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THE IMPORTANCE OF DIAZOTROPHS AS BIOFERTILISERS IN THE MAIZE AND SOYBEAN PRODUCTION

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The contemporary food production requires the preservation of soil productivity with the simultaneous maintenance of the yield level accomplished with the appropriate fertilising. The maize and soybean production is unimaginable without fertilisers and the application of information within the filed of nitrogen fixation. The application of fertilisers has been increasing. Diazotrophs are microorganisms with the ability to fix atmospheric nitrogen and to convert it in forms available to plants. Therefore, effects of different rates of mineral nitrogen (80, 120 and 160 kg N ha^{-1} in maize and half of the mentioned rates in soybean), as well as, maize seed bacterisation with the associative species (*Azotobacter chroococcum, Azospirillum lipoferum, Klebsiella planticola, Beijerinckia derxi*) and soybean with the symbiotic species

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(*Bradyrhizobium japonicum*) and their mixture on soil biogeny and yield quality and quantity were studied.

The studied parameters in maize had higher values under conditions of bacterisation and fertilisation with 80 kg N ha^{-1}, while the mixture of diazotrophs and fertilisation with 40 kg N ha^{-1} resulted in higher values of studied parameters in soybean. It is possible to produce organic/healthy food with the maintenance of soil biogeny if diazotrophs are incorporated into the soil with lower rates of mineral nitrogen. This possibility is a basic prerequisite for sustainable agriculture.

Key word: bacterisation, biogeny, maize, soybean, yield

INTRODUCTION

The development of new technologies within the industrial production and their application caused global warming and pollution of the atmosphere. However, the agricultural production also has become one of great pollutants. The increase of the human population was a trigger that initiated the race for the increase in the food scope. This resulted in the revolution in the application of agrochemicals in the agricultural production. The production of mineral fertilisers, pesticides, as well as the expansion of their utilisation are considered the third stage in the development of agriculture with numerous adverse effects on the environment, especially on the soil and waters. However, the EU concept of the food production with the maintenance of biodiversity requires the abandonment of the paradigm of modernisation of the agricultural production that was valid during the 1970s. The new pattern needs the implementation of information gained within many fields based on the employment of means and methods of the production similar to natural ecosystems. One of such information is a biological nitrogen fixation. It is known that nitrogen is an essential element for achieving high and stable yields and increased grain proteins. Also, nitrogen is an inert gas that can be converted only by fixation to the form available to plants. Biological nitrogen fixation is one of the methods of nitrogen conversion into forms available to plants. This process is clean, fix $190x10^6$ t nitrogen ha^{-1} (BABEVA and ZENOVA, 1989) and is very important for biosphere conservation as a whole (BRAY, 1983). Out of it, 80% are fixed within the terrestrial microbial system (25% by legumes and 7% in association with nonlegumes) (BURNS, 1995; HARDY *et al*., (1995); WANI *et al*., 1994). Nitrogen fixing microorganisms could be symbiotic and associative. Seed bacterisation with symbiotic diazotrophs has become a compulsory measure in the legumes production. This measure reduces the utilisation of nitrogen mineral fertilisers, preserve and increase both, nitrogen amounts in the soil and the yield quality and quantity. The application of associative diazotrophs is also of a great importance in the production of nonlegume plants, as this type of diazotrophs affects the reduction of amounts of mineral nitrogen, then, it can produce physiologically active matters, increase plant resistance to phytopathogenic fungi, as well as, it affects the reduction of pollutants in the soil. Their incorporation into the soil increases elements of biogeny and affect the increase of soil productivity.

The objective of the present study was to determine the effect of seed bacterisation of maize with associative diazotrophs and soybean with mixed populations of *Bradyrhizobium japonicum* and associative diazotrophs on the most important elements of soil biogeny in the rhizosphere of maize and soybean, but also on grain yield quality and quantity.

MATERIAL AND METHODS

The trial was carried out on slightly calcareous chernozem in the experimental field of the Maize Research Institute, Zemun Polje, at Zemun Polje during the 1999-2001 period. All cropping practices applied were of high quality and in due time. Basing fertilising was done in autumn by ploughing in a complex NPK fertiliser. Nitrogen fertiliser was used in spring prior to sowing in the amount of 80, 120 and 160 kg N ha^{-1} in maize and half of the rate in soybean. Seed bacterisation of a maize hybrid ZP 704 was done prior to sowing with a liquid inoculum, which encompassed the same amounts of the following associative diazotrophs: *Azotobacter chroococcum, Azospirillum lipoferum, Beijerincika Derx, Klebsiella planticola, Bacillus subtilis.* On the other hand, seed bacterisation of a soybean cultivar ZP 015 was performed with a mixture of different species of diazotrophs, i.e. with symbiotic *Bradyrhizobium japonicum* and the same species of associative diazotrophs used in maize seed bacterisation. The cell titer in the inoculum ranged from 10 to 25 x 10^8 per ml depending on the species of diazotrophs.

Samples of the rhizospheric soil were taken three times during the growing season - in the 5-7-leaf stage of maize and after emergence of the third trifoliate leaf of soybean (I stage), then in the flowering stage (II stage) and prior to harvest (III stage).

Standard microbiological methods (POCHON and TARDEAUX, 1962) were used to determine the number of certain groups of microorganisms. The soil enzymic activity, the dehydrogenase activity, was estimated after the modified method of THALMANN (1968).

The total proteins in the plant material were determined following the Kjeldalh method (BREMNER and MULVANEY, 1982).

The grain yield in maize, i.e. soybean was calculated at 14%, i.e. 13% moisture, respectively.

RESULTS AND DISCUSSION

The total number of microorganisms is one of the principal parameters of soil biogeny. This number in both plant species was significantly increased under conditions of bacterisation. The increase depended, first of all, on plant species, year, type of bacterisation, as well as on the incorporated amount of mineral nitrogen. The highest increase of the total number of microorganisms was determined in the variant with 80 kg N ha^{-1} in both, maize and soybean. Diazotrophs incorporated into the soil responded by their enzymatic activity to autochthonous population with the increase of their number, which can be very significant in years unfavourable for the agricultural production, such as 1999 (rainy) and 2000 (arid), as it can be observed in the Table 1. The statistical analysis of obtained results shows that the total number of microorganisms was significantly higher with bacterisation, fertilisation and interaction of these two factors.

Table 1. The total number of microorganisms in the rhizosphere (10^7 g^{-1} soil)

kg N ha^{-1}	1999		2000		2001	
	maize					
	number	I.N.	number	I.N.	number	I.N.
Control	83.86	100	93.48	100	254.21	100
80	96.62	115.22	97.88	104.71	283.11	111.37
120	74.64	89.01	100.59	107.61	261.43	102.84
160	81.53	97.22	157.42	168.40	260.55	102.49
Average 80-160	84.16	100.36	112.34	120.18	264.83	104.18
	soybean					
	number	I.N.	number	I.N.	number	I.N.
Control	86.72	100	75	100	217.73	100
40	131.39	151.51	123.12	164.16	251.13	115.34
60	142.41	164.22	96.98	129.31	180.26	82.79
80	187.73	216.48	110.12	146.83	179.06	82.24
Average 40-80	137.06	158.05	101.31	135.07	207.05	95.09

maize	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$
$A_{inocul.}$	323.63**	-	-	157.91**	-	-	926.72**	-	-
$B_{fert.}$	361.01**	-	-	135.76**	-	-	485.89**	-	-
AxB	8.45**	3.31	3.78	62.11**	9.19	12.17	37.922**	11.50	15.23
soybean	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$
$A_{inocul.}$	2017.13**	-	-	260.22**	-	-	222.39**	-	-
$B_{fert.}$	725.73**	-	-	110.58**	-	-	432.10**	-	-
AxB	114.40**	9.87	13.04	13.28**	24.35	32.16	45.23**	11.80	15.58

Azotobacter belongs to a group of the most important associative diazotrophs that can be found in our soils. Its existence was determined especially in microbial niches of the rhizosphere of maize, wheat, sugar beet and sunflower. The genus *Azotobacter* in our soils has been mainly studied (GOVEDARICA, 1986, 1992; FOJKAR, 2000; SARIĆ *et al.*, 1987) and it is found the practical application in microbiological fertilisers. Due to its susceptibility and certain requirements for the habitat, it vigorously responses, via its abundance, to all changes. Hence, it is a good indicator of all changes occurring in the soil, especially of degradation. Under the bacterisation conditions, the abundance of this group of diazotrophs in maize increased with the rate of 80 kg N ha^{-1}, while the greatest number and the highest percentage of the increase in soybean was recorded with the rates of 40 and 60 kg N ha^{-1} (Table 2). The incorporation of mineral fertilisers, especially nitrogen ones, into the soil results in the change of the number and the activity of nitrogen fixing bacteria. Based on previous studies it can be stated that higher amounts of mineral nitrogen inhibit the process of nitrogen fixation, while symbiotic species do not allow root infections with nodular bacteria (GOVEDARICA and JARAK, 1995). But, with the aim of the maximum use of the nitrogen fixation process, it is recommended to start with fertilising in the amount of 20-30 kg N ha^{-1}. This is in agreement with results obtained in our experiments. The highest percentage of the increase was recorded in the arid year of 2000. This is very important from the aspect of mitigation of adverse agro-meteorological conditions. The greatest abundance was determined in the year with the most favourable production conditions, i.e. in 2001. Also, the greatest number of *Azotobacter* was recorded in the variant without fertilising.

Table 2. The total number of Azotobacter in the rhizosphere (10^1 g^{-1} soil)

kg N ha^{-1}	1999		2000		2001	
	maize					
	number	I.N.	number	I.N.	number	I.N.
Control	90.79	100	175.96	100	237.87	100
80	83.07	91.50	208.28	118.37	253.4	106.53
120	100.8	111.03	187.21	106.39	227.26	95.54
160	71.52	78.78	180.11	102.36	198.5	83.45
Average 80-160	86.55	95.32	187.89	106.78	229.26	96.38
	soybean					
	number	I.N.	number	I.N.	number	I.N.
Control	158.14	100	147.12	100	340.29	100
40	202.67	128.16	205.98	140.01	346.04	101.69
60	160.39	101.42	193.5	131.53	340.92	97.25
80	104.53	66.10	173.8	118.13	265.59	78.05
Average 40-80	140.14	88.62	180.1	122.42	320.71	94.25

maize	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$
$A_{inocul.}$	539.71**	-	-	510.96**	-	-	308.73**	-	-
$B_{fert.}$	178.88**	-	-	441.73**	-	-	249.61**	-	-
AxB	13.302**	4.07	5.39	173.29**	-	-	2.049**	-	-
soybean	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$
$A_{inocul.}$	376.71**	-	-	1019.39**	-	-	125.74**	-	-
$B_{fert.}$	515.00**	-	-	301.43**	-	-	113.93**	-	-
AxB	16.35**	5.62	7.40	17.49**	7.99	10.56	0.319	-	-

Besides the total number of microorganisms and abundance of azotobacters, the dehydrogenase activity is considered the third, very important parameter of soil biogeny. The analysis of results shows significantly higher values of the dehydrogenase intensity under bacterisation, fertilisation and their mutual relation (Table 3). The dehydrogenase activity (DHA) is purely of the microbiological origin, and represents the oxidoreduction process in the process of respiration. The value of dehydrogenase activity indicates the intensity of mineralization of fresh organic matter and hums. According to obtained results, it was determined that DHA in maize had the highest values in the variant without the application of mineral nitrogen in 2001. The DHA intensity and its increase were correlated with the number of *Azotobacters*. This intensity in the soybean crop was the highest in the variant with 40 kg N ha^{-1}.

Table 3. Dehydrogenase activity in the rhizosphere (µgTPF 10 g^{-1} soil)

kg N ha^{-1}	1999		2000		2001	
			maize			
		I.N.		I.N.		I.N.
Control	363	100	317	100	634	100
80	327	90,08	503	158,68	614	96,85
120	306	84,30	477	150,47	508	80,13
160	340	93,66	479	151,10	571	90,06
Average 80-160	334	92,01	444	140,06	582	91,76
			soybean			
		I.N.		I.N.		I.N.
Control	408	100	577	100	505	100
40	453	111,03	635	110,05	549	108,71
60	461	112,99	635	110,05	608	120,40
80	423	103,68	618	107,11	589	116,63
Average 40-80	436	106,92	616	106,80	563	111,44

maize	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$
$A_{inocul.}$	3320.59**	4.11	5.428	5720.96**	2.77	3.67	101.255**	14.66	19.36
$B_{fert.}$	3907.30**	-	-	42835.47**	-	-	588.67**	-	-
AxB	26.44**	5.81	7.67	801.12**	3.93	5.19	28.89**	20.73	27.38
soybean	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$
$A_{inocul.}$	376.71**	-	-	1019.39**	-	-	125.74**	-	-
$B_{fert.}$	515.00**	-	-	301.43**	-	-	113.93**	-	-
AxB	16.35**	7.40	17.49**	5.62	7.99	10.56	0.319	-	-

It is well known that the yield depends on the complex genetic control, agroecological conditions, but also on the nitrogen fixation potential of soybean (MRKOVAČKI *et al.*, 1992; MOYSE, 1991), as the effect of bacterisation on the soybean yield increase is a varietal specification, i.e. a biological property of soybean.

The effect of bacterisation on the yield is presented in the Table 4. According to obtained results it was determined that bacterisation statistically significantly affected the yield increase. In years unfavourable for the agricultural production, the yield increase, on the average, was recorded for all fertilisation rates, while this increase in 2001 was detected only in the variant with 80 kg N ha^{-1} and it amounted to 1.78%. The highest yield in 1999 was determined in the variant without fertilisation, which was correlated with previously studied parameters, while the highest yield obtained in 2000 was recorded in the variant with 160 kg N ha^{-1}. This can be explained by the fact that the number of diazotrophs in the soil rhizosphere is low under unfavourable agroecological conditions and therefore, the accessible ammonium form of nitrogen is more easily taken up by plants. Similar results were obtained in the soybean crop. Statistically significant yields were obtained in the variant with 40 and 60 kg N ha^{-1} in all three years.

Maize is a starch plant, but proteins are represented with only 10% in the maize grain, which satisfy approximately 20% of total proteins in feed formulas. The analysis of obtained results shows that bacterisation statistically significantly affected the increase of the protein content in maize and soybean grain. This protein increase in soybean is quite uniform over years and ranged from 0.17 to

2.17% in dependence on fertilisation rate. In maize, the increase in the total proteins was higher and ranged from 3.14 to 27.66%.

Table 4. The effects of bacterisation on the yield

kg N ha^{-1}	1999		2000		2001	
			maize grain yield			
	t ha^{-1}	I.N.	t ha^{-1}	I.N.	t ha^{-1}	I.N.
Control	10.91	100	7.85	100	8.97	100
80	11.69	106.32	8.79	111.97	9.13	101.78
120	11.65	106.78	7.64	97.32	8.81	98.22
160	11.57	106.05	8.94	113.89	6.94	77.37
Average 80-160	11.43	104.79	8.31	105.80	8.46	94.34
			soybean grain yield			
	t ha^{-1}	I.N.	t ha^{-1}	I.N.	t ha^{-1}	I.N.
Control	2.53	100	2.18	100	2.93	100
40	2.82	111.46	2.45	112.39	3.01	102.73
60	2.98	117.79	2.38	109.17	2.94	100.34
80	2.92	115.42	2.05	94.04	2.52	86.01
Average 40-80	2.81	111.17	2.27	103.90	2.85	97.27

maize	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$
$A_{inocul.}$	303.84**	0.04	0.06	777.41**	0.13	0.20	23.44**	0.35	0.53
$B_{fert.}$	491.36**	-	-	1070.49**	-	-	3.04**	-	-
AxB	45.15**	0.19	0.27	26.32**	0.188	0.274	0.46	-	-
soybean	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$
$A_{inocul.}$	376.71**	-	-	1019.39**	-	-	125.74**	-	-
$B_{fert.}$	515.00**	-	-	301.43**	-	-	113.93**	-	-
AxB	16.35**	5.62	7.40	17.49**	7.99	10.56	0.319	-	-

Table 5. The effects of bacterisation on the total proteins content in grain

kg N ha^{-1}	1999		2000		2001	
			maize			
	%	I.N.	%	I.N.	%	I.N.
Control	8.98	100	8.28	100	9.36	100
80	9.98	111.14	8.54	103.14	9.82	104.91
120	9.29	103.45	10.57	127.66	10.34	110.47
160	9.32	103.79	10.56	127.54	10.07	107.59
Average 80-160	9.39	104.59	9.49	114.58	9.90	105.74
			soybean			
	%	I.N.	%	I.N.	%	I.N.
Control	40.12	100	40.09	100	40.78	100
40	40.64	101.30	40.48	100.97	40.85	100.17
60	40.99	102.17	40.96	102.17	40.68	99.75
80	40.81	101.72	40.78	101.72	41.26	101.18
Average 40-80	40.64	101.30	40.58	101.22	40.89	100.28

maize	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$
$A_{inocul.}$	303.84**	0.04	0.06	777.41**	0.13	0.20	23.44**	0.35	0.53
$B_{fert.}$	491.36**	-	-	1070.49**	-	-	3.04**	-	-
AxB	45.15**	0.19	0.27	26.32**	0.188	0.274	0.46	-	-
soybean	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$	F-test	$LSD_{0.05}$	$LSD_{0.01}$
$A_{inocul.}$	2.45	-	-	4.84*	0.62	-	2.18	-	-
$B_{fert.}$	10.03**	0.581	0.800	0.991	-	-	6.08*	0.60	-
AxB	0.515	-	-	0.88	-	-	0.993	-	-

The increase of total proteins in grain was almost equal in the variants with 120 and 160 kg N ha^{-1} (Table 5). Many authors (PAVLENKO, 1982; MIŠKOVIĆ *et al.*, 1977; ĐUKIĆ *et al.*, 1996, ĐUKIĆ and MANDIĆ, 1997, CVIJANOVIĆ, 2002.) found out that a high rate of mineral nitrogen did nod end in the expected results, and that even caused undesirable consequences.

CONCLUSION

Maize seed bacterisation with a mixture of different species of diazotrophs expressed various effects related to parameters of soil biogeny, yield levels, and the content of total proteins in grain. All observed parameters were significantly increased. The effect of adverse agrometeorological conditions can be mitigated in years unfavourable for the plant production by the incorporation of diazotrophs into the soil.

Based on results obtained in the course of the present study, it can be stated that a certain amount of mineral nitrogen can be reduced by bacterisation and can be replaced with nitrogen fixed by diazotrophs that is a great nitrogen potential in the soil. This is confirmed by the fact that such a system of the plant production is very important not only from the aspect of the yield level and the protein content, but also from the viewpoint of preservation of soil productivity.

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ZNAČAJ DIAZOTROFA KAO BIOFERTILIZATORA U PROIZVODNJI KUKURUZA I SOJE

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Savremena proizvodnja hrane zahteva da se sačuva proizvodna sposobnost zemljišta uz održavanje visine prinosa pravilnim đubrenjem. Proizvodnja kukuruza i soje nezamisliva je bez primene đubriva, uz saznanja iz oblasti fiksacije azota, koja imaju sve veću primenu. Diazotrofi su mikroorganizmi koji imaju sposobnost fiksacije atmosferskog azota i prevođenja u oblike dostupne za biljke. Stoga je proučavan uticaj različitih doza mineralnog azota 80, 120 i 160 kg N ha^{-1} kod kukuruza i 50% manje doze kod soje i bakterizacije semena kukuruza asocijativnim vrstama *Azotobacter chroococcum, Azospirillum lipoferum, Klebsiella planticola, Beijerinckia derxi* i soje simbioznim *Bradyrhizobium japonicum* i njihovom mešavinom, na biogenost zemljišta, kvalitet i kvantitet prinosa.

U uslovima bakterizacije i 80 kg N ha^{-1} kod kukuruza ispitivani parametri su imali veće vrednosti, dok je kod soje mešavina diazotrofa pri 40 kg N ha^{-} povećala ispitivane parametre. Pri unošenju diazotrofa u zemljište i đubrenju sa manjim dozama mineralnog azota moguća je proizvodnja zdrave hrane uz održavanje biogenosti zemljišta, što je osnovni preduslov za održivu poljoprivredu.

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