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Utjecaj benefitnih mikroorganizama na prinos i kvalitetu soje u uvjetima smanjene gnojidbe dušikom

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THE INFLUENCE OF BENEFIT MICROORGANISMS ON YIELD AND QUALITY OF SOYBEAN GRAINS UNDER CONDITIONS OF REDUCED NITROGEN FERTILIZATION

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SUMMARY

The aim of this study was to investigate the possibility to reduce the application of mineral nitrogen fertilizers through the application of beneficial microorganisms (genus Bradyrhizobium, Azotobacter, bacteria Pseudomonas fluorescens, Bacillus spp., etc.). Research was conducted during 2013 and 2014 on Eutric brown soil. The experiment was set up in a split-block scheme with 12 different variants in 4 repetitions: two soybean cultivars were used; two different treatments of nitrogen fertilizers and three different treatments of microbiological preparation were applied. Analysed parameters were soybean grain yield (kg/ha) based on 13% moisture, protein content (%), oil content (%) and hectolitre mass (kg). Given that the climatic conditions in the second year of research were more favourable than in the first year of research, all the elements of research, including control variants, achieved better results in the second year of research. All variants treated with microbiological preparations, either by application in soil or by application in soil combined with foliar treatments, also achieved statistically significant differences compared to the control variants.

Key-words: microbiological preparation, nitrogen, soybean yield, quality

INTRODUCTION

Soybean is a culture with high protein content, and aside from the grain being used for various purposes, soybean leaves behind a large amount of overhead mass that enriches the soil with organic matter. As other legumes, soybean has the possibility to create a symbiotic relationship with the nodular bacteria *Bradyrhizobium japonicum,* which can reduce the need for nitrogen fertilization. However, for effective nitrogen fixation, three conditions should be met: soil of neutral pH response, compatibility of soybean varieties and *B. japonicum* strains as well as sufficient molybdenum in soil because it is an integral part of nitrogenase and leghemoglobin (Kristek, 2001). For this reason, more and more seeds are inoculated with non-symbiotic nitrogen fixing bacteria like *A. chroococcum* and strains of the genus *Azospirillum* (Jauhri et al., 1979; Jarecki et al., 2016).

Inoculation of legumes with non-symbiotic bacteria improves the nodulation and effective nitrofixation of symbiotic bacteria with the host plant. The combined bacterial infection of *Bacillus* spp. and *B. japonicum* (Liu and Sinclair, 1993; Karaca et al., 2002; Bai et al., 2003) results not only in an increased number and weight of the nodules or higher grain yield, but they also showed antagonistic actions to pathogenic fungus *R. solani*, *S. scleotiorum*, *Phomopsis* spp. and *M. phaseolina*. Also, Kumar and Dube (1992) inoculating soybean with *P. fluorescens* recorded faster plant growth, higher nodule weights, higher yield, and smaller attack by pathogenic fungi.

Combined infection of soybean seeds with arbuscular mycorrhizal fungi and *B. japonicum* showed better nodulation, effective AMF colonization, resulted in better uptake of nitrogen, phosphorus and micronutrients (Karaca et al., 2013; Meng et al., 2015). Seed inoculation with AMF or soil treatments with AMF leads to an increase in root absorption volume, also, fungus trans-

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form nutrients to plant available forms, supply plants with water, and show antagonistic actions against pathogenic microorganisms in the soil (Sudova et al., 2007; Qiao et al.,2011; Zhu et al., 2012).

Excessive use of mineral fertilizers and chemical pesticides leads to deterioration of chemical and microbiological soil properties as well as groundwater eutrophication. The use of microbiological preparations containing various beneficial microorganisms could reduce the mineral fertilization and application of chemical pesticides, which is an economical as well as ecological goal.

MATERIAL AND METHODS

Research was conducted during 2013 and 2014 on Eutric brown soil (Table 1). The experiment was set up in a split-block scheme with 12 different variants in 4 repetitions:

A. Soybean cultivars: A1 - Ika; A2 - Vita;
B. Nitrogen fertilization: B1 - control (0 kg N_2/ha); B2 - 50 kg N_2/ha;
C. Microbiological preparation treatment: C1 - control; C2 - microbiological preparation applied into the soil (30 l/ha); C3 - microbiological preparation applied into the soil (30 l/ha) + 2 foliar treatments with microbiological preparation (2 × 6 l/ha)

Soybean was sown in the second decade of April with a pneumatic 12-row seeding machine with 50 cm space between the rows and the sowing depth was 4 cm. Total number of plants was 600 000 per hectare. Both cultivars, Ika and Vita are cultivars of the Agricultural Institute in Osijek, Croatia. Ika is a medium-early variety (0 to 1 maturity group), while Vita is an early variety (0 maturity group). The microbiological preparation Em - Aktiv was applied into the soil immediately before sowing (variants C2 and C3). In the C3 variant, at plant height of 45-50 cm the first foliar treatment was applied and the second treatment at the beginning of flowering.

Soybean was treated against weeds one day after sowing with herbicides: Dual Gold (960 g/l S-Metolachlor) 1.5 l/ha + Sencor WP 70 (700 g/kg metribuzin) 0.5 kg/ha. The protection against weeds, diseases and pests was carried out in accordance with the integrated protection, EU Directive 91/676/EEC). Cultivation of soybean was done in phase of 3-4 developed trifoliate leaves. Nitrogen fertilization was performed on two occasions: 50% in autumn during basic fertilization and 50% in spring before sowing, according to fertilization variants (Urea, 46% N_2).

The microbiological preparation Em - Aktiv (EM technology d.o.o., Valpovo, RH) was applied. Its composition includes nitrofixing bacteria of the genus *Bradyrhizobium* and *Azotobacter*, as well as the bacteria *Pseudomonas fluorescens*, *Bacillus* spp. etc.)

Harvest in both years of research was carried out in the first decade of October with a Winnesteiger Nursery combine machine. Analysed parameters were: grain yield (kg/ha) based on 13% moisture; protein content (%); oil content (%) and hectolitre mass (kg). The protein and oil content was determined by the Infratec Grain Analyser (Foss, Denmark), in the laboratory of the Agricultural Institute in Osijek.

In 2013, weather conditions during the early stages of growth and development were very favourable until the end of the second decade of July. Afterwards, high temperatures and lack of precipitation were recorded. High temperatures with simultaneous lack of precipitation in stages of increased soybean sensitivity (flowering, fertilization and grain deposition) were crucial factors for reducing the yield. Given that in 2014 the vegetation period, due to slightly elevated air temperatures and almost optimal rainfall, was almost ideal for soybean growth, the yields were high.

Table 1. Soil characteristics

Tablica 1. Svojstva tla

Layer *Dubina* (cm)	pH		Humus	Nitrogen *Dušik*	AL- P_2O_5 (mg/100 g soil)	AL- K_2O (mg/100 g soil)
	H_2O	1M KCl	%			
0 - 40	7.5	6.8	1.56	0.13	24.38	27.16

Results were processed by statistical methods (ANOVA) using the computer program StatSoft Inc. STATISTICA - data analysis software system.

RESULTS AND DISCUSSION

Grain yield (kg/ha)

The highest average yield of soybean, on the control plot, as well as on the plot with nitrogen fertilization (50 kg N_2/ha), in 2013 (Table 2) was achieved with the cultivar Ika in the variant where the microbiological preparation was incorporated into the soil and with two foliar treatments. All other varieties had statistically significantly lower average grain yields ($p<0.01$). Variants C3 (soil treatment (30 l/ha) + 2 foliar treatments (2 x 6 l/ha)) had an average grain yield of 3461 kg/ha which is 14.46% more than the variant where nitrogen fertilization was not carried out (B1C1) or 4.83% more than the variant (B2C1), where the fertilization was 50 kg N_2/ha.

Also, the cultivar Vita achieved the highest average grain yield in control variant B1 (without nitrogen fertilization) where the microbiological preparation was incorporated into soil and with two foliar treatments (C3), while in fertilized variant B2 (50 kg N_2/ha) the highest average yield of grain was obtained in variant C2 (microbiological preparation incorporated in soil, 30 l/ha). All other varieties had statistically significantly lower average grain yields ($p<0.01$).

Both cultivars achieved the highest average yield of soybean in 2014 (Table 3) on both variants of fertilization combined with the variant where the microbiological preparation was only incorporated into the soil (variant C2). All other varieties had statistically significantly lower average grain yields ($p<0.01$). Climate conditions for the plant were much more favourable in 2014, and the average grain yield of all varieties in 2014 was 15.71% higher compared to the same in 2013. This would explain why the variant C2 (microbiological preparation incorporated in the soil) achieved better results even in nitrogen fertilization variant B1. That plant growth-promoting bacteria positively influence the increase in yield elements was also determined by Jarecki et al. (2016). Liu and Sinclair (1993), using a microbiological composition containing *B. megaterium*, achieved not only higher yields of soybean grain and protein content in the grain, but also found better root system development and greater resistance to infection with pathogenic *R. solani* fungus. Cattelan et al. (1999) using plant growth-promoting bacteria in their studies showed faster initial growth of young soybean plants, increase in yield and quality elements, and resistance to *S. rolfsii* and *S. sclerotiorum*.

Protein content (%)

Both cultivars achieved the highest average protein content in the first year of the study (Table 2) in variant B2C2 (50 kg N_2/ha fertilization and soil treatment with microbiological preparation of 30 l/ha). In comparison, all other variants had a statistically significantly lower average protein content ($p<0.01$).

In the second year of research, the highest average protein content (Table 3) was obtained in variant B1C3 (without fertilization using microbiological preparation (soil treatment (30 l/ha) + 2 foliar treatments (2 x 6 l/ha)). All other variants had statistically significantly lower average protein content ($p<0.01$). In 2014, because of more favourable weather conditions, higher protein content in grains was achieved without nitrogen fertilizer as well, but using microbiological preparation.

P. fluorescens, which with the products of its metabolism, dissolves even rock phosphates and also has the ability to fix atmospheric nitrogen, in combination with *Bacillus* spp., symbiotic nitrogen fixing bacteria *Bradyrhizobium* and non-symbiotic nitrogen fixing bacteria *A. chroococcum* and *A. brasilense* provided enough nutrition for growth and development of soybean. *P. fluorescens* and *Bacillus* spp. provided also sufficient amounts of molybdenum for plant uptake, which is the basic building block of leghemoglobin and nitrogenase, on which the process of nitrofixation depends.

Table 2. Soybean grain yield and quality in year 2013

Tablica 2. Prinos i kvaliteta soje u 2013. godini

Cultivar *Sorta* (A)	Nitrogen fertilization *Gnojidba dušikom* (B)	Em – Aktiv introduction *Primjena Em-Aktiv* (C)	Grain yield *Prinos* (kg/ha)	Protein content *Sadržaj bjelančevina* (%)	Oil content *Sadržaj ulja* (%)	Hectolitre mass *Hektolitarska masa* (kg)
A1	B1	C1	3015	36.12	22.50	71.43
		C2	3251	36.87	23.53	72.17
		C3	3441*	37.53*	23.60*	72.27
	B2	C1	3292	37.03	22.48	72.30
		C2	3419	37.77*	23.27*	72.97*
		C3	3461*	37.50	23.23	72.69
Average/*Prosjek*			3353	37.14	23.44	72.20
A2	B1	C1	3064	36.95	22.98	71.75
		C2	3218	37.97	22.97	72.10
		C3	3862*	38.20*	23.47*	72.23
	B2	C1	3312	37.19	22.43	70.55
		C2	3849*	37.82*	23.10	72.67*
		C3	3513	37.65	23.30*	72.17
Average/*Prosjek*			3470	37.60	23.38	72.25
Total average/*Ukupni prosjek*			3412	37.37	23.41	72.23
$LSD_{0.05}$			16.07	0.08	0.04	0.14
$LSD_{0.01}$			30.22	0.14	0.07	0.22

Cultivar (A): A1-Ika; A2-Vita; Nitrogen fertilization (B): B1-control (0 kg N_2/ha); B2-50 kg N_2/ha; Em – Aktiv introduction (C): C1-control; C2-soil treatment (30 l/ha); C3-soil treatment (30 l/ha) + 2 foliar treatments (2 x 6 l/ha)

According to Burns et al. (1981) and the results of their researches, plant growth-promoting bacteria affect protein content. Furthermore, it was found that the non-symbiotic nitrogen fixing bacteria *A. vinelandii* enhances the symbiosis of soybean root and the symbiotic nitrogen fixing bacteria *B. japoni-*

cum (Table 3). Generally, plant growth-promoting rhizobacteria facilitates plant growth by either assisting in resource acquisition (N_2, P_2O_5 and essential minerals) or by modulating plant hormone levels, or indirectly by decreasing the inhibitory effect of various pathogens on plant growth and development in the forms of biocontrol agents (Ahemad and Kibret, 2013).

Oil content (%)

In the first year of research (Table 2) both cultivars achieved the highest average oil content (%) in variant B1C3 without fertilization using microbiological preparations (soil treatment - 30 l/ha + 2 foliar treatments - 2 x 6 l/ha). Comparatively, all other variants had a statistically significantly lower average protein content ($p<0.01$).

In the second year, the highest average oil content (%) was realized without nitrogen fertilization (B1) using only the incorporation of 30 l/ha of microbiological preparation (C2). Due to sufficient precipitation, no foliar application of the microbiological preparation was required. Incorporated beneficial bacteria adapted quickly under favourable water-air conditions and were able to grow fast and to provide all necessary nutrients for plant growth and development.

Oil content was very positively correlated with grain yield root ($r=0.946$; $p<0.01$) and protein content ($r=0.950$, $p<0.01$).

Statistically significantly higher oil content in variants treated with beneficial bacteria *P. fluorescens* was obtained by Kumar and Dube (1992), as well as by Siddiqui et al. (2005) who also registered the nematicidal activity of *P. fluorescens* on the root-knot nematode *Meloidogyne incognita*.

Hectolitre mass (kg)

Both cultivars achieved the highest average hectolitre mass in the first year of study in variant B2C2 (fertilization with 50 kg N_2/ha + soil treatment with microbiological preparation of 30 l/ha). In comparison, all other variants had a statistically significantly lower average hectolitre mass ($p<0.01$).

In the second year of research, the highest average hectolitre mass of Ika cultivar was obtained in the variant B1C3 without fertilization using microbiological preparations (soil treatment - 30 l/ha + 2 foliar treatments - 2 x 6/l ha). There were no significant differences with any other variant except with control variants (B1C1; B2C1).

Cultivar Vita achieved the highest average hectolitre mass in variant B2C2 (50 kg N_2/ha fertilization + soil treatment with microbiological preparation of 30 l/ha), again, there were no statistically significant differences with any other variant except control variants (B1C1; B2C1).

A great number of authors pointed out the positive effects of beneficial bacteria on all parameters that influence the yield and quality (Jeon et al., 2003; Vessey, 2003; Ahemad and Kibret, 2013).

Table 3. Soybean grain yield and quality in year 2014

Tablica 3. Prinos i kvaliteta soje u 2014. godini

Cultivar *Sorta* (A)	Nitrogen fertilization *Gnojidba dušikom* (B)	Em – Aktiv introduction *Primjena Em-Aktiv* (C)	Grain yield *Prinos* (kg/ha)	Protein content *Sadržaj bjelančevina* (%)	Oil content *Sadržaj ulja* (%)	Hectolitre mass *Hektolitarska masa* (kg)
A1	B1	C1	3318	38.33	21.12	69.68
		C2	4117*	38.52	21.82*	71.46
		C3	3873	38.96*	21.77	71.58*
	B2	C1	3644	38.01	21.41	70.36
		C2	3984*	38.50*	21.57*	71.57
		C3	3931	38.45	21.50	71.51
Average/*Prosjek*			3811	38.53	21.53	71.03
A2	B1	C1	3671	38.15	21.37	70.36
		C2	4149*	38.61	21.95*	71.57
		C3	3980	38.86*	21.79	71.49
	B2	C1	3704	38.26	21.50	70.83
		C2	4170*	38.56*	21.71*	71.79*
		C3	4011	38.46	21.63	71.59
Average/*Prosjek*			3948	38.48	21.67	71.27
Total average/*Ukupni prosjek*			3880	38.51	21.60	71.15
$LSD_{0.05}$			18.03	0.09	0.05	0.17
$LSD_{0.01}$			33.04	0.16	0.09	0.31

Cultivar (A): A1-Ika; A2-Vita; Nitrogen fertilization (B): B1-control (0 kg N_2/ha); B2-50 kg N_2/ha; Em – Aktiv introduction (C): C1-control; C2-soil treatment (30 l/ha); C3-soil treatment (30 l/ha) + 2 foliar treatments (2 x 6 l/ha)

CONCLUSION

Based on the obtained results of the research it can be concluded that the best results of the examined parameters were in variants treated with a microbiological preparation, and even in the variants where nitrogen fertilizer was not applied. A statistically significant effect of soybean varieties ($p<0.01$), as well as soil type ($p<0.01$) was obtained, probably due to drought in the first year of research.

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UTJECAJ BENEFITNIH MIKROORGANIZAMA NA PRINOS I KVALITETU SOJE U UVJETIMA SMANJENE GNOJIDBE DUŠIKOM

SAŽETAK

U radu je istraživana mogućnost redukcije dušičnih gnojiva uz korištenje benefitnih mikroorganizama (rodovi Bradyrhizobium, Azotobacter, bacterija Pseudomonas fluorescens, Bacillus spp., itd.), kao alternative dušičnim gnojivima. Istraživanja su provedena tijekom 2013. i 2014. godine na eutrično smeđem tlu. Pokus je postavljen prema split-blok shemi te sadrži 12 varijanti u 4 ponavljanja kako slijedi: dvije sorte soje, dvije varijante primjene mineralnoga dušičnoga gnojiva i tri varijante primjene mikrobiološkoga preparata. Elementi istraživanja bili su prinos zrna (kg/ha), preračunat po hektaru na osnovi 13% vode u zrnu; sadržaj proteina (%); sadržaj ulja (%) i hektolitarska masa (kg). S obzirom na to da su klimatske prilike u drugoj godini istraživanja bile povoljnije nego u prvoj istraživačkoj godini, svi elementi istraživanja, uključujući kontrolne varijante, druge godine istraživanja, postigli su bolje rezultate. Sve varijante tretirane mikrobiološkim preparatima, bilo aplikacijom u tlo ili aplikacijom u tlo uz folijarne tretmane, također su postigli statistički vrlo značajno bolje rezultate u odnosu na kontrolne varijante.

Ključne riječi: mikrobiološki preparat, dušik, soja, prinos, kvaliteta

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