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THE EFFECT OF BIOFERTILIZERS ON SOIL MICROBIAL ACTIVITY AND BASIC MORPHOLOGICAL CHARACTERISTICS OF *CAMELIA* SP. AND *CUPRESSUS* SP.

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Abstract

This paper examines the effect of Enteroplantin and Slavol on soil microbial activity (count of microorganisms, numbers of azotobacter, oligonitrophiles, soil fungi and actinomycetes) and growth (length of underground and aboveground plant parts) of *Camellia sp.* and *Cupressus macrocarpa gold crest*, grown in vegetative pots. Microbial counts were determined by an indirect method of growth on specific selective media. Enteroplantin and Slavol induced an increase in the number of all examined groups of microorganisms, except soil fungi. Enteroplantin was found to be particularly effective in this respect, having a more stimulating effect on the growth of roots and aboveground parts of both plants, in the course of the entire study period (2006–2008). The observed growth of the aboveground parts and roots of both plants, the established values of soil microbial parameters, and potential economic and environmental effects suggest that Enteroplantin and Slavol can be recommended for use in cultivating *Camellia sp.* and *Cupressus macrocarpa gold crest.*

Key words: biofertilizers, microorganisms, plant, soil

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Introduction. Microorganisms, as an important soil biological component, play a vital role in soil development, maintenance and improvement. They take part in the biological cycle of matter through the soil-plant system, thus affecting soil fertility. Beneficial microorganisms account for 0.1–3.0% of the total soil organic matter, depending on the soil type and other physical and chemical properties [1]. The presence and activity of microorganisms are major indicators of soil biological productivity as well as of the yield and quality of plants [2, 3]. However, in order to achieve high yields, modern agricultural practices often involve uncontrolled use of agrochemicals, most notably mineral fertilizers, which disturb the biological balance of the soil, leading to soil destruction and environmental degradation [4]. Hence the increasing need for sustainable plant production involves the maintenance of environmental quality and the preservation of natural resources both on economic and social scale. In this respect, the use of microbial inoculants – biofertilizers as alternatives and/or supplements to expensive mineral fertilizers, has gained an increasing importance [5–7]. This improves the physical, chemical and biological properties of the soil and increases the content of readily-available organic matter, phytohormones, enzymes and beneficial microorganisms, resulting in higher yields and safety of the cultivated plants [8, 9]. Moreover, nitrogen-fixing and phosphate-mineralizing microorganisms, as an active component of frequently-used biofertilizers, positively affect soil aggregation through the production of extracellular peptides, polysaccharides and lipids, leading to rapid and easy transport of water, air and nutrients in the soil [10–12]. Apart from the above, the diazotrophs introduced into the soil may increase the respiration intensity, as well as the number and enzymic activity of beneficial soil microorganisms, and can also inhibit the development of phytopathogens, intensify photosynthesis and eventually enhance yields of cultivated plants [13–15]. In this respect, the results of Emtsev et al. [16] indicate the importance of the use of *Klebsiella planticola* TSHA-91-based bioproducts towards increased yields of vegetable crops, most notably potatoes. The authors report not only their high nitrogenase activity but also their adhesion capacity, the ability to absorb at the plant roots and colonize them throughout the vegetation period, and the growth inhibition of some pathogenic fungi. Similar effects have been reported after the combined use of diazotrophs and phosphate-mineralizing bacteria [17]. However, the majority of studies have focused on the use of biofertilizers in field, vegetable, forage and industrial crop production, whereas their use in the production of ornamental plants has been given little attention. Therefore, the objective of the present study was to evaluate the effect of Enteroplantin and Slavol biofertilizers on soil microbiological activity as well as on major morphometrical characteristics (length of aboveground and underground parts) of ornamental plants *Camellia sp.* and *Cupressus macrocarpa gold crest*.

Material and methods. The three-year experiment was set up in April 2006 at Ecoplant Nursery in Podgorica (Montenegro), under controlled environment (greenhouse) conditions. Plants were planted in 10 dm^3 vegetative pots, in five replications, on a soil substrate containing 60% peat moss, 30% rice husks and 10% sand. Two ornamental plants, *Camellia sp.* and *Cupressus macrocarpa gold crest*, were used as test plants. The trial included a control (untreated soil) and two treatments with the microbial fertilizers Enteroplantin and Slavol. Enteroplantin is a pure culture of Gram-negative nitrogen-fixing bacteria *Klebsiella planticola* SL09 isolated from tomato rhizosphere and stored in the Microorganism Collection of the Laboratory of Microbiology, Faculty of Agronomy, Cacak (Serbia). The bacterial titer of the inoculum ranged from $20 - 40 \times 10^6$ cm^{-3}. Slavol is a microbial fertilizer composed of mixed cultures of nitrogen-fixing and phosphate-mineralizing bacteria (*Azotobacter chroococcum, A. vinelandi, Derxia sp., Bacillus megatherium, B. lichenformis* and *B. subtilis*) produced by Agrounik DOO (Ltd.), a Zemun-based company.

The plants were treated with 30 cm^3 of each microbial fertilizer every 15 days and were simultaneously foliarly fertilized, whereas the control was watered with the same amount of pure water. In all the three years of study, the first treatment was done on April 6. Microbiological analyses of soil, as well as growth of underground parts (roots) of cultivated plants were checked four times during 2006, i.e. (on 27 April, 6 June, 6 August, 6 October). The aboveground growth of the cultivated plants (stem) was determined at the same time, at the same time schedule, in all three studied years (2006–2008).

Microbiological analyses were performed at the Laboratory of Microbiology, Faculty of Agronomy, Cacak and included the determination of specific systematic and physiological groups of microorganisms by a method of dilution on appropriate solid media [18]. The following microbial counts were determined: total microbial count (on plate count agar, [18]), count of soil fungi (on Czapek-Dox agar, [19]), count of actinomycetes (on Krasilnikov's agar, [20]), counts of oligonitrophilic bacteria and Azotobacter (Fyodorov's medium, [21]).

Total microbial count was obtained by 10^{-6} soil dilution, the count of actinomycetes and fungi by 10^{-5} soil dilution, and the one of oligonitrophiles and Azotobacter by 10^{-4} and 10^{-2} dilution, respectively.

Incubation was followed by identification and calculation of colonies grown per gram of absolutely dry soil.

The data obtained in this study were subjected to a two-factorial (Factor A – fertilizers applied × Factor B – vegetation period) analysis of variance (Statistics SPSS 5, [22]). The Lsd test was used to evaluate the significance of differences and individual and interactive means.

Results and discussion. The effectiveness of the Enteroplantin and Slavol microbiological fertilizers on soil microorganisms and growth of the aboveground and underground parts of *Camellia* sp. and *Cupressus macrocarpa gold crest* plants was dependent on the biofertilizer type, the vegetation period (months) and the climatic conditions over the period of study.

Enteroplantin and Slavol induced a substantial increase in the total microbial count which, however, decreased considerably in June in the soil under *Camellia* sp. and in October in the soil planted with *Cupressus macrocarpa gold crest* (fertilizer/vegetation period interaction) (Tables 1, 2), being likely attributable to the negative effect of the high temperatures reported during the period and the different excretory activities of the root systems of the plants analyzed [23, 24]. The total microbial count was higher in the soil planted with *Cupressus macrocarpa gold crest*, most probably due to a larger root system, its more intensive excretory activity and higher amounts of organic harvest residues, which all stimulated the growth of microorganisms.

The microbiological fertilizers applied in this study induced a significant increase in the count of Azotobacter, particularly in the soil cultivated with *Camellia* sp. (Table 1). The Azotobacter count decreased in June after Enteroplantin treatment and in August and October after treatment with Slavol. This resulted from the specific relationship existing between this group of microorganisms, temperature and the rhizosphere effect [25].

Enteroplantin and Slavol led to a insignificant increase in the number of actinomycetes in the soil planted with each plant. Their number grew during the growing season (Tables 1, 2), except in June, which conformed to the results obtained by other authors [26]. As compared to Slavol, Enteroplantin had a stronger but insignificant effect on this group of microorganisms.

As opposed to the present study showing the highest stimulating effect of the fertilizers applied, Slavol in particular, on oligonitrophile growth in the final vegetative stage of the plants (Tables 1, 2), the research conducted by other authors indicated that the growth of these microorganisms was stimulated during the entire test period [24].

Given the ability of asymbiotic diazotrophes to produce certain fungistatics, the inhibiting effect of the applied biofertilizers on soil fungi was expectable [27]. There was a considerable decline in the counts of this group of microorganisms in June in both plants (Tables 1, 2), which was due to an effect of high temperatures, as confirmed by Mandic et al. [28].

The microbiological fertilizers applied during 2006 induced growth of the aboveground parts of *Camellia* sp. during the growing season (Table 3). This was particularly true for Enteroplantin, which stimulated the growth of the aboveground parts of the plant by 31% as compared to the control. Conversely, the fertilizers had no significant effect on *Camellia* sp. root growth.

In comparison with the control plant, in 2006, growth of the aboveground parts of *Cupressus macrocarpa gold crest* in the treatment with Enteroplantin and Slavol was observed as early as June (78 days after the first treatment) and moderate growth was maintained throughout the other stages of the growing season (Table 3). Generally, the observed growth of the above ground parts of *Cupressus macrocarpa gold crest* in the treatment with Enteroplantin and Slavol

T a b l e 1

Counts of microorganisms in the soil (g^{-1} absolutely dry soil) under *Camellia* sp. as dependent upon microbiological fertilizer (Control – Con.; Ent. – Enteroplantin; Sl. – Slavol) and vegetation period (months) during 2006

	Total microbial count ($10^6 g^{-1}$)	Azoto-bacter ($10^2 g^{-1}$)	Actino-mycetes ($10^5 g^{-1}$)	Oligo-nitrophiles ($10^5 g^{-1}$)	Fungi ($10^6 g^{-1}$)
Fertilizer (A)					
Con.	17.8 b	28.3 b	61.42	17.4 b	28.7 a
Ent.	22.6 a	35.7 a	65.92	23.7 a	24.4 ab
Sl.	22.9 a	32.7 ab	65.42	22.6 a	20.2 b
Month (B)					
IV	20.8 b	25.8 c	49.4 c	20.8 ab	28.0 a
VI	10.8 c	28.7 bc	44.7 c	16.8 b	18.6 b
VIII	24.3 ab	34.3 ab	78.4 b	22.9 a	29.9 a
X	28.6 a	40.0 a	84.4 a	24.6 a	21.4 b
Con. IV	11.0 de	20.0 e	46.0	14.3 de	30.0 b
VI	8.0 e	28.3 cde	41.0	12.0 e	19.0 de
VIII	21.3 bc	27.3 cde	76.7	20.3 bcd	44.7 a
X	31.0 a	37.3 bc	82.0	23.0 abc	21.0 cde
Ent. IV	20.0 bcd	28.3 cde	50.7	21.3 abc	28.7 bc
VI	11.3 de	26.0 de	44.7	19.3 cd	21.7 b-e
VIII	27.3 ab	40.0 ab	82.0	26.7 ab	24.0 bcd
X	31.7 a	48.3 a	86.3	27.7 a	23.3 b-e
Sl. IV	31.3 a	29.0 b-e	51.7	26.7 ab	25.3 bcd
VI	13.0 cde	31.7 bcd	48.3	19.0 cde	14.7 e
VIII	24.3 ab	25.7 de	76.7	21.7 abc	21.0 cde
X	23.0 b	34.3 bcd	85.0	23.0 abc	20.0 cde
ANOVA					
A	*	*	ns	**	**
B	**	**	**	**	**
AB	**	**	ns	**	**

Values followed by different small letters within columns are significantly different ($P < 0.05$) according to the LSD test

*F test significant at $P \leq 0.05$; **F test significant at $P \leq 0.01$; ns – insignificant

was about 10% and 7% higher, respectively, as compared to the control plant. As opposed to *Camellia* sp., Enteroplantin induced an increase in the root length of *Cupressus macrocarpa gold crest* throughout plant development. The effect

Table 2

Counts of microorganisms in the soil (g^{-1} absolutely dry soil) under *Cupressus macrocarpa gold crest* as dependent on microbiological fertilizer (Control – Con.; Ent. – Enteroplantin; Sl. – Slavol) and vegetation period (months), during 2006

	Total microbial count ($10^6 g^{-1}$)	Azotobacter ($10^2 g^{-1}$)	Actinomycetes ($10^5 g^{-1}$)	Oligonitrophiles ($10^5 g^{-1}$)	Fungi ($10^6 g^{-1}$)
Fertilizer (A)					
Con.	32.6 c	34.8	51.9	14.5 c	40.7 a
Ent.	37.3 b	38.6	55.3	22.4 a	36.2 b
Sl.	45.5 a	36.4	54.4	19.4 b	32.1 c
Month (B)					
IV	34.3 c	38.1 a	54.0 b	15.6 b	34.0 b
VI	39.0 bc	29.4 b	45.8 c	12.4 c	28.2 c
VIII	45.1 a	41.2 a	63.3 a	24.0 a	44.8 a
X	35.3 bc	37.7 a	52.3 b	23.1 a	38.2 b
Con. IV	28.7 e	36.3 abc	49.7 def	11.7 ef	39.3 b
VI	28.0 e	26.0 d	46.7 ef	8.7 f	31.0 cde
VIII	42.0 bc	38.0 abc	67.0 a	18.0 d	53.7 a
X	31.7 de	39.0 abc	44.3 f	19.7 cd	38.7 bc
Ent. IV	31.0 de	38.0 abc	54.3 cde	18.0 d	35.7 bcd
VI	31.3 de	33.0 bcd	46.3 ef	16.7 de	30.0 de
VIII	50.0 ab	45.3 a	56.3 cd	30.0 a	42.0 b
X	36.7 cde	38.0 bc	64.3 ab	25.0 b	37.0 bcd
Sl. IV	43.3 bc	40.0 ab	58.3 bc	17.0 d	27.0 e
VI	57.7 a	29.3 cd	44.3 f	12.0 ef	23.7 e
VIII	43.3 bc	40.3 ab	67.0 a	24.0 bc	38.7 bc
X	37.7 cd	36.0 abc	48.3 def	24.7 b	39.0 b
ANOVA					
A	**	ns	ns	**	**
B	**	**	**	**	**
AB	**	*	**	**	**

Values followed by different small letters within columns are significantly different ($P < 0.05$) according to the LSD test

*F test significant at $P \leq 0.05$; **F test significant at $P \leq 0.01$; ns – insignificant

T a b l e 3

Effect of microbiological fertilizers (Control – Con.; Ent. – Enteroplantin; Sl. – Slavol) and vegetation period (months) on growth (stem and root length) of *Camellia sp.* and *Cupressus macrocarpa gold crest*, during 2006

	Camellia sp.		*Cupressus macrocarpa gold crest*	
	Stem length (cm)	Root length (cm)	Stem length (cm)	Root length (cm)
Fertilizer (A)				
Con.	26.6 b	16,5	34.7 b	23.6 b
Ent.	34.9 a	17.1	38.1 a	34.0 a
Sl.	28.6 b	17.2	37.0 a	25.9 b
Month (B)				
IV	16.3 d	9.2 c	19.2 d	7.3 d
VI	28.5 c	15.8 b	24.7 c	13.3 c
VIII	34.8 b	21.0 a	45.2 b	29.8 b
X	40.5 a	21.8 a	57.5 a	60.5 a
Con. IV	16.0 d	9.0	19.0 e	7.0 f
VI	27.5 c	15.5	21.0 e	7.0 f
VIII	28.0 c	20.5	44.0 c	25.5 d
X	35.0 b	21.0	55.0 b	55.0 b
Ent. IV	17.0 d	9.5	19.5 e	8.0 f
VI	29.0 c	16.5	27.0 d	18.0 e
VIII	46.5 a	21.0	46.0 c	39.5 c
X	47.0 a	21.5	60.0 a	70.5 d
Sl. IV	16.0 d	9.0	19.0 e	7.0 f
VI	29.0 c	15.5	26.0 d	15.0 e
VIII	30.0 c	21.5	45.5 c	25.5 d
X	39.5 b	23.0	57.5 ab	56.0 b
ANOVA				
A	**	ns	**	**
B	**	**	**	**
AB	**	ns	*	*

Values followed by different small letters within columns are significantly different ($P < 0.05$) according to the LSD test

*F test significant at $P \leq 0.05$; **F test significant at $P \leq 0.01$; ns – insignificant of Slavol was somewhat lower only at the initial stages of plant development (Table 3).

The applied fertilizer significantly influenced the growth of the aboveground parts of the cultivated plants during 2007 and 2008, respectively. On the whole, the growth of *Camellia* sp. treated with Enteroplantin was by about 24% higher in the control variant during 2007, and 29% during 2008. The stimulative effect of Slavol was somewhat lower, but it was significantly higher compared to the control. Both microbiological fertilizers had the highest stimulating effect two months after the first treatment (Table 4).

As in 2006, in the next two years, the applied microbiological fertilizers influenced the growth of the aboveground parts of *Cupressus macrocarpa gold crest*. In this respect, it was evidenced that the growth of the plant treated with Enteroplantin increased by about 15% in 2007 as well as in 2008. Slavol had a similar effect, the only difference being that the growth of the aboveground part of these variants was higher by 9% compared to the control during 2007, i.e., by 6% in 2008 (Table 4).

The stimulating effects of Enteroplantin and Slavol on the stem and root growth of the test plants were attributable to a more rapid incorporation of the uptaken nitrogen forms into the plant parts, due to the pronounced activities of amino transpherase and glutamine synthetase affecting the protein production in plants [29–31] it was also reported the importance of the effect of diazotrophes and phosphate-mineralizing bacteria on initial root growth, resulting from pre-sowing inoculation, and being reflected at later stages in the growth of the aboveground plant parts. Complying with the results of the present study suggesting that the effect of the microbiological fertilizers was more pronounced at later stages of plant development, Stanojkovic et al. [32] ascribed the phenomenon to the gradual release of assimilated nitrogen by asymbiotic nitrogen-fixers during the vegetation period. Therefore, the authors underlined that this source of nitrogen could be fully used only after the decay of these microorganisms and subsequent mineralization of their plasma, suggesting that they act as accumulators of soil fertility which can be used at later stages of the vegetation period. Conversely, the results reported by some other authors suggest the positive effect of biofertilizers on the growth of Scots pine and birch only eight weeks upon their application. This effect is attributed to the ability of the introduced bacteria strains to synthesize phytohormons, fix nitrogen and stimulate cellular and pectinase activity of soil microflora [33, 34]. The observed differences in the growth of the aboveground parts and roots during the vegetation period of *Camellia* sp. and *Cupressus macrocarpa gold crest* were due to the different adaptability of these microorganisms to the effects of extreme environmental factors. In this respect, the results obtained by Venn et al. [35] suggested a reduction both in the nitrogen-fixing ability and the energy metabolism of diazotrophes under high summer temperatures.

T a b l e 4

Effect of microbiological fertilizers (Control – Con.; Ent. – Enteroplantin; Sl. – Slavol) and vegetation period (months) on growth aboveground (stem length, cm) of *Camellia sp.* and *Cupressus macrocarpa gold crest*, during 2007 and 2008

	Camellia sp.		*Cupressus macrocarpa gold crest*	
	2007	2008	2007	2008
Fertilizer (A)				
Con.	30.23 b	31.7 c	41.15 b	43.05 b
Ent.	37.60 a	40.07 a	47.5 a	49.05 a
Sl.	34.03 ab	36.65 b	45.1 a	45.75 ab
Month (B)				
IV	19.2 d	21.86 d	17.83 d	26.03 d
VI	32.8 c	34.3 c	29.7 c	32.8 c
VIII	39.6 b	40.43 b	56.96 b	58.23 b
X	44.2 a	47.03 a	67.9 a	66.73 a
Con. IV	18.5 d	21.1 d	23.5 e	25.4 d
VI	27.5 c	29.5 c	26.7 de	28.4 d
VIII	35.2 b	33.2 c	51.3 c	55.3 b
X	39.7 b	40.5 b	63.1 bc	63.1 ab
Ent. IV	20.1 cd	22.5 d	24.0 e	27.0 d
VI	37.5 b	38.4 bc	32.1 d	39.1 c
VIII	43.1 ab	47.1 ab	61.4 bc	60.3 b
X	49.7 a	52.3 a	72.5 a	69.8 a
Sl. IV	19.0 d	22.0 d	23.8 e	25.7 d
VI	33.4 bc	35.1 bc	30.3 de	30.9 d
VIII	40.5 b	41.0 b	58.2 c	59.1 b
X	43.2 ab	48.3 a	68.1 ab	67.3 ab
ANOVA				
A	**	**	**	**
B	**	**	**	**
AB	*	**	**	*

Values followed by different small letters within columns are significantly different ($P < 0.05$) according to the LSD test

*F test significant at $P \leq 0.05$; **F test significant at $P \leq 0.01$; ns – insignificant

Conclusion. Enteroplantin and Slavol led to a significant increase in the total counts of microorganisms, Azotobacter and oligonitrophiles in the soil planted with both plant species. All counts, except those of oligonitrophiles, were higher in the soil cultivated with *Cupressus macrocarpa gold crest.* The microbiological fertilizers applied had a insignificant effect on the number of actinomycetes and induced a significant decrease in the count of soil fungi under both plant species. Enteroplantin exhibited a higher stimulating effect on the test groups of microorganisms, except the total microbial count. In the course of the entire period of study (2006–2008), the applied microbiological fertilizers significantly stimulated the development of the aboveground parts of both plant species, especially in *Camellia sp.* This also goes for the growth of the underground plant part (the root) of these plants, particularly in *Cupressus macrocarpa gold crest.* In both cases, Enteroplanting had a markedly more stimulating effect.

The observed growth of the aboveground parts and roots of both plants, the established values of soil microbial parameters and potential economic and environmental effects suggest that Enteroplantin and Slavol can be recommended for use in the cultivation of *Camellia sp.* and *Cupressus macrocarpa gold crest.*

REFERENCES

[1] Lin X. G., R. Yin, H. Y. Zhang, J. F. Huang, R. R. Chen, Z. H. Cao. Environ. Geochem. Health, **26**, 2004, No 2, 119–128.

[2] Zhong W. H., Z. C. Cai. Appl. Soil Ecol., **36**, 2007, 84–91.

[3] Samuel A. D., C. Domuta, C. Ciobanu, M. Sandor. Rom. Agric. Res., **25**, 2008, 61–68.

[4] Fox C. A., K. B. Macdonald. Can. J. Soil Sci., **83**, 2003, 231–224.

[5] Parr J. F., R. I. Papendick, S. B. Hornick, R. E. Meyer. Amer. J. Alternative Agric., **7**, 1992, 5–11.

[6] Dobereiner J. Soil Biol. Biochem., **29**, 1997, Nos 5/6, 771–774.

[7] Emtsev V. T., A. Y. Sokolova, O. V. Selitskaya. Eurasian Soil Sci., **43**, 2010, No 7, 771–776.

[8] Ryu C. M., M. A. Farag, C. H. Hu, M. S. Redy, J. W. Kloepper, P. W. Pare. Plant Phisyol., **134**, 2004, 1017–1026.

[9] Sofi P., S. Wani. Asian J. Plant Sci., **6**, 2007, 203–213.

[10] Sturz A. V., J. Nowak. Appl. Soil Ecol., **15**, 2000, No 2, 183–190.

[11] Mia M. A. B., Z. H. Shamsuddin, Z. Wahab, M. Marziah. Aust. Crop Sci., **4**, 2010, No 2, 85–90.

[12] Kant C., A. Aydin, M. Turan, Y. M. Huang. Romanian Biotechnological Letters, **15**, 2010, No 6, 5754–5763.

[13] Malik K. A., B. Rakhshanda, S. Mehnaz, G. Rasul, M. Mirza. S. Plant Soil, **194**, 1997, 1775–1779.

[14] Pavez S., W. Shafiq. Asian J. Plant Sci., **6**, 2007, No 1, 203–213.
[15] Zhang H., X. Xie, M. Kim, D. A. Kornzezev, S. Holadaz. P. W. Plant J., **56**, 2008, 264–273.
[16] Emtsev V. T., O. Selitskaja, M. Bruk. In: 16th World Congress of Soil Science (ed. G. Pedro), Montpellier, France, 1998, I: 190, `http://natres.psu.ac.th/Link/SoilCongress/bdd/symp10/1602-t.pdf`
[17] Kengo Y., X. Hui-Lian. J. Crop Prod., **3**, 2000, No 1, 255–268.
[18] Pochon J., P. Tardieux. In: Techniques d'analyse en microbiologie du sol (ed. D. L. Tourelle), INRA Press, Paris, France, 1962, 111 pp.
[19] Scharlau Microbiology. In: Handbook of Microbiological Culture Media, Ref. 1–051 (Czapek-Dox), Sixt International Edition, Barcelona, Spain, 2000, 87.
[20] Scharlau Microbiology. In: Handbook of Microbiological Culture Media, Ref. 1–219, Fifth International Edition, Barcelona, Spain, 1999, 346.
[21] Anderson J. P. E., K. H. Domasch. Soil Boil. Biochem., **10**, 1958, 215–221.
[22] Statsoft. Inc., Statistica for Windows (computer program manual). Tulsa, OK: StatSoft.Inc., 1995.
[23] Bolton H. J. R., J. K. Fredrickson, L. F. Elliott. Microbial ecology of the rhizosphere. (ed. F. B. J. Metting), Marcel Dekker Inc., New York, 1992, 27–63.
[24] Hajnal T., M. Govedarica, Z. Jelicic. Acta Agric. Serb., **11**, 2001, 77–90.
[25] Whipps J. M. J. Experimental Botany, **52**, 2001, 487–511.
[26] Balakrishnan V., K. Venkatesan, K. C. Ravindran. Plant Soil Environ., **53**, 2007, No 4, 186–192.
[27] Sukhovitskaja L. A., S. V. Mokhova, H. V. Safronava, I. B. Chernetsova, N. V. Melnikova. In: Proceedings of the 9th International Symposium on Buckwheat, Prague (ed. I. Faberova), Research Institute of Crop Production, 2004, 91–94.
[28] Mandic L., D. Djukić, I. Beatovic, Z. Jovovic, M. Pesakovic, V. Stevovic. Afr. J. Biotechnol., **10**, 2011, No 36, 6954–6960.
[29] Loyola-Vargas V. M., E. Sanchez De Jimenez. J. Plant Physiol., **124**, 1986, 147–154.
[30] Rawia E. A., A. M. Nemat, H. A. Hamouda. American-Eurasian J. Agric. & Environ. Sci., **5**, 2009, No 4, 509–518.
[31] Feges J., J. F. Arsac. Plant Soil, **137**, 1991, 87–90.
[32] Stanojkovic A., D. Djukić, L. Mandic, R. Pivic. 46th Croatian and 6th International Symposium on Agriculture (ed. M. Pospisil), Opatija, Croatia, 2011, 645–649.
[33] Turjanista A. I., A. M. Petaka, M. M. Nichnik, V. P. Petrosova. Microbiol. Zh. (Ukrainian), **57**, 1995, 28–34.
[34] Hoflich G., B. Munzenberger, J. Busse. Forstw. Cbl., **120**, 2001, 68–79.
[35] Venn J. A. V., L. S. V. Overbeek, J. D. V. Elsas. Microbiol. Mol. Biol. Rev., **61**, 1997, No 2, 121–135.

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