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Effects of the application of biofertilizers on the microflora and yield of lettuce (*Lactuca sativa* L.)

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Abstract: Recently, biofertilizers are recommended as an alternative or supplement for mineral nutritients. Active agents in biofertilizers are microorganisms that are involved with their activity in the preparation of herbal assimilative and other biotic substances for plant needs. In this study, the influence of Bioaktiv bioproduct on the lettuce yield and rhizosphere microflora of lettuce was tested. „Bioaktiv" contains the active substance consisting of following microorganisms: *Bacillus subtilis, Azobacter sp., Penicillium oxalicum* and *Fusarium sp*. In the tested soil samples of lettuce rhizosphere, the total number of bacteria, *Azotobacter sp*., ammonifiers, oligonitrophyls, *actinomycetes* and fungi were determined. The total number of bacteria, *Azotobacter sp*., ammonifiers, oligonitrophyls, *actinomycetes* was increased while the number of fungi in the variant with biofertilizer was slightly lower than in the control variant. The application of this bioproduct affected on earlier formation of the lettuce head and overall higher yield.

Key words: biofertilizers, soil, lettuce, microorganisms, yield

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Introduction

Biofertilizers are microbiological fertilizer which contains selected, highly effected bacteria and fungal strains isolated from soil. Theirs input in soil activate appropriate microbiological processes which enable better and more uniform supply of plants with nitrogen, phosphorus and potassium, as well as some trace elements (Mrkovački et al., 2012). The binding of atmospheric nitrogen in the process of biological nitrogen fixation is the most important component in the nitogen cycle in nature with special significance for agriculture (Mrkovački and Milić, 2001). Depending on the strain and environmental conditions, azotobacteries can fix 50 to 80 kg of nitrogen per hectare per year (Jarak et al., 2010). Except of adopting the elemental nitrogen, *Azotobacter sp.* produce biologically active substances-auxin, gibberellin, pyridoxine, biotin and nicotinic acid, which promotes plant growth (Mrkovački and Milić, 2001). Because of these properties *Azotobacter sp.* applies as biofertilizers (Milić et al., 2004). Beside nitrogen, growth of plants directly depends of phosphorus, which is usually present in the unavailable forms for plants in the soil. Bacterial genera like *Bacillus* and *Azotobacter* can synthesized organic acid and phosphates, which will convert unavailable form of phosphorus to available form for plants. Cherr et al., 2006, Wilhelm et al., 2007, Orhan et al., 2006 in their research suggested that bacteria of the *Bacillus* genus increase yield and growth of various plants. Entering *Bacillus sp*. in the rhizosphere of pepper provide very good alternative to chemical fertilizers during the cultivation of plants in greenhouses (Garcia et al., 2004). Fungi participate in the decomposition of organic residues (*Penicillium sp*., *Fusarium sp*.). They are more efficient than bacteria because they transformed a greater amount of decomposed plant remains in available nutrients. Soil fertility significantly depends on the fungi because they continue with the decomposition of complex organic matter, even when bacteria and *actinomycetes* stopped to function (Brady, 1990).

The aim of this study was to determine the effect of biofertilizers on growth and development of lettuce in the greenhouse and the number of microorganisms in the rhizosphere of lettuce.

Materials and methods

The experiment was set in a greenhouse of the Agricultural Institute of RS in Banja Luka. Tests were conducted during winter lettuce growing season and it was used variety of lettuce „Nice", at a distance of 25 x 30 cm. The seedlings were produced in polystyrene containers in the greenhouse. Pre-crops lettuce was tomato. Harvesting lettuce was carried out at the stage of technological maturity. The average yield is determined by weighing heads of lettuce. Vegetation period was 45 days.

Bioaktiv contains the active substance consisting of the following microorganisms: *Bacillus subtilis*, *Azotobacter sp*., *Penicillium* and *Fusarium spoxalicum.* Number of cells in a gram of preparation is as follows:

- *Bacillus subtilis* - 31×10^8
- *Azotobacter sp*.-$1{,}7 \times 10^5$
- *Penicillium oxalicum* -10
- *Fusarium sp*.-10

Biofertilizer was applied by watering in the root zone. In the experiment two plots were presented: untreated lettuce and treated lettuce by biofertilizer. Each plots had twenty plants. Soil samples for microbiological analysis were taken from rhizosphere soil at the stage of technological maturity of plant and six soil samples from variants with biofertilizer and six samples from the control variant. In the soil samples using the dilution method, were determined the total number of bacteria on the surface 0.1 x TSA, a number of ammonifiers on meat peptone agar (Pochon and Tardieux, 1962). The number of sporogenic bacteria was determined by heating the inoculum at 80° C for 10 min and seeding on meat-peptone agar. Presence of oligonitrophyls was determined on medium without nitrogen by Fjodorov (Anderson and Domasch, 1958) and the number of *Azotobacter sp*. was determine on Fyodorov's medium by the fertile drop method (Anderson, 1965). The number of *actinomycetes* was determined on a synthetic medium by Krasiljnikov (1965), and fungi on Czapek-Dox medium. The time and temperature of incubation depended by the group of microorganisms. All microbiological analyses were performed in three replications and the average number of microorganisms was calculated at 1 g absolutely dry soil.

Results and Discussion

Biofertilizers gave new quantities of microorganisms in the soil which increases the amount of organic and inorganic compounds that are products of their metabolism. Also, after microbial death in the soil remains a significant amount of organic matter that is the source of nutrients for the living microbes whose number increases (Jarak et al., 2007). Some microorganisms are competitors, fighting for nutrient substrate, so that the number of certain microorganisms can be reduced (Lynch, 1983).

The total number of bacteria can be used as an indicator of general biological activity. In this study, the total number of bacteria was in the millions in a gram of soil in variant with biofertilizer and in control variant, which indicates that the soil had high biological activity. The total number of bacteria in the soil of the variant with biofertilizer was 53.15×10^6, while in the control variant total number of bacteria decreased and was 37.02×10^6 (Table 1).

Ammonifiers represent a large group of bacteria, fungi and *actinomycetes* involved in the degradation of native proteins and their transformation into new mineral or organic forms. The largest part is built into microbial proteins that are part of the humus (Jarak and Colo, 2007). After application of biofertilizer, the number of ammonifiers was increased in tested soil samples in relation to control soil samples. In the control variant, the total number of ammonifiers was 24,80 x 10^5 while the total number of ammonifiers in the variant with biofertilizer was 30,35 x 10^5.

The number of sporogenic ammonifiers was increased in the tested soil in variant with biofertilizer in relation to control variant and contributed 46,52 x 10^4. In the control variant, the number of sporogenic ammonifiers was 42,40 x 10^4.

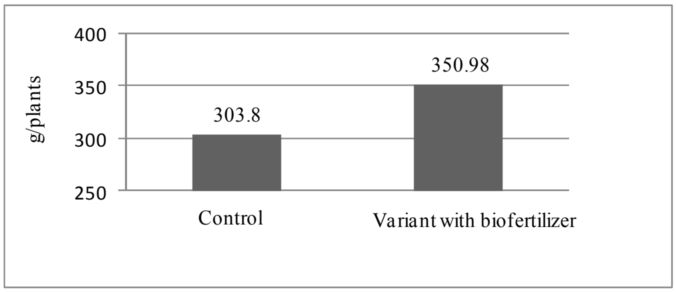
Oligonitrophyls are microorganisms that belong to the group of free nitrogen-fixing. In one gram soils of neutral and slightly acidic reaction there were several hundred thousand, and the amount of fixed nitrogen is 20-60 kg (Govedarica, 1986). The number of oligonitrophyls was increased after using of biofertilizer in the tested soil samples in relation to the control variant and was 47.36 x 10^5, while the number oligonitrophyls in the control variant was 41.00 x 10^5.

Table 1. The occurrence of total number of bacteria, ammonifying and oligonitrophilic bacteria, *Azotobacter sp., actinomycetes* and fungi in the rhizosphere of lettuce

Species of microorganisms		Variants	
		Control	Bioaktiv
		Number of microorganisms (g^{-1} absolutely dry soil)	
Total number of bacteria		37,02 x 10^6	53,15 x 10^6
Ammonifying bacteria	total	24,80 x 10^5	30,35 x 10^5
	sporogenic	42,40 x 10^4	46,52 x 10^4
Oligonitrophilic bacteria		41,00 x 10^5	47,36 x 10^5
Azotobacter sp.		57,03 x 10^2	75,24 x 10^2
Actinomycetes		62,90 x 10^4	80,90 x 10^4
Fungi		20,81 x 10^4	16,93 x 10^4

Azotobacter sp. belong to the group of free nitrogen fixing bacteria. Since these require the conditions that suit most plants, *Azotobacter sp*. are used as an indicator of soil fertility. (Govedarica, 1986). The number of *Azotobacter sp*. after using biofertilizer was increased in the tested soil and was 75.24 x 10^2, while the number of *Azotobacter sp*. in the control variant was 57.03 x 10^2.

Actinomycetes require alkaline environment and represent microorganisms that are able to break down the hardest substances such as humus. Their activity completely decomposed organic matter to end products of mineralization which make carbon dioxide and water. As organic matter is broken down, plant nutrients are released in available forms for root uptake. The number of *actinomycetes* was increased after application of biofertilizers in the tested soil compared to the control variant and contributed 80.90 x 10^4, while in the control variant the number of *actinomycetes* was 62.90 x 10^4.



Graph. 1.The yield average of the lettuce

Fungi are acidophilic microorganisms that are important in the degradation of fresh organic residues, synthesis and mineralization of humus (Govedarica and Jarak, 1995). The number of fungi in the variant with biofertilizer was slightly lower than in the control variant and was 16.93 x 10^4, while the number of fungi in the control variant contributed 20.81 x 10^4. In the variant with biofertilizer as well as in the control variant the number of fungi was less than the number of bacteria and *actinomycetes* that can be considered as a result of many biotic and abiotic factors on the abundance of this group of microorganisms in the tested soil. Belanović et al. (2004) in their research also points out that presence of certain types of microorganisms in soil depends of the presence and nature of organic matter in the soil, and many abiotic and biotic factors. Beginning of the exploration of individual types of microorganisms in the soil depends on the presence and nature of organic matter in the soil, and a number of abiotic and biotic factors. Which number of microorganisms will survive after entering in the soil largely depends on the dominated conditions in soil (Jošić, 2004).

The application of biofertilizer in this study has shown that biofertilizer acts to increase heads of lettuce, because lettuce plants had higher mass (Graph. 1), which is similar to the results of research Vernieri et al. (2002). It is considered that biofertilizers affect on higher yield (Muralidharan et al., 2000), as confirmed by the results of this research. The yield average of the lettuce in the variant with biofertilizer was 350,98 g/plants, while in the control variant the yield average of the lettuce was 303,80 g/plants.

Conclusion

In this study, use of biofertilizer influenced the increase of the total number of bacteria, ammonifiers, oligonitrophyls, *Azotobacter sp*. and *actinomycetes* in the rhizosphere of lettuce, while the number of fungi was slightly lower than in the control variant.

The application of biofertilizers influenced the increase of the yield of lettuce. There was recorded yield increase of 17.7% in variant with biofertilizer compared to the control variant.

References

Anderson, G.R. (1965): Ecology of *Azotobacter* in soil of the palouse region, I: Occurrence. *Soil Science*, 86: 57-65.

Anderson J.P.E., Domasch, K.H. (1958): A physiological method for the quantitative measurement of microbial biomas in soil. *Soil Boil. Biochem.*, 10: 215-221.

Belanović, S., Knežević, M., Miličić, B., Đorović, M. (2004): Contens of heavy metals and micro-flora in some soil of mt. Stara planina. *Bulletin of the Faculty of Forestry*, *University of Belgrade*, 89: 53-61.

Brady, N.C. (1990): *The Nature and Properties of Soils*. New York: Macmillan Publishing Company, 621.

Cherr, C.M., Scholberg, J.M.S., McSorley, R. (2006): Green manure approaches to crop production. *Agronomy Journal*, 98: 302-319.

Garcia, J.A.L., Probanza, A., Ramos, B., Palomino, M.R., Manero, F.J.G. (2004): Effect of inoculation of *Bacillus licheniformis* on tomato and pepper. *Agronomie for Sustainable Development*, 24(4): 169-176.

Govedarica, M. (1986): *Nitrogen fixing bacteria and their activity in maize*. Ph.D Thesis, University of Novi Sad, Faculty of Agriculture, Novi Sad.

Govedarica, M., Jarak, M. (1995): *Soil microbiology*. University of Novi Sad, Faculty of Agriculture, Novi Sad, 1-212.

Jarak, M., Colo, J. (2007): *Soil microbiology*. University of Novi Sad, Faculty of Agriculture, Novi Sad, 209.

Jarak, M., Đurić, S., Đorđević, B. (2010): Benefits of inoculation with azotobacter in the growth and production of tomato and pepers. *Matica srpska Journal for Natural Sciences*, 119: 71-76.

Jarak, M., Đurić, S., Simikić, M., Savin, L., Vasin, J. (2007): Microbiological activity in soil under wheat. *Traktors and power machines*, *12*(3): 49-53.

Jošić, D. (2004): *Diversity among indigenous population bacteria that nodulate clover* (*Rhizobium legominusarum bv.trifolii*). PhD thesis, University of Novi Sad, Faculty of Agriculture.

Krasiljnikov, N.A. (1965): *Биологија отедељних груп актиномицетов*. Москва, Наука.

Lynch, J.M. (1983): *Soil Biotechnology*. Oxford: Blackwell Scientific Publications.

Milić, V., Jarak, M., Mrkovački, N., Milošević, N., Govedarica, M., Đurić, S., Marinković, J. (2004): Microbiological fertilizer use and study of biological activity for soil protection purposes. *Proceedings of the Institute of Field and Vegetable Crops,* Novi Sad*,* 40: 153-169.

Muralidharan, R., Saravanan, A., Muthuvel, P. (2000): Influence of biostimulants on yield and quality of tomato (*Lycopersicon esculentum* Mill.). *Madras Agricultural Journal*, 87 (12): 625-628.

Mrkovački, N., Milić, V. (2001): Use of *Azotobacter chroococcumas* potentially useful in agricultural application. *Annals of Microbiology*, 51 (2): 145-158.

Mrkovački, N., Jarak, M., Đalović, I., Jocković, Đ. (2012): Importance of PGPR Application and Its Effect on Microbial Activity in Maize Rhizosphere. *Proceedings of the Institute of Field and Vegetable Crops*, Novi Sad, 49 (3), 335-344.

Orhan, E., Esitken, A., Ercisli, S., Turan, M., Sahim, F. (2006): Effect of Plant Growth Promoting Rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Science Horticulturae*, 111: 38-43.

Pochon, J., Tardieux, P. (1962): *Techniques d'analyse en microbiologie du sol*. Paris: Ed de la Turelle.

Vernieri, P., Malorgio, F., Tognoni, F. (2002): Use of biostimulants in production of vegetable seedlings. *Colture-Protette*, 31(1): 75-79.

Wilhelm, J., Johnson, M.F., Karlen, L., David, T. (2007): Corn stover to sustain soil organic further constrains biomass supply. *Agronomy Journal*, 99: 1665-1667.

EFEKTI PRIMJENE BIOFERTILIZATORA NA MIKROFLORU I PRINOS ZELENE SALATE (*Lactuca sativa* L.)

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Sažetak

Biofertilizacija se u novije vrijeme preporučuje kao alternativa ili dopuna mineralnim hranivima. Aktivni činioci u biofertilizaciji su mikroorganizmi koji svojom aktivnošću učestvuju u pripremanju biljnih asimilativa i drugih biotičkih materija za potrebe biljaka. U ovom istraživanju ispitan je uticaj biopreparata „Bioaktiv" na prinos zelene salate i mikrofloru rizosfere salate. Bioaktiv sadrži aktivnu materiju koju čine sledeći mikroorganizmi: *Bacillus subtilis, Azotobacter sp*., *Penicillium oxalicum* i *Fusarium sp*. U uzorcima zemljišta koji su uzeti iz rizosfere biljaka određen je ukupan broj bakterija, amonifikatora (ukupan i sporogeni), oligonitrofila, gljiva i aktinomiceta. Ukupan broj bakterija, amonifikatora, oligonitrofila, azotobaktera i aktinomiceta se povećao u zemljištu u varijanti sa biofertilizatorom u odnosu na kontrolnu varijantu, dok je broj gljiva manji u odnosu na kontrolnu varijantu. Primena ovog biopreparata je uticala na ranije formiranje glavice salate i veći ukupan prinos.

Ključne reči: biofertilizatori, zemljište, salata, mikroorganizmi, prinos