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Full Length Research Paper

Importance of microbiological fertilizer used in soybean production: Agronomical and biological aspects

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Inoculation is the best way to ensure good nodulation with the proper strain of nitrogen-fixing bacteria for increased yield in an environmentally safe manner. This investigation was conducted on three-year experiment which was set up in four replications with three-crop rotation (maize-soybean-wheat) at experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. Effects of application of microbiological fertilizer (with nitrogen-fixing bacteria *Bradyrhizobium japonicum*) on root mass, number and mass of nodes per plant, soil biogeneity (number of ammonifiers, *Azotobacter*, actinomycetes), as well as the effect on soybean yield were studied. Obtained results indicated positive impact of inoculation on nitrogen fixation parameters. The higher amounts of mineral nitrogen had negative impact on atmospheric nitrogen fixation, that is, by increasing preplanting mineral nitrogen amount in the soil by 1 kg, the amount of calculated nitrogen from the air was decreased by 1.72 kg. Increasing abundance of studied microorganisms in the rhizosphere, as well as soybean yield using microbiological fertilizer was stimulated by plowing under crop residues. The impact of meteorogical conditions on the effects of inoclutaion was very significant.

Key words: Inoculation, fertilizing, nitrogen fixation, soybean, microorganisms, yield.

INTRODUCTION

Microorganisms are widely spread around nature in the soil, water and air as well as on plants and animals and are a crucial link in the chain of matter circulation on the planet. They are the most important component of the biological soil phase and an important indicator of soil fertility and soil degradation (Milić et al., 2004). In conventional agriculture, chemical fertilizers, pesticides, and plant growth regulators are usually applied to increase the yield and quality of crops. However, the frequent excessive use of these chemicals has often resulted in adverse environmental effects, disturbing the ecological balance of soils, and making plants even more susceptible to diseases (Higa and Wididana, 1991).

The complex nature and the multitude of both biotic and abiotic interactions that occur within soils have traditionally maintained our view of the below-ground aspects of agriculture as a black box. However, as we move from high-input, conventional agriculture that is production based to sustainable systems that rely more heavily on nutrient cycling and soil microbial ecology, the elucidation of the complex interactions occurring in soils will be necessary (Schreiner and Bethlenfalvay, 1995).

Nitrogen is one of the most limiting plant nutrients for plant growth. Some rhizosphere bacteria have the ability to fix N_2 into forms that can then be used by plants. The rhizosphere conditions favor the N_2 fixation because it is carried out by heterotrophic bacteria that use organic compounds as source of electrons for the reduction of N_2. Prominent among these microorganisms are the N_2 fixers of the genera *Rhizobium, Bradyrhizobium* and others that form symbiosis with legumes. Although fixation of 5 to 25 kg N/ha per year are widely accepted, values as high as 90 kg N/ha per year have been reported (Vega, 2007). When in symbiotic association with *B. japonicum*, soybean plants can fix up to 200 kg N/ha per year,

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reducing the need for expensive and environmentally harmful nitrogen fertilizers (Javaid and Mahmood, 2010).

Therefore, it is very important to apply a good soybean cultivation practices that will increase the total number of soil microorganisms and the number of certain physiological and systematic groups of microorganisms. *Azotobacter* abundance in the soil is a good indicator of all toxicological and degradation changes in the soil (Cvijanović et al., 2011).

Many species and specific strains of bacteria residing in rhizosphere have been shown to possess plant growth promoting traits and hence they are collectively designated as plant growth promoting rhizobacteria (PGPR). Direct promotion of growth by PGPR occurs when the rhizobacteria produce metabolites that promote plant growth such as auxins, cytokinins and gibberellins (Adesemoye et al., 2008; Suresh et al., 2010).

The crop production systems may be improved by introducing strategies which are environmental friendly. Biological systems such as those involving mycorrhiza may be used to supplement the expensive chemical fertilizers (Makoi and Ndakidemi, 2009; Gao et al., 2010; Đukić et al, 2010a; Rahmanian et al., 2011).

For many years scientists have investigated the beneficial effects of microorganisms activities such as biological N fixation, organic matter decomposition, mineralization, nitrification, and fermentation. Soil productivity generally decreases as soil organic matter decreases. When this happens, the total soil microbial population and its biodiversity also tend to decrease. Microbiological fertilizers may contain one type of microorganism or a mixture of different microorganisms. In order to minimize the use of mineral fertilizers and chemicals, more attention is paid to microbiological fertilizers that are much more acceptable from the economic and ecological aspects (Milić et al., 2003).

The aim of this study was to assess the effect of inoculation of soybean on yield and soil microbiological activity, and possibility to reduce the use of mineral nitrogen fertilizer, contributes economical and environmental safety production.

MATERIALS AND METHODS

The study was conducted in the multi-year stationary field experiment at the Institute of Field and Vegetable Crops, Novi Sad, Serbia from a three-year crop rotation cycle (maize-soybean-wheat), so the maize was always used as the preceding crop for soybean. The trial was set up in 1971 within the frame of the International commission for studying the soil fertility (I.S.D.V.) and was design as a three-field plot with four replications, and the variants were distributed using a randomized block design. Mineral nitrogen feritilizer was upplied under preceding crop. Soybean (Proteinka variety) was not ferilized with nitrogen fertilizer. The trial included variants with and without plowing under crop residues (CR), and within each variant there were two sub-variants with and without soybean seed inoculation:

i. 0 kg N/ha + CR + 50 kg N/ha after wheat
ii. 50 kg N/ha + CR + 50 kg N/ha after wheat
iii. 100 kg N/ha + CR + 50 kg N/ha after wheat
iv. 150 kg N/ha + CR + 50 kg N/ha after wheat
v. 200 kg N/ha + CR + 50 kg N/ha after wheat
vi. 250 kg N/ha + CR + 50 kg N/ha after wheat
vii. 0 kg N/ha (control)
viii. 100 kg N/ha, with no crop residues
ix. 200 kg N/ha, with no crop residues

Soybean seed was inoculated just prior to planting using microbiological fertilizer *NS-Nitragin* containing a mixture of selected highly effective *Bradyrhizobium japonicum* strains.

The soil on which the trial was set is carbonate chernozem with the following characteristics: pH 7.0, 1.95% $CaCO_3$, 2.63% of humus, 11.2 mg P_2O_5/100 g soil, 20.5 mg K_2O/100 g soil. In each of the studied year agrochemical analyzes were done up to the depth of 30 cm: the pH value, content of calcium and humus, soil supply with plant-available phosphorus and potassium. Also, the movement of mineral nitrogen (N_{min}) in the soil to a depth of 90 cm was observed. These analyses were done in the spring prior to soybean planting, and in the autum immediately after harvest.

At the full bloom stage – R2, the soybean plant samples were taken to determining the root mass, number and mass of nodules per plant. At the full maturity stage – R8, the soil samples were taken from rhizosphere, and the biological activity was observed by observing abundance of some microrganisms groups. Indirect dilution method on appropriate nutritive media was used to determine the number of ammonifiers, *Azotobacter* and actinomycetes. Number of ammonifiers was determined on mesopepton agar – MPA (Pochon and Tardieux, 1962), and actinomycetes on synthetic medium according Krasiljnikov (1965). Number of *Azotobacter* was determined on nitrogen-free medium using „fertile drops“ method (Anderson, 1965). Incubation temperature was 28°C, while incubation time depended on the tested group of microorganisms.

Central four rows of each basic plot were harvested using combine for micro-trials (Wintersteiger elite). The soybean yield was presented as kg/ha (based on 14% of moisture content).

Meteorogical conditions in the studied years

In the first research year (2005) precipitation sum in vegetation period was 530 mm' which was 45% higher compared to long-term average for this region. Water deficit occured only in the first decade of June, and there was no further period of water deficit until the end of vegetation period, so the value of potential evapotranspiration (ETP) was 447 mm, and that of real evapotranspiration (ETR) 525 mm.

In the second year (2006) the sum of precipitation from April to the end of September was 420 mm or 15% higher than the long-term average. By mid July soybean was well provided with water, after which a precipitation deficit, followed by a high temperature was reported. ETP value was 468 mm, and ETR 401 mm, and in this year a water deficit of 67 mm was noted.

The third year (2007) of reasearch was also characterized by the deficit of precipitation, but the precipitation distribution was more favorable than in 2006, especially in the second decade of July and in August. Higher precipitation deficit was observed at the beginning of vegetation period (April). ETP was 499 mm, and ETR 405 mm, i.e. the water deficit of 94 mm was recorded.

Research results were statistically processed using analysis of variance, two-factorial design: factor A – doses of nitrogen fertilizer and factor B – inoculation (statistical program ˝Statistica 8.0˝), and the significance was evaluated by least significant differences (LSD) at $p < 0.05$ (only LSD for factor B was presented in results). The regression analysis for the content of mineral N in the soil, and N from the air was also done. Due to the better understanding of the results and different agrometeorological conditions in certain

Table 1. Effect of inoculation on nitrogen fixation parameters (R2 stage), average for all studied years.

Variant (kgN/ha)	Root mass (g/plant)			Nodules number/plant			Nodules mass (g/plant)		
	WMF	MF	C	WMF	MF	C	WMF	MF	C
1 (0+CR)	1.08	1.24	15	1.67	10.33	519	0.08	0.22	175
2 (50+CR)	1.05	1.28	22	1.87	7.73	313	0.09	0.24	167
3 (100+CR)	1.01	1.27	26	1.03	7.27	606	0.05	0.14	180
4 (150+CR)	1.21	1.31	8	1.17	5.40	362	0.08	0.16	100
5 (200+CR)	1.09	1.33	22	2.90	8.00	176	0.10	0.18	80
6 (250+CR)	0.93	1.31	41	1.77	6.57	271	0.06	0.17	183
7 control	1.14	1.25	10	2.73	13.93	410	0.08	0.28	250
8 (100)	1.14	1.30	14	1.67	12.20	630	0.05	0.21	320
9 (200)	1.38	1.49	8	1.03	8.37	713	0.05	0.18	260
Average	**1.11**	**1.31**	**18**	**1.76**	**8.87**	**404**	**0.07**	**0.20**	**191**

WMF – variant (1-9) whithout microbiological fertilizer.
MF – variant (1-9) + microbiological fertilizer.
C – calculated effect of inoculation (%).

years, each year was analyzed separately.

RESULTS AND DISCUSSION

Nitrogen fixation is important process which converts gaseous nitrogen in the air (N_2) to ammonium form (NH_4^+), thus increasing supply of mineral nitrogen, which is necessary for plant growth and development. The annual participation of fixed nitrogen in the yield is high. The significance of nitrogen fixation is proved by the fact that in the symbiosis of soybean and *B. japonicum* annually fixed amount of nitrogen is up to 180 kg/ha (Milošević and Jarak, 2005). Therefore it can be speak about reasonability of microbiological fertilizer application to preserve and improve soil fertility.

Effect of microbiological fertilizer on root mass, number and mass of nodules

Root mass (Table 1) in the variants with applied inoculation on the average for all three studied years was 18% higher compared to the variants without microbiological fertilizer, both with and without plowed under crop residues. The same trend was recorded in nodules number, and its mass as well, with the effect of inoculation of 404%, i.e. 191%. Numerous authors in their researches mentioned positive effect of inoculation on nitrogen fixation parameters in the field, that is root mass, number and mass of nodules per plant (Milić et al., 2002; Milić et al., 2003), while others claimed that plowed under crop residues positively influence on microbiological processes in the soil (Milošević et al., 1997).

In particular, it was noted that higher doses of nitrogen by application of mineral fertilizer reduced the number and mass of nodules in most cases. This was the most pronounced in the variants without plowed under crop residues, as well as with seed inoculation, when number of nodules was reduced by 40% in the variant with 200 kg N/ha (8.37) compared with the control without nitrogen fertilizer applied (13.93), and the reduction of nodule mass was 35% (0.18 and 0.28, respectively). Nitrates present in the soil exerted an inhibitory effect on nodulation, nodules growth and development, as well as the number and nodules dry mass, and the level of nitrogenase activity promoting premature nodule senescens (Mrkovački, 2008). Diep et al. (2002) in their research recorded the highest content of organic matter (5.31%) and total N (0.25%), after soybean harvest in the variant of fertilization with 25 kg N/ha with inoculation. The same authors concluded that application of about 50 kg N/ha together with rhizobial inoculation seemed to be an appropriate cultivation practice for soybean cultivation in soil in the Mekong Delta. If soil residual nitrogen is high, we would not expect to see as meny nodules on the roots as in a soil environment with deficient or normal nitrogen levels (Abendorth and Elmore, 2006). It is not recomended applying additional nitrogen if soil levels are over 85 kg/ha, as this will reduce the potential for any significant later season nodule activity (Franzen, 1999). The results of numerous authors proved that the application of small amounts of nitrate (20-30 kg/ha) stimulates nodulation in the early stages of soybean plant growth, the so-called „starvation for nitrogen“ period. Above these small concentrations the nodule mass is inversely propotional to the level of nitrates in the soil. If the soybean plant picks up too much nitrogen early in the season, it will delay or prevent nodulation (Bohner, 2009).

The results of the study showed that larger amount of mineral nitrogen in the soil caused reduction of the amount of nitrogen fixed from the air. For each kilogram of increased preplanting mineral nitrogen in the soil, the calculated amount of nitrogen fixed from the air was reducing by 1.72 kg (Figure 1), while it is estimated that the net loss of nitrogen which would otherwise be

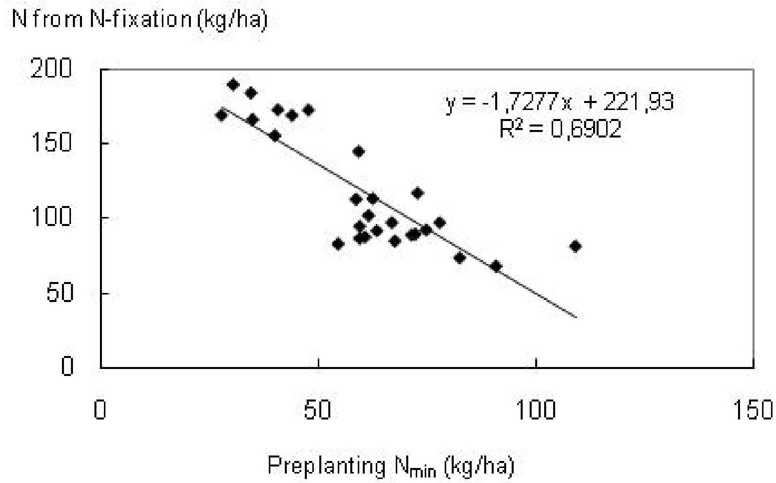

Figure 1. Influence of spring mineral nitrogen amount (preplant) on calculated amount of nitrogen fixed from the air.

available to plants was 0.72 kg.

The convertion of atmospheric nitrogen to ammonia is energetically expensive, and costs more photosynthate than simply taking up nitrate, so the plant will naturally consume nitrates before attempting to nodulate. This fundamental inability to develop and sustain nitrogen fixation in the presence of soil nitrates at greater than very small starter fertilizer rates is largely why nitrogen fertilization does not pay in soybean. Applying nitrogen fertilizer simply reduces the amount of N fixed from the air, as stated by Milić et al. (2004), and that higher amount of nitrogen in the soil adversely influenced on N_2 fixation effectiveness, i.e. plants preferably use nitrogen from the soil, and then satisfy their needs by nitrogen fixed from the air. The same authors mentioned that nitrogen fixation from the air with applied 200 kg N/ha was completely inhibited. Fertilizing with 150 kg N/ha can be defined as big amount of mineral nitrogen acting inhibitionally on nitrogen fixers, while performing enzyme inhibition, responsible for nitrogen fixation (Cvijanović et al., 2008).

The effect of microbiological fertilizer on soil biogenity

The soil is an ecological system and the habitat for different and numerous microorganisms playing the primary role in metabolic soil activity. The presence and activity of some microorganisms groups in the soil can be a good indicator of the soil fertility. Certain groups of microorganisms play a key role in the processes of the humus synthesis and some of the minerals and nutrients that are released from the humus by mineralisation process participate in plant yield formation. If mechanism of microorganisms activity is known, some microbiological processes can be directed to maintain the biological activity, that is soil biogeneity by applying of useful microorganisms.

Ammonifiers are the group of microorganisms, which use proteolytic enzymes to dissolve organic matter releasing amino acids and ammonia which are assimilated into the cells of ammonifiers and other microorganisms, as nitrogen deponents. It was noted that the use of microbiological fertilizer had significant effect on increased number of ammonifiers (Table 2) which is clearly demonstrated on variant 7 (control) that reflected influence of inoculation, only. The effect of inoculation was not observed in 2006, which was extremely unfavorable for soybean production due to the water-deficit and high temperature in the critical period of soybean vegetation (the second half of July and August). In this year, the lowest number of ammonifiers compared to the other studied years was recorded. Soybean as a legume is extremely intolerant to deficiency and excess of water in the soil, which, beside other things, may be caused by high sensitivity of the symbiotic association. Optimal soil moisture for nodule formation is 60-70% field capacity, and the optimal temperature for nitrogen fixation process is 14 to 24°C (Mrkovački, 2008). The greatest difference, i.e. incresing number of ammonifiers by inoculation was recorded in the variant with 100 kg N/ha added under the preceding crop, with plowed under crop residues (100% in 2005, and 142% in 2007), while high nitrogen doses from mineral fertilizer negatively affected the number of ammonifiers. Similar results were obtained by some other authotrs, who pointed out the positive effect of microbiological fertilizer on the ammonifiers abundance in the soybean rhizosphere. Cvijanovic (2002) concluded that inoculation with associative nitrogen fixing bacteria significantly increased the number of ammonifiers in the variants with lower nitrogen doses.

Azotobacter is one of the most significant free nitrogen fixing bacteria. Their abundance is reliable bioindicator of

Table 2. Effect of mocrobiological fertilizer on microorganisms abundance in the soil (R8 stage) (a) Ammonifiers 10^7 (instead of 107), for (b) Azotobacter 10^2 (instead of 102) and for (c) Actinomycetes 10^4 (instead of 104).

Variant (kg N/ha)	Microbiological fertilizer					
	2005		2006		2007	
	without	with	without	with	without	with
(a) Ammonifiers ×107 /g of absolutely dry soil						
1 (0+CR)	114.0	49.5	121.9	38.8	138.0	318.2
2 (50+CR)	90.3	144.8	119.4	82.0	221.8	264.3
3 (100+CR)	119.7	240.3	98.9	97.4	193.7	469.5
4 (150+CR)	115.3	81.7	161.1	61.4	364.0	385.7
5 (200+CR)	118.9	77.3	85.4	56.8	188.3	448.3
6 (250+CR)	123.4	119.4	104.6	64.7	223.8	423.3
7 control	129.1	236.4	129.6	238.6	214.7	335.7
8 (100)	114.2	120.2	109.6	106.9	236.7	413.1
9 (200)	117.9	117.2	106.4	60.4	243.9	339.0
Average	**115.9**	**131.9**	**115.2**	**89.7**	**225.0**	**377.5**
LSD 0.05	15.98		34.12		45.32	
(b) Azotobacter ×102 /g of absolutely dry soil						
1 (0+CR)	257.7	253.5	174.3	175.5	50.3	51.5
2 (50+CR)	226.3	221.1	137.2	103.4	52.4	55.6
3 (100+CR)	206.3	226.2	151.3	150.0	61.5	65.7
4 (150+CR)	115.1	109.3	89.1	121.2	50.3	53.8
5 (200+CR)	169.3	180.7	113.3	135.3	55.0	53.6
6 (250+CR)	146.9	203.3	73.8	131.3	54.7	55.3
7 control	329.3	315.5	154.5	197.0	66.0	71.4
8 (100)	288.7	261.7	124.1	104.1	52.0	58.6
9 (200)	200.4	196.7	75.3	169.2	63.7	57.9
Average	**215.6**	**218.7**	**121.4**	**143.0**	**56.2**	**58.2**
LSD 0.05	24.83		14.69		2.85	
(c) Actinomycetes ×104 /g of absolutely dry soil						
1 (0+CR)	22.4	18.7	31.3	21.4	107.4	95.4
2 (50+CR)	18.1	31.8	21.9	19.9	80.5	86.1
3 (100+CR)	16.6	23.1	23.3	28.0	81.4	122.6
4 (150+CR)	15.1	17.6	19.8	22.0	67.1	100.3
5 (200+CR)	12.5	18.6	20.1	25.1	96.5	118.4
6 (250+CR)	14.7	21.0	21.2	23.9	59.2	105.5
7 control	13.0	26.0	14.7	15.8	58.4	76.4
8 (100)	14.1	17.8	16.4	19.7	78.4	108.8
9 (200)	12.6	25.0	18.2	13.3	93.2	89.5
Average	**15.5**	**22.2**	**20.8**	**21.0**	**80.2**	**100.3**
LSD 0.05	2.22		3.79		10.83	

biological activity in soil. The obtained results revealed that inoculation, had positive influence on *Azotobacter* abundance, which was especially evident during unfavorable year, such as 2006. In the mentioned year, the increased number of *Azotobacter* in variants with applied microbiological fertilizer was 18%, compared to the variants without inoculation. It is very important to note that application of microbiological fertilizer can compensate the adverse environmental conditions, as well as improve the microbiological activity even in the soil water deficit. Increased number of *Azotobacter* with applied inoculation has been reported in other investigations (Milić et al., 2004; Dozet, 2009). In the meteorological conditions that are less favorable for plant production, the influence of inoculation is very significant for *Azotobacter* number increasing, especially on variants fertilizing with 90 and 120 kg N/ha (Cvijanović et al., 2011).

Actinomycetes play an important role in the processes of humification and dehumification, production of vitamins and some metabolites that influence the physiological processes of other beneficial microorganisms and plants. Inoculation increased the number of actinomycetes in the soil (Table 2), with significance in the studied years favorable for soybean production. This increase was 44%, on average for 2005 (15.5 on variants without and 22.2 on variants with inoculation), in which precipitation sum during vegetation period was higher compared to long-term average with lower daily temperatures, that is 25% in 2007 (80.2 and 100.3, respectively) with water deficit and higher mean daily temperatures. These results are in agreement with the findings of others (Cvijanović, 2002; Milić et al., 2003) that inoculation can affect the increase in the actinomycetes number, which are included in the humification processes of soil, and that was confirmed by increasing of humus percentage in the given soil. Greater number of actinomycetes was recorded in the variants with plowed under crop residues when microbiological fertilizer was applied (average of variants 2-6), compared to the variants where only mineral fertilizer was added (average of variants 8 and 9), especially in unfavorable year. This difference was 44% in 2006, and 14% in 2007. Plowing under crop residues in addition to mineral fertilizer stimulated microbiological processes in soil. Depending on the type of soil and climate conditions, microbes immobilize 7 to 15 kg N/ha during straw degradation, which may explain the positive impact of plowing under crop residues on the microbial number and activity (Milošević et al., 1997).

Soil is a dynamic environment due to microorganisms involved in all synthesis and decompositions, and make connection between plant and soil. Great number of microorganisms, wide range of present species, and enzymatic activities of microbe population in the soil are indicators that the soil characteristics are favorable for plant production. Application of microbiological fertilizer can significantly influence the above mentioned characteristics.

Our results revealed that seed inoculation had positive effect on abundance of important microorganisms groups, such as ammonifiers, *Azotobacter* and actinomycetes, but those higher doses of nitrogen mineral fertilizer negatively affected the number of studied microorganisms. Entered diazotrophs in conditions of various presence of mineral nitrogen, affect to increasing dynamics of bacteria total number, and some systematic and physiologic groups of microorganisms (Cvijanović et al., 2008). In all studied years, meteorological conditions influenced the effect of inoculation as was also confirmed by other authors (Abendorth and Elmore, 2006; Bohner, 2009), who claimed that nitrogen fixation is highly sensitive to soil drying. Nitrogen fixation is more sensitive to water-deficit stress then all other phisyological processes in the plant and initially, as soil dry (especially if soil moisture is below 50% field capacity) the total bacteria count decreases.

It is evident from the present studies, as well as from the erlier investigations, that application of microbiological fertilizer can contribute to reducing the use of mineral fertilizers, which has not only economic benefit, but also a very important cultivation practice in terms of environmental safety production. Milošević et al. (2003) stated that the term “healthy soil” was determined by an ecological approach to studying the environment in the function of defining the quantity and the quality of agricultural plant production, while maintaining the biological balance in the nature.

Soybean yield and mineral nitrogen uptake from the soil

Comparing the studied years, 2005 was the most favorable for soybean production, when the highest soybean yield was achieved (Figure 2). Application of inoculation, on average for all variants with plowed under crop residues, increased the yield (3947 kg/ha) by 7% compared to the variants without inoculation (3688 kg/ha). The yield was higher where the mineral fertilizer and crop residues plowed under preceding crop. Soybean yield was the highest in this year, regardless of the lowest difference between preplant amount of mineral nitrogen and quantity after harvesting, compared to the other studied years. This fact can be explained by weather conditions during soybean vegetation period in the given year, when the precipitation sum was 165.5 mm higher compared to the long-term average, and winter water reserves was also higher then the long-term average. Such conditions caused substantial mineralizetion of nitrogen from soil organic matter, which was available to plants, thus the difference between the preplanting quantity of mineral nitrogen and after harvesting was minimal.

Mineral nitrogen uptake from the soil was lower in the variants with inoculation, and significantly higher soybean yield was achieved due to increased nitrogen fixation in these variants. The highest soybean yield (4061 kg/ha) was recorded when the crop residues and 150 kg ha^{-1} of nitrogen fertilizer were plowed under preceding crop, with applying inoculation. The highest uptake of mineral nitrogen was noted in the variant with applied 200 kg N/ha, without plowed under crop residues, and without inoculation. Obtained soybean yield was reduced to the level of control or the variants with lower nitrogen fertilizer doses.

In the second research year the lowest soybean yield (3084 kg/ha) was achieved. Deficit of winter moisture reserves in comparison to long-term average was 31 mm, and precipitation distribution during vegetation period was very uneven, with very high daily temperature. In the third

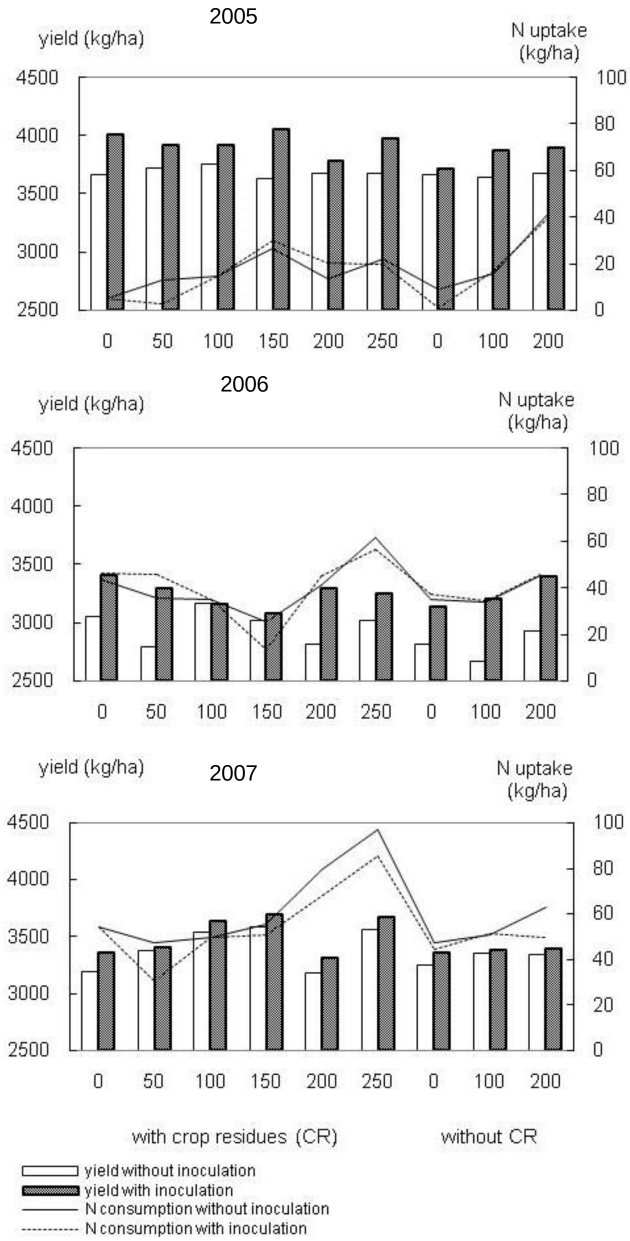

Figure 2. Soybean yield and mineral nitrogen uptake from the soil, during vegetation period.

decade of July the temperature was 4°C higher compared to the long-term average, and only 0.1 mm of precipitation was recorded. Soybean was in the stages of ending pod formation and grain filling, and considerable rejection of pods from lower nodes occurred, causing reduction in yield. Application of inoculation increased the soybean yield by 10.5% on average for the variants with plowed under crop residues, compared to the variants without inoculation. The highest soybean yield (3408.8 kg/ha) was recorded when microbiological fertilizer was applied in the variant with plowed under crop residues, but with no nitrogen applied under preceding crop, while the highest yield without inoculation (3165.8 kg/ha) was achieved in the variant in which 100 kg N/ha and crop residues were plowed under preceding crop. The least difference between the quantity of mineral nitrogen prior to planting and after harvesting was noticed in the variant with 150 kg N/ha applied under preceding crop, and with plowed under crop residues, while the highest consumption of mineral nitrogen was recorded when 250 kg N/ha was applied, both with and without inoculation.

Application of microbiological fertilizer positively affected soybean yield in 2007, too (Figure 2). On average for all variants with plowed under crop residues, the highest yield was achieved with applying inoculation, which was 3% higher compared to the variants without inoculation. Consumption of mineral nitrogen was higher compared to the previous year, especially in the variant without inoculation, in which 250 kg N/ha and crop residues were plowed under the preceding crop. The highest soybean yield (3691 kg/ha) was achieved in application of 150 kg N/ha added under the preceding crop and with plowed under crop residues, with (3691 kg/ha), and without (3578 kg/ha) inoculation. Also, high yield was noted in the variant with added 250 kg N/ha' with plowed under crop residues, but in this variant significantly higher consumption of mineral nitrogen from soil was recorded. This year of research in the variants without inoculation, the difference between the quantity of mineral nitrogen prior to planting and after harvesting was greater, indicating increased consumption of mineral nitrogen from the soil.

Effect of microbiological fertilizer on the soybean yield increasing was expressed especially in the year with unfavorable conditions for soybean production, which is very important with the agronomic aspect, i.e. soybean yield stability even under less favorable conditions. Mrkovački et al. (2002) also confirmed increase in soybean yield caused by inoculation, even by 25%, but they also mentioned significant influence of meteorological conditions during the year, which was also concluded by other authors (Nedić et al., 2004).

High doses of applied mineral fertilizer had a negative impact on soybean yield, due to poor nitrogen fixation from the air, regardless of increased mineral nitrogen uptake from the soil. Obtained results are in accordance with the results reported by Diep et al. (2002), who concluded that soybean yield in the variant with added 25 kg N/ha with inoculation was equal with that in the variant with 100 kg N/ha without inoculation. They also pointed out that application of the high nitrogen levels lowered organic matter and nitrogen derived from air. Many other researches also confirmed the mentioned results (Pušić et al., 2008; Đukić et al., 2009; 2010b). Since a 3000 kg/ha soybean crop requires nearly 230 kg N/ha to reach maturity, a large contribution by the nodules is necessary. Soybean can be groun without inoculation if nitrogen is supplied, but regarding the fertilizer expenses it is better to apply more modest levels of nitrogen and inoculate (Franzen, 1999).

Regarding intensive production practices, agricultural lands worldwide have become vulnerable to degradative processes such as soil erosion, nutritient depletion and loss of organic matter, and have suffered a consequent decline in soil productivity. The great, positive effect of using microbiological fertilizer in soybean production is one of the important items in solving the problems regarding soil protection, taking into account economic effects of production. Inoculation is a low cost way to provide sufficient amount of nitrogen that plants required, without high doses of nitrogen from mineral fertilizer, as well as to preserve soil biogenity, and increase yield in an environmentally frendly production.

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