title, In: *source in Italics*, Date of access, Available from: <Available URL>

Use n.d. (no date) where no publication date is available.

Where no author is available, transfer the organisation behind the website, or the title, to the author space.

5.2. Референце у тексту треба да укључе презиме аутора и годину издања. Ако има два аутора, треба навести обојицу, а у случају три или више аутора треба навести првог аутора и назначити “et al.”.

5.3. Ако се наводе два или више радова истог или истих аутора, објављених у истој години, потребно је у тексту и списку литературе ставити а, б, ц, итд. иза године објављивања.

5.4. Имена часописа треба скраћивати према “Bibliographic Guide for Authors and Editors” (BIOSIS, Chemical Abstracts Service and Engineering Index, Inc.,).

5.5. Референце се не превode на језик рада. Наслови цитираних домаћих часописа дају се у оригиналном, скраћеном облику. Ако је референца нпр. на српском језику на крају се стави (Sr).

6. Јединице, имена, скраћенице и формуле

6.1. Треба користити SI ознаке за јединице (SI Systeme International d’Un.); изузетно се могу користити и друге званично прихваћене јединице.

6.2. Називе живих организама на латинском треба писати италиком.

6.3. При коришћењу скраћеница у тексту, пун термин треба навести приликом првог спомињања, а скраћеницу додати у загради.

6.4. Хемијске структурне формуле и сложене једначине треба нацртати и припремити за фотографску репродукцију.

7. Илустрације

7.1. За илустрације могу се користити црно беле фотографије и цртежи доброг квалитета.

7.2. Свака илустрација треба да има текст (легенду) који објашњава садржај прилога (испод слике).

8. Табеле

8.1. Табеле треба куцати на одвојеним страницама и приложити их на крају рада.

8.2. Табеле се означавају арапским бројевима.

8.3. Свака табела треба да почне насловом који објашњава њен садржај (изнад табеле).

8.4. Места табела у тексту треба означити на левој маргини.

9. Копија рада у електронској форми

9.1. После прихватања рада потребно је доставити CD са коначном верзијом рада. Приложити и једну копију одштампаног рада ради лакше техничке обраде. Рукопис треба слати на адресу: Уредништво Зборника Матице српске за природне науке, Матица српска, Ул. Матице српске, 21000 Нови Сад. Рукописи се шаљу у Word формату.

9.2. Пре уласка рада у штампу ауторима се доставља рукопис за коначну ревизију. Исправљање текста припремљеног за штампу треба ограничити на штампарске грешке. Значајне промене текста ће се наплаћивати. Кориговани текст треба вратити Уредништву у најкраћем могућем року.

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