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The Effect of Different Kinds of Fertilizers on the Number of Azotobacters in the Smonitza Type of Soil Under Maize and the Yield of Maize

L. Mandic, D. Djukic, V. Stevovic
Faculty of Agronomy, Cacak, Serbia and Montenegro

Abstract: Lower nitrogen rates stimulated the development of azotobacters in the soil during almost the entire investigation period. Solid barnyard manure exerted a similar effect. Liquid manure had a somewhat shorter active impact on the growth of azotobacters. High nitrogen rates had a non-stimulating effect on the investigated group of microorganisms, particularly in the initial growing stages and in the period of low soil humidity (mid vegetation). The toxic effect of high nitrogen rates was considerably lower in the rhizospheric soil, as well as in the periods of increased humidity.

The most significant increase of the yield of maize silage during the first research year was obtained by the medium nitrogen rate (120 kg ha^{-1}), whereas in the other years, a higher yield of maize silage and grain in the variant with 150 kg N ha^{-1} was statistically insignificant. Except for the first, in the two following investigation years the organic fertilizers did not significantly increase the yield of maize silage and grain. The application of Azotobacter strain resulted in the statistically insignificant increase of the mentioned productive traits of this field crop.

Key words: fertilizer, soil, bacteria, maize, yield.

Introduction

Among the cultural operations (conventional and modern), special attention should be paid to a timely and appropriate application of various fertilizers and fertilization systems in order to realize the desired effects. Investigations in this field are mostly focused on the increase of the yield of crops whereas the traits of the cumulative effect of fertilizers (the change of biological and chemical soil properties, the content of biogenic elements and heavy metals etc.) have often been disregarded (Bogdanovic et al., 1997). Therefore, the problem of a rational and effective use of various fertilization systems can be solved only on the basis of a complex attitude with microbiological investigations as an important factor, because microorganisms are a necessary component of each agro- ecosystem. They have a powerful enzymic system, perform diverse functions in the circulation of mater and energy, providing a constant functioning of the ecosystem as a whole.

The introduction of mineral fertilizers, as a radical way of improving the soil nutrient balance, strongly and rapidly intensifies microbiological processes. That can be considered to be positive up to a certain degree if the main aim is to increase the yield. Nevertheless, excessive overactivation of soil microorganisms can be also harmful, because the processes aimed at reestablishing the disturbed equilibrium cause the mineral fertilizers loss, reduction of the physicochemical and biological soil properties and other serious ecological consequences. The use of ever increasing nitrogen fertilizer rates results in a rapid mineralization of humus and other soil nitrogen compounds, the increase of gaseous nitrogen losses during ammonification and denitrification, nitrate accumulation in the soil, plants and underground waters, causing methemoglobinemy and other diseases in animals and humans (Knowles, 1982).

The coefficient of nitrogen utilization by crops is low (30-60%) and is being reduced even more with the increase of nitrogen fertilizers rates. Therefore, from the point of view of the environmental protection and the increase of plants yields, the issue of determining the optimum nitrogen fertilizer rates and kinds for different soils is of current interest. In addition, it is essential to increase the share of "biological" nitrogen in plant nutrition and reduce the level of gaseous losses to the minimum.

The aforementioned problems should be overcome by a partial substitution of these fertilizers with microbiological and organic ones. To what extent and in what way could this be done? We hope that these investigations will provide an answer to at least part of the question.

Material and method

The experiment was set up on the smonitza type of soil by the randomized block design with four replications. The investigated soil was characterized by acid reaction (pH 5.01), medium nitrogen and humus supply, being 0.134% and 2.68%, respectively, good easily available potassium supply (26.4 mg/100g soil) and low phosphorus content (2.9 mg/100g).

Tables 1. and 2. present the climatic characteristics of the investigated region of Cacak.

Maize - NSSC-640 (FAO 600) hybrid - was used as a test plant. Besides the control (the unfertilized soil), the following fertilization variants were examined:

- N_1PK (90:75:60 $kg \cdot ha^{-1}$);
- N_2PK (120:75:60 $kg \cdot ha^{-1}$);
- N_3PK (150:75:60 $kg \cdot ha^{-1}$);
- solid manure (45 $t \cdot ha^{-1}$);
- liquid manure (80 $t \cdot ha^{-1}$);
- inoculation with *Azotobacter chroococcum* strain 84 (10^9 active cells/ml) - 1l/10 kg seed.

Tab. 1. Precipitation sum (l/m^2) over the period 1996-1998

Month	Year			$\overline{X}$
	1996	1997	1998	1965-1994
I	14.2	20.5	61.5	50.2
II	63.5	35.1	38.5	44.8
III	50.5	54.7	28.6	53.8
IV	61.4	64.8	34.0	57.8
V	138.3	45.8	68.8	88.6
VI	46.5	22.9	58.8	98.2
VII	10.2	129.9	44.6	76.0
VIII	38.8	116.3	45.4	59.5
IX	141.4	32.9	85.7	56.5
X	54.2	111.7	112.9	47.8
XI	42.1	13.2	84.8	58.6
XII	99.7	80.3	42.7	57.6
Total	760.8	728.1	715.8	749.4

Tab. 2. Average monthly air temperatures (^{0}C) over the period 1996-1998

Month	Year			$\overline{X}$
	1996	1997	1998	1965-1994
I	0.0	0.7	0.2	-3.3
II	- 0.9	4.0	4.7	2.4
III	1.9	5.6	4.3	6.4
IV	11.2	6.7	13.6	11.5
V	17.5	17.0	15.5	16.2
VI	20.4	21.2	21.4	19.5
VII	21.4	20.9	23.0	20.9
VIII	21.7	19.6	22.6	20.5
IX	13.9	15.6	16.5	16.9
X	11.5	8.4	12.7	11.8
XI	8.2	7.4	3.9	5.8
XII	0.8	2.8	- 3.0	1.5
Average	10.6	10.8	11.3	11.1

The liquid beef manure used was obtained by the cow breeding system in a grated-floor cow barn without bedding and the solid manure from a 6-8-month combined manure storage process. Table 3 presents chemical characteristics of the fertilizers mentioned.

Tab. 3. Chemical composition of organic fertilizers applied (%)

Composition	Solid manure	Liquid manure
Dry matter	17.40	9.10
Organic matter	13.30	8.20
Nitrogen	0.37	0.41
Phosphorus	0.23	0.17
Potassium	0.48	0.40

The maize sowing was done by a machine at planting density of 60 000 plants per hectare (70x24 cm). The maize harvest was performed at the milk-waxy (for ensilaging) and full grain maturity stages.

The microbiological analysis consisted in the determining of the number of azotobacters (by the method of dilution on specific solid nutrient media) in the rhizospheric zone soil and the maize edaphosphere over the all three periods (intensive plant growth stage - 7-8 leaves, milk-waxy maturity stage, full grain maturity stage). The number of azotobacters was determined by adding of 0.2 ml 10^{-2} soil dilution to the Fjodorov's medium (the fertile drops method).

The maize and grain yield was determined at the milk-waxy maturity (by measuring the weight of the aboveground plant part) and full grain maturity stages (expressed in t/ha - at 14% grain humidity).

The data obtained were processed by the method of the analysis of variance of the three-factorial trial of the 7x3x2 ratio (treatment x period x sampling zone) for microbiological analyses, and on the other hand, of the two-factorial trial of the 7x3 form (treatment x year), for the maize yield. The significance of the differences between individual and interaction media was checked through the LSD-test.

Results and discussion

The fertilizers investigated had exerted a highly significant effect on the number of azotobacters in the soil. The metabolic activity of plants and numerous other factors take an important part in standardizing its quantitative occurrence. It was only in the second investigation year that a highly significant interaction effect between the fertilizers investigated and the occurrence of azotobacters in the soil zones studied (A x B) was expressed, whereas their effect in the first year of the study and in the year 1998 was significant and statistically insignificant, respectively (tab. 1, 2, 3)

In the year 1996, the maize seed inoculation with *Azotobacter chroococcum* resulted in the highest increase in the number of azotobacters in the soil. The similar effect was produced by the solid manure, which caused stimulation of biological parameters - soil respiration, cellulolysis, nitrification etc. (Djordjevic, 1993). Low and medium rates of the mineral fertilizers applied had an insignificant effect and the higher nitrogen rates exerted a highly depressive effect on the group of microorganisms studied, which was in accordance with the results obtained by other researchers (Govedarica et al., 1991; Cvijanovic et al., 2000; Pahnenko et al., 2001). The similar effect, as could have been expected, was produced by the liquid manure (tab. 1), since the nitrogen concentration in it was very high. Some authors consider this occurrence to be secondary because in such situations a change in the dynamics of the other groups of microorganisms, particularly of fungi, is being initiated, which is characteristic of some domestic researches (Saric, 1971). A special depression of the nitrogen regime bacteria growth was characteristic of the second and third research years, which was due to the cumulative effect of the continually applied nitrogen fertilizer and the soil cultivating level (Djukic, Mandic, 2000; Mandic et al., 2001). Similar to our observations, the authors stressed out that the highest inhibition in the soils investigated related to azotobacters, both in the soils previously fertilized and in the unfertilized ones.

Tab.1. Numbers of azotobacter (10^2 g^{-1} of absolutely dry soil) in the soil under maize as affected by the fertilizers applied (A), sampling zone (B) and vegetation period (C), 1996.

A		Control		Azotobacter		N_1PK		N_2PK		N_3PK		Solid manure		Liqid manure		$\overline{X}$
B		Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	
Periods (C)	I	19.3	20.0	23.0	41.3	19.0	27.3	17.0	25.6	7.0	11.3	22.3	31.6	11.0	13.3	20.66
	II	16.3	18.3	20.0	28.3	14.3	12.0	16.3	12.0	11.3	11.3	26.3	28.3	8.0	8.6	16.52
	III	23.3	27.3	27.6	34.3	28.3	31.0	24.3	30.0	20.3	22.6	30.3	35.0	13.3	17.3	26.09
$\overline{X}$		20.77		29.11		22.03		20.88		13.94		29.00		11.94		
$\overline{X}$	Edaphosphere	18.98														
	Rhizosphere	23.21														

Lsd

Lsd	**A**	**B**	**C**	**A×B***	**A×C**	**B×C***	**A×B×C**
0.05	1.35	0.71	0.87	1.91	2.34	1.25	3.31
0.01	1.78	0.94	1.15	2.52	3.09	1.65	4.38

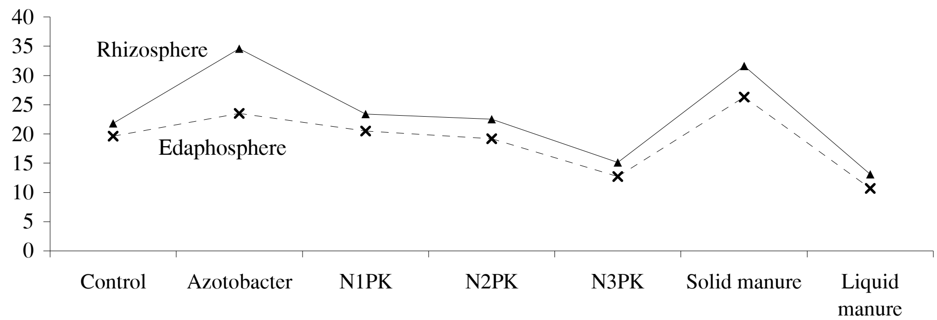
Ed.- Edaphosphere; Rh. - Rhizosphere

The toxic effect of nitrogen as far as azotobacter was concerned decreased throughout the vegetation period so that eventually only its highest rates (150 kg·N ha^{-1}) had an adverse effect.

The maize rhizosphere was found to be more biogenous than the edaphosphere. During the growing season, the lowest number of azotobacters was established in the summer period and the highest one towards the end of vegetation.

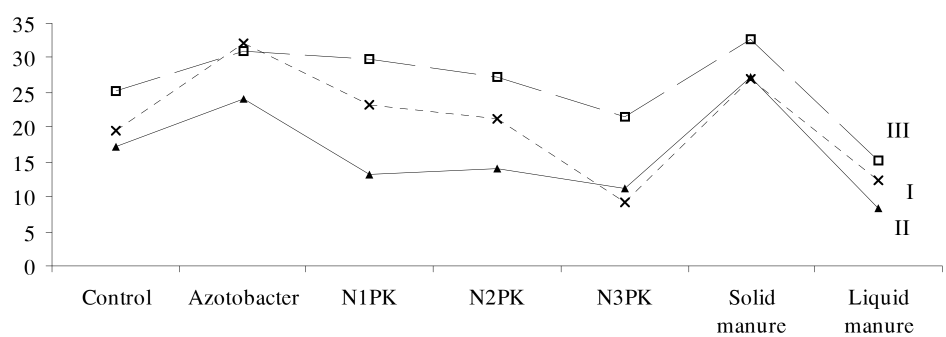
The importance of the mentioned interaction (AxB) in 1996 was noticeable only in the soil part related to the introduced Azotobacter strain, because in the rhizospheric soil part its stimulation was markedly higher. In the other variants in both soil zones the individual effects of the mineral and organic fertilizers were expressed (Fig. 1).

Fig.1. Interaction (AxB) of fertilizers applied (A) and sampling zone (B) and the effect on azotobacter numbers in the soil (10^2 g^{-1} of absolutely dry soil), 1996.



The highest stimulation of the number of azotobacters in the smonitza as influenced by the effect of its strain introduced was obtained in the first period of the investigation, whereas the increase of its number in the second and third periods was at a relatively equal level. At the beginning and towards the end of vegetation, low and medium nitrogen rates did not have a depressive impact on this group of microorganisms, as opposed to the growing season with low precipitation sum (the mid vegetation). Almost the same level of the depressive impact of the liquid manure on the number of azotobacters during the growing season was observed, whereas the solid manure caused the highest stimulation at the end of the maize vegetation (Fig. 2), which was due to the increase in the content of organic matter in the soil and improvement of its water-air, heat and nutrition regime (Jarak et al., 1991).

Fig.2. Interaction (A x C) of fertilizers applied (A) and maize vegetation perid (C) and the effect on azotobacter numbers in the soil (10^2 g^{-1} of absolutely dry soil), 1996.



During 1997, the number of azotobacters in the smonitza type of soil was considerably higher than in the previous year, which was particularly noticeable in the second investigation period. The depressive impact of the high and medium nitrogen rates was even more pronounced than in the previous year, whereas the effect of the liquid manure was significantly lower (tab. 2).

The stimulating effect of the maize seed inoculation with Azotobacter and low nitrogen rates in this year was somewhat more marked in the rhizospheric soil whereas high nitrogen fertilizer rates had a depressive impact. The solid manure had an almost equal stimulating effect in both soil zones investigated. The liquid manure exerted a somewhat greater influence towards decreasing the number of azotobacters in the maize rhizosphere, but the effect, as in the edaphosphere, was statistically significant (Fig. 3).

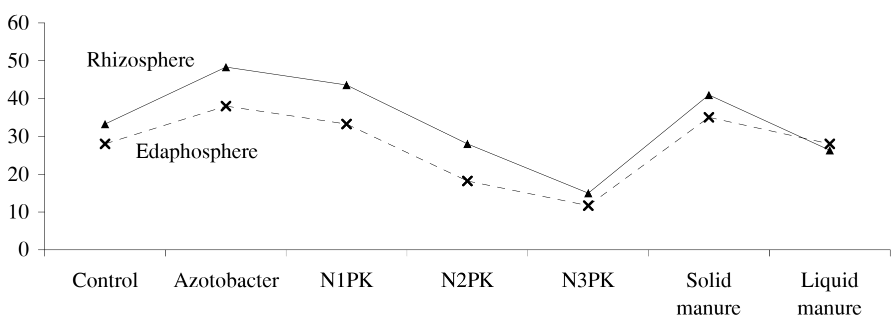
Tab.2. Numbers of azotobacter (10^2 g^{-1} of absolutely dry soil) in the soil under maize as affected by the fertilizers applied (A), sampling zone (B) and vegetation period (C), 1997.

A		Control		Azotobacter		N_1PK		N_2PK		N_3PK		Solid manure		Liquid manure		$\overline{X}$
B		Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	
Periods (C)	I	28.3	32.0	34.3	48.3	29.6	40.3	19.3	26.6	7.3	13.0	33.0	46.6	29.0	20.0	29.07
	II	38.3	40.3	58.6	67.0	37.3	39.3	21.6	28.3	21.0	21.0	32.6	36.3	35.0	38.6	36.81
	III	17.7	27.6	21.0	30.3	33.3	51.0	14.0	29.3	7.0	11.3	39.0	40.3	20.3	20.3	25.81
$\overline{X}$		30.66		43.17		38.50		23.11		13.36		38.31		27.18		
$\overline{X}$	Edaphosphere	27.46														
	rhizosphere	33.67														

L s d							
Lsd	**A**	**B**	**C**	**A×B**	**A×C**	**B×C**	**A×B×C**
0.05	2.26	1.21	1.49	3.19	3.92	2.09	5.52
0.01	2.98	1.60	1.97	4.22	5.19	2.78	7.31

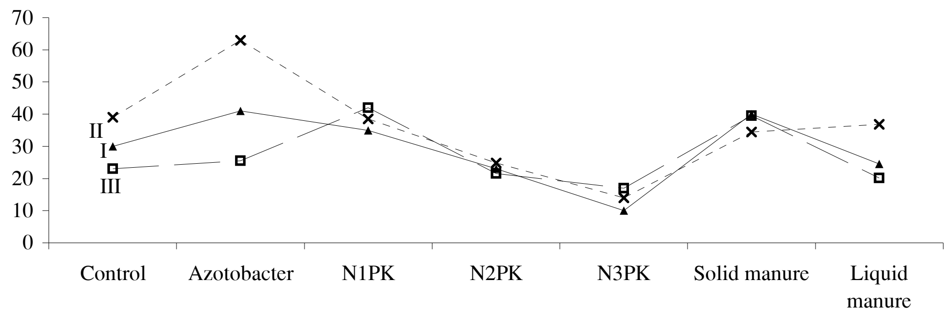
Ed.- Edaphosphere; Rh. - Rhizosphere

Fig.3. Interaction (AxB) of fertilizers applied (A) and sampling zone (B) and the effect on azotobacter numbers in the soil (10^2 g^{-1} of absolutely dry soil), 1997.



Similarly to the previous year, in the first two investigation periods the number of azotobacters in the variant with introduced *Azotobacter chroococcum* was particularly increased, whereas the low nitrogen rate caused the highest increase at the end of the maize vegetation. Medium and high nitrogen rates caused a decrease in the number of azotobacters during vegetation with a tendency to gradually decrease over time. In all the periods of the investigation, except in the second one, the solid manure significantly stimulated the growth of azotobacter, whereas the liquid manure during the two initial periods had a non-stimulating effect on this group of asymbiotic nitrogen fixers. As opposed to that, towards the end of the maize vegetation, its number was almost at the level of the control variant (Fig. 4).

Fig.4. Interaction (A x C) of fertilizers applied (A) and maize vegetation perid (C) and the effect on azotobacter numbers in the soil (10^2 g^{-1} of absolutely dry soil), 1997.



According to the trend in the number of azotobacters during the maize vegetation, the last study year (1998) was most similar to the year 1996, though on the whole its number in 1998 was lower than in the initial year of the investigation. Thus, apart from the insignificant effect of organic fertilizers and ever stimulating impact of the introduced Azotobacter strain, all the other effects were equal to those in the aforementioned study year. We can also state that in this year insignificant differences in the number of azotobacters were observed in the investigated soil zones (tab. 3).

According to our investigations, during the first year of the study, the application of the mineral and organic fertilizers resulted in a significant increase of maize silage yield. A considerable yield increase trend in the following years resulted from the mineral fertilizers only, whereas the effect of the organic fertilizers was statistically insignificant. Similar results of a positive effect of mineral fertilizers as well as of prolonged insignificant effect of organic fertilizers were obtained by other authors as well (Singh et al. 1986; Amorywa et al., 1987).

As seen from the trial results (tab. 4, 5), the maize yield increase, particularly at the milk-waxy maturity stage, did not fully correlate with the increase of the rate of nitrogen used for the maize fertilization, because the highest nitrogen rates (150 kg· ha^{-1}) had not always produced significantly higher yields as compared to the lower rates (120 kg·ha^{-1}). If we know that such nitrogen fertilizer rates have a depressive effect on the greatest number of soil microorganism groups too, the insignificant maize yield increase in such conditions could have been therefore expected.

Tab.3. Numbers of azotobacter (10^2 g^{-1} of absolutely dry soil) in the soil under maize as affected by the fertilizers applied (A), sampling zone (B) and vegetation period (C), 1998.

A		Control		Azotobacter		N_1PK		N_2PK		N_3PK		Solid manure		Liquid manure		$\bar{X}$
B		Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	
Periods (C)	I	17.3	13.6	39.0	38.0	19.3	14.3	11.0	12.3	7.0	8.3	19.3	17.0	18.3	14.3	17.78
	II	9.0	10.3	14.0	16.3	13.3	12.0	10.3	10.0	5.3	8.3	14.0	13.3	10.0	7.3	10.90
	III	30.0	22.0	35.3	31.6	29.3	31.3	19.0	21.3	16.0	19.3	29.3	25.6	27.7	21.0	25.64
$\bar{X}$		17.06		28.94		19.94		14.05		10.67		19.78		16.39		
$\bar{X}$	Edaphosphere	18.71														
	Rhizosphere	17.51														

L s d

Lsd	**A**	**B**	**C**	**A×B**	**A×C**	***B×C***	**A×B×C**
0.05	2.08	1.11	1.35	2.93	3.56	1.92	5.09
0.01	2.75	1.47	1.78	3.88	4.72	2.54	6.73

Ed.- Edaphosphere; Rh. - Rhizosphere

Tab. 4. Maize silage yield ($t \cdot ha^{-1}$) as affected by the kind and rate of fertilizers (1996-1998)

Fertilizers (A)	Year (B) 1996.	1997.	1998.	$\bar{X}$
Control	52.8	48.2	30.4	43.8
Azotobacter	53.6	49.1	31.5	44.9
N_1	59.5	65.8	38.9	54.7
N_2	63.4	69.6	41.3	58.1
N_3	62.7	71.4	44.2	59.4
Solid manure	61.5	49.9	30.5	47.3
Liquid manure	60. 8	49.5	29.2	46.5
$\bar{Y}$	59.2	57.6	35.1	50.6

Lsd	A	B	AB
0.05	2.97	2.21	5.38
0.01	3.96	2.93	6.96

Tab.5. Maize kernel yield ($t \cdot ha^{-1}$) as affected by the kind and rate of fertilizers (1996-1998)

Fertilizers (A)	Year (B) 1996.	1997.	1998.	$\bar{X}$
Control	6.69	13.76	6.59	9.01

Azotobacter	6.81	13.94	6.86	9.20
N_1	8.19	16.73	7.13	10.68
N_2	8.70	17.48	7.65	11.27
N_3	9.27	18.61	10.67	12.85
Solid manure	8.32	14.00	6.96	9.76
Liquid manure	8.57	13.91	6.97	9.82
$\overline{Y}$	8.08	15.49	7.55	10.37
Lsd	A	B		AB
0.05	0.74	0.52		1.32
0.01	0.99	0.69		1.78

The application of "biological nitrogen" (through maize seed inoculation with *Azotobacter chroococcum*) resulted in an insignificant maize yield increase, which was not in accordance with the results obtained by other authors (Foges, Arsac, 1991; Milosevic et al., 1999), who underlined the significant contribution of associative nitrogen fixers to regulation and balancing of plant nitrogen nutrition, bearing in mind the soil, climatic and other properties of the agro-ecosystems examined, as well as physiological characteristics and genetic predisposition of the used strains of biological nitrogen fixers.

Acid soil reaction (which is characteristic of the soil where these investigations had been conducted) does not necessarily reduce the number of azotobacters (though it often does), but according to the results obtained by many authors (Werner, 1995) it considerably inhibited its energy metabolism and nitrogenase activity, which affected the yield of the grown plants as well.

Conclusion

High mineral nitrogen rates (120 and 150 kg$\cdot$ ha^{-1}) considerably decreased the number of azotobacters in the smonitza under maize. The similar effect was produced by liquid manure during the first two study years, whereas solid manure caused the highest stimulation in the same period.

The rhizospheric soil was found to be more biogenous than the edaphospheric one. The toxic effect of the high nitrogen rates was considerably lower in the rhizospheric soil as well as in the periods of increased soil humidity.

The most important increase in the maize silage yield during the first study year resulted from medium nitrogen rate (120 kg$\cdot$ ha^{-1}), whereas in the other years, the higher yield of maize silage and grain in the variant with 150 kg$\cdot$ ha^{-1} was statistically insignificant. In all the study years, except in the first one, organic fertilizers did not significantly increase the yield of maize silage and grain. The applied Azotobacter strain caused the statistically insignificant increase in the mentioned productive traits of this crop.

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UTICAJ RAZLIČITIH VRSTA ĐUBRIVA NA BROJNOST AZOTOBAKTERA U SMONICI POD KUKURUZOM I PRINOS KUKURUZA

- originalni naučni rad -

L. Mandić, D. Đukić, V. Stevović
Agronomski fakultet Čačak

Rezime

Niže doze azota su tokom čitavog perioda ispitivanja stimulisale razvoj azotobaktera u zemljištu. Slično dejstvo je ispoljio i čvrsti stajnjak. Nešto brži gubitak aktivnog uticaja na azotobakter ispoljio je tečni stajnjak. Visoke doze azota delovale su depresivno na proučavanu grupu mikroorganizama, posebno u početnim vegetacionim fazama i u periodu niske vlažnosti zemljišta (sredina vegetacije). Toksični efekat visokih doza azota je znatno niži u rizosfernom zemljištu, kao i u periodima povećane vlažnosti.

Najznačajnije povećanje prinosa silokrme kukuruza tokom prve godine istraživanja ispoljila je srednja doza azota (120 kg ha^{-1}), dok je u ostalim godinama veći prinos silokrme i zrna kukuruza na varijanti sa 150 kg ha^{-1}, statistički neznačajan. Izuzev prve, u naredne dve godine istraživanja organska đubriva nisu značajno povećala prinos silokrme i zrna kukuruza. Primenjeni soj azotobaktera je delovao na statistički neznačajno povećanje pomenutih proizvodnih karakteristika ove biljne kulture.