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ANTIOXIDATIVE AND ANTIMICROBIAL POTENTIALS OF *Parmelia saxatilis* AND *Pseudoevernia furfuracea*

ABSTRACT: Even though numerous lichen species possess significant medical potentials they are still unexplored, and particularly species and strains originating from Serbia. Therefore, the aim of this study was to evaluate the antioxidative and antimicrobial potential of ethanol extracts of *Parmelia saxatilis* and *Pseudoevernia furfuracea* collected in Serbia. The tested extracts were good scavengers of DPPH radicals, with capacities ranging from 14.76% to 79.76% in *P. saxatilis* and from 21.39% to 90.04% in *P. furfuracea*. In *P. saxatilis* level of DPPH• neutralisation was highly correlated with phenol content ($r^2 = 0.9981$) and in *P. furfuracea* with amount of total flavonoides ($r^2 = 0.9641$). The extract of *P. furfuracea* inhibited the growth of all tested microorganisms with exception of *Aspergillus flavus*, while *P. saxatilis* extract affected only growth of bacterial species. Among tested microorganisms, *Staphylococcus aureus* and *Klebsiella pneumoniae* were the most sensitive, while *Enterococcus faecalis*, *Pseudomonas aeruginosa* as well as micromycetes were the least sensitive to tested extracts. Because of these potentials and the fact that their long term usage does not have any negative side effects on organism and development of microbial resistance, the extracts could be included in conventional therapy.

KEYWORDS: antioxidative potential, antimicrobial potential, *Parmelia saxatilis*, *Pseudoevernia furfuracea*

INTRODUCTION

Lichens as symbiotic organisms have specific characteristics which are not typical for their components, i.e. algae and fungi. They are cosmopolits that colonize even extreme habitats due to the ability to synthesize specific secondary metabolites (aliphatic, cycloaliphatic, aromatic and terpenic compounds) known as lichen substances [Güvenç *et al.*, 2012; Kosanić *et al.*, 2012; Oettl *et al.*, 2014]. These compounds protect lichens from pathogens, predators, intense UV light and oxidative stress, but also could have some beneficial effects on human health

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[Güvenç *et al*., 2012; Kosanić *et al*., 2012; Oettl *et al*., 2014; Fernández-Moriano *et al*., 2015]. Traditionally, lichens are used as food and feed, in folk medicine, in the perfume and dye industries and as bioindicators, but recently more attention is given to their medical potentials, such as antimicrobial, antioxidative, anti-inflammatory, antiproliferative, cytotoxic, neuroprotective activity, etc. [Ingólfsdóttir *et al*., 1998; Kosanić *et al*., 2013; Fernández-Moriano *et al*., 2015].

Frequent diseases and desorders of a modern man, such as cancer, neurodegeneration, aging, and so on, are usually consequences of oxidative stress because of imbalance between concentration of free radicals and the capacity of internal defence system [Ćilerdžić *et al*., 2013]. Therefore, the organism needs the assistance of exogenous antioxidants. Given that a number of synthetic antioxidants also have side effects, there is a growing need for new natural resources of antioxidative agents, among which lichenes are very important [Kosanić and Ranković 2011]. Another serious problem of modern medical practice is the of numerous resistant strains of microorganisms because of uncontrolled and long term usage of antibiotics/antimycotics. Lichens could also have an important role in solving that problem, as new alternative sources of natural antimicrobial compounds.

The usage of *Pseudoevernia furfuracea* dates back to ancient Egypt where it was used in the mummification process to prevent the odour [Güvenç *et al*., 2012]. Nowadays, about 2,000 tons of this lichen have been processed per year in the perfume industry [Güvenç *et al*., 2012]. Although species of the genus *Parmelia* have been used traditionally in the treatment of pulmonary and cranial deseases, there are just a few studies revealing their medicinal properties [Gulluce *et al*., 2006; Goel *et al*., 2011; Ranković *et al*., 2011]. During the last few years numerous studies have shown the existence of significant medical potentials of these species, but they are still unexplored, and particularly species and strains originating from Serbia. Therefore, the aim of our study was to evaluate the antioxidative and antimicrobial potential of *Parmelia saxatilis* and *Pseudoevernia furfuracea* collected in Serbia.

MATERIALS AND METHODS

Lichen samples

The samples of *Parmelia saxatilis* (L.) Ach. and *Pseudoevernia furfuracea* (L.) Zopf. were collected at Tara mountain, Serbia, in May 2011. The voucher specimen of the lichens are deposited in the Institute of Botany, Faculty of Biology, University of Belgrade.

Preparation of the lichen extracts

Finely pulverized dried thalli of *P. saxatilis* and *P. furfuracea* (10.0 g) were extracted with 300.0 mL of 96% ethanol by stirring on the magnetic stirrer

(150 rpm) for 72 h. The obtained extracts were centrifuged (20 ºC, 3,000 rpm, 10 min) (Hettich Universal 32R, Germany) and supernatants were filtered through Whatman No. 4 filter paper, concentrated under reduced pressure in a rotary evaporator (Büchi, Rotavapor R-114, Germany) at 40 °C to dryness, and redissolved in 96% ethanol (for antioxidative assay) or in 5% dimethyl sulphoxide (DMSO) (for antimicrobial assay) to the initial concentration of 20.0 mg/mL.

Antioxidative activity assay

The free radical scavenging activity of the extracts was determined spectrophotometrically (CECIL CE 2501) by measuring the methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH•) reduction [Blois, 1958]. The mixture of 200.0 µL of extract (series of double dilutions from 20.0 mg/mL to 0.312 mg/mL) and 1800.0 µL of 4% methanol solution of DPPH• was shaken vigorously, incubated for 30 min in the dark and absorbance was measured at 517 nm. The scavenging effect was calculated using the equation:

$$\text{DPPH• scavenging effect (\%)} = [(A_0 - A_{sample})/A_0] \times 100$$

A_0 – absorbance of the negative control (all reagents except the extract);
A_{sample} – absorbance of the reaction mixture.

Extract concentration (mg/mL) providing 50% of DPPH• reduction (EC_{50}) was obtained by interpolation from linear regression analysis. Butylated hydroxyanisole (BHA), a commercial antioxidant, in a concentration range from 10.0 mg/mL to 0.02 mg/mL, was used as a positive control.

Determination of total phenol content

Total phenol compounds in the extracts of tested lichens were estimated by a colorimetric assay based on procedure described by Singleton and Rossi [1965], using gallic acid as standard. 200.0 µL of extract (1.0 mg/mL) and 1000.0 µL of 10% Folin-Ciocalteu reagent were reacted in the dark for 6 min before addition of 800.0 µL of 7.5% aqueous solution of Na_2CO_3. The reaction mixture was vortexed vigorously and incubated on a rotary shaker (100 rpm) in the dark at room temperature (22 ± 2 ºC) for 2 h. The absorbance of each reaction mixture was measured spectrophotometrically at 740 nm. The blank was a mixture where extract was substituted by sterile distilled water. The total phenol content was determined as µg of gallic acid equivalent (GAE) per mg of dried extract using an equation that was obtained from a standard gallic acid graph:

$$\text{Absorbance} = 0.013 \times \text{total phenols (µg of gallic acid)} + 0.165 \ (R^2 = 0.996).$$

Determination of total flavonoid content

Total flavonoid content was determined by the method of Park et al. (1997) using quercetin as the standard. 1.0 mL of extract (1.0 mg/mL) was diluted with 4.3 mL mixture containing 4.1 mL of 80% ethanol, 0.1 mL of 10% aluminium nitrate ($Al(NO)_3$ x $9H_2O$) and 0.1 mL of 1.0 M aqueous potassium acetate (CH_3CO_2K). The reaction mixture was incubated at room temperature for 40 min and absorbance was measured spectrophotometrically at 415 nm. The mixture with ethanol instead of extract was used as the blank. Total flavonoid concentration was expressed as μg of quercetin equivalent (QE) per mg of dry extract using an equation obtained from standard quercetin hydrate graph:

Absorbance = 0.006 x total flavonoid (μg quercetin hydrate) – 0.017 ($R^2 = 0.995$).

Antimicrobial activity

Microorganisms and cultivation

Antimicrobial activity was tested against 5 bacterial species (*Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumoniae* ATCC 70063 and *Enterococcus faecalis* ATCC 29212) and 7 micromycetes (*Acremonium strictum* BEOFB10m, *Aspergillus glaucus* BEOFB21m, *A. flavus* BEOFB22m, *A. fumigatus* BEOFB23m, *A. nidulans* BEOFB24m, *A. niger* BEOFB25m and *A. terreus* BEOFB26m). The tested bacteria are part of the culture collection of the Department of Biology, Faculty of Science, University of Kragujevac, while mycromycetes are deposited in the culture collection of the Institute of Botany, Faculty of Biology, University of Belgrade. Bacteria were cultivated on Müller-Hinton agar (MHA) at 37 °C overnight and inocula were prepared by washing agar surfaces with sterile 0.85% saline and brought up by dilution according to the McFarland standard to approximately 10^8 colony-forming units (CFU) per mL. Micromycetes were grown on malt agar at 30 °C for 3–7 days and spore suspensions were obtained by washing with saline enriched with 0.1% Tween 80 (v/v) and turbidity was determined spectrophotometrically at 530 nm and adjusted to 10^6 CFU/mL with saline.

Microdilution method

Series of double extract dilutions (from 20.0 mg/mL to 0.312 mg/mL) were prepared in Tryptic Soy broth (TSB) for bacterial cultures and Sabourad dextrose broth (SDB) for fungal cultures. 96-well microtiter plates were used. Each well comprised TSB/SDB, cell/spore suspension and extract of a defined concentration. The mixture without extract was used as the negative control, while the positive control contained commercial antibiotic (streptomycin) or fungicide (ketoconazole) instead of extract. The effect of 5% DMSO on the bacterial cell growth and fungal spore germination was also analyzed by its addition in the mixture instead of nutritional medium. Microtiter plates with bacteria were

incubated at 35 ± 2 °C for 24 h and plates with fungi at 25 ± 2 °C for 72 h. The lowest extract concentration without visible bacterial/mycelium growth was defined as minimal inhibitory concentration (MIC) and determined by colorimetric microbial viability assay based on reduction of resazurin. Resazurin is an oxidation/reduction indicator used for the detection of microbial growth. Namely, it is a blue non-fluorescent dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells.

Statistical analysis

The assays were carried out in triplicate and the results are expressed as mean ± standard error. One-way analysis of variance (ANOVA) and Tukey`s test were performed using STATISTICA, version 6.0 (StatSoft, Inc., Tulsa, USA) to test any significant differences among means. Statistical significance was declared at $p<0.05$.

RESULTS AND DISCUSSION

The tested lichen extracts showed considerable potentials for scavenging DPPH radicals, which were statistically significant difference ($p<0.05$). The level of DPPH• reduction ranged from 14.76% to 79.76% in *Parmelia saxatilis* and from 21.39% to 90.04% in *Pseudoevernia furfuracea* (Figure 1).

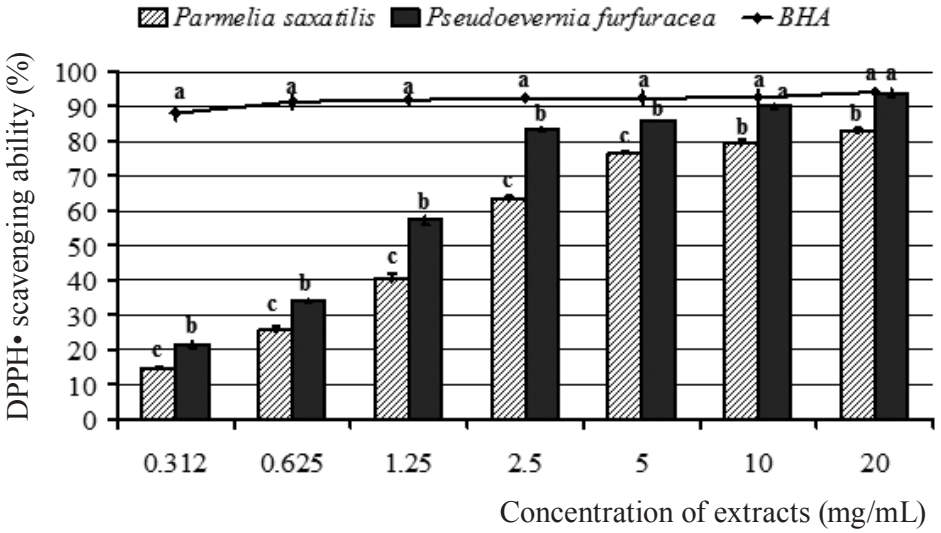

Figure 1. DPPH• scavenging capacity of *Parmelia saxatilis* and *Pseudoevernia furfuracea* extracts and commercial antioxidant (Data represent mean value of activities of three different samples. Variations are given as standard errors. Values within each concentration group with different superscripts differ significantly ($p<0.05$) according to Tukey's test).

The extract of *P. furfuracea* showed higher antioxidative potential than *P. saxatilis*, and at higher concentrations it did not differ significantly from that in the positive control. Antioxidative activity of the lichenes extracts gradually increased from the concentration of 0.312 mg/mL to 20.0 mg/mL while BHA reached the peak of activity at the smallest concentrations which affected the EC_{50} values (0.11 mg/mL for BHA, 1.05 mg/mL for *P. furfuracea* extracts and 1.79 mg/mL for *P. saxatilis* extracts).

Contrary to a high potential of ethanol extract of *P. saxatilis* to reduce DPPH•, level of the radical neutralisation with methanol extract of the same species originated from Turkey was zero [Gulluce *et al.*, 2006]. On the other hand, the tested *P. saxatilis* extract showed much lower DPPH• scavenging capacity than extracts of other lichen species, for example *Lecanora atra* acetone extract (94.7%) and *Umbilicaria polyphylla* methanol extract (90.08%) [Kosanić and Ranković 2011; Ranković *et al.*, 2011]. In the case of studied *P. furfuracea* extract, DPPH radicals scavenging ability was significantly lower than that of acetone extract of the species (EC_{50} values were 1.08 mg/mL and 0.402 mg/mL, respectivelly), as well as various extracts of other species [Kosanić *et al.*, 2013; Kosanić and Ranković 2011; Ranković *et al.*, 2011].

The amounts and ratio between total phenols and flavonoides in the studied extracts were significantly different (Table 1).

Table 1. Content of total phenols and flavonoids in *Parmelia saxatilis* and *Pseudoevernia furfuraceae* ethanol extracts.

Extracts	Phenol content (μg GAE/mg of dry extract)	Flavonoid content (μg QE/mg of dry extract)
P. saxatilis	72.48 ± 1.02^{a}*	9.41 ± 0.22^{a}
P. furfuraceae	6.15 ± 0.06^{b}	70.66 ± 5.98^{b}

* Means within a column with different superscripts differ significantly ($p<0.05$) according to Tukey`s test.

P. saxatilis extract contained almost 8-fold higher amount of total phenols than flavonoides, while in *P. furfuracea* extract the ratio of 1:11 was noted in favor of flavonoids. In *P. saxatilis* extract the correlation (r^2) between DPPH• scavenging activity and concentration of total phenols was 0.9981, contrary to *P. furfuracea* whose phenolic content was in low correlation with its antioxidative potential ($r^2 = 0.6826$). On the other hand, in *P. furfuracea* extract the level of DPPH• neutralisation was highly correlated ($r^2 = 0.9641$) with the amount of total flavonoides, while low correlation was observed for total phenol compounds ($r^2 = 0.6634$).

These results were not in accordance with those obtained by Kosanić *et al.* [2013] and Ranković *et al.* [2010] who showed that the main carriers of antioxidative activity in acetone extract of *P. furfuracea* and *Parmelia centrifuga* were phenols present in significant concentrations (76.42 μg PE/mg of extract and 49.8 mg GA/g of liophylisate, respectivelly). Another important fact in our

study is that flavonoids are also very important antioxidant agents, i.e. their concentrations are in high correlation with level of radical neutralisation in *P. furfuracea*.

The obtained results have clearly showed the existance of antibacterial and/or antifungal potential of tested lichen species (Table 2).

Table 2. Antimicrobial activity of *Parmelia saxatilis* and *Pseudoevernia furfuracea* ethanol extracts and commercial antibiotic/fungicide.

Extracts, antibiotic & fungicide / Tested bacteria and micromycetes	*Parmelia saxatilis*	*Pseudoevernia furfuracea*	Streptomycin	Ketoconazole
	MIC (mg/mL)			
Staphylococcus aureus	2.5	10.0	0.031	–**
Enterococcus faecalis	20.0	20.0	0.031	–
Escherichia coli	10.0	10.0	0.031	–
Pseudomonas aeruginosa	20.0	20.0	0.062	–
Klebsiella pneumoniae	5.0	2.5	0.062	–
Acremonium strictum	/*	20.0	–	0.025
Aspergillus glaucus	/	20.0	–	0.025
Aspergillus flavus	/	/	–	0.025
Aspergillus fumigatus	/	20.0	–	0.025
Aspergillus nidulans	/	20.0	–	0.050
Aspergillus niger	/	20.0	–	0.050
Aspergillus terreus	/	20.0	–	0.025

* MIC was not detected; ** not tested

The extract of *P. furfuracea* inhibited the growth of all tested organisms with the exception of *Aspergillus flavus* which was indifferent toward the extract, while *P. saxatilis* extract proved to be good antibacterial but not antifungal agent because any extract concentration did not present MIC for any tested micromycetes. The tested microorganisms showed differences in susceptibility on lichen extracts and MICs ranged from 2.5 mg/mL to 20.0 mg/mL. Among tested bacteria, *Staphylococcus aureus* and *Klebsiella pneumoniae* were most sensitive to extract of *P. saxatilis* and *P. furfuracea*, respectivelly, while the growth of *Enterococcus faecalis* and *Pseudomonas aeruginosa* was inhibited only with the highest tested concentration of the extracts. The situation was different with tested microfungi which were susceptible only to *P. furfuracea* extract in the concentration of 20.0 mg/mL (Table 2). Contrary to the extracts, the commercial antibiotic and antimycotic were more effective than tested extracts, with a MICs ranging between 0.031 and 0.062 mg/mL for streptomycin and from 0.025 to 0.050 mg/mL for ketoconazole.

Previous studies also demonstrated that various lichen species and their extracts have antibacterial and antifungal potentials which differ depending on the species, type of extract, and microorganism species/strain [Ingólfsdóttir *et al*., 1998; Goel *et al*., 2011; Manojlović *et al*., 2012; Ćilerdžić *et al*., 2013]. Results of this study, as well as some others, showed greater sensitivity of bacteria than micromycetes to the lichen extracts which can be explained by differences in the composition and permeability of bacterial and fungal cell wall [Manojlović *et al*., 2012]. Likewise, noted MIC values of *P. saxatilis* and *P. furfuracea* ethanol extracts were similar to previously obtained for some other lichen crude extracts but expectedly lower than those for clear active compounds like salazinic acid from *P. saxatilis* (concentration of 250 μg/mL was MIC for *Mycobacterium aurum*) or physodic acid from *P. furfuracea* (concentration of 0.0075 mg/mL inhibited growth of *K. pneumoniae*) [Ingólfsdóttir *et al*., 1998; Kosanić *et al*., 2013]. Antimicrobial activity of crude lichen extract, which represents a complex mixture of different chemical compounds, can depend on a type of action among its components, i.e. antagonisticaly, synergisticaly or additively [Kosanić *et al*., 2012].

The results obtained in our study showed that ethanol extracts of *Parmelia saxatilis* and *Pseudoevernia furfuracea* have a great ability of DPPH• reduction as well as significant potential of microorganism growth inhibition. Although these activities of the extracts were much weaker comparing to the commercial drugs, significance of the results was not reduced because of the possibility of their long term usage without any negative side effects on oraganizam and apperiance of microbs resistence. If we add to these facts that the lichen species have not yet been fully explored, a complete picture about the importance of the study can be obtained. Therefore, further studies should move in the direction of chemical analysis of these extracts, isolation of active components, testing their action with commercial drugs (synergistic or additive), and inclusion of the extracts/compounds in the conventional therapy.

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АНТИОКСИДАТИВНИ И АНТИМИКРОБНИ ПОТЕНЦИЈАЛ *Parmelia saxatilis* И *Pseudoevernia furfuracea*

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РЕЗИМЕ: Иако је познато да бројне врсте лишајева поседују значајан медицински потенцијал, још увек нема довољно података за врсте и сојеве пореклом из Србије. Због тога, циљ овог рада је процена антиоксидативног и антимикробног потенцијала етанолних екстраката *Parmelia saxatilis* и *Pseudoevernia furfuracea* прикупљених са неких локалитета у Србији. Тестирани екстракти су показали добар антиоксидативни потенцијал редукујући DPPH радикал, у распону од 14,76% до 79,76% (*P. saxatilis*), односно од 21,39% до 90,04% (*P. furfuracea*). Антиоксидативни потенцијал тестираних екстраката одразио се и на EC_{50} вредност која је за *P. saxatilic* била 1,79 mg/mL а за *P. furfuracea* 1,05 mg/mL. Степен неутрализације DPPH• екстрактом *P. saxatilis* био је у високом степену корелације са садржајем фенолних једињења ($R^2 = 0,9981$) док је код *P. furfuracea* садржај укупних флавоноида корелисао са редукцијом DPPH радикала ($R^2 = 0,9641$). Тестирани екстракти су се такође показали и као добри антимикробни агенси. Наиме, екстракт *P. furfuracea* је инхибирао раст свих тестираних микроорганизама са изузетком *Aspergillus flavus*, док је екстракт *P. saxatilis* негативно утицао само на раст бактеријских врста. Међу тестираним микроорганизмима, *Staphylococcus aureus* је била најосетљивија на екстракт *P. saxatilis*, *Klebsiella pneumoniae* на екстракт *P. furfuracea*, док су *Enterococcus faecalis*, *Pseudomonas aeruginosa* као и микромицета били најмање осетљиве на тестиране екстракте лишајева.

КЉУЧНЕ РЕЧИ: антиоксидативни потенцијал, антимикробни потенцијал, *Parmelia saxatilis, Pseudoevernia furfuracea*

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SUCCEPTIBILITY OF SOME FUNGI TO *Boswellia carteri* Birdw. ESSENTIAL OIL

ABSTRACT: Antifungal activity of commercial sample of *Boswellia carteri* essential oil against selected micromycetes was evaluated *in vitro* using a microatmosphere method. When compared with biocide Sanosil S003, used as positive control, the tested essential oil showed moderate antifungal activity. The most susceptible fungi to oil treatment were *Stachybotrys chartarum* and *Trichotecium roseum*. For both fungi, mycelia growth inhibition of 85% was recorded at oil concentration of 100 μL mL^{-1}. The tested essential oil caused inhibition of *S. chartarum* sporulation as well as depigmentation of conidia, which is very significant since melanin contributes to virulence, survival and endurance of pathogenic fungi spores. *Aspergillus niger* was the least susceptible isolate to essential oil treatment. Mycelial growth of this fungus was not inhibited by any oil concentrations used in the experiment.

KEYWORDS: antifungal activity, *Boswellia carteri* Birdw., essential oil, micromycetes

INTRODUCTION

The genus *Boswellia* (order Sapindales; family Burseraceae) consists of 19 species, mostly distributed in tropical regions [Niebler and Buettner 2015]. *Boswellia carteri* Birdw. (syn. *Boswellia sacra* Flueck.), commonly known as frankincense or olibanum tree, is a deciduous middle sized tree which inhabits arid woodland and eroding slopes in Oman, southern Yemen and northern Somalia [Thulin 1998]. Although major botanical and scientific references currently regard two scientific names for frankincense, *B. carteri* and *B. sacra*, as being synonymous [Woolley *et al*., 2012], according to Niebler and Buettner [2015], *B. sacra* refers to frankincense population originating from Oman and Yemen, while the scientific name *B. carteri* is related to the plants of Somalian

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origin. Since the essential oil (EO) used in this study originated from Somalian plants, the name *Boswellia carteri* Birdw. was chosen. Frankincense resin has been widely used in folk medicine for the treatment of rheumatic and other inflammatory diseases, and ulcerative colitis [Prajapati *et al*., 2003]. High assortment of biological activity of *B. carteri* resin and essential oil is related to their chemical composition and presence of active ingredients such as α-pinene, linalool, and 1-octanol [Li *et al*., 2016].

The aim of this study was to estimate antifungal potential of *B. carteri* EO against selected mould species. Literature reports regarding the antifungal properties of *B. carteri* EO are scarce. However, El-Nagerabi *et al*. [2013] indicated significant antifungal properties of *B. carteri* EO against aflatoxin-producing *Aspergillus* species belonging to section *Flavi*. Also, Prakash *et al*. [2014] confirmed antifungal activity of frankincense against toxigenic *Aspergillus* species.

MATERIALS AND METHODS

Essential oil

The *Boswellia carteri* EO used in the study was a commercial sample obtained from Herba, d.o.o, Belgrade, Serbia (serial number: 8606103256300), as a product imported from France. The frankincense resin originated from Somalia, and was hydrodistilled in France in order to yield high quality EO.

Biocide

Biocide, Sanosil S003 (Sanosil Ltd.), used as a positive control in antifungal assay, was obtained from the Institute for Protection of Cultural Monuments in Serbia, as a water solution of the final concentration 2.7% (silver nitrate 0.2%, and hydrogen peroxide 2.5%).

Tested fungi

Fungi used in antifungal assay (*Aspergilus melleus* Yukawa (BEOFB 351m), *Aspergillus niger* Tiegh (BEOFB 342m), *Emericella nidulans* (Eidam) Vuill. (BEOFB 331m), *Stachybotrys chartarum* (Ehrenb.) S. Hughes (BEOFB 1410m), and *Trichotecium roseum* (Pers.) Link (BEOFB 1510m)) belong to the fungal collection of the Department for Algology, Mycology and Lichenology, Institute of Botany, Faculty of Biology, University of Belgrade. Fungal isolates were maintained on malt extract agar (MEA), potato dextrose agar (PDA), stored at 4 °C and subcultured once a month. All tested fungi are human, animal or plant pathogens.

Microatmosphere method

For studying the effect of the volatile fractions of the *B. carteri* EO, modified microatmosphere method, described by Maruzzella and Sicurella [1960], was used. The assay was performed in sterile Petri dishes (85mm, Ø) containing MEA (20ml). After inoculation of tested fungal isolates in the center of MEA, Petri plates were overturned. Sterilized filter paper (1cm^2, surface area) sodden with *B. carterii* EO at final concentrations of 5, 25, 50, 75 and 100 µL mL^{-1} were placed in the center of the Petri dish lid. Inoculated Petri dishes were then incubated in incubator (Memmert) at the temperature of 24 ± 1 °C. Colony growth of tested fungi was measured after 7 days. Effect of antifungal activity was expressed in terms of mycelial growth inhibition (MGI) and calculated according to Pandey et al. (1982) formula:

$$MGI\ (\%) = 100\ (DC - DT)/DC$$

DC = average diameter of fungal colony in control (mm);
DT = average diameter of fungal colony in treatment (mm).

Agar dilution method

To investigate the antifungal activity of the biocide Sanosil S003, agar dilution method, with MEA as medium, was used [Ishii 1995]. The stock solution of biocide (2.7%) was further diluted in melted MEA in Petri dishes to achieve final concentrations of 1, 5, 10, 20, 50 and 100 µL mL^{-1}. The tested fungi were then transferred to the center of MEA, and Petri dishes were incubated for 7 days (24 ± 1 ^{o}C). MGI of the biocide was determined in the same manner as for the micro-atmosphere method.

Microscopic analysis

After the incubation period, mycelium samples were taken from the margin of a colony grown on MEA enclosed with evaporated *B. carteri* EO (microatmosphere method) or on MEA enriched with different concentrations of Sanosil S003 (agar dilution method). The samples were dyed and fixed with Lactophenol cotton blue and observed under a light microscope (Zeiss Axio Imager M.1, with AxioVision Release 4.6 software) to examine the occurrence of morphological abnormalities. Samples from the control plates were also stained and observed.

Statistical analysis

One-way ANOVA (Microsoft office Excel 2007) was performed for mycelial growth assay, and p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The tested fungi exposed to *Boswellia carteri* EO displayed different susceptibility. The least susceptible species was *Aspergillus niger,* with MGI not documented for any of the EO concentrations used in the experiment. On the other hand, *Stachybotrys chartarum* and *Trichotecium roseum* were the most sensitive fungal isolates ($p < 0.05$), with the highest documented MGI (85 ± 1.88% and 85.0 ± 1.24%, respectively) at the concentration of 100 µL mL^{-1} (Figure 1a). Biocide Sanosil S003, used as positive control, exhibited stronger antifungal activity compared with *B. carteri* EO. Although the tested fungi showed different susceptibility to biocide, in case presented here it can also be concluded that *S. chartarum* was the most sensitive fungi. Sanosil S003 at concentration of 5 µL mL^{-1} caused 100% of MGI for this mould. On the other hand, *A. melleus* and *Emericella nidulans* were the least sensitive fungi in biocide treatment ($p < 0.05$) (Figure 1b).

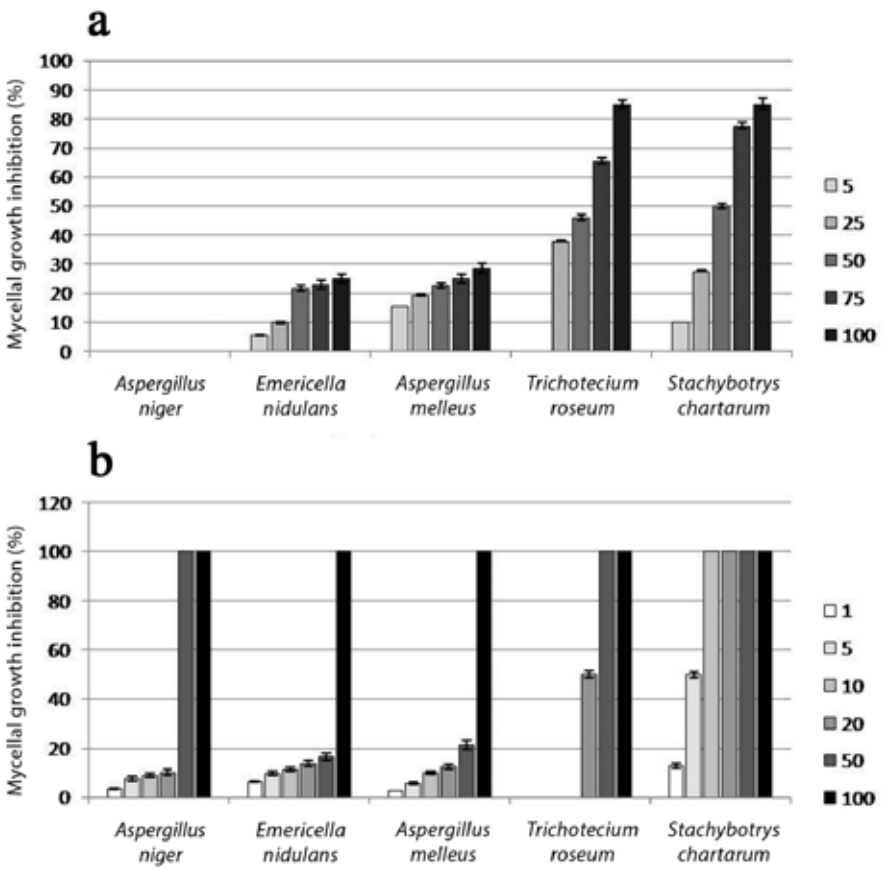

Figure 1. Susceptibility of the tested fungi to (a) *Boswellia carteri* essential oil and (b) biocide Sanosil S003. The concentration of oil and biocide is expressed in µL mL^{-1}.

In addition to MGI, variations in morphological aspects were observed for some fungi grown in essential oil enriched microatmosphere, such as different growth dynamics, absence of sporulation depigmentation, etc. (Figure 2). The highest concentration of *B. carteri* EO (100 µL mL^{-1}) caused scarce sporulation in *A. niger* colonies (Figure 2c). It appears that *B. carteri* EO can prevent *A. niger* to complete its life cycle by interfering with conidia formation. Similar variations in *A. niger* colonies, due to interaction with different EOs, have already been reported. Visible lack of sporulation and pigmentation of *A. niger* colonies grown with essential oil isolated from *Citrus sinensis* (L.) epicarp were reported by Sharma and Tripathi [2008], while Stupar *et al*. [2014] pointed out *Helichrysum italicum* (Roth) G. Don EO sporulation-inhibiting activity against *A. niger*.

Figure 2. Colony growth of (a) *Stachybotrys chartarum*, (b), *Trichotecium roseum* and (c) *Aspergillus niger* in *Boswellia carteri* essential oil enriched microatmosphere.

Likewise, scarce sporulation in *S. chartarum* colonies was recorded in the presence of *B. carteri* EO (75 and 100 µL mL^{-1}) (Figure 2a). Also, *B. carteri* EO caused depigmentation of *S. chartarum* conidia, probably due to inhibition of melanin synthesis during development of hyphae and conidia. Since melanin production by certain pathogenic fungi contributes not only to their virulence [Butler *et al*., 2001], but also to survival and endurance of fungal spores [Wheeler and Bell 1988], demelanization induced by interaction with *B. carteri* EO is a very significant result. Furthermore, *S. chartarum* is a well known producer of toxic secondary metabolites (atranones, dolabellanes, satratoxins, roridins and trichodermin) and exposure to this fungus leads to rashes, mucosal irritation and bleeding [Samson *et al*., 2010]. In recent years, this fungus has attracted attention as a possible causative agent of the so called "sick building syndrome"

[Mahmoudi and Gershwin 2000]. To our knowledge, there are no scientific reports regarding the *S. chartarum* susceptibility to *B. carteri* EO.

In case of *E. nidulans*, morphological alterations included formation of both teleomorphic and anamorphic fungal reproductive structures. Teleomorphic state of *E. nidulans* included formation of cleistotecial ascocarps. It is well known that formation of cleisthotecia involves the coordinated development of two quite different tissue types: ascogenous cells that ultimately give rise to asci and the network of sterile hyphae that surround the asci forming peridium [Sohn and Yohn 2002]. Since the observations obtained with light microscopy revealed the abundant presence of cleisthotecia surrounded by Hülle cells in the highest tested EO concentration (100 μL mL^{-1}) (Figure 3b), it can be assumed that increasing EO concentrations favored the formation of ascocarps. At lower oil concentration only conidiophores bearing the conidial heads were present (asexual state *Aspergillus nidulans*) (Figure 2a).

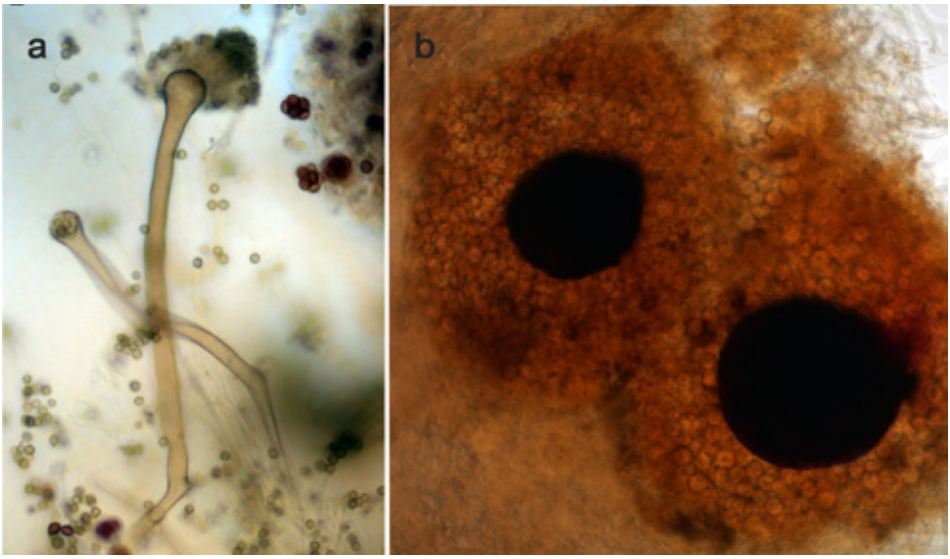

Figure 3. Influence of *Boswellia carteri* essential oil on anamorph/teleomorph occurrence of *Emericella nidulan*: a) anamorphic stage, *Aspergillus nidulans*, dominant in control colony, b) favorized cleisthotecia formation, documented at oil concentration of 100 μL mL^{-1}

The antifungal activity of *B. carteri* EO obtained in this experiment can be considered moderate and significantly lower than antifungal effect of Sanosil S003. However, documented MGI and morphophysiological variations suggested that investigated EO can interfere with fungal metabolism. Application of EOs against fungi may lead to cytoplasm retraction and hyphal wall disintegration [Carmo *et al*., 2008]. Also, EOs components can interfere with enzymatic reactions within the hyphae, and as such affect

fungal growth and morphogenesis [Souza *et al*., 2010]. On the other hand, antifungal activity of biocide Sanosil S003 could be ascribed to synergistic activity of its main components: silver ions and hydrogen peroxide. The main mechanisms of action of this biocide include oxidizing of lipids, proteins and DNA [Bienert *et al*., 2007], as well as functional alterations of cell membrane and hyphal walls [Jo *et al*., 2009], and enzyme inactivation [Feng *et al*., 2000].

In general, essential oils can be good alternative for nowadays commonly applied biocides, due to low mammalian toxicity, susceptibility to biodegradation and consequently low impact on the environment.

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ОСЕТЉИВОСТ МИКРОМИЦЕТА НА РАЗЛИЧИТЕ КОНЦЕНТРАЦИЈЕ ЕТАРСКОГ УЉА *Boswellia carteri* Birdw.

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РЕЗИМЕ: Антифунгална активност комерцијалног препарата етарског уља *Boswellia carteri* испитивана је методом ароматичне коморе. У поређењу са биоцидом Sanosil S003, коришћеним као позитивна контрола, испитивано етарско уље тамјана показало је умерену антифунгалну активност. Најосетљивије микромицете на испитивано етарско уље тамјана биле су *Stachybotrys chartarum* и *Trichotecium roseum*, код којих 85% инхибиције раста мицелије забележено при концентрацији уља 100 μL mL^{-1}. Такође, код врсте *S. chartarum* забележено је смањење интензитета спорулације, као и депигментација конидија. Меланин присутан у конидијама доприноси вурулентности и опстанку патогених врста. Врста *Aspergillus niger* показала је најмању осетљивост на испитивано етарско уље с обзиром да инхибиција раста мицелије ове врсте није забележена при највећој концентрацији уља коришћеном у експерименту.

КЉУЧНЕ РЕЧИ: антифунгална активност, *Boswellia carteri* Birdw., етарска уља, микромицете

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INFLUENCE OF GREEN ALGAE *Chlorella vulgaris* ON INITIAL GROWTH OF DIFFERENT AGRICULTURAL CROPS

ABSTRACT: The aim of this research was to evaluate the effect of green algae *Chlorella vulgaris* on the initial growth of wheat, maize, bean and lettuce and the microbiological activity of rhizospheric soil. The experiment was conducted in controlled conditions. The inocula were applied as foliar fertilizer by spraying. Plant material was taken 30 days after plant emergance. *Chlorella vulgaris* affected positively the length (28.5% increase) and fresh mass (17.9% increase) of maize root, stem length of wheat (24.2% increase) and stem mass of lettuce (56.34% increase). Application of *Chlorella vulgaris* led to the increase of the total number of bacteria and the number of aminoheterotrophs in the maize rhizosphere, total bacterial number in the wheat rhizosphere, and the number of fungi in the rhizosphere of bean. The number of other investigated groups of microorganisms did not change significantly. The activity of dehydrogenase enzyme was not affected by inoculation with green algae.

KEYWORDS: green algae, foliar treatment, stimulation of growth

INTRODUCTION

Green algae *Chlorella vulgaris* stimulates plant growth by production of growth hormones, vitamins, macronutrients (N, P, K) and micronutrients (Fe) [Bajguz and Piotrowska-Niczyporuk 2013]. *Chlorella* sp. enhances soil structure, soil aeration, and absorbs heavy metals. Mode of application varies from foliar treatment to seed coating or only algal extracts could be used [Faheed and Abd-El Fattah 2008]. Green algae based microbial fertilizers are used in Hungary, India, Egypt, Portugal and in Scandinavia. *Chlorella vulgaris* can be successfully used as single treatment or in combination with different rhizobacteria [Raposo and De Morais 2011; Gonzales and Bashan 2000].

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The aim of this research was to investigate the influence of *Chlorella vulgaris* based microbial fertilizer on the initial growth of wheat, maize, been, and lettuce, as well as on the microbiological activity in the rhizospheric soil.

MATERIAL AND METHODS

The experiment was set up in vegetation pots filled with humus substrate. Plant material was as follows: maize (NSSC6010), been (Žuta olovka), wheat (NSR-5) and lettuce (Majska kraljica). The above ground plant parts were treated foliarly by spraying two times within 30 days with 1.6% water suspension of green algae microbial inocula (Natur Plasma – liquid fertilizer containing *Chlorella vulgaris*, $3x10^7$ CFU/ml, Hungary). First application was carried out seven days after emergence, second treatment 25 days after emergence. Sampling of the plant material was performed five days after second treatment. Stem length (cm), stem fresh mass (g), root length (cm) and root fresh mass (g) were measured. In the rhizospheric soil, the total number of bacteria (TNB), number of actinobacteria (ACT), fungi (FNG), aminoheterotrophs (AMH) and azotobacter (AZB) were determined. Standard method of agar plates was used [Trolldenier 1996]. Dehydrogenase (DHA) activity was measured by spectrophotometric method [Lenhard 1956; Thalmann 1968]. Statistical analyses were performed using STATISTICA 10.0 (Hamburg, Germany).

RESULTS AND DISCUSSION

Application of *Chlorella vulgaris* affected differently the initial growth of the plants. The initial maize growth parameters increased by 4.5 and 28.5% (Table 1). Maize root length was the highest regardless of the foliar treatment of *Chlorella vulgaris*. The highest stem length was obtained in wheat. Stem and root of bean plants grew uniformly, the increase in length was around 10%, respectively. *Chlorella vulgaris* affected positively the growth of lettuce, which is of great importance because it is mainly grown for leaves and used as salads in human diet.

The significant increase of growth parameters recorded in this research is in accordance with the works of many authors worldwide. Faheed and Abd-El Fattah [2008] studied the effect of green algae on growth parameters and some physiological response of lettuce (*Lactuca sativa*) seed germination and growth. Our results show that the fresh mass of lettuce increased by 56.34% after foliar treatment with *Chlorella vulgaris*. The stimulatory effects of algae as biofertilizer on some growth parameters of lettuce are also in accordance with the results obtained by Rani and Sathiamoorthy [1997]. Mahmoud and Amara [2000] found that all treatments significantly increased plant growth parameters compared with untreated plant.

Table 1. Effect of *Chlorella vulgaris* on investigated plant growth parameters*

	Treatment	Stem length	Root length	Stem fresh mass	Root fresh mass
	Control	47.25	41.50	2.14	0.94
Maize	*Chlorella vulgaris*	51.00	53.33	2.23	1.11
	% increase ordecrease	7.9	28.5	4.5	17.9
	Control	29.62	14.0	130.0	31.0
Wheat	*Chlorella vulgaris*	36.8	16.6	174.0	38.4
	% increase ordecrease	24.2	18.57	33.0	23.8
	Control	33.5	32.0	4.25	0.791
Bean	*Chlorella vulgaris*	37.0	35.5	4.40	1.11
	% increase ordecrease	10.44	10.9	3.5	40.0
	Control	17.25	14.25	5.85	0.816
Lettuce	*Chlorella vulgaris*	18.75	19.12	9.14	1.46
	% increase ordecrease	8.6	34.2	56.34	78.3

* Plant growth parameters: stem length (cm), root length (cm), stem fresh mass (g/plant), root fresh mass (g/plant)

Table 2. Effect of *Chlorella vulgaris* on microbiological activity in rhizospheric soil (log No)*

	Treatment	TNB	ACT	FNG	AMN	AZB	DHA
Maize	Control	9.37^{bc}	6.21^{a}	5.07^{bd}	9.20^{ab}	4.84^{a}	474^{a}
	Chlorella vulgaris	9.95^{d}	6.35^{a}	5.66^{b}	9.63^{c}	4.83^{a}	440^{a}
Wheat	Control	9.41^{a}	6.35^{a}	4.76^{a}	9.36^{a}	4.72^{a}	1424^{a}
	Chlorella vulgaris	9.84^{b}	6.26^{ab}	5.38^{a}	9.88^{a}	4.77^{a}	674^{a}
Bean	Control	9.86^{d}	6.24^{a}	3.46^{a}	9.69^{c}	4.79^{a}	551^{a}
	Chlorella vulgaris	9.59^{be}	6.28^{a}	4.74^{cd}	9.44^{ac}	4.56^{a}	342^{a}
Lettuce	Control	9.19^{c}	6.28^{a}	4.42^{c}	9.14^{ab}	3.70^{b}	893^{a}
	Chlorella vulgaris	8.91^{a}	6.20^{a}	4.75^{cd}	8.93^{b}	3.57^{b}	2004^{a}

* Note: different letters in superscript indicate the statistically significant difference among investigated parameters; LSD test (p=0.05); DHA-µg TPF /10 g soil

Plants foliar treatment with *Chlorella vulgaris* led to the increase of total bacterial number in the rhizosphere of maize and wheat (Table 2). Actinomycetes and azotobacter were not affected significantly by algalization. Abundance of saprophytic fungi was increased only in the rhizosphere of bean. The number of aminoheterotrophic bacteria was increased in the rizosphere of maize. Dehydrogenase activity was negatively affected by algal inoculation.

In contrast to stimulated plant growth, algalization with *Chlorella vulgaris* did not influence significantly the microbiological activity in the rhizosphere of the investigated plants. This was expected, since the algal inoculum was applied as foliar treatment. Few algal cells reached the soil surface and could not interact with soil microorganisms.

Green algae as biofertilizers are a promising alternative to agrochemicals in order to avoid soil pollution. Also, they recover the nutrients content in soil as they secrete exo-polysaccharides that improve soil structure and bio-active substances that enhance the plant growth. Algae are known to be one of the most promising source of bio-control agents, thereby having positive impact on human health [Silva *et al*., 2000].

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ПРИМЕНА АЛГЕ *Chlorella vulgaris* КАО МИКРОБИОЛОШКО ЂУБРИВО

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РЕЗИМЕ: Циљ истраживања био је да се испита утицај зелене алге *Chlorella vulgaris* на почетни раст пшенице, кукуруза, пасуља и салате као и на микробиолошку активност ризосферног земљишта испитиваних биљака. Оглед је постављен у контролисаним условима. Микробиолошки препарат је примењиван фолијарно прскањем биљака. Биљни материјал за анализу узет је 30 дана након клијања биљака. Примена *Chlorella vulgaris* позитивно је утицала на дужину и свежу масу корена кукуруза, дужину надземног дела пшенице и салате. Инокулација зеленом алгом довела је до повећања укупног броја бактерија и аминохетеротрофа у ризосфери кукуруза, укупног броја бактерија у ризосфери пшенице, као и броја гљива у ризосфери пасуља. Бројност осталих група микроорганизама као и активност ензима дехидрогеназе није статистички значајно промењена.

КЉУЧНЕ РЕЧИ: *Chlorella vulgaris*, фолијарни третман, стимулација раста

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SOIL MICROBIAL ACTIVITY UNDER CONVENTIONAL AND ORGANIC PRODUCTION OF BEAN AND MAIZE

ABSTRACT: The objective of this study was to compare the effects of conventional and organic production system on microbial activity in the soil cultivated with bean and maize crops. The trial in Đurđevo was set up according to the conventional farming system, while organic farming system was used in Futog. Two maize hybrids and two bean cultivars were used in the trial. Soil samples were collected in two periods during 2014 (before sowing, at flowering stage of bean crops, and at 9–11 leaf stage of maize) at two depths, at both locations. The following microbiological parameters were tested: the total number of microorganisms, number of ammonifiers, *Azotobacter* sp., free nitrogen fixing bacteria, fungi, actinomycetes, and activity of dehydrogenase enzyme. The results showed that the total number of microorganisms, number of free N-fixers and dehydrogenase activity were higher within organic production, while *Azotobacter* sp. was more abundant in conventional production. Variations in the number of ammonifiers, fungi and actinomycetes in relation to the type of production were not obtained. Significant differences in microbial activity were also obtained between period and depths of sampling.

KEYWORDS: bean, conventional and organic production, dehydrogenase activity, maize, microbial abundance

INTRODUCTION

Microorganisms account for 0.1 to 3.0% of total soil organic matter, and their biomass in soil ranges from 1 to 5 t ha^{-1} on average. Microorganisms play the key role in the mineralization of organic compounds to inorganic and mobilization of less soluble inorganic compounds in the soil, thus providing plants with nutrients. In addition to the mineralization and nutrient cycling, soil microorganisms

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are also involved in other important ecosystem functions, such as the formation and preservation of soil structure [Bloem and Breure 2003].

Routine monitoring of soil from the biological aspect was launched in several countries in the early nineties. One or more microbiological parameters were included in these monitoring programs in most countries [Stenberg 1999]. Today, due to the increasing pollution, the European Union and many countries around the world are working on introducing laws that would impose microbiological monitoring of soil as an obligation for the most effective protection of the environment [Bloem *et al*., 2006].

For each soil type there are characteristic communities of microorganisms with a specific number and proportion of different physiological groups [Marinković *et al*., 2007]. Cultivation practices lead to the disturbance of these relationships, which is manifested by a reduced number and enzymatic activity of microorganisms, especially because modern agricultural production involves the use of large amounts of pesticides and fertilizers [Đurić *et al*., 2006].

Information about the general microbiological activity, potential soil fertility and general causes of a certain condition of soil can be obtained by determining the presence of certain systematic and physiological groups of microorganisms, the abundance of some genera and species as well as the activity of microbial enzymes [Milošević 2008].

The objective of this study was to compare the effects of conventional and organic production system on microbial number and dehydrogenase activity in the soil cultivated with bean and maize crops.

MATERIALS AND METHODS

Field trials were set up during 2014 at production plots in Futog and Đurđevo. The trial in Đurđevo was set up according to the conventional farming system, while organic farming system was used in Futog. Trials at both localities were set on chernozem soil using a randomized block design with three replications. Two maize hybrids and two bean cultivars (Institute of Field and Vegetable Crops, Novi Sad) were used for the trial. Maize hybrid NS 444 and bean cultivar "Dvadesetica" were used in Futog, while maize hybrid NS 609B and bean cultivar "Maksa" were used in Đurđevo. Sowing was conducted during the optimal sowing period, using all the necessary cultivation practices. Bean seeds were inoculated with *NS-Nitragin* for beans and string beans (Institute of Field and Vegetable Crops, Novi Sad).

Soil microbial properties were determined according to the number of different systematic and physiological groups of microorganisms and the activity of the enzyme dehydrogenase (EC 1.1.1.). At both localities, soil samples for microbial analyses were taken before sowing (March) and once during the vegetation period – at the stage of flowering in beans and at the 9–11 leaf stage in maize. Soil samples were taken from two depths, 0–30 cm and 30–60 cm. Before sowing, the main soil chemical properties were determined at both locations.

The number of microorganisms was determined using the method of agar plates on a suitable nutrient medium, while soil suspension was prepared using a dilution series. The total number of microorganisms was determined on an agarised soil extract, and the number of ammonifiers on the meat-peptone agar (MPA) [Pochon and Tardieux 1962]. The presence of free N-fixers was determined on a N-free agar, and "fertile drops" method was used for the number of *Azotobacter* sp. [Anderson 1965]. The number of actinomycetes was determined on synthetic agar [Krasilnikov 1965], and the number of fungi on Czapek-Dox agar. Incubation temperature was 28 °C, while incubation time depended on the tested group of microorganisms [Jarak and Đurić 2006]. All microbiological analyses were performed in three replications and the average number of microorganisms was calculated at 1.0 g of absolute dry soil.

Dehydrogenase activity was determined using the spectrophotometric method according to the standard [Casida *et al*., 1964], which is based on measuring the extinction of triphenyl formazan (TPF) created by the reduction of TTC (2,3,5-triphenyltetrazolium chloride).

The data were analyzed in accordance with three–way model of analysis of variance (ANOVA) using Statistica software (StatSoft Inc. 2012), followed by mean separation according to Fisher's LSD test.

RESULTS AND DISCUSSION

The chemical soil properties of experimental fields are presented in Table 1. According to pH reaction of soil solution, soils from both localities can be placed into the group of slightly alkaline soils. Soil at Đurđevo locality is humic and contains an optimal supply of easily accessible phosphorus, while the content of easily accessible potassium is high. Soil at Futog locality is slightly humic, poorly supplied with easily accessible phosphorus, and supplied with an optimal level of easily accessible potassium.

Table 1. Soil chemical properties

Experimental Field	pH		$CaCO_3$ %	Humus %	Total N %	AL-P_2O_5 mg/100g	AL-K_2O mg/100g
	in KCl	in H_2O					
Đurđevo	7.26	8.07	1.51	3.14	0.215	24.8	48.0
Futog	7.48	8.31	6.78	2.08	0.155	6.7	21.8

Microorganisms are one of the indicators of the overall soil biogeny since they are actively involved in the processes of transformation of organic matter, assimilation of mineral elements, and formation of humus [Đukić *et al*., 2003]. Some microbial groups can be used as indicators of soil fertility because of their great sensitivity to changes of nutrient concentration in soil solution, water content, etc. [Jarak *et al*., 2010].

The largest parts of soil enzymes have a microbial origin. Their activity is primarily related to catalysis of the reaction of synthesis and mineralization of organic matter, which can be used as a valid indication of soil fertility. Since dehydrogenases are constitutive enzymes of most microorganisms, a general assessment of microbial activity in soil can be given on the basis of dehydrogenase activity [Jarak and Đurić 2006].

In these trials, the number of microorganisms and dehydrogenase activity depended on the farming system, period and depth of soil from which the samples were taken. Soil samples from deeper soil layers had a lower number of microorganisms of the tested microbial groups and a weaker dehydrogenase activity in both sampling periods (Tables 2 and 3). Numerous previous studies [Govedarica *et al.*, 2000; Tintor *et al.*, 2007; Marinković *et al.*, 2008; Milošević *et al.*, 2010] have confirmed that the number of microorganisms decreases with the depth of sampling, which is in accordance with the obtained results. The same conclusion was reached by Samuel *et al.* [2008]. Microbial activity is higher at the soil surface (0–30cm) which contains more organic matter, as well as enough moisture and oxygen. Aerobic microorganisms are most commonly found, and their activity is the most significant to agriculture [Jarak and Čolo 2007]. Deeper soil layers have less favourable ecological conditions, which results in the lower number and weaker enzymatic activity of microorganisms.

The number of microorganisms and microbial activity are seasonal in our climatic region. They are the highest in spring and early autumn, when soil moisture is at a suitable level, and when temperatures range between 20–30 ºC. High temperatures and low soil moisture level during summer, as well as low temperatures during winter, cause decrease in the number of microorganisms and microbial activity.

Sampling period significantly affected the number of microorganisms within the studied groups, and enzymatic activity in soil. Favourable climatic factors during second sampling period caused the increase in the number of microorganisms within the studied groups, while greatest differences were observed in the system of organic farming at the soil surface (0–30 cm). A higher number of ammonifiers, azotobacters, free nitrogen fixers, actynomicetes, and the total number of microorganisms, was determined in soils under bean and maize crops, whereas the number of fungi was not significantly changed. Dehydrogenase activity was significantly higher in second sampling period, in both conventional and organic production, when compared with the period before sowing (Tables 2 and 3).

In the period before sowing, the total number of microorganisms and the number *Azotobacter* sp., free N-fixing bacteria and actinomycetes was higher at the surface of soil under conventional production compared with the organic production system. However, dehydrogenase enzymatic activity at the soil surface layer was significantly higher under organic production system (Tables 2 and 3).

Table 2. Number of microorganisms and dehydrogenase enzymatic activity in the soil cultivated with bean crops at the flowering stage

Production system		Microbial group (CFU ml^{-1} g^{-1} absolutely dry soil)						Dehydrogenase activity (µg TPF g^{-1} soil 24 h^{-1})
		Total microbial number x 10^7	Ammonifiers x 10^6	*Azotobacter* x 10^2	Free N-fixers x 10^6	Fungi x 10^6	Actinomycetes x 10^4	
Before sowing								
Conventional	0–30 cm	92^{b}	42^{abc}	99^{cd}	80^{b}	17^{bc}	10^{a}	306^{cd}
	30–60 cm	47^{c}	31^{cde}	62^{e}	33^{cd}	7^{e}	1^{cd}	151^{d}
Organic	0–30 cm	43^{cd}	36^{bcd}	68^{e}	48^{c}	20^{ab}	6^{b}	717^{b}
	30–60 cm	22^{d}	23^{de}	38^{e}	19^{d}	11^{cde}	0^{d}	253^{cd}
During the vegetation period								
Conventional	0–30 cm	51^{c}	53^{ab}	173^{a}	46^{c}	16^{bcd}	11^{a}	675^{b}
	30–60 cm	44^{c}	36^{cd}	149^{ab}	28^{cd}	9^{cde}	2^{cd}	235^{cd}
Organic	0–30 cm	217^{a}	56^{a}	127^{bc}	224^{a}	26^{a}	11^{a}	914^{a}
	30–60 cm	104^{b}	19^{e}	62^{e}	78^{b}	8^{de}	3^{bc}	313^{c}

The different letter above the number indicates a significant difference at $P < 0.05$

Table 3. Number of microorganisms and dehydrogenase enzymatic activity in the soil cultivated with maize crops at the 9–11 leaf stage

Production system		Microbial group (CFU ml^{-1} g^{-1} absolutely dry soil)						Dehydrogenase activity (µg TPF g^{-1} soil 24 h^{-1})
		Total microbial number x 10^7	Ammonifiers x 10^6	*Azotobacter* x 10^2	Free N-fixers x 10^6	Fungi x 10^6	Actinomycetes x 10^4	
Before sowing								
Conventional	0–30 cm	92^{b}	42^{b}	99^{c}	80^{bc}	17^{bc}	10^{a}	306^{c}
	30–60 cm	47^{c}	31^{bcd}	62^{d}	33^{de}	7^{d}	1^{c}	151^{d}
Organic	0–30 cm	43^{cd}	36^{bc}	68^{d}	48^{de}	20^{ab}	6^{b}	717^{a}
	30–60 cm	22^{d}	23^{d}	38^{e}	19^{e}	11^{cd}	0^{c}	253^{c}
During the vegetation period								
Conventional	0–30 cm	85^{b}	54^{a}	185^{a}	57^{cd}	26^{a}	12^{a}	483^{b}
	30–60 cm	41^{cd}	23^{d}	163^{ab}	30^{de}	14^{bcd}	3^{bc}	162^{d}
Organic	0–30 cm	206^{a}	65^{a}	156^{b}	158^{a}	13^{bcd}	11^{a}	690^{a}
	30–60 cm	107^{b}	28^{cd}	79^{cd}	98^{b}	8^{d}	2^{c}	308^{c}

The different letter above the number indicates a significant difference at $P < 0.05$

The total number of microorganisms and free nitrogen fixers was significantly higher at both surface and deeper layers of soil cultivated with bean and maize plants, under organic production. However, the number of *Azotobacter* was significantly higher under conventional production of both plant species, as well as the number of fungi in soils cultivated with maize plans. Number of ammonifiers and actinomycetes did not vary enough to produce a statistically significant change between the two production systems (Tables 2 and 3).

Similar results were obtained by Mrkovački *et al.* [2012], who determined a significantly higher number of microorganisms under organic production system compared with conventional. Significant differences in microbial abundance between plant species, growing systems and sampling periods were also obtained by Bjelić *et al.* [2015]. Opposite results were obtained in the research of Perez-Brandan *et al.* [2014], which can be attributed to different agrichemical soil properties, climate, and different production management. A research conducted in Brazil by Bettiol *et al.* [2002] and a research conducted in Vojvodina [Vasin *et al*., 2013] indicated no great variations in the number of microorganisms depending on the production system.

Species of the genus *Azotobacter* are one of the most significant free aerobic nitrogen fixers. Number of *Azotobacter* depends on pH reaction of the environment, organic matter and phosphorus content, and it represents an important indicator of soil fertility. Orr *et al.* [2012] indicate the possibility of higher number of *Azotobacter* under conventional production, especially during the initial phases of the vegetation period, due to higher initial concentrations of phosphorus in mineral P fertilizers. This assumption was confirmed by the agrichemical analyses of soil conducted in Đurđevo and Futog, where plots under the conventional production system had significantly higher phosphorus concentration. A higher number of some groups of microorganisms before sowing, which was exhibited in the research, as well as the increased number of *Azotobacter* sp. in the second sampling period within the conventional production system, can be explained by a higher content of humus, phosphorus, and potassium at this production plot.

Production system proved to have a significant effect on microorganism enzymatic activity in the second sampling period. Dehydrogenase activity ranged from 151 to 914 μg TPF g^{-1} soil, and similar results were obtained by Serra-Wittling *et al*. [1995], and Januszek *et al.* [2007; 2015]. At the soil surface layers, cultivated with bean and maize crops, and deeper soil layers cultivated with maize crops, a significantly higher dehydrogenase activity was detected under organic production system (Tables 2 and 3).

Dehydrogenases are enzymes which transport hydrogen between donor and acceptor in the respiration process, while their origin in soils is mainly microbial. Higher dehydrogenase activity indicates higher respiration intensity or larger microbial activity. Microbial activity in soil can be increased by adding fresh organic matter through the application of organic fertilizers (manure, compost, green manure, etc.), which ultimately leads to higher dehydrogenase activity in soils. Research conducted by Vasin *et al.* [2013] showed that soils under conventional growing system, or those undergoing conversion, have lower dehydrogenase activity compared with organic agriculture.

CONCLUSION

Differences in microbial activity between production systems were recorded for the total number of microorganisms, number of free N-fixers, *Azotobacter* sp. and dehydrogenase activity. The number of microorganisms as well as dehydrogenase activity significantly decreased with the increase of soil depth. The increase of the total number of microorganisms, number of free N-fixers and dehydrogenase activity in soil under two different crops grown within organic production confirm the positive effect of this agricultural practice on microbial activity and biological health of soil compared with conventional management.

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МИКРОБИОЛОШКА АКТИВНОСТ ЗЕМЉИШТА У КОНВЕНЦИОНАЛНОЈ И ОРГАНСКОЈ ПРОИЗВОДЊИ ПАСУЉА И КУКУРУЗА

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РЕЗИМЕ: Циљ ових истраживања био је да се упореде ефекти конвенционалног и органског система гајења на микробиолошку активност у земљишту које је под пасуљем и кукурузом као усевима. Оглед у Ђурђеву постављен је у систему конвенционалне пољопривредне производње, а оглед у Футогу у систему органске производње. У огледима су коришћена два хибрида кукуруза и две сорте пасуља Института за ратарство и повртарство у Новом Саду. Узорци земљишта за микробиолошке анализе узети су током 2014. године (пре сетве и у фази цветања пасуља, као и у фази 9–11 листова кукуруза), са две дубине, на оба локалитета. Микробиолошка активност праћена је на основу заступљености укупног броја микроорганизама, амонификатора, *Azotobacter* sp., слободних азотофиксатора, гљива, актиномицета и активности ензима дехидрогеназе. Резултати су показали да су укупан број микроорганизама, број слободних азотофиксатора и дехидрогеназна активност били већи у систему органске производње, док су врсте из рода *Azotobacter* sp. биле заступљеније у систему конвенционалне производње. Нису забележене разлике у бројности амонификатора, гљива и актиномицета у зависности од система гајења. Такође, значајне разлике у микробиолошкој активности утврђене су између периода и дубине узорковања.

КЉУЧНЕ РЕЧИ: пасуљ, конвенционална и органска производња, дехидрогеназна активност, кукуруз, бројност микроорганизама

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THE EFFECT OF INOCULATION WITH *Azotobacter chroococcum* ON MICROORGANISMS IN RHIZOSPHERE AND SUGAR BEET YIELD IN ORGANIC FARMING

ABSTRACT: The effect on sugar beet yield parameters and microbiological soil status was studied using two techniques of sugar beet inoculation with strains of *Azotobacter chroococcum*. Cultivar "Drena" was used in the study, and field trial was set under the conditions of organic farming system in Bački Petrovac. A mixture of three strains of *Azotobacter chroococcum* was used as microbial fertilizer. Inoculation was performed by: (A) incorporation of strains into soil before sowing; and (B) repeated incorporation of strains into soil two weeks after sowing. PGP characterization of the strains confirmed the ability of producing indole-3-acetic acid (IAA) from 12.63 μg ml^{-1} to 14.95 μg ml^{-1}, nitrogen fixation, and P-solubilization. Positive effects on the number of azotobacter and free nitrogen fixers in rhizosphere were obtained by inoculation, as well as positive effects on the tested sugar beet yield parameters. The largest increase in root yield, yield of crystal sugar, and yield of polarised sugar compared with the control was obtained by repeated soil inoculation, ranging from 22 to 23%.

KEYWORDS: abundance of microorganisms, *Azotobacter chroococcum*, organic production, root yield, sugar beet, sugar yield

INTRODUCTION

Production of mineral fertilizers requires a significant amount of non-renewable energy sources and great financial expenses, with negative effects of their application on the environment. Rationalization of mineral fertilizer application can be achieved using N-fixing and P-solubilizing bacteria as microbial fertilizers, which transform the macroelements essential for plant nutrition (nitrogen and phosphorus) into plant-accessible forms [Milić *et al.*, 2004; Mi-

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lošević *et al*., 2006]. Interaction between plants and microorganisms are becoming increasingly significant in the systems of sustainable organic agriculture, above all for the purpose of transformation and mobilisation of nutrients from limited soil nutrient supply, so that plants can adopt these nutrients in order to achieve their full genetic potential. Therefore, more work has recently been made in the use of microbial preparations as addition or replacement for mineral fertilizers and pesticides, and increased efficiency of these preparations using the best combinations of useful bacteria.

Among the plant growth-promoting rhizobacteria – PGPR, bacteria of the genus *Azotobacter* is well known for promotion of growth of non-leguminous plants, resulting in increased plant growth, increased dry weight, higher total nitrogen content, and often in significant yield increase [Govedarica *et al*., 1993; Jarak *et al*., 2012; Milošević *et al*., 2012]. The results of our previous research revealed a significant effect of *Azotobacter chroococcum* on productive and technological traits of sugar beet [Čačić *et al*., 2003; Mrkovački *et al*., 2008], and a significant increase in biogenicity of sugar beet rhizosphere [Mrkovački and Mezei 2003; Kuzevski *et al*., 2011]. Besides the ability to bind atmospheric nitrogen, bacteria of the genus *Azotobacter* have a positive influence on growth and yield of plants due to their P-solubilization ability as well as the ability to produce phytohormones, exopolysaccharides, siderophores, and antibiotics [Bjelić *et al*., 2015].

With the application of PGPR a limited yield increase can be achieved, due to variability of the factors which contribute to the survival of PGPR strains in soil. In addition to the selection of optimal bacterial strains and defining their useful traits, it is necessary to additionally examine different techniques of inoculant application. Therefore, the aim of our research was to examine the effect of inoculation and repeated inoculation with *Azotobacter chroococcum* strains on microbial abundance in rhizosphere and yield of sugar beet grown in the system of organic farming.

MATERIALS AND METHODS

Bacterial strains. Strains of *Azotobacter chroococcum* (strains 5, 8, 14) used in this study were taken from the collection maintained at the Department of Microbiological Preparations, Institute of Field and Vegetable Crops, Novi Sad (WDCM754). *A. chroococcum* was cultured for 72 hrs in Burk's N-free broth, at optimal temperature of 28 °C, at a shaking rate of 150 rpm.

PGPR properties of Azotobacter strains. Quantitative analysis of IAA production was performed as described by Glickman and Dessaux [1995]. The potential of strains to grow on Döbereiner nitrogen-free culture medium [Döbereiner 1988] indicated their N_2-fixation ability. Phosphate solubilization capacity was determined by spot inoculations on Pikovskaya medium – PVK [Pikovskaya 1948] and National Botanical Research Institute's phosphate medium – NBRIP [Nautiyal 1999] with 0.5% TCP [$Ca_3(PO_4)_2$].

Experimental design. Research of the effects of different inoculation techniques using *Azotobacter chroococcum* strains on the parameters of yield and microorganisms of sugar beet rhizosphere was carried out at the locality of Bački Petrovac. The experiment was set in the system of organic production as a randomized block design with four replications, using basic plots 10 m long and 2 m wide. Sowing was done mechanically, using inter-row spacing of 50 cm x 10 cm, with the correction of planting density after sprouting to inter-row spacing of 20 cm. Seed of sugar beet cultivar "Drena" developed at the Institute of Field and Vegetable Crops in Novi Sad was used for sowing. Two techniques for inoculation with *Azotobacter chroococcum* strains were used in the trial: (A) incorporation of strains into soil before sowing, (B) repeated incorporation of strains into soil two weeks after sowing. A mixture of liquid cultures of *Azotobacter chroococcum* strains was used for soil treatment (strains 5, 8 and 14). The capacity of inoculum (density of 10^9 cells per ml) was calculated per trial surface (1 l inoculum + 300 l water ha^{-1}). Untreated soil was used as the control.

Microbiological analysis. Rhizosphere soil samples were taken for microbiological analyses at two dates (June and September) during 2015. Samples were analysed by the serial–dilution method followed by plating on different selective media. A total number of microorganisms (TNM) was determined on an agarized soil extract (dilution 10^7). Nitrogen–free medium was used for determination of free N-fixing bacteria (N-fix) (dilution 10^6) and *Azotobacter* sp. (AZT) (dilution 10^2). Ammonifiers (AMN) were determined on a mesopeptone agar (dilution 10^6). All microbiological analyses were performed in three replications and the average number of microorganisms was calculated at 1.0 g of absolutely dry soil [Jarak and Đurić 2006].

Soil chemical analysis. Soil samples were taken for determination of soil chemical characteristics at the end of the experiment, in late October. Samples were collected from the depth of 0–30 cm, air-dried and ground to a particle size <2 mm, after which the basic chemical characteristics were determined in the laboratory of the Institute of Field and Vegetable Crops.

Yield analysis. Plants were dug up at the end of October, after which root weight and number of plants were determined. The samples containing twenty sugar beet plants from each replication were examined for their sugar content and non-sugar content (K, Na, and amino N), which was determined in the laboratory of the Institute of Field and Vegetable Crops in Novi Sad for the purpose of sugar beet root analysis. The obtained data were used for calculation of root yield per surface unit, yield of polarized sugar, and yield of crystal sugar.

Statistical analysis. The variables were analysed in accordance with the analysis of variance (ANOVA) using software *STATISTICA* (StatSoft Inc. 2012). Means between the levels of the factors were separated by Duncan's multiple range test (DMRT) and letter groupings was generated using 0.05 level of significance.

RESULTS AND DISCUSSION

Azotobacter is one of the most widely reported among the different bacterial genera that have been established as PGPR [Mrkovački and Milić 2001].

Azotobacter represents the main group of heterotrophic free living nitrogen-fixing bacteria present in rhizosphere of many plants (free nitrogen fixation), and occasionally at the root surface (associative nitrogen fixation) [Wani *et al*., 2013]. The isolated culture of *Azotobacter* fixes about 10 mg nitrogen g^{-1} of carbon source under *in vitro* conditions [Jnawali *et al*., 2015]. The amount of nitrogen taken by *Azotobacter* under field conditions is about 20–60 kg ha^{-1} per year [Hajnal *et al*., 2012], depending on soil conditions. PGP characteristics of strains used in this research are shown in Table 1. Strains produced IAA on the agar with added L-triptophan. N-fixing ability was determined for all strains, while P-solubilizing ability was recorded in AC5 and AC8 strains. Similarly, the variability within the PGPR properties in different isolates was recorded by Cakmakci *et al*. [2009], while plant-growth response was variable and dependent on the inoculant strain, plant species, and evaluated growth parameters.

Table 1. Plant growth promoting properties of *Azotobacter* strains

Strain	IAA (μg ml^{-1})		N_2-fix	P – sol	
	0 μg ml^{-1}	250 μg ml^{-1}		PVK	NBRIP
AC5	0.37 ± 0.07	14.95 ±0.13	+	+	+
AC8	0.07 ± 0.12	12.99 ± 0.22	+	+	+
AC14	0.32 ± 0.09	12.63 ± 0.35	+	–	–

IAA: values are average of three replicates (mean ± SD); N_2-fixation: (-) negative reaction; (+) positive reaction; P-solubilization: (-) without clear zone (+) 1–4 mm diameter of clear zone formed around the bacterial colony as a result of solubilization of tri-calcium phosphate

The presence of *Azotobacter* sp. in soils has beneficial effects on plants, but the abundance of these bacteria is related to many factors, soil physico-chemical (e.g. organic matter, pH, temperature, soil moisture) and microbiological properties. *Azotobacter* presence in our climatic region goes from several hundred to several thousand cells, primarily inhabiting neutral or alkaline soils. The population of *Azotobacter* is generally low in the rhizosphere of crop plants, and in uncultivated soils. However, a higher presence of *Azotobacter* sp. was recorded in rhizosphere comparing with the surrounding soil. Previous results have confirmed that the application of bacteria in plant production increases the number and enzymatic activity of microorganisms, which results in higher production ability of soil [Đurić *et al*., 2004; Jarak *et al*., 2012].

Our research revealed that the incorporation of strains in soil before and after sowing lead to increase in the number of *Azotobacter* sp. and free nitrogen fixers in sugar beet rhizosphere. The number of *Azotobacter* increased compared with the control in both sampling periods, and the largest number was obtained in the variant of repeated incorporation of strains into soil in the second period, which was higher than control by 23.2%. In the first period, the number of ammonifiers was higher compared with the control in both variants of inoculation. However, unlike the number of azotobacters and similar to the

total number of microorganisms, the number of ammonifiers decreased in the second sampling period. The highest total number of microorganisms and of ammonifiers was obtained in the control variant in the second sampling period. The number of free nitrogen fixers increased in the first sampling period compared with the control, and decreased in the second sampling period. These microorganisms were the most abundant (higher than control by 33.9%) in the variant with repeated inoculation, similarly to the number of azotobacters in the second period (Table 2). Increase in the abundance of microorganisms in sugar beet rhizosphere as a result of inoculation with *Azotobacter chroococcum* was also obtained in our previous studies [Mrkovački *et al.*, 2012].

Table 2. Effect of inoculation with *Azotobacter* on microbial number in sugar beet rhizosphere

Treatment	Sampling	Microbial group (CFU ml^{-1} g^{-1} absolutely dry soil)			
		TMN x 10^7	AZT x 10^2	AMN x 10^6	N-fix x 10^6
Ø	I	68 ± 44 abc	63 ± 46 b	111 ± 20 a	247 ± 89 a
	II	119 ± 29 a	102 ± 33 ab	221 ± 10 a	99 ± 50 b
Inoculation	I	97 ± 18 ab	87 ± 19 ab	129 ± 14 a	251 ± 73 a
	II	39 ± 19 c	109 ± 21ab	148 ± 40 a	65 ± 74 b
Repeated Inoculation	I	79 ± 19 abc	70 ± 15 b	116 ± 2 a	330 ± 25 a
	II	53 ± 30 bc	126 ± 8 a	117 ± 29 a	41 ± 31 b

Values are average of four replicates (mean ± SD); means followed by the same letter are not statistically different at 0.05 level according to Duncan's multiple range test (DMRT)

Chemical analyses of soil have shown that humus content and total nitrogen content were higher in inoculated variants compared with the control. Therefore, the repeated incorporation of strains into soil had the best effect on these traits (Table 3), suggesting that microorganisms play a very important role in supplying nutrients to crop plants by improving soil fertility through a number of processes. Microorganisms enable processes of humification and dehumification, nitrogen fixation, and release of certain nutrients present in organic matter (N, P, C, S). They also affect plant nutrition by the products of their life activity, thereby participating in the creation and maintaining of soil fertility, growth, yield and health of plants [Milošević *et al.*, 2006].

Table 3. Soil chemical properties

Treatment	pH		$CaCO_3$ %	Humus %	Total N %	AL-P_2O_5 mg/100g	AL-K_2O mg/100g
	in KCl	in H_2O					
Ø	7.55	8.29	2.82	2.61	0.194	29.8	26.8
Inoculation	7.53	8.19	2.11	2.79	0.207	27.6	26.4
Repeated Inoculation	7.50	8.24	1.61	3.18	0.218	24.8	26.8

Incorporation of *Azotobacter chrooccum* strains in soil before and after sowing significantly affected the studied parameters of sugar beet yield (Table 4). Root yield was increased by inoculation compared with the control (7.52–8.47 t ha^{-1}). Inoculation affected the increase in root yield by 20%, and repeated inoculation increased it by 23%. Higher yields of polarized and crystal sugar obtained after inoculation ranged between 20–21%, while repeated inoculation caused the increase of the studied parameters by 22–23%.

Table 4. Effect of inoculation with *Azotobacter* on the yield of sugar beet roots (t ha^{-1})

Treatment	Root yield (t ha^{-1})	Polarized sugar yield (t ha^{-1})	Crystal sugar yield (t ha^{-1})
Ø	37.43 ± 6.10 b	5.19 ± 0.64 b	4.34 ± 0.76 b
	100%	100%	100%
Inoculation	44.95 ± 1.08 a	6.23 ± 0.14 a	5.27 ± 0.26 a
	+ 20%	+ 20 %	+ 21%
Repeated Inoculation	45.90 ± 1.79 a	6.37 ± 0.16 a	5.28 ± 0.23 a
	+ 23%	+ 23%	+ 22%

Values are average of four replicates (mean ± sd); means followed by the same letter are not statistically different at 0.05 level according to Duncan's multiple range test (DMRT)

Previous results [Mrkovački and Mezei 2003] obtained after two years of testing the effects of inoculation with *Azotobacter* in several sugar beet cultivars showed the increase in root yield by about 5.9%, and increased yield of crystal sugar by 7.9–8.2% (536–660 kg ha^{-1}). The results of Čačić *et al.* [2003] showed a statistically significant increase in crystal sugar yield of three sugar beet cultivars at two localities after inoculation with *A. chroococcum*. Yield increase due to *Azotobacter* inoculation ranged from 2–45% in vegetables, 9–24% in sugar cane, and 0–31% in maize, sorghum etc. [Pandey and Kumar, 1989]. Results of Amirhandeh *et al.* [2012] suggested that *Azotobacter chroococcum* is a suitable inoculant, due to its positive response in crop production and it could be part of a strategy in achieving sustainable agriculture. It is necessary that some future researches further explore the potentiality of *Azotobacter* in crop production, especially in organic growing systems. The success of PGPR inoculants will depend on our ability to manage the rhizosphere in order to enhance survival and competitiveness of these beneficial microorganisms.

CONCLUSION

The research confirmed that incorporation of *Azotobacter chroococcum* strains into the soil affected the increase in sugar beet production and soil biogenicity. Incorporation of strains into the soil before and after sowing lead to the increase in abundance of *Azotobacter* sp. and free nitrogen fixers in sugar beet rhizosphere. Root yield increased after inoculation compared with

the control (7.52–8.47 t ha^{-1}). Inoculation increased root yield by 20%, and repeated inoculation caused a 23% increase. Increase in the yield of crystal sugar obtained after inoculation was 21%, while repeated inoculation caused the increase of 22–23%.

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УТИЦАЈ ИНОКУЛАЦИЈЕ СА *Azotobacter chroococcum* НА МИКРООРГАНИЗМЕ У РИЗОСФЕРИ И ПРИНОС ШЕЋЕРНЕ РЕПЕ У ОРГАНСКОЈ ПРОИЗВОДЊИ

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РЕЗИМЕ: Испитан је ефекат два начина инокулације шећерне репе са сојевима *Azotobacter chroococcum* на параметре приноса шећерне репе и микробиолошки статус земљишта. У испитивањима је коришћена сорта Дрена, а експеримент је постављен у систему органске производње у Бачком Петровцу. Као микробиолошко ђубриво коришћена је смеша три соја *Azotobacter chroococcum*. Инокулација је извршена на два начина: (А) инкорпорација сојева у земљиште пре сетве, (Б) поновљена инкорпорација сојева у земљиште две недеље након сетве. PGP карактеризацијом коришћених сојева утврђена је способност продукције индол-3-сирћетне киселине (IAA) од 12.63 μg ml^{-1}до 14.95 μg ml^{-1}, азотофиксације и фосфосолубилизације. Инокулацијом је добијен позитиван ефекат на број азотобактера и слободних азотофиксатора у ризосфери, као и на испитиване параметре приноса шећерне репе. Највеће повећање приноса корена, приноса кристалног и поларизационог шећера добијено је на варијанти поновљене инокулације земљишта и кретало се од 22 до 23% у односу на контролну варијанту.

КЉУЧНЕ РЕЧИ: *Azotobacter chroococcum*, бројност микроорганизама, органска производња, принос корена, принос шећера, шећерна репа

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VASCULAR FLORA OF THE PROMETANJ SITE (MOKRA GORA, NORTHERN PROKLETIJE MT.)

ABSTRACT: Floristic research of the Prometanj site, located in the northwestern part of Mokra Gora Mt. along the right bank of the Ibar River, was conducted during 2011. A total of 340 species and five subspecies of vascular plant taxa were registered. Families with the largest number of species were Asteraceae, Fabaceae, Rosaceae, Lamiaceae, Ranunculaceae, while the most numerous genera were *Trifolium*, *Acer*, *Campanula*, *Geranium*, *Veronica*, *Ranunculus* and *Vicia*. Floral elements of analyzed plant taxa were grouped into ten areal types, with domination of Central European and Eurasian and significant participation of Mediterranean-Submediterranean. The biological spectrum was characterized by the dominance of hemicryptophytes. Five strictly protected and 43 protected species were registered. Prometanj is the only remaining locality in Serbia for tertiary species *Adenophora liliifolia*. Floristic research of Prometanj should be extended to entire area of Mokra Gora Mt. together with the Ibar River gorge, in order to explore the whole botanical richness of this area.

KEYWORDS: arel types spectrum, biological spectrum, Prometanj, Prokletije, vascular flora

INTRODUCTION

Serbian part of the Prokletije mountain range is located in the extreme southwest of the country, on the tripoint with Montenegro and Albania. Length of the main mountain range, situated between Lake Skadar, the lower course of the Drim and the Cijevna rivers in the southwest, and the valley of the Ibar River near Kosovska Mitrovica in the northeast, is about 170 km. This is the southernmost part of the Dinaric Alps, rising at the periphery of the Adriatic

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Sea, in the wider area of Mediterranean fold mountains. As a consequence, they represent a high mountain barrier, open for influences from the south, but also from the north and an obstacle for further penetration of the same. As a separate mountain system includes 24 mountain groups with 152 peaks over 2,000 m.a.s.l. [Belij 2003], organized into three relief units − north, central and south [Knežević 1998]. Hajla, Žljeb, Rusolija, Mokra Gora and Mokra Planina mountains are situated in the north, with Koprivnik and Lumbardske Planine mountains in the central part, whereas Đeravica, Bjelič and Karanfili belong to the southern part [Knežević 1998].

Research locality covers northwestern part of Mokra Gora Mt. along the right bank of the Ibar River. It extends from the village of Leskoviće in the east to Mazgale saddle in the southwest and from the mouth of the Ibar River in Gazivoda Lake (726 m.a.s.l.) to Veprnja Mt. (1,200 m.a.s.l.) (Figure 1). The investigated area is mainly located in a gorge, which gradually passes into a canyon. Mokra Gora massif consists almost entirely of Triassic carbonate rocks, predominantly of limestone and dolomite [Šehovac 2003]. Dominant soil types are limestone and dolomite black soil, brown soil on limestone and terra rossa [Škorić *et al*., 1985]. Climate is modified continental with somewhat warmer summers and colder and wetter winters [Anonymous 2013; Martinović and Markišić 2002]. The study area is dominated by forest vegetation of the order *Quercetalia pubescentis* Br.-Bl. 1932 and *Fagetalia sylvaticae* Paw. 1928 with different secondary communities and chasmophytic elements in rock crevices.

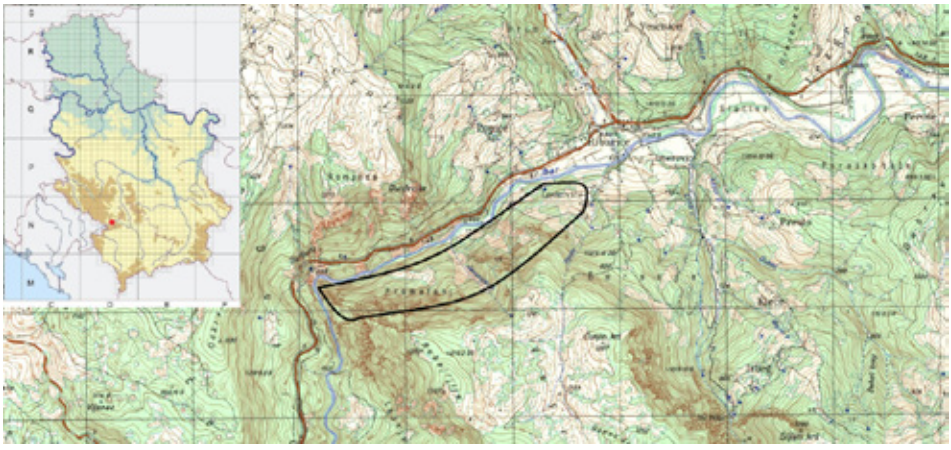

Figure 1. Position and map of the investigated locality [Anonymus 1972; modified]

In his work *Flora of the Principality of Serbia*, Pančić had not provided data for Mokra Gora Mt., only mentioning the Ibar River in a wider geographical context [Pančić 1874; Janković 2003]. However, floristic data about Sandžak region can be found in some regional floras [Beck 1906–1923; Vandas 1909]. Igor Rudski [1949] pioneered research on flora and vegetation of

Mokra Planina Mt. as adjacent part of the Prokletije mountain range, while later the same massif was the subject of several studies by Koviljka Stanković Tomić [1969, 1970, 1974]. The latter author has also contributed to the floristic and vegetation research of Ibarski Kolašin, a wider area to which Prometanj belongs (1967, 1975). Meadow and pasture vegetation of Tutin was studied by Branimir Petković [1985], Prokletije peaks in Rožaje surrounding by Martinović and Markišić [2002], while the serpentine flora of the middle course of the Ibar River was subject of interest of Prodanović and his colleagues [2008, 2010]. Prometanj, as a part of Mokra Gora Mt. in the upper course of the Ibar River, has not been the subject of floristic researches so far.

MATERIALS AND METHODS

Plant material was collected during 2011 in all growing seasons. Plants were herbarized using standard method [Nikolić 1996] and deposited in the Herbarium of the University of Novi Sad (BUNS). Determination was performed using regional and national floras and iconographies [Domac 1973; Fiori and Paoletti 1970; Jávorka and Csapody 1975; Josifović 1972–1977; Sarić 1986, 1992; Stevanović 2012; Tutin *et al*., 1968–1980; Tutin *et al*., 1993]. Taxonomic status was harmonized according to reference databases [Euro+Med, 2006; International Organization for Plant Information, 2012; The International Plant Names Index, 2012; The Plant List, 2013]. Grouping of angiosperm, ferns, horsetails and gymnosperms taxa in higher systematic categories were done according to selected authors [Christenhusz *et al*., 2011; Smith *et al*., 2006; Takhtajan 2009]. Plant material was subjected to standard floristic analysis. Floral elements were determined according to Meusel and associates [1965, 1978] and Meusel and Jäger [1992] and classified into defined areal types for the territory of Serbia according to Stevanović [1992a]. The life forms were defined according to Raunkier [1934], adapted according to Mueller-Dumbois and Ellenberg [1974], and further for the Flora of Serbia according to Stevanović [1992b].

RESULTS AND DISCUSSION

Floristic survey of Prometanj site revealed the presence of 345 plant taxa, of which 340 at the species and 5 at the subspecies level (Table 1).

Table 1. Vascular flora of the Prometanj site

Taxa	Life form	Areal type
Equisetopsida C. Agardh 1825		
Equisetaceae Michx. ex A. DC. 1804		
Equisetum arvense L. 1753	a Mes-Meg G rhiz	Hol
Equisetum hyemale L. 1753	a Meg-Alt G rhiz	Bor
Polypodiopsida Cronquist, Takht. & Zimm. 1966		
Aspleniaceae Newman 1840		
Asplenium adiantum-nigrum L. 1753	fo semp Ch herb caesp	CEv
Asplenium ruta-muraria L. 1753	fo semp Ch herb caesp	Hol
Asplenium scolopendrium L. 1753	fo semp Ch herb semiros	Hol
Asplenium trichomanes L. 1753	fo semp Ch herb caesp	Cos
Ceterach officinarum Willd. 1804	fo semp Ch herb caesp/semiros	MSm
Woodsiaceae Herter 1949		
Gymnocarpium dryopteris (L.) Newman 1851	Mes G rhiz	Bor
Dryopteridaceae Ching 1965		
Polystichum aculeatum (L.) Roth ex Mert. 1800	fo semp Ch herb semiros	Cos
Polypodiaceae Bercht. & J. Presl 1820		
Polypodium vulgare L. 1753	fo semp Ch herb caesp	Hol
Pinopsida Burnett 1835		
Pinaceae Spreng. ex F. Rudolphi 1830		
Abies alba Mill. 1759	ac semp Mes P scap	CEv
Picea abies (L.) H. Karst. 1881	ac semp Mes P scap	Bor
Pinus sylvestris L. 1753	ac semp Mes P scap	Bor
Pinus nigra J.F. Arnold 1785	ac semp Mes P scap	MSm
Cupressaceae Gray 1822		
Juniperus communis L. 1753	ac semp Mi P caesp/Mi-Mes P scap	Hol
Magnoliopsida Brongn. 1843		
Aristolochiaceae Juss. 1789		
Asarum europaeum L. 1753	v fo semp Ch herb rept	EAs
Ranunculaceae Juss. 1789		
Anemone nemorosa L. 1753	v Mi-Mes G rhiz	Hol
Anemone ranunculoides L. 1753	v Mi-Mes G rhiz	CEv
Aquilegia vulgaris L. 1753	a Mes-Meg H semiros	EAs
Clematis recta L. 1753	a Alt H scap	PSs
Clematis vitalba L. 1753	a Alt S lig	MSm
Ficaria verna Huds. 1762	v Mi-Mes G tub	CEv
Helleborus odorus Waldst. & Kit. ex Willd. 1809	v Meg G rhiz	MSm
Hepatica nobilis Mill. 1768	v semp Mi-Mes H semiros	Hol
Isopyrum thalictroides L. 1753	v Mes G rhiz	PSs
Ranunculus acris L. 1753	a Meg H scap semiros	EAs
Ranunculus bulbosus L. 1753	a Mes-Meg H scap	CEv
Ranunculus millefoliatus Vahl 1791	v-a Mes H scap/G tub	MSm

Ranunculus polyanthemos L. 1753	a Meg H scap semiros	PSs
Ranunculus repens L. 1753	a Mes-Meg H rept	EAs
Thalictrum minus L. 1753	a Mes-Alt H scap	EAs
Papaveraceae Juss. 1789		
Chelidonium majus L. 1753	v-a Mes-Meg H semiros	EAs
Fumariaceae Marquis 1820		
Corydalis cava (L.) Schweigg. & Körte 1811	v Mes G tub	CEv
Corydalis solida (L.) Clairv. 1811	v Mes G tub	CEv
Pseudofumaria alba (Mill.) Lidén 1986 subsp. *acaulis* (Wulfen) Lidén 1986	v fo dec Mes-Mac Ch herb rept/caesp	MSm
Fagaceae Dumort. 1829		
Fagus sylvatica L. 1753	fo dec Mes P scap	CEv
Quercus cerris L. 1753	fo dec Mes P scap	MSm
Quercus petraea (Matt.) Liebl. 1784	fo dec Mes P scap	CEv
Betulaceae Gray 1822		
Alnus glutinosa (L.) Gaertn. 1790	fo dec Mes P scap	CEv
Betula pendula Roth 1788	fo dec Mes P scap	EAs
Corylus avellana L. 1753	fo dec Mi P caesp/Mi P scap	CEv
Ostrya carpinifolia Scop. 1772	fo dec Mi-Mes P scap/caesp	MSm
Juglandaceae DC. ex Perleb 1818		
Juglans regia L. 1753	fo dec Meg P scap	MSm
Caryophyllaceae Juss. 1789		
Dianthus sylvestris Wulfen 1786	a Mi-Mes fo dec Ch herb semipulv-pulv	MSm
Petrorhagia saxifraga (L.) Link 1831	a Mes H caesp/fo dec Ch herb caesp	MSm
Saponaria officinalis L. 1753	a Meg H scap	EAs
Silene viscaria (L.) Jess. 1879	a Mes-Meg H scap semiros	EAs
Silene vulgaris (Moench) Garcke 1869	a Mes H scap/G rad	EAs
Stellaria graminea L. 1753	a Mes-Meg H scap	EAs
Stellaria holostea L. 1753	v-a Mes fo dec Ch herb scap rept	EAs
Stellaria media (L.) Vill. 1789	v-aut Mi T rept	Cos
Polygonaceae Juss. 1789		
Persicaria maculosa Gray 1821	a-aut Mac-Meg T caesp/scap	EAs
Rumex acetosa L. 1753	a Meg H scap	EAs
Rumex acetosella L. 1753	a Mes-Meg H scap	Hol
Balsaminaceae A. Rich. 1822		
Impatiens glandulifera Royle 1835	a Meg-Alt T scap	Adv
Hypericaceae Juss. 1789		
Hypericum hirsutum L. 1753	a Mes-Meg H scap	EAs
Hypericum perforatum L. 1753	a Mes-Meg H scap	EAs
Hypericum richeri Vill. 1779	a Mes-Mac H scap	SEm
Primulaceae Batsch ex Borkh. 1797		
Lysimachia nummularia L. 1753	v N-Mi fo dec Ch herb rept	CEv
Lysimachia punctata L. 1753	v-a Mac-Meg H scap	PSs
Primula elatior (L.) Hill 1765	v Mi-Mes H ros	EAs
Primula veris L. 1753	a Mi-Mes H ros	CEv

Primula vulgaris Huds. 1762	v Mi H ros	MSm
Salicaceae Mirb. 1815		
Populus tremula L. 1753	fo dec Mes P scap	EAs
Salix caprea L. 1753	fo dec Mi-Mes P scap/caesp	EAs
Salix purpurea L. 1753	fo dec Mi P caesp	EAs
Violaceae Batsch 1802		
Viola arvensis Murray 1770	v-aut Mi-Mac T scap	EAs
Viola canina L. 1753	v Mi-Mes H scap semiros	Hol
Viola odorata L. 1753	v Mi-Mes H rept ros	MSm
Brassicaceae Burnett 1835		
Alliaria petiolata (M.Bieb.) Cavara & Grande 1913	v-a Meg H scap bienn	CEv
Arabis alpina L. 1753	v-aut N-Mac fo dec Ch herb rept	AAl
Capsella bursa-pastoris (L.) Medik. 1792	v-aut Mi-Meg T ros/H ros bienn	Cos
Cardamine bulbifera (L.) Crantz 1769	a Mes-Meg G rhiz	CEv
Cardamine enneaphyllos (L.) Crantz 1769	v-a Mes H scap	CEv
Draba lasiocarpa Rochel 1810	v Mi-Mes fo dec Ch herb pulv	PSs
Erophila verna (L.) DC. 1821	v N-Mi T ros	Hol
Rorippa sylvestris (L.) Besser 1821	a Mi-Mes H scap	EAs
Sisymbrium officinale (L.) Scop. 1772	a Meg T scap	EAs
Thlaspi arvense L. 1753	v-a Mes T scap	EAs
Tiliaceae Juss. 1789		
Tilia cordata Mill. 1768	fo dec Mes P scap	CEv
Tilia platyphyllos Scop. 1771	fo dec Mes P scap	CEv
Malvaceae Juss. 1789		
Malva moschata L. 1753	a-aut Mes-Meg H scap	MSm
Cistaceae Juss. 1789		
Helianthemum nummularium (L.) Mill. 1768	a Mes-Meg fo dec Ch suffr caesp	CEv
Thymelaeaceae Juss. 1789		
Daphne alpina L. 1753	fo dec N P caesp	MSm
Daphne blagayana Freyer 1838	fo semp N P caesp	MSm
Daphne mezereum L. 1753	fo dec N P caesp	EAs
Ulmaceae Mirb. 1815		
Ulmus glabra Huds. 1762	fo dec Mes P scap	CEv
Urticaceae Juss. 1789		
Parietaria officinalis L. 1753	a Mes-Meg H scap	MSm
Urtica dioica L. 1753	a Meg-Alt H scap	EAs
Euphorbiaceae Juss. 1789		
Euphorbia amygdaloides L. 1753	a Mi-Mes H scap	MSm
Euphorbia cyparissias L. 1753	a Mes-Meg H scap	EAs
Mercurialis perennis L. 1753	a Mes-Meg H scap	CEv
Crassulaceae J. St.-Hil. 1805		
Sedum acre L. 1753	v-a N-Mi Ch herb caesp succ	EAs
Sedum album L. 1753	a Mi-Mes Ch herb scap succ	CEv
Sedum telephium L. 1753	a Mes Ch herb scap succ	EAs
Sempervivum globiferum L. 1753 subsp. *hirtum* (L.) 't Hart & Bleij 1999	a Mi-Mes fo semp Ch herb ros succ	MSm

Saxifragaceae Juss. 1789		
Chrysosplenium alternifolium L. 1753	v-a Mi-Mes T scap	Hol
Saxifraga paniculata Mill. 1768	v-a N-Mes fo semp Ch herb ros pulv	AAl
Saxifraga rotundifolia L. 1753	v-a Mes-Meg H ros	MSm
Saxifraga tridactylites L. 1753	a-aut Mes T scap	CEv
Rosaceae Juss. 1789		
Aremonia agrimonoides (L.) DC. 1825	a Meg H ros	MSm
Aruncus dioicus (Walter) Fernald 1939	v-a Mac-Alt H scap	Hol
Crataegus monogyna Jacq. 1775	fo dec N-Mi P caesp/Mi P scap	CEv
Filipendula vulgaris Moench 1794	a Meg H scap	EAs
Fragaria vesca L. 1753	a Mes H rept	EAs
Geum urbanum L. 1753	a Meg H scap	EAs
Malus sylvestris (L.) Mill. 1768	fo dec Mi-Mes P scap	EAs
Potentilla micrantha Ramond ex DC. 1805	a Mi-Mes H scap	MSm
Potentilla recta L. 1753	a Mes-Meg H scap	PSs
Potentilla reptans L. 1753	a Mi-Mes H rept	EAs
Potentilla supina L. 1753	a-aut Mes-Mac T rept/H rept	EAs
Prunus avium (L.) L. 1755	fo dec Mes P scap	CEv
Prunus spinosa L. 1753	fo dec N P caesp	PSs
Pyrus pyraster (L.) Burgsd. 1787	fo dec Mes P scap	CEv
Rosa canina L. 1753	fo dec N P caesp	CEv
Rosa glauca Pourr. 1788	fo dec N-Mi P caesp	CEv
Rosa spinosissima L. 1753	fo dec N P caesp	CEv
Rosa × *nitidula* Besser 1815	fo dec N P caesp	CEv
Rubus hirtus Waldst. & Kit. 1804	fo dec N P rept	CEv
Rubus idaeus L. 1753	fo dec N P caesp	Hol
Rubus plicatus Weihe & Nees 1822	fo dec N P caesp	CEv
Sanguisorba minor Scop. 1771	a Mi-Mes H ros	EAs
Sorbus aria (L.) Crantz 1763	fo dec N-Mes P scap	CEv
Sorbus aucuparia L. 1753	fo dec Mes P scap	CEv
Sorbus torminalis (L.) Crantz 1763	fo dec Mes P scap	MSm
Spiraea media Schmidt 1792	fo dec N P caesp	EAs
Onagraceae Juss. 1789		
Circaea lutetiana L. 1753	a Mac H scap	Hol
Epilobium angustifolium L. 1753	a Mes-Alt H scap	Hol
Epilobium montanum L. 1753	a Mes-Meg H scap	EAs
Epilobium palustre L. 1753	a-aut Mes-Meg H scap	Hol
Epilobium parviflorum Schreb. 1771	a-aut Mes-Meg H scap	EAs
Fabaceae Lindl. 1836		
Anthyllis vulneraria L. 1753	a Mes-Meg H scap caesp	CEv
Astragalus glycyphyllos L. 1753	a Mes-Meg H scap rept	PSs
Colutea arborescens L. 1753	fo dec Mi P caesp	MSm
Cytisus austriacus L. 1763	v-a Mes-Mac fo dec Ch suffrut caesp	PSs
Cytisus hirsutus L. 1753	v-a Mi-Meg fo dec Ch frut caesp	MSm
Dorycnium herbaceum Villar 1779	a Mes fo dec Ch suffr caesp	MSm

Genista pilosa L. 1753	v-a Mi-Mes fo dec Ch suffrut caesp	MSm
Hippocrepis emerus (L.) Lassen 1989	a fo dec N P caesp	MSm
Laburnum anagyroides Medik. 1787	fo dec Mi-Mes P caesp/scap	MSm
Lathyrus aphaca L. 1753	a Mes ST herb/T scap	PSs
Lathyrus latifolius L. 1753	a Meg-Alt SH herb/H rept	MSm
Lathyrus pratensis L. 1753	a Meg H scap	EAs
Lathyrus venetus (Mill.) Wohlf. 1892	a Mes-Meg H scap	PSs
Lotus corniculatus L. 1753	a Mes H scap	EAs
Medicago lupulina L. 1753	a Mes T scap/H scap	EAs
Melilotus officinalis (L.) Pall. 1776	a Meg-Alt H scap bienn	EAs
Onobrychis viciifolia Scop. 1772	a Mes-Meg H scap	MSm
Robinia pseudoacacia L. 1753	fo dec Mes P scap	Adv
Securigera elegans (Pančić) Lassen 1989	a Mac-Alt H rept/scap	PSs
Securigera varia (L.) Lassen 1989	a Meg H scap	PSs
Trifolium campestre Schreb. 1804	a Mes T scap	CEv
Trifolium dubium Sibth. 1794	v Mes-Mac T rept/scap	CEv
Trifolium incarnatum L. 1753	a Mes-Meg T scap	MSm
Trifolium medium L. 1759	a Mi-Mes H scap	CEv
Trifolium montanum L. 1753	a Mes H scap	PSs
Trifolium pallidum Waldst. & Kit. 1799-1802	a Mes T scap/H scap bienn	MSm
Trifolium pratense L. 1753	a Mes H scap	EAs
Trifolium repens L. 1753	a Mi H rept	EAs
Vicia cracca L. 1753	a Meg-Alt SH herb/H scap	EAs
Vicia hirsuta (L.) Gray 1821	a Mes-Meg ST herb/T scap	EAs
Vicia sativa L. 1753	a Mes-Meg ST herb/T scap	EAs
Vicia sepium L. 1753	v-a Mac-Meg SH herb/H scap	CEv
Vicia sylvatica L. 1753	a Alt SH herb/H scap	EAs
Polygalaceae Hoffmanns. & Link 1809		
Polygala alpestris Rchb. 1823	a Mi-Mes H caesp	AAl
Polygala amara L. 1759	v-aut Mi-Mes H caesp ros	CEv
Oxalidaceae R. Br. 1818		
Oxalis acetosella L. 1753	a Mi G rhiz	Hol
Staphyleaceae Martinov 1820		
Staphylea pinnata L. 1753	fo dec Mi P caesp/scap	MSm
Aceraceae Durande 1782		
Acer campestre L. 1753	fo dec Mes P scap	CEv
Acer heldreichii Orph. ex Boiss. 1856	fo dec Mes P scap	MSm
Acer monspessulanum L. 1753	fo dec Mi-Mes P scap	MSm
Acer opalus Mill. 1768 subsp. *obtusatum* (Waldst. & Kit. ex Willd.) Gams 1925	fo dec Mes P scap	MSm
Acer platanoides L. 1753	fo dec Mes P scap	CEv
Acer pseudoplatanus L. 1753	fo dec Mes P scap	CEv
Anacardiaceae R. Br. 1818		
Cotinus coggygria Scop. 1771	fo dec Mi P caesp/scap	PSs

Geraniaceae Juss. 1789		
Geranium lucidum L. 1753	a Mi-Meg T scap	MSm
Geranium macrorrhizum L. 1753	v-a Mes-Mac H scap ros	MSm
Geranium molle L. 1753	a Mi-Mes T scap/H scap bienn	EAs
Geranium phaeum L. 1753	a Mes-Meg G rhiz/H scap	CEv
Geranium robertianum L. 1753	a Mi-Meg T scap/H scap bienn	Hol
Geranium sanguineum L. 1753	a Mi-Mes H rept	PSs
Linaceae DC. ex Perleb 1818		
Linum tenuifolium L. 1753	a Mes-Mac H scap	PSs
Celastraceae R. Br. 1814		
Euonymus verrucosus Scop. 1771	fo dec Mi P caesp/scap	PSs
Rhamnaceae Juss. 1789		
Frangula alnus Mill. 1768	fo dec Mi P caesp/scap	CEv
Rhamnus alpina L. 1753 subsp. *fallax* (Boiss.) Maire & Petitm. 1908	fo dec N-Mi P caesp	MSm
Rhamnus cathartica L. 1753	fo dec Mi P caesp/scap	PSs
Cornaceae Bercht. & J. Presl 1825		
Cornus mas L. 1753	fo dec Mi P caesp/Mi-Mes P scap	PSs
Cornus sanguinea L. 1753	fo dec Mi P caesp	CEv
Viburnaceae Raf. 1820		
Viburnum lantana L. 1753	fo dec N-Mi P caesp/Mi P scap	MSm
Viburnum opulus L. 1753	fo dec N-Mi P caesp	EAs
Sambucaceae Batsch ex Borkh. 1797		
Sambucus ebulus L. 1753	a Alt G rad/H scap	PSs
Sambucus nigra L. 1753	fo dec Mi P caesp/Mi-Mes P scap	CEv
Sambucus racemosa L. 1753	fo dec N-Mi P caesp/scap	Hol
Adoxaceae E. Mey. 1839		
Adoxa moschatellina L. 1753	v Mi G rhiz	Bor
Caprifoliaceae Juss. 1789		
Lonicera caprifolium L. 1753	a Alt S lig	MSm
Lonicera xylosteum L. 1753	fo dec Mi P caesp	EAs
Valerianaceae Batsch 1802		
Valeriana montana L. 1753	a Mes-Mac H scap	CEv
Valeriana officinalis L. 1753	a Meg-Alt H scap	EAs
Dipsacaceae Juss. 1789		
Knautia arvensis (L.) Coult. 1823	a Mes-Meg H scap bienn	CEv
Araliaceae Juss. 1789		
Hedera helix L. 1753	aut semp Alt S lig	MSm
Apiaceae Lindl. 1836		
Aegopodium podagraria L. 1753	a Meg-Alt G rhiz	EAs
Astrantia major L. 1753	v-a Mes-Meg H scap	CEv
Athamanta turbith (L.) Brot. 1804	a Mac H scap	MSm
Carum carvi L. 1753	v-a Mac-Meg H bienn scap	EAs
Daucus carota L. 1753	a Meg H scap/T scap	EAs

Eryngium palmatum Pančić & Vis. 1870	a Mes-Meg G rad	MSm
Laserpitium siler L. 1753	a Mac-Alt H scap	MSm
Myrrhis odorata (L.) Scop. 1771	v-a Mac-Alt H scap	CEv
Sanicula europaea L. 1753	a Mes-Meg H scap	EAs
Torilis arvensis (Huds.) Link 1821	a Mes T scap	EAs
Campanulaceae Juss. 1789		
Adenophora liliifolia (L.) A. DC. 1830	a Mac-Meg H scap	EAs
Campanula glomerata L. 1753	a Mes-Meg H scap	EAs
Campanula lingulata Waldst. & Kit. 1801	a Mes-Meg H scap bienn	MSm
Campanula patula L. 1753	a Mes-Meg H scap	CEv
Campanula persicifolia L. 1753	v-a Mac-Meg H scap	EAs
Campanula secundiflora Vis. & Pančić 1861	a Mes-Alt H scap semiros	SEm
Campanula rapunculoides L. 1753	a-aut Mes-Meg H scap	EAs
Edraianthus graminifolius (L.) A. DC. 1839	a N fo dec Ch suffr pulv	MSm
Phyteuma spicatum L. 1753	a Mac-Meg H scap	CEv
Asteraceae Bercht. & J. Presl 1820		
Achillea ageratifolia (Sibth. & Sm.) Benth. & Hook. f. 1873	v-a Mi-Mes H scap	MSm
Achillea millefolium L. 1753	a Meg H scap	EAs
Bellis perennis L. 1753	a Mes H ros	CEv
Centaurea reichenbachii DC. 1838	a-aut Mes-Alt H scap	MSm
Centaurea scabiosa L. 1753	a Meg H scap	PSs
Cichorium intybus L. 1753	a-aut Meg-Alt H scap	EAs
Cyanus triumfettii (All.) Dostál ex Á. Löve & D. Löve 1961	a Mes-Meg H scap	CEv
Doronicum columnae Ten. 1811	v Mes-Meg H scap/G rhiz	MSm
Erigeron acris L. 1753	a Mes-Meg T scap	Hol
Erigeron annuus (L.) Pers. 1807	a Mes-Meg T scap/H scap bienn	Adv
Eupatorium cannabinum L. 1753	a-aut Mac-Alt H scap	CEv
Gnaphalium uliginosum L. 1753	a-aut Mi-Mes T scap	EAs
Hieracium murorum L. 1753	a Mes-Mac H scap	CEv
Inula britannica L. 1753	a Mes-Meg H scap	CEv
Inula ensifolia L. 1753	a Mi-Mac H scap	PSs
Inula oculus-christi L. 1753	a Mes-Meg H scap	PSs
Lactuca pancicii (Vis.) N. Kilian & Greuter 2003	a Meg-Alt H scap	MSm
Lapsana communis L. 1753	a Meg-Alt T scap	CEv
Leontodon hispidus L. 1753	a Mes-Meg H ros	CEv
Leucanthemum vulgare (Vaill.) Lam. 1779	v-aut Mes-Meg H scap	EAs
Matricaria chamomilla L. 1753	v-aut Mes-Mac T scap	EAs
Petasites hybridus (L.) G. Gaertn., B. Mey. & Scherb. 1801	v Mes-Alt G rhiz	CEv
Pilosella officinarum Vaill. 1754	a Mi-Mac H ros	CEv
Prenanthes purpurea L. 1753	a-aut Mes-Alt H scap	CEv
Senecio nemorensis L. 1753	a Mac-Alt H scap	Bor
Solidago virgaurea L. 1753	a-aut Mi-Meg H scap	Bor
Tanacetum corymbosum (L.) Sch. Bip. 1844	a Mac-Alt H scap	PSs

Tanacetum macrophyllum (Waldst. & Kit.) Sch. Bip. 1844	a Alt H scap	MSm
Taraxacum officinale Weber 1780	v-aut Mes H ros	EAs
Taraxacum serotinum (Waldst. & Kit.) Poir. 1816	a-aut Mi-Mes H ros	PSs
Telekia speciosa (Schreb.) Baumg. 1816	a-aut Meg-Alt H scap	MSm
Tragopogon pratensis L. 1753	a Meg H scap	EAs
Tussilago farfara L. 1753	v Mi-Mes G rhiz	EAs
Rubiaceae Juss. 1789		
Asperula taurina L. 1753	a Mes-Meg H scap	PSs
Cruciata laevipes Opiz 1852	v-a Mes-Mac H scap	CEv
Galium rubrum L. 1753	a Mes-Mac H scap	MSm
Galium verum L. 1753	a Mes-Meg H scap	EAs
Gentianaceae Juss. 1789		
Centaurium erythraea Rafn 1800	a Mi-Mes H bienn/T scap	CEv
Gentiana asclepiadea L. 1753	a-aut Mes-Meg H scap	CEv
Gentiana cruciata L. 1753	a Mes-Meg G rad	EAs
Gentianopsis ciliata (L.) Ma 1951	a-aut Mi-Mes H bienn scap	CEv
Apocynaceae Juss. 1789		
Vincetoxicum hirundinaria Medik. 1790	a Mes-Meg H scap	PSs
Solanaceae Juss. 1789		
Atropa belladonna L. 1753	a Meg-Alt H scap	CEv
Solanum dulcamara L. 1753	a Meg-Alt S lig	EAs
Physalis alkekengi L. 1753	v-a Mes-Meg H scap	PSs
Convolvulaceae Juss. 1789		
Calystegia sepium (L.) R. Br. 1810	a Alt rhiz SG herb	EAs
Convolvulus arvensis L. 1753	a Alt rhiz SG herb	Cos
Boraginaceae Juss. 1789		
Anchusa officinalis L. 1753	a Meg H scap bienn	CEv
Lithospermum officinale L. 1753	v-a Mac-Meg H scap	EAs
Myosotis arvensis (L.) Hill 1764	a Mes H scap bienn	EAs
Myosotis discolor Pers. 1798	v-a Mi-Mes T scap	CEv
Pulmonaria officinalis L. 1753	v Mi-Meg H scap	CEv
Symphytum tuberosum L. 1753	a Mi-Meg G tub	PSs
Oleaceae Hoffmanns. & Link 1809		
Fraxinus excelsior L. 1753	fo dec Mes P scap	CEv
Fraxinus ornus L. 1753	fo dec Mi-Mes P scap	MSm
Syringa vulgaris L. 1753	fo dec Mi P caesp	MSm
Scrophulariaceae Juss. 1789		
Chaenorhinum minus (L.) Lange 1870	a-aut Mi-Mes T scap	CEv
Digitalis ferruginea L. 1753	a Mes-Alt H ros	MSm
Digitalis grandiflora Mill. 1768	a Mes-Alt H ros	CEv
Digitalis laevigata Waldst. & Kit. 1804	a Mac-Alt H ros	MSm
Linaria vulgaris Mill. 1768	a-aut Mes-Meg H scap	CEv
Melampyrum nemorosum L. 1753	a-aut Mes-Mac dec ep Semipar T scap	EAs
Rhinanthus minor L. 1756	a Mi-Mes dec ep Semipar T scap	CEv

Scrophularia nodosa L. 1753	a Meg-Alt H scap	EAs
Veronica anagallis-aquatica L. 1753	v-aut Mes-Meg H scap	Hol
Veronica austriaca L. 1759	a Mi-Meg H scap	PSs
Veronica beccabunga L. 1753	a Mes-Meg H rept	EAs
Veronica chamaedrys L. 1753	v-a Mi-Mes H scap	CEv
Veronica officinalis L. 1753	a Mes H rept	Bor
Veronica urticifolia Jacq. 1773	a Mes-Mac H scap	CEv
Gesneriaceae Rich. & Juss. 1816		
Ramonda serbica Pančić 1874	v-a Mi poik H ros	MSm
Plantaginaceae Juss. 1789		
Plantago lanceolata L. 1753	a Mi-Meg H ros	EAs
Plantago major L. 1753	a Mes-Meg H ros	EAs
Plantago media L. 1753	a Mes-Meg H ros	EAs
Verbenaceae J. St.-Hil. 1805		
Verbena officinalis L. 1753	a Mes-Meg H scap	Cos
Lamiaceae Martinov 1820		
Ajuga genevensis L. 1753	a Mi-Mes H semiros	EAs
Ajuga reptans L. 1753	a Mes H rept	CEv
Ballota nigra L. 1753	a Meg H scap	PSs
Clinopodium alpinum (L.) Kuntze 1891	a Mes-Mac H scap	MSm
Clinopodium grandiflorum (L.) Kuntze 1891	a-aut Mes-Mac H scap	MSm
Clinopodium nepeta (L.) Kuntze 1891 subsp. *glandulosum* (Req.) Govaerts 1999	v-aut Mes-Mag H scap/fo dec Ch suffr caesp	PSs
Clinopodium vulgare L. 1753	a Mes-Meg H scap	Hol
Lamium galeobdolon (L.) L. 1759	v-a Mes-Meg H scap	CEv
Lamium garganicum L. 1763	v-a Mes-Meg H scap	MSm
Melissa officinalis L. 1753	a Mi-Meg H scap	MSm
Mentha longifolia (L.) L. 1756	a Mes-Meg H scap	CEv
Origanum vulgare L. 1753	a Mes-Meg H scap	EAs
Prunella laciniata (L.) L. 1763	a Mi-Mes H scap	PSs
Prunella vulgaris L. 1753	a Mi-Mes H scap semiros	EAs
Salvia glutinosa L. 1753	a-aut Mac-Meg H scap	CEv
Salvia verticillata L. 1753	a Mes-Meg H scap	PSs
Scutellaria altissima L. 1753	a-aut Mac-Meg H scap	PSs
Stachys germanica L. 1753	a Meg H scap	PSs
Stachys officinalis (L.) Trevis. 1842	aut Meg H scap	CEv
Stachys recta L. 1767	a Mes-Meg H scap	PSs
Stachys sylvatica L. 1753	a Mes-Meg H scap	CEv
Teucrium chamaedrys L. 1753	v-a Mes-Mac fo dec Ch suffr rept	PSs
Thymus odoratissimus Mill. 1768	a Mi-Mes fo dec Ch suffr rept	PSs
Thymus serpyllum L. 1753	v-aut Mi fo dec Ch suffr rept	CEv
Liliopsida Batsch 1802		
Trilliaceae Chevall. 1827		
Paris quadrifolia L. 1753	a Mes-Meg G rhiz	Bor
Colchicaceae DC. 1804		
Colchicum autumnale L. 1753	aut Mi-Mes G bulb	CEv

Liliaceae Juss. 1789		
Erythronium dens-canis L. 1753	v Mi-Mes G bulb	MSm
Lilium martagon L. 1753	a Meg-Alt G bulb	EAs
Orchidaceae Juss. 1789		
Anacamptis morio (L.) R.M.Bateman, Pridgeon & M.W.Chase 1997	v-a Mi-Mac G tub	CEv
Cephalanthera damasonium (Mill.) Druce 1906	v-a Mes-Mac G rhiz	CEv
Cephalanthera rubra (L.) Rich 1817	v-a Mes-Meg G rhiz	CEv
Dactylorhiza maculata (L.) Soó 1962	a Mes-Meg G tub	EAs
Neottia nidus-avis (L.) Rich. 1817	v Mes-Mac Sapr G tub	CEv
Orchis purpurea Huds. 1762	v-a Mac-Meg G tub	MSm
Platanthera bifolia (L.) Rich. 1817	v-a Mes-Mac G tub	EAs
Hyacinthaceae Batsch ex Borkh. 1797		
Ornithogalum pyrenaicum L. 1753	v Mes G bulb	MSm
Scilla bifolia L. 1753	a Mi-Mes G bulb	MSm
Alliaceae Borkh. 1797		
Allium carinatum L. 1753	a Mes-Meg G bulb	MSm
Allium ursinum L. 1753	v Mes-Mac G bulb	CEv
Convallariaceae Horan. 1834		
Convallaria majalis L. 1753	v Mes G rhiz scap	Hol
Polygonatum odoratum (Mill.) Druce 1906	a Mes-Meg G rhiz	EAs
Dioscoreaceae R. Br. 1810		
Dioscorea communis (L.) Caddick & Wilkin 2002	v Alt SG herb rhiz	MSm
Juncaceae Juss. 1789		
Luzula sylvatica (Huds.) Gaudin 1811	a Meg-Alt H caesp	CEv
Cyperaceae Juss. 1789		
Carex vulpina L. 1753	v-a Mac-Meg H caesp	EAs
Poaceae Barnhart 1895		
Briza media L. 1753	a Meg H caesp	EAs
Dactylis glomerata L. 1753	a Meg H caesp	EAs
Hordeum murinum L. 1753	v-a Mes T caesp	MSm
Poa bulbosa L. 1753	a Mes-Meg H caesp	EAs
Poa trivialis L. 1753	a Mes-Meg H caesp	EAs
Sesleria caerulea (L.) Ard. 1764	v-a Mi-Mac H caesp	CEv
Stipa capillata L. 1762	a Mac-Meg H caesp	PSs

Legend: Life form: a – summerly, ac – needle-leaved, alt – tall, aut – autumnal, bienn – biannual, bulb – bulbous, caesp – caespitose, Ch – Chamaephytes, dec – deciduous, fo – foliose, frut – fruticose, G – Geophytes, H – Hemicryptophytes, herb – herbaceous, lig – ligneous, Mac (Macro) – big/tall/long, Meg (Mega) – big, large, Mes (Meso) – intermediate, Mi (Micro) – small/low, N (nano) – dwarf, P – Phanerophytes, poik – poikilohydrous, pulv – pulvinate, rad – root-bud plant, rept – reptant, rhiz – rhizomatous, ros – rosulate, S – Scandentophytes, Sapr – Saprophytes, scap – scapose, Semipar – Semiparasitophytes, semiros – semirosulate, semp – evergreen, suffr – suffruticose, succ – succulent, T – therophytes, tub – tuberous, v – vernal; Areal type: AAl – Arcto-Alpian, Adv – Adventitious, Bor – Boreal, CEv – Central European, Cos – Cosmopolitan, EAs – Euroasian, Hol – Holarctic, MSm – Mediterranean-Submediterranean, PSs – Pontic-South Siberian, SEm – South European mountain

Taxonomic analysis

Recorded plant taxa were classified into 224 species, 77 families, five classes and four phyla. Phylum Equisetophyta was represented by only two species (0.58%), while Polypodiophyta, represented by one class (Polypodiopsida), contained four families and five genera with eight species (2.32%). Phylum Pinophyta included equally small number of species − two families, four genera and five species (1.45%). With 330 taxa at the species and subspecies level, or 95.7% of the total number of taxa, phylum Magnoliophyta absolutely dominated. Within this phylum, class Magnoliopsida was represented by 303 taxa at the species and subspecies level (87.8%), grouped into 59 families, 190 genera and 27 species, whereas class Liliopsida (7.83%) encompassed 11 families and 24 genera.

Families with the largest number of genera were Asteraceae (26), Fabaceae (17), Rosaceae (15), Lamiaceae (13) and Apiaceae (10). All other families had less than ten genera, with absolute domination of families with only one, 42 of them, or two genera (17). Families with the largest number of species were Asteraceae (33), Fabaceae (33), Rosaceae (26), Lamiaceae (24), Ranunculaceae (15), Scrophulariaceae (14), Apiaceae (10), Brassicaeae (10), Campanulaceae (9) and Caryophyllaceae (8). This taxonomic structure significantly corresponds to flora of Serbia. A large number of species belonging to families Ranunculaceae, Brassicaceae and Apiaceae indicates strong Central European and Eurasian influences, while Mediterranean impact could be attributed to families Caryophyllaceae, Fabaceae and Lamiaceae [Stevanović *et al*., 1995]. Eight of ten families that dominate in Serbia were also the most numerous in Prometanj, but with a slightly different representation. Families Campanulaceae and Rosaceae had a somewhat higher representation at the Prometanj site, while the families Liliaceae and Poaceae had a much smaller number of species than it is the case with the Flora of Serbia [Stevanović *et al*., 1995].

The most species-rich genus was *Trifolium* (8), followed by four genera (*Acer*, *Campanula*, *Geranium* and *Veronica*) with six species and seven (*Asplenium*, *Clinopodium*, *Epilobium*, *Lathyrus*, *Potentilla*, *Rosa* and *Stachys*) with four species. Most of these are among the species-richest genera in the taxonomic spectrum of the Flora of Serbia [Stevanović *et al*., 1995]. Genera *Trifolium*, *Ranunculus* and *Vicia*, widespread in the Holarctic and *Campanula* which is Mediterranean genus in a broader sense [Stevanović *et al*., 1995], indicate transitional character of the research area and importance of its geographical position in terms of phytogeography. When it comes to the number of species, the presence of a large number of *Acer* species, the genus which is not in the first twenty of the Serbian flora, is very interesting. However, given that Prometanj is almost entirely a woodland locality and taking into consideration its biogeographic characteristics, this data was expected.

Phytogeographical analysis

Floral elements of the analyzed plant taxa were grouped into ten areal types (Figure 2). Central European areal type with 93 species (27%) was the most

abundant in the investigated site. This areal type is typical for montane and subalpine regions in which various types of deciduous forests grow [Gajić 1984]. Given that the research site is largely covered with beech and beech-coniferous forests at higher altitude, and polydominant deciduous forests in lower areas, such large contribution of this areal type is not surprising. Proportion of Eurasian species is equally large (27%). Eurasian, Holarctic (7%) and cosmopolitan (2%) species together make about 36% of all vascular plant species of the investigated locality. Despite of forest dominance in the investigated area, these species can survive in various types of habitats, fragmentary occur in the form of rocky areas, mountain meadows, ruderal and arable land.

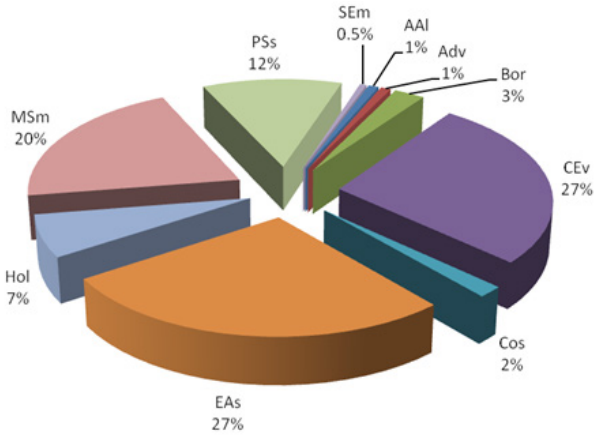

Figure 2. Areal types spectrum of the flora of the Prometanj site

Legend: AAl – Arcto-Alpian, Adv – Adventitious, Bor – Boreal, CEv – Central European, Cos – Cosmopolitan, EAs – Euroasian, Hol – Holarctic, MSm – Mediterranean-Submediterranean, PSs – Pontic-South Siberian, SEm – South European mountain

Mediterranean-Submediterranean areal type, with 71 species (20%) is the third most abundant type. Refugial character of the Ibar River gorge, with temperate continental climate, warmer summers and colder winters, as well the presence of xerophilic rocky areas, enable the growth of these species. Pontic-South Siberian areal type, represented by 41 species (12%), also occurres in the rocky areas. Among them, the most dominant are those with Subpontic and Pontic--Submediterranean floral elements. They live in habitats similar to those of Submediterranean species of Mediterranean-Submediterranean areal type.

A small percentage of Boreal (3%), Arcto-Alpian (1%) and South European mountain (0.5%) species was expected, due to the limited presence of suitable habitats acceptable for species of northern and high mountain regions, such as peaks and isolated stone blocks on a higher terrain. Adventitious areal type was represented by only three species (1%), which indicates naturalness and good conditions in this locality, regardless of the strong human activity present in this area.

The presence of 16 tertiary relicts was registered (*Adenophora liliifolia*, *Asarum europaeum*, *Isopyrum thalictroides*, *Ostrya carpinifolia*, *Juglans regia*, *Staphylea pinnata*, *Syringa vulgaris*, *Cotinus coggygria*, *Aremonia agrimonoides*, *Acer opalus* subsp. *obtusatum*, *A. heldreichii*, *Hedera helix*, *Ramonda serbica*, *Rhamnus alpina* subsp. *fallax*, *Campanula secundiflora*, and *Erythronium dens-canis*). Domination of forest habitats is suitable for tertiary relicts [Stevanović 1995], but on the other hand a relatively large uniformity of habitats resulted in a small number of endemics. Only four Balkan endemic species (*Eryngium palmatum*, *Campanula secundiflora*, *Ramonda serbica*, *Achillea ageratifolia*) and two subendemic species (*Lamium garganicum*, *Edraianthus graminifolius*) have been registered so far.

Biological spectrum

The biological spectrum of the Prometanj site is characterized by seven life forms (Figure 3). Flora of the studied locality has hemicryptophytic character with contribution of 46.8% in the biological spectrum. If we compare biological spectrums of the Prometanj and Serbia [Diklić 1984], it is evident that most life forms contribute similarly. There is a difference in the percent of phanerophytes, abundantly present in Prometanj (19%) compared to Serbia (2.5%). This disproportion is caused by the predominance of forest ecosystems in the research site, whereby the increase in the number of phanerophytes was followed by the reduction of therophytes. The biological spectrum of the flora of Serbia was

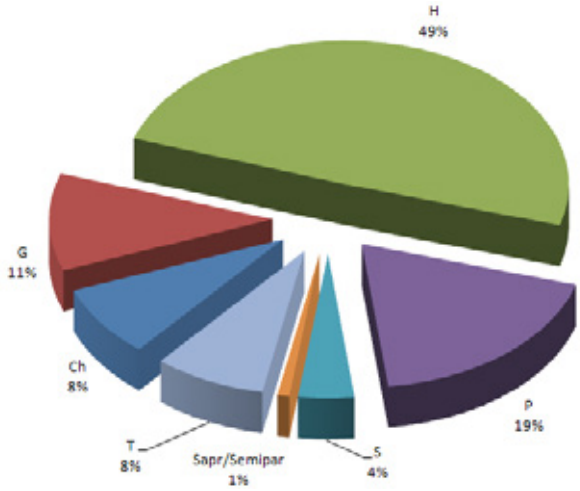

Figure 3. Biological spectrum of the flora of the Prometanj site

Legend: Ch – Chamaephytes, G – Geophytes, H – Hemicryptophytes, P – Phanerophytes, S – Scandetophytes, Sapr/ Semipar – Saprophytes/Semiparasitophytes, T – therophytes

characterized by 18.5% of therophytes, while on the study site they comprise only 8%. This number can be slightly increased if annual herbaceous scandentophytic (4%), saprophytic and semiparasitic (1%) plants are also taken into consideration. Species that by their other environmental and biological characteristics belong to geophytes, therophytes or hemikryptophytes were registered among scandentophytes. Given that the study area did not include aquatic habitats, hydrophytes were not registered.

Threatened status

Five strictly protected species (*Acer heldreichii*, *Campanula secundiflora*, *Dactylorhiza maculata*, *Laburnum anagyroides,* and *Ramonda serbica*) and 57 protected species [Anonymous 2010a] were registered. Also, many protected species, 43 of them, are listed in the Directive on Control of Use and Trade of Wild Plant and Animal Species [Anonymous 2010b]. Prometanj is the only remaining locality for tertiary species *Adenophora liliifolia* [Stevanović and Lakušić 1999] in the territory of Serbia.

CONCLUSION

In the past 100 years Prokletije massif has been subject of more or less intensive floristic and vegetation researches. Whether because of extraordinary botanical wealth or impressive geomorphological and geographical characteristics, these studies were oriented towards the high mountainous areas of Prokletije peaks. From that point of view, studies of specific marginal areas, which at the first sight seem of minor botanical interest, have been sidelined. Among those areas certainly were Mokra gora Mt. and the upper stream of the Ibar River, so the results of our research are a contribution to the floristic researches of such areas. On a very small area and during only one year of research, 345 taxa of vascular flora at the level of species and subspecies were registered in Prometanj. Phytogeographical analysis and analysis of biological spectrum, with a large number of Mediterranean-Submediterranean species and domination of hemicryptophytes, show the importance of this study area as a connection between the Mediterranean, whose influence through the gorges of the Southern Dinarides and Pindos Mt. reaches Mokra gora Mt., and the inner, continental areas whose impact is reflected in a large number of species, primarily of the Eurasian and Central European areal types. In addition, significant presence of Subpontic and Pontic-Submediterranean elements connect this area with the steppe areas of the Kosovo-Metohija valley. For consideration of the overall picture, floristic research of Prometanj should be extended to the entire Mokra gora Mt. with the Ibar River gorge, which will undoubtedly lead to some changes in the results presented in terms of number of species and ecological and phytogeographical characteristics of flora.

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ВАСКУЛАРНА ФЛОРА ЛОКАЛИТЕТА ПРОМЕТАЊ (МОКРА ГОРА, СЕВЕРНЕ ПРОКЛЕТИЈЕ)

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РЕЗИМЕ: Током 2011. године спроведена су флористичка истраживања локалитета Прометањ, северозападног дела Мокре горе уз десну обалу Ибра. Утврђено је присуство 345 биљних таксона, од чега је 340 на нивоу врсте и пет на нивоу подврсте. У васкуларној флори Прометања, фамилије са највећим бројем врста су Asteraceae, Fabaceae, Rosaceae, Lamiaceae, Ranunculaceae, а родови *Trifolium*, *Acer*, *Campanula*, *Geranium*, *Veronica*, *Ranunculus* и *Vicia*. Флорни елементи анализираних биљних таксона груписани су у десет ареал типова са доминацијом средњеевропског и евроазијског, као и значајним учешћем медитеранско-субмедитеранског. Биолошки спектар карактерише доминација хемикриптофита. На истраживаном подручју регистровано је пет строго заштићених и 43 заштићене врсте. Прометањ представља једини преостали локалитет на подручју Србије за терцијарну врсту *Adenophora liliifolia*. Флористичка истраживања локалитета Прометањ треба проширити на читаву Мокру гору са клисуром Ибра ради сагледавања целокупног флористичког богатства ове области.

КЉУЧНЕ РЕЧИ: спектар ареал типова, биолошки спектар, Прометањ, Проклетије, васкуларна флора

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POLLEN MORPHOLOGY OF THE BALKAN-CARPATHIAN ENDEMIC *Campanula lingulata* Waldst. & Kit. (Campanulaceae)

ABSTRACT: Palynomorphological characteristics of *Campanula lingulata*, the Balkan-Carpathian endemic species growing in Serbia, have been investigated using light microscopy and scanning electron microscopy for the first time, in order to provide some information helpful for a better understanding of the taxonomic position of this species within the genus, as well as to contribute to the pollen atlas of Serbian apiflora. The pollen grains are radially symmetrical, isopolar, 3-zonoporate and medium-sized monads oblate-sphaeroidal in shape. Mean of the polar axis (P) is 27.6±1.9 µm, while the average length of the equatorial axis (E) is 28.8±1.6 µm. The apertures are operculate. The sculpturing pattern of the exine is microreticulate–microechinatae. The exine surface is covered with evenly distributed supratectal spinules of variable length and sparse granules. The longest supratectal spinules are 0.64±0.05 µm in length and the smallest sculptural elements are less than 0.2 µm high. The microechinae density per sample area of 5 µm x 5 µm averages 17.4±2.4.

KEYWORDS: pollen morphology, *Campanula lingulata*

INTRODUCTION

Genus *Campanula* (Campanulaceae), comprising about 420 species worldwide, is distributed almost throughout the temperate regions of the Northern Hemisphere, with the greatest diversity in the Mediterranean region, whereby the distribution area extends to the Caucasus [Lammers 2007; Khansari 2012]. Centers of endemism are found in the Eastern Mediterranean, the Balkan Peninsula, the Caucasus and Turkey [Borsch *et al*., 2009]. Twenty-eight species of *Campanula* occur in the Serbian flora inhabiting various habitats such as calcareous grassy and stony places, roadsides, field margins and woodland

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edges, from dry rocky slopes of river gorges to meadows and pastures at high altitudes in hilly and mountain areas [Obradović 1974].

Campanula lingulata Waldst. & Kit. (section *Campanula*; Campanulaceae) is the Balkan-Carpathian endemic species [Škondrić *et al*., 2014], which grows in Serbia most commonly in gorges, on rocky slopes exposed to the sun, in some sunlit shrubby habitats, and eroded terrains. For the purpose of the present study, plant specimens were collected in the gorge of the Tišnica River located in eastern Serbia. This locality was found to be very interesting from the aspect of biodiversity and the presence of relic and endemic species [Gajić 1985]. *C. lingulata* is categorized as protected in Serbia (Official Gazette of the Republic of Serbia, No. 35/10).

The earliest extensive palynological studies of the family Campanulaceae were carried out by Erdtman [1952, 1969] and Chapman [1967]. Avetisjan [1967, 1973] studied pollen morphology of this and closely related families from the aspect of taxonomy and phylogeny and also discussed an evolutionary trend in the development of apertures within Armenian Campanulales. The palynomorphological studies of different Campanulaceae species were conducted in India [Sahay 1969], Pakistan [Perveen and Qaiser 1999], and Turkey [İnceoğlu 1975, 1976; Oybak and Pınar 1995; Ocak 2003; Akçiçek *et al*., 2005; Erkara *et al*., 2008; Alçitepe 2012]. In Iran, variability and possible systematic implication of various palynomorphological characters of *Campanula* and allied genera were examined by Khansari *et al*. [2012]. Detailed palynomorphological researches on Campanulaceae, focusing on the surface ultrastructure using scanning and transmission electron microscopy, were performed by Dunbar [1973, 1975a,b, 1981, 1984].

Given that there are no previous reports dealing with the pollen morphology of *C. lingulata*, the present study represents an integral part of the extensive palynological studies, aiming at possibly providing some new information, which would be helpful for assessing the taxonomic position of this Balkan-Carpathian endemic species within the genus, as well as to contribute to the pollen atlas of Serbian apiflora.

MATERIALS AND METHODS

Research area. Plant specimens were found in the chasmophytic vegetation of the Tišnica River gorge located in temperate-continental climate area of eastern Serbia (Figure 1). Specific orography of the gorge also modifies local hygro-thermal conditions [Mišić 1981]. These modifications involve the formation of a strong thermal gradient in a small area, increase of relative humidity, frequent formation of haze, and attenuation of hygro-thermal extremes. Such climate conditions enabled protection and development of endemo-relict plants.

Study species. C. lingulata was recorded at the forest edges, in the shrub vegetation zone, as well as on a south-facing rocky slope of the gorge exposed to the sun, growing at the altitude of 450–500 m. The flower specimens of *C. lingulata* and data on the distribution and flowering phenology were collected during the vegetation period of 2013. Pollen material was obtained from

the flowers at full flowering stage from 10 plants of wild populations. *Flora of Serbia* [Obradović 1974] was used for species identification and the voucher specimens were deposited in the herbarium of the Institute for Biological Research "Siniša Stanković" in Belgrade.

During the flowering period (May–June) plants produce an inflorescence in terminal dense few-flowered heads. The pollen-collecting hairs are present on the style of protandrous flowers and on the distal surface of the stigmatic lobes. Hairs serve as a secondary pollen presentation mechanism, facilitating the transfer of pollen from the flower to insect pollinators while seeking nectar produced by floral nectaries at the top of the ovary [Nyman 1993].

Light and scanning electron microscopy. The pollen morphology of *C. lingulata* was examined by both light microscopy (LM) and scanning electron microscopy (SEM). For LM study, the pollen grains from mature anthers, prepared according to the standard acetolysis method (Erdtman 1952), were mounted in glycerine jelly and observed with a Leica DMSL microscope equipped with a digital camera (Leica DC 300) and Leica IM1000 software. The number of pores was determined and exine thickness was measured under LM. For SEM study the samples were mounted directly on metallic stubs using double sided adhesive tape and coated with gold (in BAL-TEC SCD 005 Sputter Coater, 100 seconds in 30 mA) in a sputtering chamber. The tectal sculptural elements and the aperture characteristics were examined using JEOL JSM-6390 LV electron microscope at an acceleration voltage of 20 kV. SEM micrographs were used mainly for studing the shape and size of grains, the length of polar (P) and equatorial axis (E), number, position and diameter of pores and the interporal distance, as well as for getting more detailed information on the ornamentation referring to ground sculpture and spinule size and density. The pollen grains were photographed in polar and equatorial views, and observations and measurements were performed on a sample of 50 grains for each morphological character. The terminology used for pollen description is in accordance with Erdtman [1952] and Punt *et al.* [2007].

RESULTS

The pollen morphological features of the species examined are shown in Figure 2. a-g. The pollen grains of *C. lingulata* are shed as monads and are radially symmetrical and isopolar. The aperture type is 3-zonoporate. Equatorially distributed round operculate pores average 4.9±0.4 μm in diameter. The mean distance between two pores is 21.6±0.2 μm. The average operculum height is 2.1±0.05 μm and it had a rough, non-ornamented surface. The grains are medium-sized. Mean of the polar axis length (P) is 27.6±1.9 μm, while the length of the equatorial axis (E) averages 28,8±1.6 μm. The average P/E ratio (shape index) is 0.96±0.04, which defines the shape as oblate-sphaeroidal. In LM analyses, the outline is circular in the polar view and elliptic in the equatorial view (Figure 2. a–d). The mean exine thickness apart from microechini as measured under light microscopy is 0.9±0.1 μm.

The sculpturing pattern of exine is microreticulate-microechinate. SEM micrographs reveal clearly visible microreticulate ground sculpture of the exine covered with predominant supratectal spinules of variable size and sparse granules, more or less evenly distributed (Figure 2. g). Supratectal spinules are smooth and obtuse but sometimes with curved apices. The longest supratectal spinules are 0.64±0.05 μm in length and the smallest sculptural elements are less than 0.2 μm high. The microechinae density (spinules of all sizes) per sample area of 5 μm x 5 μm is in average 17.4±2.4.

DISCUSSION

The results of the present study, regarding the basic palynomorphological characteristics such as polarity, symmetry, size, shape, ornamentation and apertures, are completely or partially in accordance with previous palynomorphological investigations of some *Campanula* species [Nowicke *et al*., 1992; Erkara *et al*., 2008; Khansari *et al*., 2012]. The most common shape of pollen grains in Campanuloideae was reported as oblate-spheroidal [Dunbar 1975a; Erkara *et al*., 2008]. However, in subfamilies Lobelioideae [Price and Ayers 2008] and Cyphioideae the prolate shape type is present [Dunbar 1975b]. According to Erkara *et al*. [2008], who presented detailed palynomorphological features of 12 Turkish *Campanula* taxa, pollen grains are triporate (and/or tetraporate) and more or less oblato-sphaeroidal, which agrees with the current study. The oblato-sphaeroidal shape of pollen grains found in *C. lingulata,* as well as in the majority of *Campanula* species analyzed previously, is confirmed by the shape index (P/E) which is a constant feature for this and other studied Campanulaceae genera [Erkara 2008; Khansari *et al*., 2012]. As pointed out elsewhere [Khansari *et al*., 2012], the shape character within this family is not enough variable to provide a reliable feature for diagnosing supraspecific taxa at any level. The shape index for the majority of previously investigated taxa ranged from 0.92 to 0.97 [Erkara *et al*., 2008], or from 0.88 to 0.99 [Khansari *et al*., 2012] being slightly over 1.00 only in three species (*C. patula, C. phyctidocalyx, C. humilima*). Among 47 taxa examined by the latter authors, larger pollen grains (E > 40 μm) were recorded in five taxa of *Asyneuma* and six taxa of *Campanula*. In the latter study, the pollen grain size of *C. kermanica* (P-27.84±1.93 μm; E- 29.40±1.78 μm) are mostly in accordance with polar and equatorial axis measurements in *C. lingulata*.

The ornamentation in Campanuloideae is basically defined as rugulate-echinate or according to another terminology as rugulate-spinulose/verrucose [Dunbar 1975a; Perveen and Qaiser 1999]. According to Erkara *et al*. [2008] characterized the exine sculpture of 12 examined Turkish taxa of *Campanula* was described as granulate-scabrate or rugulate-scabrate and microechinate, whereas Khansari *et al*. [2012] reported that most of 47 analyzed taxa of Campanulaceae including 35 taxa of *Campanula* is characterized by rugulate-echinate or rugulate-microechinate type of ornamentation. Concerning this, the findings of the current study related to microreticulate and microechinate sculpturing

pattern of *C. lingulata* are more in accordance with those of Khansari *et al.* [2012]. Moreover, they completely correspond to those of Nowicke *et al.* [1992], who defined microreticulate tectum in *C. americana*, a species native to eastern and central North America.

The present results pertaining to the sculptural represented by spinules are in agreement with those reported by Perveen and Qaiser [1999] and Khansari *et al.* [2012], but appear contradict previous results of İnceoğlu [1975, 1976], who referred granulate sculpturing pattern. According to Khansari *et al.* [2012], the most valuable palynomorphological characters for subgeneric classification of *Campanula* are the size and density of echinae. According to the mentioned authors, the taxa in sect. *Campanula* are characterized by have the longest echinae of all analyzed species (1.00–2.10 μm) but distributed with low density (3–7/25 μm^2). Authors also found that the increase in the number of echini per unit area (5 μm x 5 μm) causes the reduction of their length. Thus, for example, *C. sclerotricha* has the lowest echini density (2 per unit area) of the maximum length (2.10 μm), and in *C. erinus* the situation is reversed (65; 0.1 μm). Also, in *C. incanescens* and *C. strigosa* was determined the same number of echini (17) per sample area (5 μm x 5 μm) as in *C. lingulata*. The microechine length in *C. lingulata* corresponds to those found in *C. lamondiae* and *C. lourica* defined by Khansari *et al.* [2012].

It has been reported that some aperture features and exine structures are among the essential criteria for determination of the phylogenetic relationships of *Campanula* species [Kuprianova 1967; Cronquist 1968; Walker 1974a,b; Takhtajan 1980]. Based on earlier studies [Dunbar 1984], all three major aperture types in Campanulaceae exist: colpate, colporate and porate and the basic number of apertures is three. According to Nowicke *et al.* [1992], only five species of *Campanula* have pantoporate polen grains (*C. americana, C. californica, C. exigua, C. griffinii* and *C. sharsmithiae*) and all other species examined have 3–4(7) pores distributed equidistantly and equatorially. However, their research revealed that in some cases, pores are unevenly distributed like in zonoporate grains of *C. angustifolia*, or they are not always uniform in size or distribution as in the pantoporate grains of *C. californica*. The results of the present study related to type, number and position of apertures corresponds to those of some Turkish *Campanula* species studied by Erkara *et al.* [2008], or some Iranian species examined by Khansari *et al.* [2012], who reported that all studied members of sect. *Rupestres* (Boiss.) possessed triporate pollen grains, while in sect. *Campanula apart from* triporate (*C. bononiensis* and *C. latifolia*), tetra- to pentaporate grains (*C. rapunculoides* and *C. trachelium)* also occurred. The latter authors pointed out that, since pore number can be variable even among different individuals of certain species, it was not a reliable character for infrageneric classification of *Campanula* and its allies.

The similarity of the pollen morphology between *C. lingulata*, the Balkan--Carpathian endemic species growing in Serbia, and *C. americana*, tne American endemic, in terms of ground sculpture of the exine, and on the other hand, some European and Asian *Campanula* species in terms of the type and number of apertures, indicates that from palynological standpoint, the taxonomic status

of the species examined might be reconsidered. The present study represents a significant contribution to the pollen atlas of Serbian apiflora, providing valuable palynological information useful in the upcoming taxonomic investigations of *Campanula* and allied genera.

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Figure 1. Map of Serbia showing the study area

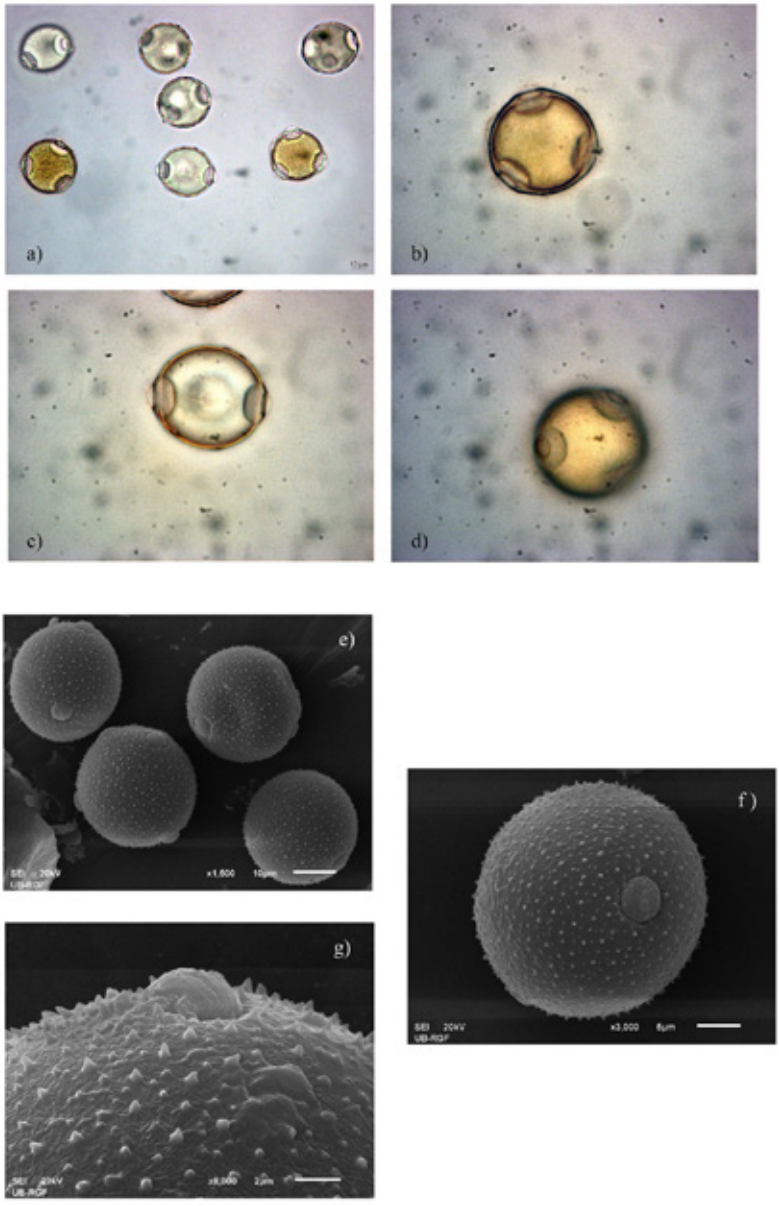

Figure 2. 3-zonoporate pollen grains of *Campanula lingulata*:
a-d) Light photomicrographs: a) general view; b) polar view in the meridional optical section; c) equatorial view; d) surface view.
e-g) Scanning electron photomicrographs: e) general view showing oblate-spheroidal pollen with porate apertures placed equidistant on the equator of the grains; f) equatorial view of pollen grain possessing sparse granules and supratectal spinules on exine surface; g) detail of exine surface showing microreticulate ground pattern of exine and circular pore covered with operculum.

МОРФОЛОГИЈА ПОЛЕНА БАЛКАНСКО-КАРПАТСКОГ ЕНДЕМИТА *Campanula lingulata* WALDST. & KIT. (Campanulaceae)

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РЕЗИМЕ: Палиноморфолошке карактеристике *Campanula lingulata*, балканско-карпатске ендемичне врсте проучене су уз помоћ светлосне и скенирајуће електронске микроскопије, у циљу бољег разумевања таксономске позиције врсте унутар рода, као и доприноса атласу полена апифлоре Србије. Поленова зрна су средње величине, облатно-сфероидног облика, радијално симетрична, изополарна и 3-зонопоратна. На порама се уочава оперкулум. Просечна дужина поларне осе (P) износи 27,6±1,9 μm, а екваторијалне (E) 28,8±1,6 μm. Површина тектума је микроретикулатна са микроехинатном орнаментацијом. Површина егзине је прекривена равномерно распоређеним супратекталним спинулама варијабилне дужине, као и ретким гранулама. Најдужи супратектални елементи износе 0,64±0,05 μm, а најкраћи су дужине до 0,2 μm. Број микроехина по јединици површине 5 μm x 5 μm износи 17,4±2,4.

КЉУЧНЕ РЕЧИ: морфологија полена, *Campanula lingulata*

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PHYSIOLOGICAL AND CHEMICAL CHARACTERISTICS OF SAFFLOWER (*Carthamus tinctorius* L.) GROWN IN THE PRESENCE OF LOW SALT CONCENTRATIONS

ABSTRACT: Safflower (*Carthamus tinctorius* L.) is highly regarded in the world as an aromatic, spicy, medicinal and oilseed crop, which can be used in all kinds of industries. It inhabits arid and semiarid areas of the world. The influence of the relatively low NaCl concentrations found in soils and irrigation waters on the growth and metabolism of safflower, grown under semi-controlled conditions, was examined in this work. It was found that increased concentrations of NaCl affected the number of leaves per plant and dry leaves mass/area ratio. The transpiration intensity was reduced in plants grown in the presence of NaCl and stomatal diffusive resistance increased following an increase in NaCl concentration.

KEYWORDS: safflower, sodium chloride, stress, growth, water regime

INTRODUCTION

Soil salinity is considered to be one of the most important abiotic, external, environmental factors that affects the quality and yield of grown crops in arid and semi-arid areas of the world. Under these conditions, the process of evapotranspiration is highly intensive and very often exceeds the rainfall. As a result, the accumulation of salt in the soil increases. Parent substrate and the presence of salts in the irrigation water also contribute to this problem. The result of salt accumulation, beside soil salinity, is also soil alkalinisation, which both can jeopardize its productivity and consequently agriculture. Soil salinity can be controlled through meliorative measures and irrigation advancement, but this processes are often unprofitable and considered to be short-term solutions. Therefore, more attention will be paid to the significance of growing crops with higher salt tolerance in the future [Arzani 2008].

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The FAO data [1997] show that more than 6% of world soil contains excessive concentrations of salt or sodium. Also, over 400 million hectares of soil contain increased concentrations of sodium, which is 3.4%. In Serbian soils, salt usually does not prevent the plant growth, but it can affect it by changing the quality of plant tissue. Although sunflower, soya bean, and oilseed rape are mainly used as a source of oil in Serbia, some other plant species like castor oil, linen, safflower, coriander, camelina, and many others can also be used as oilseed crops [Schuster 1992].

Safflower is hardy, shrub species, mostly unknown in our country, but it is highly regarded in the world as an oilseed crop which can be used in all kinds of industries. It is considered to be salt tolerant crop, although its mechanisms of tolerance are poorly known [Zhao *et al.*, 2014]. Even though safflower has strong spindle-shaped root system, which makes it drought and salt tolerant, a further study of salt effect on physiological and chemical properties of safflower is necessary. Also, breeding methods can increase its salt tolerance [Dajue and Mündel 1996].

The aim of this experiment was to examine the effect of relatively low concentrations of NaCl, which can be found in the soils in our country, on the growth, water regime and biochemical characteristics of safflower.

MATERIAL AND METHODS

The seed of safflower (*Carthamus tinctorius* L.), used in this experiment was obtained from the Institute of Field and Vegetable Crops in Novi Sad.

The unsorted seed of safflower was sown in shallow, round dishes filled with previously sterilized sand and watered with deionized water. After germination, plants were transfered to pots containing half-strength Hoagland solution [Hoagland and Arnon 1950]. After 14 days, different amounts of NaCl were added into nutrient solution (0-control, 0.2, 0.6, and 1.2 g NaCl/l). There were 7 replications of each treatment, with 8 plants per replication. Analyses were done 21 days after the beginning of the experiment.

Fresh and dry weight were measured, number of leaves per plant and dry leaf mass/leaf area ratio. The intensity of transpiration was measured gravimetrically and the diffusive resistance of stomata by an automatic porometer.

Concentration of free proline was assessed as described by Bates [1973] and the concentration of photosynthetic pigments in acetone extracts as described by Holm [1954] and Von Wettstein [1957].

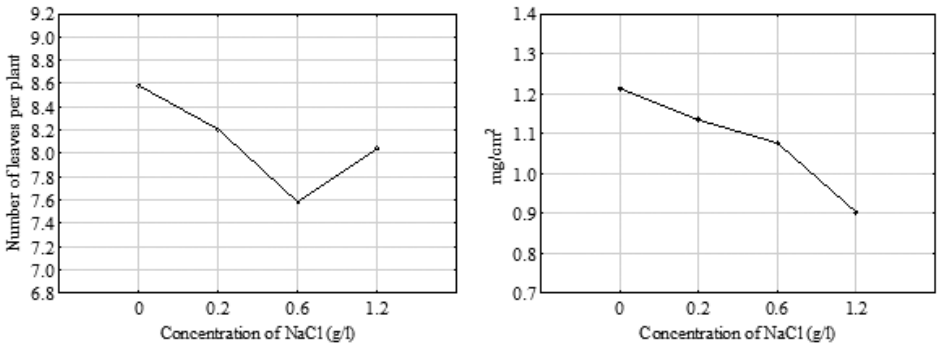
Statistical analysis of data was performed by Statistica 12. Significance of obtained differences between means was established by LSD test for all the parameters.

RESULTS AND DISCUSSION

The salt influence on number of leaves per plant and the dry mass/leaf area ratio

The plants that were treated with 0.6 g NaCl/l had 11.6% lower number of leaves per plant as compared to control treatment (Figure 1). Also, the other treated plants had lower number of leaves per plant relative to the control. This decrease indicates the influence of increasing concentration of NaCl on the growth of safflower.

The dry mass/leaf area ratio showed the lowest values when it comes to plants that were treated with 1.2 g NaCl/l (Figure 1). This value was 25.6% lower as compared to plants that were not treated. It is also significant that the plants that were treated with 0.6 g NaCl/l had 11.5% lower dry mass/leaf area ratio as compared to the control. In the presence of higher concentrations of salt in the soil, plants reduce the growth of their shoot and root [Romero-Aranda *et al.*, 2001]. In the most number of cases, this inhibition of growth can first be seen in shoots, because of commonly greater tolerance of roots to excess salts. The decreased intensity of the plant growth, as a consequence of the presence of a higher concentration of salt, can first be noticed in mass and leaf area of the treated plants (Lauchli and Epstein, 1990). When it comes to studyng the stress influence on the grown plants, the growth analyses become very important [Nidhal *et al.*, 2013]. The stress induced by saline soils affects the plant metabolism and the outcome of agricultural production in many different ways [Marschner 1995].



A B

Figure 1. The influence of NaCl on the number of leaves per plant (A) and the dry mass/leaf area (B)

The salt influence on the transpiration intensity and stomatal diffusive resistance

The increasing NaCl concentration lowered the transpiration intensity (Figure 2). It is significant that the plants treated with 0.2 g NaCl/l had 8.7%

lower intensity of transpiration as compared to the control. Also, there were significant differences between the plants grown in the presence of 0.6 g NaCl/l which had 17.4% lower intensity of transpiration relative to the control. We observed similar differences when it comes to plants grown in the presence of 1.2 g NaCl/l, which had 24.4% lower transpiration intensity as compared to the control. Similar results were obtained by Lu *et al.* [2002]. Their research showed that transpiration intensity of halophyta *Suaeda salsa* decreased due to increasing NaCl concentrations. The salt effect on the water regime of the plants is considered to be one of the main causes of decreased plant growth. The osmotic pressure of the soil solution increases with the increase in NaCl concentration and, consequently, water uptake by plants is often prevented. As a result, water becomes unavailable for plants despite its presence. This phenomenon is called 'physiological drought' [Ayers and Westcot 1976]. High concentration of salts aggravates water apsorption and leads to decrease of apsorptive root area, which is necessary for water uptake. Along with this, the leaf area increases, which reduces the whole transpiration process. In the saline conditions plants shorten their vegetation, their water regime is disturbed and the yield is reduced, as it was shown for pea by Maksimović *et al.* [2008 and 2010].

The stomatal diffusive resistance increases due to higher NaCl concentrations. The highest value of this parameter was recorded in plants treated with 0.6 and 1.2 g NaCl/l (Figure 2). Although the concentrations of NaCl that we used in this experiment were relatively low, they still caused the changes in stoma. The immediate plant response to the effects like this is stomatal closure, which was found in many other plant species [Hasegawa *et al*., 2000].

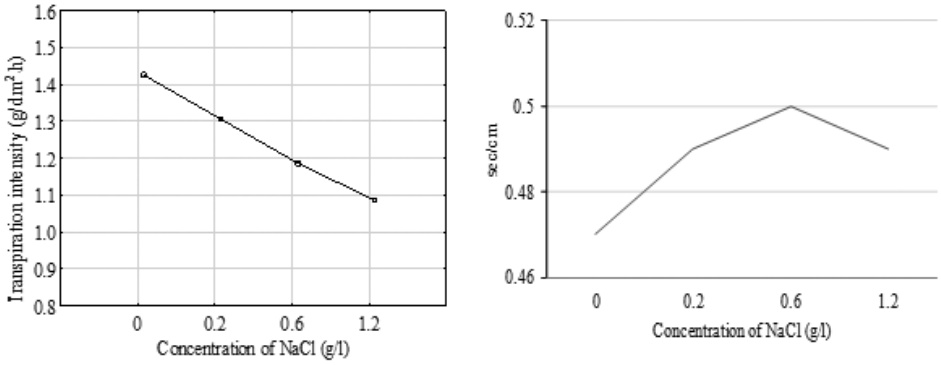


Figure 2. The influence of NaCl on the transpiration intensity (A) and stomatal diffusive resistance (B)

The salt influence on concentrations of free proline and photosynthetic pigments

Plants treated with 0.6 g NaCl/l had the highest free proline concentration in fresh leaf mass (Figure 3). They had 53% higher free proline concentration as compared to the control. A significant difference was also detected between plants treated with 0.2 g and those treated with 0.6 g NaCl/l. Plants treated with 1.2 g NaCl/l had 40.2 % higher proline concentration relative to the control. Seküre and Hüsnü [2014] showed that the free proline concentration increased in fresh leaves of 4 grown types of safflower (Dincer, Yenice, Remzibey-05, and LSD 5) due to increasing NaCl concentrations in substrate. Also Liu and Zhu [1997] showed that the free proline concentration increased in leaves of mutant *Arabidopsis* (sos1), which is considered to be hypersensitive to salt presence, due to higher NaCl concentrations added to nutrient solution, in comparison with a wild type of *Arabidopsis.*

When it comes to chlorophyll a, b and carotenoids content in dry leaf mass, the obtained results showed variability. Plants treated with 0.6 g NaCl/l had 13.9% lower concentration of chlorophyll a, as compared to the control. They also had 23% lower chlorophyll a concentration as compared to plants treated with 0.2 g NaCl/l (Figure 3). Analyses of chlorophyll b concentration showed that the plants treated with 0.6 g NaCl/l had significantly lower chlorophyll b content relative to the control (15.5%) (Figure 3). Carotenoids are, next to ascorbic acid, non-enzimatic antioxidants which play very important role in plant osmotic stress [Mane *et al*., 2011]. Plants treated with 0.6 g NaCl/l had 19.9% lower carotenoid concentration as compared to the plants threated with 0.2 g NaCl/l and this difference was statisticaly significant. Salem *et al.* [2013] showed that the carotenoid content in leaves of two treated genotypes of safflower (104 and Jawhara) had the highest values when 10 g NaCl/l was added to the nutrient solution and the lowest values when the plants were treated with 15 g NaCl/l.

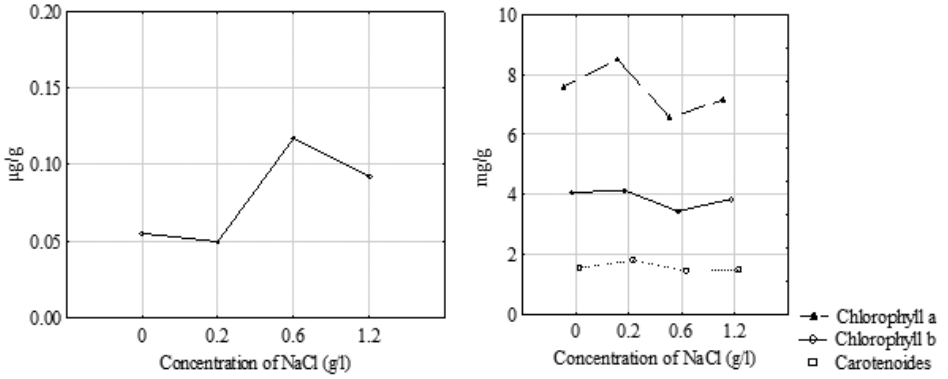


Figure 3. The influence of NaCl on free proline content in fresh leaves (A) and the content of photosynthetic pigments in dry leaf mass (B)

CONCLUSION

When it comes to soil salinity, safflower is considered to be a tolerant species. However, relatively low concentrations of NaCl (up to 1.2 g NaCl/l) affected growth, water regime and metabolism of the treated plants.

The number of leaves per plant and the leaf dry mass/leaf area ratio decreased in the presence of the highest NaCl concentrations.

The decreased transpiration intensity and the increased diffusive stomatal resistance inhibited the production of organic matter in plants.

As a response to the higher NaCl concentrations, free proline content in leaves increased, and the concentration of photosynthetic pigments decreased.

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ФИЗИОЛОШКЕ И ХЕМИЈСКЕ ОСОБИНЕ ШАФРАНИКЕ (*Carthamus tinctorius* L.) ГАЈЕНЕ У ПРИСУСТВУ НИСКИХ КОНЦЕНТРАЦИЈА СОЛИ

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РЕЗИМЕ: Шафраника (*Carthamus tinctorius* L.) изузетно је цењена у свету као ароматична, зачинска биљка и уљана култура која се користи у различитим гранама индустрије. Сматра се првенствено биљком аридних и семиаридних предела. У овом раду испитан је утицај релативно ниских концентрација NaCl, које могу да се нађу у земљиштима или водама за наводњавање, на биљке шафранике гајене у полуконтролисаним условима. Утврђено је да повећане концентрације NaCl утичу на параметре раста, као што су, бројност листова по биљци и однос суве масе и површине листа, али и на водни режим третираних биљака. Присуство соли довело је до смањења интензитета транспирације и до повећања дифузног отпора стома.

КЉУЧНЕ РЕЧИ: шафраника (*Carthamus tinctorius* L.), натријум хлорид, стрес, раст, водни режим

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SEED YIELD AND PROTEIN CONTENT IN SUNFLOWER DEPENDING ON STAND DENSITY

ABSTRACT: The aim of this research was to investigate the effect of stand density on seed yield and protein content in sunflower hybrids. The field experiment was carried out at Rimski Šančevi location. Six NS sunflower hybrids were examined. Five hybrids are confectionery (NS Goliat, NS Slatki, NS Gricko, Vranac and Cepko), and one is used for bird food (NS-H-6485). The trial was arranged as randomized complete block design (RCBD) with four replications. Sowing was done with six different densities (from 20,000 to 70,000 plants per hectare, with an increment of 10,000 plants per hectare). Analysis of variance (ANOVA) showed that the effect of hybrid, stand density and hybrid × stand density interaction were highly significant for seed yield and protein content. The highest seed yield, on the basis of average for all densities, was found in NS-H-6485 (4.77 t ha^{-1}) and in NS Gricko (4.43 t ha^{-1}). Average seed yield of hybrids significantly increased up to 50,000 plants per ha^{-1}, when it reached the value of 4.50 t ha^{-1}, and then decreased. Significantly higher protein content, taking into account all stand densities, showed hybrid Cepko (16.94%). Protein content, above the overall average value, was achieved in hybrid Vranac (16.11%). The highest protein content in the average for all six hybrids was at the lowest stand density (20,000 plants per ha^{-1}), and then decreased up to higher densities. The results showed that stand density had significant effect on seed yield and protein content in sunflower hybrids.

KEYWORDS: hybrid, interaction, oil content, seed yield, stand density, sunflower

INTRODUCTION

One of the most important annual plant species, whose seeds are used for extraction of edible oil, is sunflower (*Helianthus annuus* L.). Except in oil, sunflower seeds are rich in protein, and minerals such as calcium and phosphorus. There are two main types of sunflower: oilseed and non-oil seed sunflower [Salunkhe *et al*., 1991; Jocic *et al*., 2015]. Non-oil seed type *Helianthus*

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annuus L. var. *macrocarpus* (DC) Ckll is also called confectionery, protein, or big seed sunflower. Demand for confectionery sunflower is increasing both in the world and in Serbia, considering the rising possibility of using protein from the seeds in the food and confectionery industry. In the human diet it is used as seed with shell and as hulled kernels. The sunflower seed is used for preparing over 100 different food products: special types of bread, semi-prepared and ready meals, cakes, ice cream, chocolate, peanut butter, mixtures with butter, honey and salt as a spread, and as a substitute for nuts in cakes, addition to salads, and yogurt supplement [Jovanović 2001; Dijanović *et al*., 2003; Cold *et al*., 2012; Jocić *et al*., 2012]. The protein content in 17 hybrids of confectionery type sunflower ranged from 17.3% to 21.1% according to the results reported by Jovanović *et al*. [2008]. A number of authors found a significant correlation between protein content and other seed traits in sunflower [Joksimović *et. al*., 1999; Radić *et al*., 2009, Cold *et al*., 2012; Hassan *et al*., 2013; Ramzan *et. al*., 2015, Hladni *et al*., 2015]. The protein content was correlated with seed yield, weight of 1,000 seeds, dry matter content, oil content and other traits, communicated these authors.

Stand density affects plant architecture, and it is one of the most important cultural practices which determines seed yield, as well as other agronomic attributes of the crop. It is very important to determine the optimum stand density in order to get high seed yield. According to Villalobos *et al*. [1994], as well as Diepenbrock *et al*. [2001], optimal sowing density in sunflower is influenced by several factors, such as temperature, soil fertility, availability of moisture and genotype.

The aim of this study was to investigate the seed yield and protein content in sunflower hybrids depending on the stand density.

MATERIAL AND METHODS

In the experiment conducted in 2012 at Rimski Šančevi location, seed yield and protein content in six NS sunflower hybrids were examined. Five hybrids are confectionery (NS Goliat, NS Slatki, NS Gricko, Vranac and Cepko), and one is used for bird food (NS-H-6485).

NS Goliat is a confectionery sunflower hybrid used for nutrition and peeling. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 190 to 195 cm. The genetic potential for seed yield is higher than 4.5 t ha^{-1}. The oil content is less than 33%, and protein content in the kernel is greater than 23%. It has a low content of the shell. It is resistant to rust and sunflower moth, and tolerant to *Phomopsis*. NS Goliat is attractive to pollinators, and is also well adaptable to different environmental conditions and soil types.

NS Slatki is a confectionery sunflower hybrid used for nutrition and peeling. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 185 to 195 cm. The genetic potential for seed yield is higher than 4.5 t ha^{-1}. The oil content in the seed is less than 35%; protein content in the kernel is higher than 25%. It has a low content of the shell. This hybrid is resistant to

rust and sunflower moth, and tolerant to *Phomopsis*. It is attractive to pollinators, and is also well adaptable to different environmental conditions and soil types.

NS Gricko, a confectionery sunflower hybrid, is used for nutrition and peeling. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 185 to 195 cm. The genetic potential for seed yield is higher than 4,5 t ha^{-1}. The oil content in the seed is less than 35%; protein content in the kernel is higher than 25%. It has a low content of the shell. It is resistant to rust and sunflower moth, and tolerant to *Phomopsis*. NS Gricko is attractive to pollinators, and is also well adaptable to different environmental conditions and soil types.

NS-H-6485 is a sunflower hybrid for bird feed. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 180 to 190 cm. The genetic potential for seed yield is higher than 4.5 t ha^{-1}. Seed oil content is 37-40% and the protein content in the kernel is about 25%. The seed is black with gray lines marked at the ends. It is resistant to rust.

Vranac is a confectionery sunflower hybrid for food and peeling. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 175 to 180 cm. The genetic potential for seed yield is higher than 4 t ha^{-1}. The oil content is from 44 to 48%. It has a low content of the shell. It is resistant to rust and sunflower moth, and tolerant to *Phomopsis*. Vranac is attractive to pollinators, and is also well adaptable to different environmental conditions and soil types.

Cepko, a confectionery sunflower hybrid, is used for peeling and bird food. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 180 to 185 cm. The genetic potential for seed yield is higher than 4.5 t ha^{-1}. The oil content is less than 42%, and seed protein content is more than 16%, and therefore this hybrid is suitable for bird food. It is resistant to rust and sunflower moth and tolerant to *Phomopsis*. This hybrid is attractive to pollinators, and is also well adaptable to different environmental conditions and soil types.

In hybrids Vranac and Cepko the genes responsible for high genetic potential for seed yield and good technical-technological traits of the seeds are successfully combined. Hybrids Vranac and Cepko are suitable for peeling and production of kernels. In addition, Cepko is suitable for bird food [Jovanović and Škorić 2006].

The experiment was set up in a randomized complete block design (RCBD) with four replications. Sowing was done with six different densities (from 20,000 to 70,000 plants per ha^{-1}, with an increment of 10,000 plants per ha^{-1}). Seed was sown in four rows at spacing of 70 cm between the rows. Row length was 10 m. The first and fourth row served as protection, and two inner rows were used for analysis. Seed yield was measured after the harvest from two inner rows, without first and last plants in a row. The seed yield is calculated in t ha^{-1} and corrected to 11% moisture. Seed protein content (%) was determined by Kjeldahl method (VAP-50-Gerhardt).

Statistical analysis (two factorial ANOVA) was performed using the program STATISTICA 12.0. Differences between the treatments were determined by LSD range test at 0.05 and 0.01 level.

RESULTS AND DISCUSSION

Seed yield

Sunflower seed yield is a complex trait and it is strongly under the influence of environmental factors. Yield depends on the genetic potential of hybrids for yield, and yield stability depends on the ability of hybrids response to the environmental conditions [Jocić 2003; Škorić 2012].

Observing the results of the experiment conducted at Rimski Šančevi location, sunflower seed yield depended on hybrid, stand density and hybrid × stand density interaction. All sources of variation were significant. Hybrids had the largest impact on seed yield (50.07%), while the stand density and hybrid × stand density interaction almost equally contributed to seed yield (Table 1). The stand density showed a relatively large effect on seed yield of confectionery sunflower seed, and had a smaller effect on oil content and seed size, according to Zubriski and Zimmermann [1974].

Table 1. ANOVA for seed yield in sunflower hybrids

Source of variation	df	SS	SS (%)	*P*
Hybrid (H)	5	10.86	50.07	< 0.001
Stand density (SD)	5	4.88	22.50	< 0.001
H × SD	25	5.95	27.43	0.044
Error (E)	105			

*$P < 0.05$; **$P < 0.01$

On the basis of average for all densities, the highest seed yield was found in NS-H-6485 (4.77 t ha^{-1}) and in NS Gricko (4.43 t ha^{-1}), while the lowest yield was in Cepko (Table 2). According to Balalić *et al*. [2013], in 2012 the average yield for all hybrids and densities was significantly higher (4.29 t ha^{-1}) than in 2011 (3.72 t ha^{-1}). This could be explained by favorable weather conditions during the growing season of 2012. Based on the test results of 97 confectionery sunflower hybrids in Turkey, Kaya *et al.* [2008] reported that the two-year average yield was 2.24 t ha^{-1}, which is significantly lower than the yield achieved in our experiment. Average seed yield of hybrids significantly increased up to 50,000 plants per ha^{-1}, when it reached the value of 4.50 t ha^{-1}, and then decreased (Table 2). In the average, for three years and all hybrids (NS-Dukat, NS-H-111, and NS-H-103), seed yield in oil sunflower type grew up to 60,000 plants per hectare, significantly up to 50,000 plants per hectare, while the regression maximum was at 55,000 plants per hectare [Dušanić *et al*., 2004]. Crnobarac *et al*. [2006] reported that an average seed yield for four oil sunflower hybrids in 2005 increased to the maximum density. Seed yield at densities of 60,000 and 70,000 plants per hectare was significantly higher than at lower densities, while in 2004 only the lowest (30,000) and the highest density (80,000) had

significantly low yield. According to Barros *et al*. [2004], in Mediterranean region optimum stand density for achieving the maximum sunflower yield was between 3 and 4 plants per m^2.

Variability of seed yield was 8.9%.

Table 2. Seed yield (t ha^{-1}) in sunflower hybrids

Hybrid	Stand density (plants/ha^{-1})						Mean
	20,000	30,000	40,000	50,000	60,000	70,000	
NS Goliat	3.49	3.60	4.21	4.24	4.33	4.26	4.02
NS Slatki	3.79	4.19	4.53	4.56	4.57	4.41	4.34
NS Gricko	4.31	4.48	4.24	4.84	4.38	4.31	4.43
NS-H-6485	3.94	4.69	4.88	5.16	4.93	5.05	4.77
Vranac	4.18	4.42	4.39	3.99	4.10	4.14	4.20
Cepko	3.75	4,12	3.88	4.19	4.03	3.71	3.95
Mean	3.91	4.25	4.35	4.50	4.39	4.31	4.29

LSD	H	SD	H × SD
0.05	0.22	0.22	0.53
0.01	0.29	0.29	0.71

CV= 8.9%

Protein content

One of the indicators of sunflower seed quality is protein content. This is a quantitative trait, determined poligenically. The protein content varies depending on the genotype, agro-ecological conditions, as well as on the interaction between genotype and environmental conditions. Confectionery or protein sunflower type is characterized, among other traits, by lower oil content and increased seed protein content. Radić [2006] reported that, in the process of maturing, protein synthesis previously stabilized in relation to biosynthesis of oil in sunflower seed.

Table 3. ANOVA for protein content in sunflower hybrids

Source of variation	df	SS	SS (%)	*P*
Hybrid (H)	5	243.46	70.07	< 0.001
Stand density (SD)	5	60.77	18.74	< 0.001
H × SD	25	20.08	6.19	< 0.001
Error (E)	105			

*$P < 0.05$; **$P < 0.01$

For seed protein content additive (hybrid, stand density) and non-additive (hybrid × stand density interaction) sources of variation showed high significance. Hybrids showed the highest impact on protein content at Rimski Šančevi location (70.07%), followed by stand density (18.74%), while the lowest proportion was observed in hybrid × stand density interaction (Table 3). According to Dijanović *et al*. [2004], genotype (26.42%) and genotype × year × location interaction (20.32%) almost equally contributed to protein content. In addition to genotype, environmental factors have a large impact on seed protein content, as reported by some authors [Merriam *et al*., 1988; Dušanić 1994]. Stanojević *et al*. [1998] stated that protein content in sunflower depended on environmental factors and locations. Kandil *et al*. [1990], examining the content of protein and oil in five sunflower hybrids and varieties at two locations (Germany and Egypt), came to the conclusion that the protein content was strongly influenced by the location. Dijanović *et al*. [2004] also noted that the protein content in the lines of confectionery sunflower seed also varied depending on the location. Radić *et al*. [2009] examined the seed protein content in two lines (L2 and L4) grown in two locations. The line L4 in the location 2 had significantly lower protein content (15.07%) compared with the location 1 (26.74%).

Table 4. Protein content (%) in sunflower hybrids

Hybrid	Stand density (plants/ha^{-1})						Mean
	20,000	30,000	40,000	50,000	60,000	70,000	
NS Goliat	14.08	13.11	13.33	13.03	12.76	12.91	13.20
NS Slatki	16.39	16.24	14.82	15.11	13.34	14.08	15.00
NS Gricko	16.98	15.47	15.46	14.57	14.38	14.61	15.24
NS-H-6485	15.37	14.31	13.42	12.72	13.10	12.93	13.64
Vranac	16.70	16.46	15.87	15.84	15.99	15.82	16.11
Cepko	17.92	17.10	16.92	16.55	16.77	16.40	16.94
Mean	16.24	15.45	14.97	14.64	14.39	14.46	15.02

LSD	H	SD	H × SD
0.05	0.29	0.29	0.71
0.01	0.38	0.38	0.93

CV = 3.3%

Significantly higher protein content, taking into account all stand densities, showed hybrid Cepko (16.94%). Above the overall average value, protein content is achieved in hybrid Vranac (16.11%). Other hybrids had significantly lower protein content compared with the overall average, which was 15.02% (Table 4). Observing the influence of stand density on protein content it can be seen that the highest content in the average for all six hybrids was at the lowest density (20,000 plants per ha^{-1}), and then decreased up to higher densities.

There were no significant differences in protein content between the last three stand densities, whereby the protein content was significantly lower than the overall average (Table 4). Crnobarac *et al*. [2013] suggested, based on two-year results of the protein content in three confectionery sunflower hybrids grown in eight sowing dates, that the protein content was higher in the medium terms compared with earlier planting dates. Taking into account three hybrids, protein content had the highest value (15.78%) at planting date on 20.04. Dijanović *et al*. [2004] examined the protein content in three inbred lines of confectionery sunflower at three locations (Zaječar, Leskovac and Požarevac) and in three generations (S3, S4 and S5). Cultivar Kolos was taken as a control. The highest protein content had the line Rs4I10-S3 (21.75% in Zaječar and Požarevac, and 22.24% in Leskovac). The minimum protein content showed line D4441-S5 (Zaječar 18.85%, 19.44% Leskovac, Požarevac 19.18%). Hladni *et al*. [2009] reported that the protein content in the seed of new confectionery hybrids ranged from 11.9% (NS-H-6309) to 14.0% (NS-H-6309), while by standards Cepko and Vranac it was 12.8% i.e. 13.2%. The results obtained in 2008 [Hladni *et al*. 2011a] at two locations (Rimski Šančevi, Vojvodina region and Kula, central Serbia) expressed higher seed yield in comparison with standards (Vranac and Cepko), though with a lower seed oil content. In the experiment were used 13 confectionary hybrids and Vranac and Cepko as the control. The protein content ranged from 10.7% (NS-H-17) to 14.2% (NS-H-04). Vranac had 13.1% and Cepko 13.7% protein content. The same authors reported a very strong positive correlation between seed yield and seed protein content, kernel content, and mass of 1,000 seeds. Hladni *et al*. [2011b], based on two-year results at Rimski Šančevi location with three confectionery sunflower hybrids, concluded that the seed protein content ranged from 13.6% (NS-H-6316) to 15.8% (NS-H-6320). According to the results of Hladni *et al*. [2012], the lowest mean value of seed protein content was found in NS-H-6487 (14.4%), and the highest in NS-H-1206 (20.1%). Vranac had 17.5%, and Cepko 20.0% seed protein content. The protein content in the seed ranged from 21.7% (NS-H-1206) to 27.5% (NS-H-6317). The average protein content in the seed of confectionery hybrid Proteinac 94 was 21.43%, according to the results of Dijanović *et al*. [2003]. Dimitrov [1990] talked about the impact of selection on high sunflower seed protein content. The resulting cultivar was Obitel which had an average seed protein content of 22.3%, oil content of 42.6% and seed yield of 2.8 tha^{-1}.

Variability of protein content was rather low (3.3%).

CONCLUSION

According to the results obtained in this paper, the following conclusions can be reached:

- Results of ANOVA for seed yield showed highly significant differences for all sources of variation. Hybrids had the highest impact on seed yield (50.07%), while stand density (22.50%) and hybrid × stand density interaction (27.43%) almost equally contributed to seed yield.

– The highest average seed yield for all stand densities were found in NS-H-6485 (4.77 t ha^{-1}) and NS Gricko (4.43 tha^{-1}).
– The average seed yield for all hybrids significantly increased up to 50,000 plants per ha^{-1}, when it reached a value of 4.50 tha^{-1}, and then decreased.
– The content of seed protein additive (hybrid, stand density) and non-additive (hybrid × stand density) sources of variation showed high significance. Hybrids have the highest impact on the protein content at Rimski Šančevi location (70.07%), followed by stand density (18.74%), while the lowest proportion showed hybrid × stand density (5.19%).
– Significantly higher protein content was obtained in hybrid Cepko (16.94%), taking into account all stand densities. Above the general average protein content was achieved in hybrid Vranac (16.11%). Other hybrids had significantly lower protein content compared with the overall average, which stood at 15.02%.
– The highest protein content in the average for all six hybrids was at the lowest stand density (20,000 plants ha^{-1}), and then decreased up to highest densities. Between the last three stand densities (50,000, 60,000, 70,000 plants per ha^{-1}) there were no significant differences in protein content, but it was significantly lower than the overall average.

Study results may be helpful in recommending optimal sunflower stand density in this region.

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ПРИНОС СЕМЕНА И САДРЖАЈ ПРОТЕИНА СУНЦОКРЕТА У ЗАВИСНОСТИ ОД ГУСТИНЕ СЕТВЕ

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САЖЕТАК: Циљ истраживања био је да се испита ефекат густине сетве на принос семена и садржај протеина код хибрида сунцокрета. Пољски оглед изведен је на локалитету Римски шанчеви. Испитивано је шест хибрида сунцокрета. Пет хибрида су конзумног типа (НС Голиат, НС Слатки, НС Грицко, Вранац и Цепко), а један је за исхрану птица (NS-H-6485). Оглед је постављен по потпуно случајном блок систему (RCBD), у четири понављања. Сетва је изведена са шест

густина (од 20.000 до 70.000 биљака по хектару, са кораком од 10.000 биљака по хектару). Анализа варијансе (ANOVA) је показала да су ефекти хибрида, густине сетве и интеракције хибрид × густина сетве били високо значајни за принос семена и садржај протеина. Највећи принос семена, на основу просека свих густина сетве, показали су хибриди NS-H-6485 (4,77 t ha^{-1}) и НС Грицко (4,43 t ha^{-1}). Просечан принос хибрида значајно се повећавао до 50.000 биљака по хектару, када је постигнут принос од of 4,50 t ha^{-1}, а затим је опадао. Узимајући у обзир све густине сетве, значајно највећи садржај протеина постигао је хибрид Цепко (16,94%). Садржај протеина изнад општег просека имао је и хибрид Вранац (16,11%). Највећи садржај протеина у просеку за свих шест хибрида био је код најмање густине сетве (20.000 биљака по хектару), а затим се смањивао до већих густина. Резултати су показали да густина сетва има значајан утицај на принос семена и садржај протеина код хибрида сунцокрета.

КЉУЧНЕ РЕЧИ: хибрид, интеракција, садржај уља, принос зрна, густина сетве, сунцокрет

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BREEDING OF THE COMMON KINGFISHER *Alcedo atthis* AT THE BORAČKA RIVER

ABSTRACT: The breeding population of the common kingfisher *Alcedo atthis* was studied in the Boračka River area in 2006 and 2007. A high breeding density was documented in 2007 with five breeding pairs recorded along the 1.4 km section of a stream habitat. All of the studied nests were placed in vertical banks without excessive riparian vegetation, while the distance between adjacent nests ranged 120–430 m. The same nest holes were used in both years, although birds excavated a couple of new ones in 2007. One pair bred in two consecutive years; the same pair had two breeding attempts in 2007, while three breeding attempts were recorded for one male. Birds used the same holes for subsequent clutches or excavated new nests. Also, one nest was used by different pairs in the same breeding season. In the study period 21 individuals were banded – none of the juvenile individuals was recaptured, suggesting that the fledglings dispersed shortly after they had left their nests. Also, none of the breeding individuals was recaptured at the river outside the breeding season.

KEYWORDS: Kingfisher, *Alcedo atthis*, Serbia, breeding, population density

INTRODUCTION

The common kingfisher, *Alcedo atthis*, is widely distributed throughout the Palearctic and Indo-Malayan region where it inhabits different types of water habitats with available aquatic prey and suitable nest sites, such as streams, rivers, canals, drains, lakes, fishponds and occasionally seashore bays [Cramp 1985; Fry *et al.* 1992]. Kingfishers nest in tunnels, usually excavated in steep stream or river banks, occasionally in holes in walls or tree stumps [Eastman 1969; Morgan and Glue 1977]. In Europe, breeding season starts in mid-April and ends at the beginning of September. In that period, birds typically have two broods, occasionally three [Cramp 1985].

Populations of the common kingfisher have been experiencing long term declines across the European part of the species range, most likely due to river

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pollution. For that reason, the common kingfisher is classified as a vulnerable species in Europe [BirdLife International 2015]. In Serbia, the common kingfisher has the status of a breeding species and year round resident. During the period 1990–2003, its breeding population was estimated at 1,200–1,800 breeding pairs with stabile population trend [Puzović *et al.,* 2003]. However, common kingfishers were rarely subjects of ornithological research in Serbia, hence, information on this species can be found mainly in ornithofaunistic reports. Matvejev [1950] confirmed the presence of common kingfishers at 37 locations in Serbia, while Simić [2004] examined seasonal changes in the occurrence of kingfishers on the Danube near Belgrade, in the period 1993–1996, concluding that these birds are the most abundant from September to November and very rare from February to June. In the EMERALD network in Serbia, the presence of the common kingfisher had been confirmed at 144 locations [Sekulić and Šinžar-Sekulić 2010].

Breeding of common kingfishers has never been investigated in Serbia. Therefore, the major goals of this study are to examine breeding habits of this species and to determine how many pairs of kingfishers can breed along a stream habitat that is, due to presence of steep banks and available fish prey, suitable for their nesting. Better understanding of breeding biology of common kingfishers is necessary for accurate estimates of their population trends and sizes.

MATERIAL AND METHODS

The Boračka River is ~9 km long tributary of Gruža accumulation, central Serbia, that along the entire course flows through arable land [Čomić and Ostojić 2005]. In the study section, shallow main stream alternates with deeper pools, with rocks that dominate the bottom in riffles, gravel and sand in pools, while mud is the prevalent substrate close to the accumulation. Turbidity varies from low in shallow pools to high after heavy rains. In the lower reaches, the river is surrounded by high banks and tree cover mainly of willow (*Salix* spp.), poplar (*Populus* spp.) and black locust (*Robinia pseudoacacia*).

I examined breeding habits of kingfishers along the 1.9 km section of water course close to the junction of the river and accumulation, during the breeding seasons in 2006 and 2007. In 2006 I examined 1 km of the water course beginning from the accumulation, while in 2007 I examined additional 900 m of the water course. Kingfishers were captured in nylon mist nets set across the river, close to kingfisher nests, and banded with metal bands (issued by the National Center for Animal Marking). Sex and age of captured birds were determined following Cramp [1985] and Baker [1993]. To examine if birds used their breeding areas outside the breeding season, I continued mist-netting throughout the fall and winter period.

All nests were located by walking along the stream; I found six nests that are from now on referred to as nests A – F. I used several parameters to confirm the breeding activity of individuals within nest tunnels – adult birds that where flying in or flying out of nests, usually with fish in their beaks, fresh feces on

the bank bellow nest holes and calls of chicks. In addition, three nest tunnels were examined with a camera during the breeding season in 2007. I assumed that adult individuals that were captured two or more times in mist nets close to a particular nest were a breeding pair of that nest. On several occasions, individuals flew into mist nets directly from their nests, which verified the occupancy of nests. The distance between nests was determined using global positioning system (GPS; Garmin eTrex Vista).

RESULTS

During the study period 21 individuals were banded, nine adults (four males and five females) and 12 juveniles. None of the adult birds and juveniles banded during the breeding season was recaptured on the river during the fall and winter period. Two nests were located in 2006, but only one breeding pair was identified, while six nests were detected in 2007, when four breeding pairs were identified and assumed the presence of a fifth one (Table 1). All of the studied nests were placed in vertical banks without excessive riparian vegetation. The distances between recorded nests were: A – B 150 m, B – C 120 m, C – D 450 m, D – E 430 m and E – F 260 m, while the distances from the accumulation were: 340 m (A), 490 m (B), 610 m (C), 1,060 m (D), 1,490 m (E) and 1,750 m (F) (Figure 1).

Figure 1. The position of kingfisher nests (A-F) in the study area (Boračka river)

Breeding season 2006

Along the 1 km long section of the river, only nests B and C were detected. A breeding pair from the C-nest was identified; a male (further in text m_1) and female (f_1) were captured twice from June 15 – July 7. The activity of the C-nest was not registered throughout August. The activity of the B-nest was confirmed on May 26, although the breeding pair was not identified. That nest was inactive in July, but its activity was documented again at the beginning of August, when chirping of chicks was recorded and the female f_1 was captured by the nest. A single recapturing of the female f_1 by the B-nest does not confirm that she bred there, but it indicates that she may have laid the second clutch in that nest.

Breeding season 2007

Along the 1.9 km long section of the river, all six nests were recorded. Throughout May the activity of four nests was confirmed – A, B C and E; the A-nest was newly excavated (not recorded in 2006), while the E-nest was detected in the section that was not examined in 2006. In that period, two breeding pairs were identified; a male (m_2) and female (f_2) that bred in the B-nest and the male m_1 and female f_1 that bred in the C-nest. In addition, a male (m_3) was captured with a fish in his beak in front of the E-nest, which suggests that he may have bred there. A breeding pair from the A-nest was not identified. However, on June 1 fledglings were recorded with a camera, both in nests A and C, while eggs were registered in the B-nests. This indicated the presence of a distinct breeding pair in the A-nest, given that it is less likely that the m_1-f_1 and m_2-f_2 pairs were able to raise two broods simultaneously.

During June and July three nests were active. In June, a breeding pair m_1-f_1 started their second brood in a newly excavated D-nest, while a new pair, a male (m_4) and female (f_4) inhabited the C-nest. The F-nest was registered in March 2007, although its activity was confirmed for the first time at the end of June when the male m_3 was captured there. The same male was captured in front of that nest at the beginning of July again, but he was also captured once in June and at the end of July at the E-nest. Thus, it is possible that after he had his first brood in the E-nest (in May and June) he stared his second brood in the F-nest at the beginning of July and then returned to the E-nest for the third brood at the end of July. His partner at the end of July in the E-nest was a female f_3.

In the breeding season 2007 nine hunting perches were recorded, they were recognized based on the presence of bird feces containing fish scales. Perches were placed 20–150 cm above the water level, while the depth of water pools below them ranged 20–105 cm, although measurements were conducted at the end of June, when the water level was very low.

Table 1. The nest activity in the breeding seasons 2006 and 2007

nest		A	B	C	D	E	F
2006	I brood	Did not exist	Active, breeding pair unidentified	Active, breeding pair m_1-f_1	Did not exist	Unknown	Unknown
	II brood	Did not exist	Active, breeding pair unidentified (possibly f_1)	Inactive	Did not exist	Unknown	Unknown
2007	I brood	Active, breeding pair unidentified	Active, breeding pair m_2-f_2	Active, breeding pair m_1-f_1	Did not exist	Active, breeding pair unidentified (possibly m_3)	Inactive
	II brood	Inactive	Inactive	Active, breeding pair m_4-f_4	Active, breeding pair m_1-f_1	Inactive	Active, male m_3
	III brood	Inactive	Inactive	Inactive	Inactive	Active, breeding pair m_3-f_3	Inactive

DISSCUSION

The Boračka River is suitable for breeding of common kingfishers, providing that five pairs bred there along the 1.4 km of water course. I was not able to determine the exact sizes of their breeding territories; however, based on flight directions of captured birds, I concluded that kingfishers captured prey upstream and downstream from their nests. Most likely, the birds captured all prey at the Boračka River, given that it is the only water course in that area and that the Gruža accumulation near the river junction lacks appropriate hunting perches.

The breeding densities of common kingfishers are variable. Typically, breeding pairs are separated around 1–2 km along a water course or even more [Kumari 1978; Glutz and Baurer 1980; Čech 2006]. However, in spite of documented territorial behavior of these birds [Eastman 1969; Kumari 1978], the breeding density recorded at the Boračka River is not unusual. Thus, simultaneously active nests were separated 150 m in Scotland [Brown 1935], 125 m and 200 m in Sweden [Svensson 1978], 300 m in Estonia [Kumari 1978], 100 m in Japan [Sayako and Tatsuhiko 2002], 50 m in Czech Republic [Čech 2006], while four breeding pairs were recorded along 650 m of a water course in Belgium [Libois 1994]. Breeding territory size of the common kingfisher depends on the availability of food and nest sites together with the overall population level [Libois 1997]. In the Boračka River area, breeding kingfishers reached the high density most likely due to presence of river banks suitable for nesting, as well as due to available prey and foraging sites. It is not known how such nest distribution affected reproductive success of individuals as overcrowding

often leads to increased incidence of aggressive encounters between neighboring breeding pairs [Clancey 1935; Boag 1982].

Common kingfishers can use suitable nest sites many years [Eastman 1969; Kumari 1978]. At the Boračka River, the same nest was used in consecutive breeding seasons by one breeding pair. Thus, the breeding pair m_1-f_1 bred in the C-nest in both study years, while in 2007 the same pair excavated a new nest for the second brood. Excavation of additional nesting tunnels within the breeding season is not uncommon among kingfishers and thus subsequent broods may overlap [Brown 1935; Eastman 1969; Kumari 1978]. The pair m_1-f_1 most likely started building a new tunnel while still taking care of the young from the first brood, given that I recorded fledglings in the C-nest on June 1 2007, whereas their parents were captured in front on the D-nest on June 13.

Under favorable environmental conditions, birds can continue using breeding territories as feeding territories throughout the fall and winter [Boag 1982]. However, I did not recapture any of the breeding individuals outside the breeding season even though the Boračka River was mainly ice-free during the winter 2006/07. It is not known where these birds spent the winter. In addition, I did not recapture any of juvenile individuals banded throughout the breeding period as the young disperse from the nesting place just a few days after leaving their nests [Clancey 1935; Eastman 1969; Boag 1982].

CONCLUSION

Stream habitats with suitable vertical banks, foraging areas and available fish prey can support relatively high breeding densities of common kingfishers in spite of documented territoriality of these birds. Providing documented breeding dynamics of kingfishers, such as excavation of new nest tunnels for the second clutch, as well as utilization of one nest by different pairs within the same breeding season, more research is necessary for correct estimates of the size and trends of their populations.

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ГНЕЖЂЕЊЕ ВОДОМАРА *Alcedo atthis* НА ПОДРУЧЈУ БОРАЧКЕ РЕКЕ

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РЕЗИМЕ: Гнездилишна популација водомара *Alcedo atthis* праћена је на Борачкој реци у току 2006. и 2007. године. Високу густину популације забележила сам 2007. године, када се пет парова гнездило дуж 1.4 km речног тока. Сва гнезда су лоцирана на вертикалним земљаним одесцима без вегетације, приликом чега су растојања између гнезда износила 120–430 m. Иста гнезда су коришћена током обе сезоне, а током лета 2007. године птице су издубиле неколико нових дупљи. Један гнездилишни пар имао је два покушаја гнежђења у 2007. години, док су исте године забележена три покушаја гнежђења једног мужјака. За поновљена легла птице су користиле иста гнезда или су издубиле нова. Такође, током сезоне 2007. забележила сам коришћење једног гнезда од стране два различита пара. У току истраживачког периода маркирана је 21 јединка, ниједна јувенилна јединка није поново уловљена након датума прстеновања, што имплицира да полетарци напуштају гнездилишне територије врло брзо након напуштања гнезда. Такође, ниједна адултна јединка није поново уловљена на реци ван сезоне гнежђења.

КЉУЧНЕ РЕЧИ: водомар, *Alcedo atthis*, Србија, гнежђење, густина популације

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WITHERS HEIGHT OF PIG – *Sus scrofa domestica* L. 1758, DOMESTIC COW – *Bos taurus* L. 1758 AND SHEEP – *Ovis aries* L. 1758 AT THE "GORNJA ŠUMA" ARCHAEOLOGICAL SITE (NOVI SAD)

ABSTRACT: In spring 2012, osteological material was collected at the "Gornja Šuma" site (site no. 47), located in the territory of Novi Sad, and it was dated to the early 9th century. The withers heights of pig – *Sus scrofa domestica,* domestic cow – *Bos taurus* and sheep – *Ovis aries*, as the three most dominant species at this archaeological site, were analysed based on the length of bones and according to various authors [Boessneck 1956; Zalkin 1960; Matolcsi 1970; Teichert 1975]. It was determined that in these three species the withers heights mostly corresponded to the data from the Middle Ages.

KEYWORDS: withers height, *Sus scrofa domestica, Bos taurus, Ovis aries, "*Gornja Šuma" archaeological site

INTRODUCTION

Osteological material has been collected at the archaeological sites in Vojvodina since 1930s. This material comes from different periods, such as: **Neolithic Age** – New Stone Age (6000–3200 B.C.), **Eneolithic Age** – Copper Age (3200–2000 B.C.), **Bronze Age** (2000–950 B.C.), **Early Iron Age** (950–300 B.C.), **Later Iron Age** (4th century B.C. – 1st century A.D.), **Roman period** (1st-4th century A.D.), **Migration period** (4th–9th century A.D.), and **Middle Ages** (9th century – 1526 A.D.) [Cerović *et al.*, 1997].

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Certain animal species, due to their characteristics useful for humans, were subjected to the process of domestication. Main characteristics of domestication are, predominantly, anatomical-morphological changes related to the reduction in body dimensions, changes in the structure and density of bones, thinning of cortical part of bones, expanding of modular channel, accompanied by changes in behaviour and physiology. The domestication process is long and depends on many factors [Bökönу 1974]. Changes occur due to anthropogenic effect on species, artificial selection and reproductive isolation. Through selection, humans favoured certain animal characteristics that suited them, and they tried to pass these characteristics to following generations, while 'unfavourable' characteristics were eliminated over time. The human influence and reproductive isolation led to differentiation between domestic and wild populations.

The domestication process started as early as the Neolithic Age (circa 6,000 B.C.). The first examples of domestication of animals in Europe can be found in Thessaly in the 7th century B.C. (first goats and sheep). Only animals living in herds could be domesticated (large herbivores), most likely in several phases: non-systemic hunting → selective hunting → tracking herds → enclosing herds → herding. Certain species (such as dog and pig) were probably domesticated through certain symbiosis with humans, especially where permanent settlements started to appear [Clutton et Brock 1999].

The process of domestication of animals did not have the same direction and intensity. Differences that can be observed in archaeological material are the result of different conditions, of which the most important ones are ecological factors and characteristics of breeding that are specific for certain cultures [Blažić 2005 a].

The goal of this paper is to analyse values of the withers height of pig – *Sus scrofa domestica* L. 1758, domestic cow – *Bos taurus* L. 1758, and sheep – *Ovis aries* L.1758, as three most common species at the "Gornja Šuma" medieval archaeological site (site no. 47), located in the territory of Novi Sad, and to draw comparisons with values from other archaeological sites of the same and earlier dates, in order to track changes in body dimensions of these animals throughout history.

DESCRIPTION OF THE SITE

The "**Gornja Šuma**" site (site no. 47) is located in north-west part of Novi Sad city area, in the zone of E–75 motorway. It is situated on southern part of Bačka loess plateau, in the hinterland of old high Danube bank.

During the construction of the new energy corridor around Novi Sad (gas and oil pipelines), archaeological excavations and researches were done at this site under supervision of the Institute for the Protection of Cultural Monuments of the City of Novi Sad. These works included partial excavation of a medieval settlement dated to the 9th century in the length of circa 800 metres. Apart

from household items, a large amount of bones of domesticated animals was also found.

MATERIAL AND METHODS

The osteological material at the "Gornja Šuma" site (site no. 47) was collected and analysed in the period March–April 2012.

Determination of Vertebrata species was done using the key Schmidt [1972] and comparative osteological collections.

Measurement of the osteological material was done according to proposed guidelines given by Driesch [1976]. Calliper of 0.1 mm precision and digital calliper of 0.01 mm precision were used for measurement. The measurement box was used for bone parts that were not in the same plane.

The withers height of *Sus scrofa domestica* was calculated according to Teichert [1969], for the species *Bos taurus* this parameter was calculated based on the coefficient given by Boessneck [1956], Zalkin [1960] and Matolsci [1970], while the withers height of *Ovis aries* was calculated according to Teichert [1975].

RESULTS AND DISCUSSION

The "Gornja Šuma" site (site no. 47) hosts remains of a settlement dated to the early Middle Ages, more precisely to the 9th century. After the analysis of animal bone remains, the members of the following classes were recorded: Mammalia, Aves and Osteichthyes. Similarly to other sites in Vojvodina [Radmanović *et al*., 2013; 2014 a,b] and Europe [Bökönу 1974] mammals are dominant, and the total share of pig – *Sus scrofa domestica*, domestic cow – *Bos taurus* and sheep – *Ovis aries* equals 94.15%. With 45.45% of the total sample of bone fragments, pig was the dominant species, which is not usual for sites from this period because then pork was less used in diet due to religious reasons [Nedeljković 2008]. Therefore, this archaeological site in the territory of Novi Sad is very interesting. The second most numerous species was domestic cow (26.88%) and sheep was the third one (21.82%).

The withers height, as one of the characteristics of domestication, can be calculated only using whole bones.

Withers Height of Pig – *Sus scrofa domestica*

There were only two whole bones in the entire osteological material of *Sus scrofa domestica* from the "Gornja Šuma" site (site no. 47): femur and tibia. By multiplying their maximum length with the coefficient given by Teichart [1969], it was calculated that the withers height based on femur was 69.9 cm, and based on tibia 82.1 cm.

Blažić [1988] analysed the withers height of *Sus scrofa domestica* from the Early Iron Age from the "Gomolava" site (Hrtkovci), and stated that this parameter was 67.2 cm based on the calcaneus length, 69.8 cm based on astragalus length, and 65.2 cm based on scapulae length. The same author analysed in 2010 osteological material from the "Asfaltna Baza" site (Zemun), also from the Early Iron Age, and based on measurements of astragalus and calcaneus stated that the withers height of this domesticated species was 79.26 cm and 77.52 cm respectively.

Based on astragalus of domesticated pig from the "Vranj" site (Hrtkovci), which belongs to the Roman period, Blažić [1993] calculated the withers height in the range between 68 and 77 cm, and concluded that these values were closer to autochthonous individuals than to so called improved Roman race.

Nedeljković [2008] stated that at the "Sirmium 85" site (Sremska Mitrovica) the withers height of domestic pig in the Roman period was 76.23 cm, and this result was based on calcaneus length.

Bartosiewitz [1996] stated that the withers height of pig from the Middle Ages in Hungary was 66.1 cm, based on the length of humerus.

Blažić [1999] stated that, at the "Ras-Gradina" medieval site (Novi Pazar), the value of withers height of domestic pig was in the range between 52 and 91 cm with mean value of 73 cm, but it was not stated which bones were used to calculate it. The values from the "Gornja Šuma" site fit these data.

Withers Height of Domestic Cow – *Bos taurus*

In Central and Eastern Europe, domestic cow (*Bos taurus*) is almost always the most dominant or one of the most dominant species of bred animals. This is also the case with sites in the territory of Vojvodina [Radmanović *et al*., 2013; 2014 a,b; 2015] and the entire Pannonian Basin. Its presence at sites from various periods in Romania is discussed by Stanc *et al.* [2010], noting that in the Medieval Ages its contribution in the mammal fauna was between 35% and 65%, and in domesticated mammal fauna between 45% and 65%. Such high share is the result of multi-functionality of this animal – its meat and milk are used in diet, strength for pulling, and horns and bones for producing various objects. No other species of domestic animals has surpassed the economic value of domestic cow so far [Bökönyi 1974].

As already mentioned, the withers height of an animal is calculated using whole bones, and in case of domestic cow, by multiplying their maximum length with coefficients given by Boessneck [1956], Zalkin [1960] and Matolcsi [1970]. In the entire *Bos taurus* sample from the "Gornja Šuma" site (site no. 47), there were only four bones where maximum length could be measured. These were three metatarsal bones and one tibia. Some earlier works [*Archaeozoologie*, 1989] state that distal epiphysis of metatarsus shortens between 2 and 2.5 years of age. Based on the fact that all three of these bones had epiphyses, we can conclude that the given individuals were younger than 2 years.

Bökönyi [1974] made an overview of osteological material found at sites in Central and Eastern Europe, on the basis of historical periods. Using the

above-mentioned data, mean values of lengths of long bones were calculated on the basis of special periods, and after that withers heights were calculated according to the given mean values. The results are in Table 2.

Table 1. Calculated values of withers heights (expressed in cm) of domestic cow *Bos taurus* from the **"Gornja Šuma" archaeological site (site no. 47)**

Author and year	Bone		
	Metatarsus		Tibia
	Min	Max	
Boessneck [1956]	109.6	116.8	
Zalkin [1960]	104	108.7	
Matolcsi [1970]	102.8	109.5	114

Table 2. Calculated values of the withers height of *Bos taurus* based on the data by Bökönyi [1974], expressed in cm

Bone	Copper Age	Bronze Age	Iron Age	Roman period	Migration period	Avar period	10^{th}–13^{th} centuries	14^{th}–17^{th} centuries
Humerus			110.5		107.6	111.9	98.5	
Radius	135.5	119.5	112	126	107.5	119.2	123	117.8
Metacarpus	121.5	116.7	112.6	125.8	112.3	119.8	109	120
Femur			102.4			110		
Tibia	127.7		118.3	122		106		122
Metatarsus		117	111	122.7	120.4	120	116	136.4

The results of the withers height of domestic cow calculated using the long bones from the "Gornja Šuma" site (site no. 47) are expected, taking into consideration their date, and they correspond to the values from other sites in the region from the same period. Domestic cow from the Copper Age was bigger, like the one from the Roman period (it is believed that bigger races were introduced from the territory of present-day Italy in this period). The lowest withers height was calculated according to the average length of humerus from the period between the 10^{th} and 13^{th} centuries (98.5 cm), and the highest was calculated according to the radius from the Copper Age (135.5 cm) and metatarsus from the period between the 14^{th} and 17^{th} centuries (136.4 cm) (Table 2). These results show the tendency of decreasing of withers heights of domestic cow over time. The withers heights in the Roman period and period between the 14^{th} and 17^{th} centuries deviate from this rule, which can be related to the introduction of larger cow races from other areas by Romans and Turks.

El Susi [2007] collected data on domestic animals that were excavated during the first decade of this century at archaeological sites from the Early Neolithic in the territory of Romanian Banat and Transylvania. Using four

figures for the length of metacarpus, a mean withers height was calculated, and it was 125.6 cm, which is 10–13 cm higher than the withers height of domestic cow at the "Gornja Šuma" site.

El Susi [1998] determined the withers height of domestic cow at medieval sites in the territory of Romanian Banat. The withers height of males was between 112 and 118 cm, and of females between 107 and 108 cm.

Blažić [1992 c] worked with remains of animal bones from the sites along the motorway through Srem. At the "Zlatara" site (Ruma) from the Neolithic period, the calculated withers height of domestic cow was between 102.3 and 108.2 cm. At the "Livade" site (Sremska Mitrovica) from the Eneolithic period (Copper Age), the withers height was estimated according to metacarpus length and it was 115 cm. At the "Vrtlozi" site (Šimanovci) from the Early Iron Age, the calculated withers height of domestic cow was between 109.6 and 112.3 cm. In the Late Iron Age, the withers height was between 106 and 109 cm at the "Tromeđa" site (Pećinci) and 118 cm at the "Vrtlozi" site (Šimanovci). The withers height increased in the Roman period due to the introduction of cows from other parts of Europe. Therefore, the authors stated that at the "Malo Kuvalovo" site (Krnješevci) the withers height of indigenous cows was 123 cm, and of the introduced ones 142 cm. At other sites in Vojvodina, the withers height of indigenous cows was between 104.6 and 123.8 cm, and of introduced ones between 125.5 and 136.3 cm. Blažić [1993] also discussed animal bone remains from the "Vranj" site from the Roman period and determined that the withers height of indigenous cows was between 109 and 118 cm, and of introduced ones between 124 and 131 cm.

Blažić [1988] discussed material from the "Gomolava" site from the Early Iron Age and determined the withers height according to the length of metacarpus and metatarsus. The withers height of these domestic cows was 109.8 and 106 cm, which is similar to the height of cows from our research site.

Bökönyi [1988], in the analysis of the osteological material from the "Kalakače" site (Beška), a settlement from the Early Iron Age, used the measures of radius, metacarpus, tibia and metatarsus to calculate the withers height. Based on the mean length of radius, the calculated height was 116.5 cm, based on the length of metacarpus it was 116.3 cm, on the length of tibia 107.2 cm, and on the length of metatarsus 117.9 cm. The withers heights in this period do not significantly differ from the withers heights calculated at the "Gornja Šuma" site.

Bökönyi [1976] discussed animal bone remains from the site in the southern part of Hungary, which was inhabited by the Sarmatians. Based on his data on the length of radius, tibia and metatarsus, the following withers heights of domestic cow were calculated: for radius the height was 115 cm, for tibia 117 cm, and for metatarsus 122 cm. The above-mentioned sites were dated to the Migration period. The withers height of domestic cow from these sites is somewhat larger that the withers height of domestic cow whose remains were found at the "Gornja Šuma" site.

Bartosiewitz [1996] published data on the length of bones from the medieval sites and those from the Ottoman period. The withers height was calculated according to the length of the long bones (humerus, radius, metacarpus, femur,

tibia, metatarsus) in the 14th century (118 and 120 cm), in the Late Middle Ages (119 cm), while the Ottoman period showed increase in the withers height (between 118 and 165 cm).

Considering the withers height of domestic cow, the osteological material from the "Otok" medieval site in Slovenia [Bartosiewitz 2006] corresponds with the "Gornja Šuma" site. The mean height of domestic cow from this site was circa 108 cm in layers of unknown date, 106.5 cm in layers from the 12th century; the lowest specimens were in layers from the 13th century (96 cm), and the highest ones in layers from the 14th century (117 cm).

Clason [1979] discussed the osteological material from the "Gomolava" site from the Vinča (Neolithic) and La Tène (Late Iron Age) periods, and determined the withers height between 102 and 125 cm in Vinča, and between 92 and 113 cm in La Tène period. The same author [1980] determined the withers height of domestic cow from the "Starčevo" Neolithic site (Starčevo culture), which was between 120 and 116.4 cm.

Withers Height of Sheep – *Ovis aries*

In the entire sample from the "Gornja Šuma" archaeological site, there are 17 bone fragments of sheep – *Ovis aries* with maximum lengths measured for: 8 calcaneus bones, 3 radius bones, 2 metacarpus bones, 2 astragalus bones, and one tibia and metatarsus. The withers height was calculated by multiplying their maximum lengths with the coefficient given by Teichert [1975], and it is given in Table 3. Teichert gives coefficients for sub-adult and adult individuals. In order to calculate the withers height based on bones of unknown age, the mean value of both above-mentioned coefficients was used.

Table 3. Calculated values of the withers height of *Ovis aries* from the **"Gornja Šuma" archaeological site (site no. 47)** (expressed in cm)

Bone	N (number of bones)	Min–Max	$\bar{X}$
Radius	3	62.44–68.57	66.53
Metacarpus	2	57.06–63.79	60.42
Tibia	1	60.95–60.95	60.95
Astragalus	2	60.23–61.27	60.75
Calcaneus	8	54.12–70.07	62.73
Metatarsus	1	62.38–62.38	62.38
$\bar{X}$			62.77

Out of seventeen whole bones from the researched site, eleven are of unknown age. The remaining six bones belong to individuals of known age, one bone (metacarpus) belongs to a sub-adult individual, one bone (radius) belongs to a sub-adult/adult individual, and four bones (three calcaneus bones and one tibia) belong to adult individuals.

The withers height of sheep from the "Gornja Šuma" archaeological site is between 54.12 and 70.07 cm, and the mean value is 62.77 cm. The smallest and largest values are calculated according to the calcaneus length of adult individuals (Table 3).

As it was already stated, El Susi [2007] analysed the bones of domestic animals from the Early Neolithic period in the territory of Romanian Banat and Transylvania. The withers height was calculated according to the length of scapula, radius, metacarpus and metatarsus bones. In the territory of Banat, the withers height of sheep was between 56.9 and 60.3 cm (mean value – 58.9 cm), while in the territory of Transylvania, the height was between 48.5 and 65 cm (mean value – 56.7 cm). The above-mentioned values in this period are somewhat lower in comparison to the values from the "Gornja Šuma" site. The preliminary analysis of sheep bones indicated that these animals were smaller during the Early Neolithic period, the withers height being 62–65 cm (possibly of rams). The author stated that the obtained data corresponded with the withers height of sheep in south-eastern Europe.

Blažić [1992a] analysed fauna remains from the "Donje Branjevine" site (Deronje) from the Neolithic period and determined withers height of sheep on the basis of the length of metacarpus. The calculated withers height, according to Zalkin method [1960] was 58 cm. The author stated that the calculated values were somewhat smaller than mean values in this part of the Pannonian Basin.

Blažić and Radmanović [2011] discussed the osteological material from the late Vinča settlements of "Crkvine" and "Belež" (Kolubara basin) from the Neolithic period and cited Dimitrijević [2006], according to whom the estimated withers height of sheep was between 47.8 and 56 cm at the "Vinča – Belo Brdo" site.

Blažić [1992c] calculated the withers height of sheep from the "Bregovi – Atovac" site (Kuzmin) from the Early Iron Age using Zalkin method [1960]. Based on the longest length of metacarpus, the withers height was 65 cm, and based on the longest length of metatarsus it was 70 cm. In this paper, the author cited Bökönyi [1974], according to whom the withers height of sheep from the Neolithic was between 57 and 60 cm, and from the Eneolithic (Copper Age) between 57 and 74 cm.

Blažić [1988] analysed the osteological material from the "Gomolava" site from the Late Iron Age, and based on the length of metacarpus, determined the withers height of sheep to be 57.3 cm. In 2010, the same author estimated the withers height of sheep on the basis of three whole metatarsus bones, one metacarpus bone and one calcaneus bone of sheep from the "Asfaltna Baza" site, also from the Early Iron Age, and it was between 52.47 and 60.1 cm (mean value 56.9 cm).

Bökönyi [1988] calculated the withers height of sheep from the "Kalakača" site (settlement from the Early Iron Age) according to the length of metacarpus and using the Zalkin method [1960]. He came to the conclusion that it was between 56.62 and 60.61 cm, and that it corresponded to the mean height of this animal from the Carpathian basin in the above-mentioned period.

Bökönyi [1981] also analysed material from the Early Iron Age in the territory of ex-Yugoslavia Danube basin, and stated that the withers height of one individual sheep from the "Gradina" site (Vašica) was 62.71 cm, which is similar to the mean height of sheep from the "Gornja Šuma" site.

Blažić [2005 b] analysed the osteological material from the "Kale" site (Krševica) from the Late Iron Age, and used measures of whole metacarpus, metatarsus and radius bones of adult individuals for calculating the withers height. Based on the length of these bones, the calculated mean value of the withers height was 62.21 cm, which is similar to values obtained from the "Gornja Šuma" site. The author cited Bökönyi [1974], according to whom the mean withers height of metacarpal bone from the Iron Age in Central and Eastern Europe was 57.5 cm, and of metatarsal bone 61 cm.

Blažić [1992 b] stated that the withers height of sheep in the Iron Age was between 51 and 69 cm, on the basis of ten analysed sites in Vojvodina.

Blažić [1993] also analysed animal remains from the "Vranj" site from the Roman period, and on the basis of the whole metacarpus and metatarsus bones estimated that the withers height of sheep was between 51.2 and 73.3 cm.

Nedeljković [2008] analysed fauna remains from the "Sirmium 85" site and stated that the mean withers height of sheep from the Roman period was 60.79 cm. These data were based on the length of whole metacarpus, metatarsus and radius bones. The author cited Bökönyi [1982], who stated that the new, higher races of sheep, which replaced the indigenous populations in the Roman period, most likely originated from Greece. Nedeljković [2008] also cited Bökönyi [1984], who stated that the difference in the withers height of Roman races can be up to 10 cm when compared to the indigenous specimens. Sheep bone fragments from the 5th and 6th centuries (Migration period) were also found at the "Sirmium 85" site. Two whole metacarpal bones indicated the withers height of 59.83 cm, and one metatarsal bone the height of 61.54 cm. The mean value of 60.68 cm deviates insignificantly from the mean value of the withers height of Avar sheep that is up to 60 cm. Osteological material originating from the period between the 16th and 18th centuries was also found at the "Sirmium 85" site. The withers height was calculated according to the two whole metacarpal bones of adult sheep, and it was 70.65 cm and 63.74 cm, and the withers height based on tibia was 67.45 cm. The mean height of sheep in the Middle Age was 67.28 cm, based on this sample. The values from the "Gornja Šuma" site are somewhat lower when compared to the above-mentioned data from the "Sirmium 85" site.

Blažić [1999] analysed the osteological material from the "Ras-Gradina" medieval site, and calculated the withers height of sheep according to the long bones (metacarpus, metatarsus and radius) and using the coefficients given by Teichert [1975]. The calculated withers height based on metacarpus length was between 58.56 and 69.29 cm, based on metatarsus length between 57.3 and 69.16 cm, and based on radius length between 52.8 and 58.4 cm. The mean value of the withers height calculated according to the length of metacarpus and metatarsus was 62.58 cm, which corresponds to the values obtained from the "Gornja Šuma" site. The author stated that sheep reached the lowest withers

height in the Middle Ages, after the Copper Age. The withers height of domestic races was decreasing during the Migration period and wars, when almost all results of selection and development from the Roman period were destroyed. The mean height decreased by circa 5 cm when compared to the Roman period and it is between 51 and 64 cm in the Central and Eastern Europe [Bökönyi 1974].

Bartosiewitcz [1996] published data on the length of sheep bones from the medieval sites and those from the Ottoman period in Hungary. The withers height was calculated according to the length of metacarpus and metatarsus bones and it was between 55.2 and 71.7 cm in the 14th century, and between 63 and 74.5 cm in the late Middle Ages. The withers height of sheep in the Ottoman period was calculated according to metacarpus length and it was between 57.4 and 71.1 cm.

CONCLUSION

Osteological material was collected at the "Gornja Šuma" medieval site (site no. 47), located in the territory of Novi Sad, in spring 2012.

Domestic pig *Sus scrofa domestica* dominates in this material, which is unusual because pork was used less in diet during the Middle Ages due to religious reasons.

The withers height of **pig – *Sus scrofa domestica*** was calculated according to the length of whole femur and tibia bones and it was 69.9 cm and 82.1 cm respectively, which corresponds with the data from the Middle Ages.

The withers height of **domestic cow – *Bos taurus*** was calculated according to the length of one tibia and three metatarsus bones given by various authors [Boessneck 1956; Zalkin 1960; Matolcsi 1970]. The estimated withers height was between 102.8 and 116.8 cm, which corresponds with the general picture of small medieval cows.

The withers height of **sheep** – ***Ovis aries*** was calculated according to the length of 8 calcaneus, 3 radius, 2 metacarpus, 2 astragalus, one tibia and one metatarsal bones given by Teichert [1975], and it was between 54.12 and 70.07 cm, while mean value was 62.77 cm. Mean value of the withers height of sheep from the "Gornja Šuma" site either corresponds with or is slightly smaller than the values from the archaeological sites from the same period in Serbia and Hungary.

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ВИСИНА ГРЕБЕНА СВИЊЕ – *Sus scrofa domestica* L. 1758, ГОВЕЧЕТА – *Bos taurus* L. 1758 И ОВЦЕ – *Ovis aries* L. 1758 СА АРХЕОЛОШКОГ ЛОКАЛИТЕТА „ГОРЊА ШУМА" (НОВИ САД)

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РЕЗИМЕ: Са средњовековног локалитета „Горња шума" (локалитет бр. 47) који се налази у атару Новог Сада, у пролеће 2012. године сакупљен је остеолошки материјал. У овом материјалу доминирала је домаћа свиња *Sus scrofa domestica* што је необично јер је се свињско месо током средњовековног периода из религиозних разлога мање користило у исхрани. На основу дужине целих костију фемура и тибије, израчуната је висина гребена **свиње – *Sus scrofa domestica*** која је износила 69,9 cm, односно 82,1 cm, што се уклапа у литературне податке из средњовековног периода.

На основу дужине једне tibia-е и три metatarsus-а израчуната је висина гребена **говеда – *Bos taurus*** према различитим ауторима [Boessneck 1956; Zalkin 1960; Matolcsi 1970]. Процењена висина гребена износи између 102,8 и 116,8 cm, што одговара општој слици малих средњовековних говеда.

На основу дужине осам calcaneus-а, три радијуса, два metacarpus-а, два astragalus-а, једне tibia-е и једне metatarzal-не кости **овце – *Ovis aries*** према Teichert-у [1975] израчуната је висина гребена која се кретала између 54,12 и 70,07 cm, а средња вредност износила је 62,77 cm. Просечна вредност висине гребена овце са локалитета „Горња шума" уклапа се или је нешто нижа у односу на израчунате вредности са археолошког локалитета истог датовања са подручја Србије и Мађарске.

КЉУЧНЕ РЕЧИ: висина гребена, *Sus scrofa domestica*, *Bos taurus*, *Ovis aries*, археолошки локалитет „Горња шума"

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AN EXAMPLE OF DIFFERENT AHP STRUCTURING IN A FOREST MANAGEMENT PROBLEM

ABSTRACT: The paper investigates how different hierarchy structuring in analytic hierarchy process (AHP) may affect the final results in the decision-making process. This problem is analyzed in a case study of the Rila monastery forest stands in Bulgaria. There were three similar and mutually overlapped hierarchies defined. A decision maker evaluated all of them and after analyzing final results and consistency performance, he selected and revised the most appropriate hierarchy structure. Consistency check assisted in detecting the judgments which have strongly violated evaluation procedure. These mistakes are interpreted as a consequence of a large number of required pair-wise comparisons. The paper emphases the importance of properly defining hierarchy structure and recommends using consistency analysis as a guide and not as a directive for the revision of judgments.

KEYWORDS: analytic hierarchy process, Rila monastery forest, hierarchy structures, consistency parameters

INTRODUCTION

Analytic hierarchy process (AHP) [Saaty 1980] is widely used in forestry decision making [Balteiro and Carlos 2008; Samari *et al.*, 2012]. Usually, a single hierarchy structure is defined and the goal is to obtain the final decision [Leskinen and Kangas 1998; Coulter *et el.*, 2006; Srdjevic *et al.*, 2013]. In this paper, the aim is different; the focus is to address the problem of different hierarchy structuring and to analyze its influence on the final results. A real case study from the Rila monastery forest, Bulgaria, is used in this research. Chief forest manager (the third author of the paper) identified decision elements, defined problem structures – hierarchies and performed required pair-wise comparisons, expressing in this way his judgments about mutual importance of elements at given level versus elements in the upper level. The decision

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maker was faced with AHP methodology for the first time, while other authors regularly use AHP in their research and they were in charge for processing decision maker's evaluations. Hierarchy structures, developed by decision maker, were mutually overlapping to a major extent, i.e. they were considering the same goal, criteria, sub-criteria and alternatives sets, but these hierarchy elements were differently organized and not all levels were considered every time. There were three hierarchies defined: a three-level hierarchy with three criteria, a four-level hierarchy with three criteria and six sub-criteria, and a three--level hierarchy with six criteria. The goal and alternatives were always the same. The goal was stated as assessing forest stands' functionality in the Rila monastery forest. Along with recognition of the most effective stand in this regard, the obtained results should be a baseline for planning of allocation of future investments for four selected forest stands. In order to check the reliability of performed evaluations, consistency parameters were calculated for each matrix and for the hierarchies as a whole.

The research shows the major obstacles during the decision-making process. Conclusions delivered should be a guideline for defining hierarchy structures in AHP and they should improve its real life applications in forestry. General recommendation would be to define and evaluate several mutually overlapped hierarchy structures and to analyze which one is the most appropriate for the stated decision-making problem. Analysis can be guided by decision maker's personal opinion and expertise, but also by performing a consistency check for each matrix of comparison. Besides checking the parameter *CR* (consistency ratio), it is strongly recommended to check parameters *MV (*minimum violation)*, ED* (Euclidean distance) and *CM* (consistency measure) because they provide closer insight of the matrix evaluation. By taking into account the consistency performance and professional opinion, the decision maker can choose the most appropriate hierarchy structure and recheck his evaluations in order to conduct a reliable decision.

METHODS

AHP requires a well-structured problem, represented as a hierarchy. At its top is a goal; the lower levels moving downward contain criteria and sub-criteria, while the alternatives lie at the bottom level. The decision making process involves the evaluation of criteria, sub-criteria and alternatives in pair-wise manner, always with respect to the superior elements in the hierarchy. Comparisons of all elements in the hierarchy (criteria with respect to a goal, sub-criteria with respect to criteria, and alternatives with respect to sub-criteria) are made by using an appropriate ratio scale. Although several well-known scales are in use, the one known as the Saaty's scale [Saaty 1980] is most commonly used and referenced as the fundamental ratio scale.

Comparisons of elements in a certain level of a hierarchy with respect to a certain element in a higher level are made by filling the upper triangle of the symmetric positive reciprocal matrix $A(a_{ij})$ with numeric values from Saaty's scale.

The reciprocals of values from the upper triangle are inserted automatically into the lower triangle of the matrix. Values 1 are posted on the main diagonal.

$$A = \begin{bmatrix} 1 & a_{12} & \cdot & \cdot & a_{1n} \\ a_{21} & 1 & \cdot & \cdot & a_{2n} \\ \cdot & & & & \cdot \\ \cdot & & & 1 & \cdot \\ a_{n1} & & & & 1 \end{bmatrix} \tag{1}$$

The vectors of weights (w) of compared elements (with respect to superior element in a hierarchy) are extracted by the so-called prioritization method from the comparison matrix A. The procedure is identical in all nodes of a hierarchy, and matrices and belonging vectors are treated as local. In this paper, eigenvector method was used for calculating local priorities:

$$Aw = \lambda w,\ eT = 1 \tag{2}$$

where: w is the priority vector and λ is the principal eigenvalue of matrix A.

At the end, synthesis consists of obtaining the overall priority vector of alternatives with respect to a goal by multiplying local priority vectors of alternatives by the priority of their parent nodes and adding for all such nodes [Saaty 2008].

Consistency parameters

In the paper, four parameters were used for the consistency check. The parameter *CM* was calculated for each matrix separately, while parameters *CR, ED* and *MV* were calculated for each matrix and for the entire hierarchies.

Consistency ratio (*CR*) is the most widely used consistency parameter. It is associated with eigenvector method and has a defined threshold value of 0.1. The procedure for calculating the value is defined by Saaty [1980].

The Euclidean distance (*ED*) is a common measure of consistency contained in matrix of comparison. It shows overall distance between all the judgment elements in the comparison matrix and associated ratios of the weights from the derived vector w [Srdjevic 2005].

$$ED = \left[\sum_{i=1}^{n} \sum_{j=1}^{n} \left(a_{ij} - w_i / w_j \right)^2 \right]^{1/2} \qquad i, j = 1,2,...,n \tag{3}$$

The minimum violation (*MV*) sums up all violations related to the computed priority vector w and is calculated as (Golany and Kress, 1993):

$$MV = \sum_{i=1}^{n} \sum_{j=1}^{n} I_{ij} \qquad i, j = 1,2,...,n \tag{4}$$

where the rule for obtaining violations I_{ij} is

$$I_{ij} = \begin{cases} 1 \text{ if } w_i > w_j \text{ and } a_{ji} > 1 \\ 0.5 \text{ if } w_i > w_j \text{ and } a_{ji} \neq 1 \\ 0.5 \text{ if } w_i \neq w_j \text{ and } a_{ji} = 1 \\ 0 \text{ otherwise} \end{cases} \quad i, j = 1,2,\ldots,n \qquad (5)$$

The consistency measure (C*M*) is related to a single comparison matrix and shows how strongly evaluations within matrix violate transitivity rule (Koczkodaj, 1993):

$$CM(A) = \max\left\{\min\left\{\left|1 - \frac{b}{ac}\right|, \left|1 - \frac{ac}{b}\right|\right\} \text{ for each triad } (a, b, c) \text{ in } A\right\} \qquad (6)$$

Number of possible triads (judgments interrelated by transitivity rule) in *nxn* size matrix *A* is equal to $\frac{n(n-1)(n-2)}{3!}$ [Duszak and Koczkodaj 1994; Bozóki and Rapcsák 2008].

Case study description

Selected case study is located in the Rila monastery forest, in the south west of Bulgaria. Forest stands evaluated within AHP framework are labeled as: 1021b, 1023a, 1024g and 1024z. They are situated along an asphalt road with an easy access for tourists and harvesting teams. This was done deliberately to give the stands equal starting point. The stands area varies from 10 to 25 ha. A brief description of selected forest stands is presented in Table 1.

Table 1. Brief description of forest stands in the Rila monastery forest, southwestern Bulgaria

Stand №	Area [ha]	Species composition	Age of main canopy, [years]	Altitude, [m] a.s.l.	Growing stock, [m^3/ha]
1021b	15.4	Beech 10	160	1250	224
1023a	23.6	Fir 5 Spruce 1 Scots pine 1 Beech 3	100	1400	476
1024g	10.3	Fir 7 Spruce 2 Beech 1	170 150	1450	239
1024z	24.7	Beech 7 Fir 2 Spruce 1	80 100	1500	297

Due to AHP requirements, decision-making problem is structured as hierarchies and there were three hierarchy structures defined (Figure 1). Two

hierarchies have three level structures (Figure 1a and 1c), and the remaining is a four-level hierarchy (Figure 1b). As Figure 1 shows, criteria in structure (a) are divided into sub-criteria in structure (b). After that, sub-criteria in hierarchy structure (b) are turned into criteria in hierarchy structure (c); while the criteria set from structure (b) is neglected when defining structure (c). This way, three hierarchies that are overlapping to a major extent are considered.

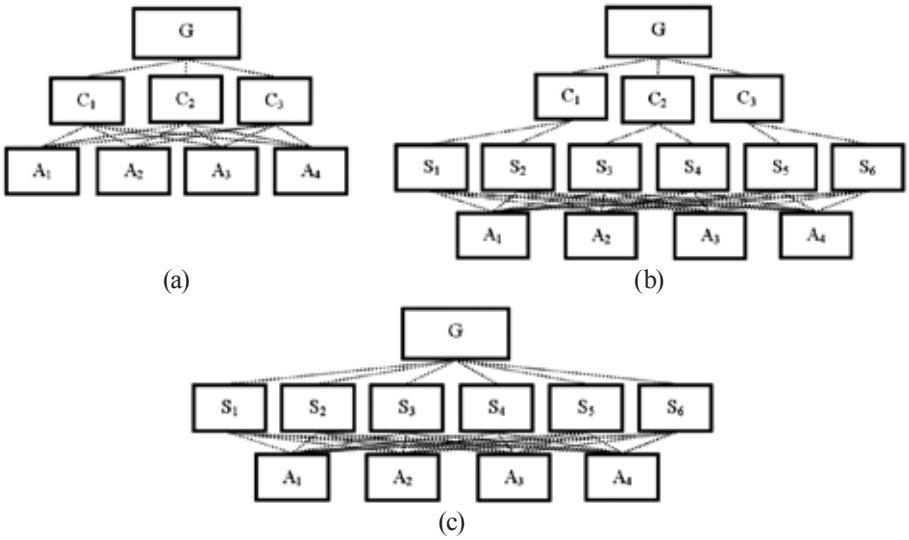

Figure 1. Hierarchy structures

Table 2. Hierarchy elements (Description of the elements presented in Figure 1.)

Hierarchy level	Definition	Label	Link to superior element(s)
Goal	Assessing forest stands' functionality	G	–
Criteria	Environmental	C_1	G
	Social	C_2	
	Economic	C_3	
Sub-criteria	Biodiversity	S_1	C_1
	Age of main canopy	S_2	C_1
	Recreation potential	S_3	C_2
	Park infrastructure	S_4	C_2
	Harvesting costs	S_5	C_3
	Timber value	S_6	C_3
Alternatives	Forest stand № 1021b	A_1	C_1, C_2, C_3
	Forest stand № 1023a	A_2	
	Forest stand № 1024g	A_3	
	Forest stand № 1024z	A_4	

Presented hierarchies were defined and evaluated by the chief forest manager of the Rila monastery forest who holds a Ph.D. in forestry sciences. The evaluations were done consequently. The pauses between evaluations of hierarchies lasted three days each. After performing all evaluations, decision maker analyzed all of them in order to identify the most suitable one.

RESULTS AND DISSCUSSION

The decision maker defined and evaluated three defined hierarchy structures (Figure 1). Number of pair-wise comparisons varied; there were 21 pair-wise comparisons for hierarchy (a), 42 for hierarchy (b) and 51 for hierarchy (c). When the number of pair-wise comparisons gets larger, the evaluation becomes more challenging. If a decision maker encounters AHP methodology for the first time, like in this research, evaluation of a large number of pair-wise comparisons can be tiresome. Checking consistency parameters is in that case especially useful, because it helps in detecting possible misjudgments.

The procedure for AHP calculation was as follows: each hierarchy pair-wise comparison at all nodal points was subjected to the eigenvector method to obtain local weights of corresponding decision elements, and then standard AHP synthesis was performed to obtain global (overall) weights of alternatives versus goal (Table 3). Interpretation of the results in Table 3 shows that the alternative weights differ for different hierarchy structures, but that the final ranking is the same.

Table 3. Overall weights

Alternatives	Overall weights *W* and ranks		
	Structure (a)	Structure (b)	Structure (c)
1021b	0.197 (2)	0.240 (2)	0.187 (2)
1023a	0.082 (4)	0.083 (4)	0.107 (4)
1024g	0.590 (1)	0.572 (1)	0.569 (1)
1024z	0.131 (3)	0.105 (3)	0.137 (3)

Table 4 shows the consistency parameters for analyzed hierarchy structures. Based on the results presented in this table, it can be concluded that evaluation of the structure (a) was the most consistent (values of *CR*, *ED* and *MV* parameters are the smallest). Evaluation of the structure (c) was the least consistent and in this evaluation overall *CR* parameter exceeded the threshold value of 0.1 [see Zeshui 2004; Coulter *et el*., 2006]. These results are expected; the consistency was decreasing as there were more pair-wise comparisons to perform.

Table 4. Overall consistency parameters

Parameter	Value		
	Structure (a)	Structure (b)	Structure (c)
CR	0.042	0.080	0.151
ED	12.29	34.17	42.84
MV	2.0	3.0	5.0

When the results were obtained, the decision maker was asked to select the most appropriate hierarchy structure. Based on his opinion, that is hierarchy (c). Since consistency performance for the hierarchy (c) was not within acceptable limits (*CR*>0.1), the whole hierarchy evaluation was again shown to the decision maker and it was accompanied with data regarding consistency parameters, local and overall weights. According to his personal opinion, he had an equal right to keep his previous evaluations or to modify them. Table 5, which was shown to the decision maker, represents local consistency parameters for hierarchy structure (c).

Table 5. Local consistency parameters – structure (c)

Matrix	Consistency parameters			
	CR	ED	MV	CM
G vs. S	0.144	11.21	1.0	0.96
A vs. S_1	0.222	3.81	1.0	0.89
A vs. S_2	0.040	2.74	1.0	0.44
A vs. S_3	0.122	6.28	0.0	0.80
A vs. S_4	0.073	6.35	1.0	0.74
A vs. S_5	0.110	4.65	1.0	0.67
A vs. S_6	0.184	7.80	0.0	0.80

The decision maker revised his previous comparisons and decided to re-evaluate following matrices: matrix of comparison of alternatives with respect to sub-criteria S_1 (Figure 2a) and matrix of comparison of alternatives with respect to sub-criteria S_6 (Figure 3a). These matrices had the lowest consistency performance (Table 5). By analyzing consistency parameters in detailed inspection, the decision maker concluded that he violated the rank of elements in both matrices (Figure 2 and 3). This mistake can be related to a large number of pair-wise comparisons that decision maker was supposed to do for the first time. Revised, modified matrices are shown in Figure 2b and 3b.

S_1	A_1	A_2	A_3	A_4
A_1	1	3	1/3	1/3
A_2		1	1/3	1
A_3			1	5
A_4				1

Figure 2a. Original matrix of comparison of alternatives with respect to sub-criteria S_1, hierarchy (c)

S_1	A_1	A_2	A_3	A_4
A_1	1	3	1/3	3
A_2		1	1/3	2
A_3			1	5
A_4				1

Figure 2b. Revised matrix of comparison of alternatives with respect to sub-criteria S_1, hierarchy (c)

S_6	A_1	A_2	A_3	A_4
A_1	1	1/3	1/7	5
A_2		1	1/7	3
A_3			1	7
A_4				1

Figure 3a. Original matrix of comparison of alternatives with respect to sub-criteria S_6, hierarchy (c)

S_6	A_1	A_2	A_3	S_4
A_1	1	3	1/7	5
A_2		1	1/7	3
A_3			1	7
A_4				1

Figure 3b. Revised matrix of comparison of alternatives with respect to sub-criteria S_6, hierarchy (c)

Figure 2. and *Figure 3.*

Revised comparison matrices were included in the structure (c), by taking the place of original ones. Re-calculated overall priorities presented in Table 6 are considered as final. As Table 6 shows, revision led to changes in both overall weights and the final ranking of alternatives. Presented results express relative functionality of forest stands in the Rila monastery forest and they can be a good starting point for future allocation of investments. In that regard, it is especially important to state properly the rank along with overall weights of alternatives.

Table 6. Overall weights and ranks – final results

Alternatives	Overall weights *W* and rank	
	Structure (c), original	Structure (c), revised
1021b	0.187 (2)	0.243 (2)
1023a	0.107 (4)	0.110 (3)
1024g	0.569 (1)	0.570 (1)
1024z	0.137 (3)	0.076 (4)

Table 7 shows consistency check for the revised structure (c). Based on all parameters' values in Table 7, it can be concluded that revised structure (c) has a better consistency than the original one. Also, overall *CR* parameter is minorly exceeding the threshold value of 0.1 which means that matrix re-evaluation contributed in reaching more acceptable consistency of evaluation.

Table 7. Overall consistency parameters – final results

Parameter	Value	
	Structure (c), original	Structure (c), revised
CR	0.151	0.112
ED	42.84	41.22
MV	5.0	4.0

It is worthy to mention that the similar procedure can be repeated even if the other prioritization methods are applied. In that case, *ED, MV* and *CM* parameters can be used again, while *CR* parameter should be replaced by corresponding ones, depending on a method used [Aguarón *et al*., 2003; Srdjevic 2005]. Nevertheless, there are numerous high-quality methods that provide reaching acceptable consistency by direct changing of inconsistent judgments without decision maker's interference [Zeshui 2004; Bagla *et al*., 2013; Jandová and Talašová 2013; Jalao *et al*., 2014]. The proposal is to let the decision maker perform the changes by him/herself.

CONCLUSION

AHP is one of the most commonly used decision-making tools in forestry. For a successful AHP application, it is essential to properly define a decision--making problem. This research shows an example on how different hierarchy structuring alters the final results, even when the hierarchies are similar and mutually overlapped. Analyses of the obtained results have shown that a large number of pair-wise comparisons can be tiresome for a decision-maker, especially if he/she is dealing with the AHP for the first time. Due to various reasons during the decision-making process misjudgments might occur. A possible way to detect wrong judgments is to check the consistency of decision maker by calculating specific consistency indicators such as *CR*, *ED*, *MV* and *CM*. Consistency check should point out which matrices (and even pair-wise comparisons) should be revised from the theoretical point of view. Sometimes, consistency can be promoted by fine tuning of judgments, but the proposal is to use consistency parameters to check if the consistency was strongly violated (by violating the rank of elements and so on), and to revise these judgments more closely. In order to avoid perfectly consistent matrices without a real decision maker's opinion and expertise, a decision maker should have equal right to keep or to modify inconsistent matrices and judgments. In that regard, consistency check should offer a proposition, but not a directive on the judgments' revision.

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ПРИМЕР РАЗЛИЧИТОГ СТРУКТУИРАЊА АХП ХИЈЕРАРХИЈЕ ЗА РЕШАВАЊЕ ПРОБЛЕМА У УПРАВЉАЊУ ШУМАМА

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РЕЗИМЕ: Рад анализира пример различитог структуирања проблема одлучивања у Аналитичком хијерархијском процесу и његов утицај на коначне резултате. Проблем је приказан на студији случаја манастирске шуме Светог Јована Рилског у Бугарској. Доносилац одлука дефинисао је и вредновао три хијерархије одлучивања. На основу анализе добијених резултата и параметара конзистентности, доносилац одлука одабрао је једну хијерархију и за њу извршио проверу свих вредновања. Параметри конзистентности су служили за препознавање вредновања која су нарушавала доследност у процесу одлучивања. Претпоставка је да је до наведених одступања дошло услед већег броја поређења које је доносилац одлука обавио приликом примене АХП метода. Рад истиче значај правилног дефинисања АХП хијерархије и препоручује да се параметри конзистентности користе само као препорука за накнадну проверу вредновања о чему се коначно изјашњава доносилац одлука.

КЉУЧНЕ РЕЧИ: Аналитички хијерархијски процес, манастирска шума Светог Јована Рилског, структурирање хијерархије, параметри конзистентности

BOOK REVIEW / ПРИКАЗ КЊИГЕ

UDC 598.2(497.11)(049.32)
DOI: 000

Marko Šćiban, Draženko Rajković, Dimitrije Radišić, Voislav Vasić & Uroš Pantović (2015): *Птице Србије – критички списак врста : Birds of Serbia – critical list of species*. The Institute for Nature Conservation of Vojvodina Province and the Bird Protection and Study Society of Serbia, Novi Sad. ISBN 978-86-915199-6-4. p. 194. Available from publishers after ordering. E-mail for ordering: sekretar@pticesrbije.rs; info@pzzp.rs

Ornithologists in Serbia finally have a complete updated list of birds registered in their country! This definitely can be concluded after reading this book. There have been several attempts so far at making the complete lists (sometimes called catalogs) of birds occurring in the territory of today's Republic of Serbia, starting from Matvejev's *Ornithogeographia Serbica* (1950), followed by *Catalogus Faunae Yugoslaviae* (Matvejev and Vasić, 1973), to Vasić's chapter on birds diversity in a monograph titled *Biodiversity of Yugoslavia* (1995). Quite intensive ornithological field research works were done in the periods between publishing these publications, mainly by ornithologists from the Bird Protection and Study Society of Serbia (BPSSS) and the Institute for Nature Conservation of Vojvodina Province (co-publishers of this book), but also by other Serbian ornithologists. Results are extensively published, mainly in *Ciconia*, the only Serbian ornithological journal.

One of the main principles applied in this book is a critical analysis of a quite large set of available data. This principle includes several criteria that were not used in previous lists/catalogs. The inventory was made on the basis of the existing published and unpublished data, stored within BPSSS's database, but also according to the data obtained by detailed search through bird collections in museums and private collections. References are ranging back to the early 17th century, the time when the first reliable data on birds occurring in today's Serbia were published. Some of the previously widely accepted data are rejected after extensive discussions within the team of authors, which included some species considered to be breeders, but whose breeding was inadequately or wrongly documented. Final inventory contains Serbian and scientific names, occurrence and breeding categories (internationally standardized) and a condensed description of the main elements of species' ecology: hystorical and current occurrence, distribution in Serbia, and habitats. The text is in Serbian with English abstracts (for each species). Inadequately documented species are listed separately, but these data are not considered less valuable.

Serbian ornithologists can have certain difficulties with scientific bird names. The simple reason for this is that names used in this book are new and follow official scientific bird names used since 2015 by BirdLife International, a leading global partnership of bird conservation organizations.

These names reflect the most recent nomenclature changes based on results of phylogenetic research, and relatively conservative Serbian ornithologists (at least regarding this issue) have a serious task to remember and use them from now on. This is at least an intention of this book – to become a standard for scientific and also popular use, considering that a presumed majority of its readers are not academic scientists.

The size of this inventory demonstrates current capacities of ornitology in Serbia. It is expected that this branch of zoology will continue to be very dynamic, and this book will certainly give its valuable contribution to this dynamic. It is also expected that Serbian scientists, bird conservationists, decision makers and interested public will use it and update it more regularly, and that publishing of the so called „bird nomenclators" will become more regular. Let us also hope that critical attitute in presentation of ornithological knowledge in Serbia will continue to be deliberately applied.

Marko Tucakov
Secretary of the Bird Protection and Study Society of Serbia

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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
(g) World Wide Web Sites and other electronic sources: Author's last name, Author's 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 organization behind the website or the title to the author space.

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 документ 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 A4 (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 радови 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 абецедним 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 радови 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 абецедним 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 Engineerings Index, Inc.,).
5.5. Референце се не преводе 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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