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Leka G. Mandić, Dragutin A. Đukić, Vladeta I. Stevović

Faculty of Agronomy Čačak, Cara Dusana 34,
32000 Čačak, Serbia and Montenegro

THE SOIL PROTEOLYTIC ACTIVITY AND ORGANIC PRODUCTION OF CORN UNDER THE CONDITIONS OF APPLYING DIFFERENT NUTRITION SYSTEMS

ABSTRACT: The effects of increasing amounts of mineral nitrogen (90, 120, 150 kg·ha^{1}), liquid (80 t·ha^{-1}) and solid manure (45 t·ha^{-1}) those of inoculation with *Azotobacter chroococcum* (the strain 84) on the soil proteolytic activity and corn yield grown in monoculture were surveyed over the 3-year-long research work.

The research results indicated that proteolytic activity and corn yield depended highly on the fertiliser sorts, amounts and on the research year, too.

Over the first research year, the highest proteolytic activity was recorded on the variants with organic fertilisers, whereas the fertiliser stimulating effect markedly decreased, particularly with liquid manure over the following two years of the research. Mineral fertilisers were also found to remarkably stimulate the biological parameters we have been concerned above, particularly the mean N amount (120 kg · ha^{-1}). While the highest corn yield could be obtained using 120 kg N · ha^{-1} throughout all the research years, its insignificant rise and barely altered soil proteolytic activity using corn seed inoculation with 84 *Azotobacter chroococcum* could be noticed.

KEY WORDS: Azotobacter, corn, yield, liquid manure, mineral fertilisers, proteinase activity, soil, solid manure

INTRODUCTION

Of the whole array of agrotechnical steps taken (from the conventionally to the currently utilised ones) the timely and appropriately used fertilisation systems deserve mention for enabling their effects to come into being fully. The research trends in this field mainly tend to raise the yield of agricultural crops, whereas the basis of their cumulative impact (the changes in biological and in chemical properties of the soil) are often disregarded. Therefore, a rational and an efficient application of the mineral (particularly nitrogen) and organic fertilisers may be attained only if they are approached holistically with a significant role of the microbiological studies taken into consideration, too.

The number and the activity of the soil microorganisms, as the significant indices of the biological soil productivity, may indicate an economical justifiability of using differing fertiliser sorts and amounts (Đukić, Mandić, 2000, Acosta-Martinez and Tabatabai, 2000).

Narrowing the ratio C: N by introducing nitrogen fertilisers may exert and favour mineralization processes, thereby suiting the soil proteolytic activity as well as the amount of the available nitrogen compounds (Soloveva et al., 2001). The level and the value of these processes primarily depends on the amount and the type of nitrogen fertilisers. However, the increased hydrolityc ability of the soil, brought about by N amount, may result in weakening of the soil physico-chemical properties, thereby leading to an array of serious environmental disruptions (Gostkowska et al., 1998), which suggests that such dimension of fertiliser application should be regarded with utmost care.

The issues put forth may be overcome by partial replacement of these fertilisers with the microbiological and organic ones. This will help improve the physico-chemical and biological soil properties, introduce certain amounts of other nutritional elements, phytohormones, enzymes and some useful microorganisms (Šlimek et al., 1999). In contrast, an uncontrollable utilisation of organic fertilisers, particularly that of liquid manure, may even exhibit certain undesirable effects on the biocenosis and on cultivated plants, as well (Đukić and Mandić, 1993).

The objective of the paper was, aided by a continual surveilance of the proteolytic activity, to establish the most optimal N fertiliser amounts as well as the possibilities of their replacement with the organic and microbiological fertilisers.

MATERIALS AND METHOD

The 3-year-long research was performed at the trial field of the Faculty of Agronomy, Cacak on the smonitza type of soil with its chemical characters outlined in tab.1.

Tab. 1. Agrochemical characteristics of the studied soil

Depth	pH		Humus %	N %	mg / 100 g	
	H_2O	nKCl			P_2O_5	K_2O
0—20 cm	6.12	5.01	2.68	0.134	2.9	26.4

Environmental characteristics of the area under way as being over the research period are given in Tabs. 2 and 3.

Tab. 2. Precipitation sum (l/m²) over the period 1996—1998

Month	Year			$\overline{X}$ 1965—1994
	1996	1997	1998	
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 (I faza)	46,5	22,9	58,8	98,2
VII	10,2	129,9	44,6	76,0
VIII (II faza)	38,8	116,3	45,4	59,5
IX	141,4	32,9	85,7	56,5
X (III faza)	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. 3. Average monthly air temperatures (°C) over the period 1996—1998

Month	Year			$\overline{X}$ 1965—1994
	1996	1997	1998	
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 trial was set up following the random-split-block design with four replications. The elementary plot amounted to 21.25 m², the inter-row 0.5 m and each block of 1 m spacing.

The corn hybrid NSSC-640, cultivated in the 3-year long monoculture, was utilised as a test plant over the research work.

The following fertilisation variants were surveyed: N1PK (90:75,60 kg · ha^{-1}), N_3PK (150:75:60 kg · ha^{-1}), solid manure (45 t · ha^{-1}), liquid manure (80 t · ha^{-1}) and inoculaation with *Azotobacter chroococcum* (the strain 84) — 11/10 kg of seed. This strain of Azotobacter was obtained from the microorganism collection of the Microbiology laboratory of the Agriculture faculty in Novi Sad. The cell titration in the inoculum amounted to $40 \cdot 10^6$/ml.

These amounts of N as well as those of P and K and inoculum were added in the pre-sowing stage each year, while the organic fertilisers were introduced in the first research year with the aim of surveying their extended effects.

Proteolytic activity (R o m e i k o, 1969) was determined in edaphosphere over the three different corn growth vegetation stages (the intensive plant growth, milk-waxy and full maturity of corn).

Corn yield was determined in the stage of its full maturity, calculated to have 14% moisture.

The obtained results were worked out through the three-factorial trial analysis $7 \times 2 \times 3$ (fertiliser x sampling zone x vegetation stage) — for the purpose of microbiological analyses, i.e., the two-factorial trial 7×3 (agent $\times$ year) — for corn green matter.

RESULTS AND DISCUSSION

Analysing the experimental data (tab 4, 5 and 6), the proteolytic activity was found to highly significantly statistically depend on both, fertiliser sorts and on their concentrations (A), on the soil zone used for taking samples intended for analysis (B) and on the corn vegetation period (C).

In the first year of studies, the proteolytic activity increased throughout corn vegetation, which was in agreement with results of numerous authors (Kandeler, et al., 1990). This finding suggested higher dependence of the soil proteolytic activity on the plant root metabolitic activity and its lower dependence on the environmental factors existing over the plant vegetation.

The lower doses of the mineral (90 and 120 kg $N \cdot ha^{-1}$) and particularly organic fertilisers notably increased proteolytic activity, whereas the effect of the high N amount (150 kg $\cdot$ ha^{-1}) compared to its previously used amounts seemed to be insignificant, statistically (Tab. 4). This is in full agreement with results obtained by other authors, who emphasized a positive impact of both, organic and lower mineral fertiliser amounts on the majority of hydrolytic soil enzymes (G o m o r o v a, 1986, B l e c h a r c z y k et al., 1993, B e l i n s k a, 1999).

The corn seed inoculated with *Azotobacter chroococcum* (the strain 84) didn't significantly alter the soil proteolytic activity.

Further, over the whole research period, the activity of enzymes proved to be highly higher in the rhizosphere soil rather than being in the edaphosphere of the corn (tab. 4). Fertilisers causing no changes in differences level being reinstated. The obtained results infer metabolites to be of the plant and microbiological origin, showing higher dominance in the root system zone and visibly exerting the production and activity of the extra-cellular enzymes of the protease type (J a r a k et al., 1991).

Tab. 4. The average proteolytic activity (gel. units g^{-1} of soil) during 1996

A		Control		Azoto-bacter		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 I	I	24.0	26.0	24.3	26.0	29.0	29.3	30.0	40.0	35.0	41.2	40.0	42.0	28.0	30.5	31.81
	II	34.6	40.0	35.0	37.5	33.0	40.0	36.0	41.0	36.5	45.0	62.5	68.3	57.5	66.0	45.21
	III	60.0	70.5	59.0	70.0	76.0	95.0	72.8	75.0	70.0	71.3	85.0	90.0	80.0	81.5	75.43
$\overline{X}$		42.53		41.97		50.39		49.14		49.75		64.64		57.25		
$\overline{X}$	Edaphosphere									48.49						
	Rhizosphere									54.10						

L s d

Lsd	A	B	C	A×B	A×C	B×C	A×B×C
0.05	2.19	1.17	1.44	3.13	3.82	2.04	5.41
0.01	2.93	1.55	1.91	4.14	5.05	2.70	7.15

Ed. — Edaphosphere; Rh — Rhizosphere
A — fertilizers applied; B — sampling zone; C — vegetation period

Over the second year, the vegetation growth trend of proteolytic activity was established only in the first two periods of studies (Tab. 5), its fall recorded in the final vegetation phases of corn, as the consequence of an extreme soil humidity over that stage, which, on behalf of anaerobity, visibly decreased the number of microorganisms responsible for the production of numerous enzymes and subsequently of the proteolytic ones, too (E m t s e v and Đ u k i ć, 2000).

Tab. 5. The average proteolytic activity (gel. units g^{-1} of soil) during 1997

A		Control		Azoto-bacter		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	62.5	62.5	60.0	60.3	57.5	60.0	57.5	60.0	58.0	59.3	70.3	75.3	60.0	65.0	62.01
	II	50.0	70.3	67.6	65.3	67.5	68.0	70.0	73.0	62.3	68.0	70.3	75.0	52.5	65.3	66.07
	III	62.5	65.3	67.5	60.3	52.8	60.0	55.0	57.5	58.3	63.5	65.3	72.5	65.0	70.0	62.53
$\overline{X}$		62.19		61.86		61.21		62.16		61.61		71.47		62.97		
$\overline{X}$	Edaphosphere									60.68						
	Rhizosphere									64.93						

L s d

Lsd	A	B	C	A×B	A×C	B×C	A×B×C
0.05	1.01	0.53	0.65	1.45	1.76	0.93	2.49
0.01	1.34	0.71	0.86	1.91	2.33	1.23	3.30

Ed. — Edaphosphere; Rh — Rhizosphere
A — fertilizers applied; B — sampling zone; C — vegetation period

Unlike being in the previous year, in 1997, neither the three N amounts contained in N fertilisers nor the liquid manure had any effects on the soil activity. The results of some authors (Š ć e r b a k o v a, 1983) indicated that, in conditions of high soil humidity and somewhat lower temperatures over the summer months, the application of mineral fertilisers was not always favoring soil biological activity. Compared with the preceding research year, the decline in proteolytic activity on the variant with liquid manure could be attributed to rather swift mineralisation processes having taken place in the first year of its introducing (S t e v a n o v i ć and R a k o č e v i ć - B o š k o v i ć, 2001). Additionally, the effect of corn seed initially inoculated with the strain 84 *Azotobacter chroococcum* was, similarly to the preceding year, insignificant statistically.

Excepting the variants with organic fertilisers, the last research year confirmed the soil proteolytic activity to be very much the same that recorded in 1996 (Tab. 6.). The fall in proteinase on the variants with organic fertilisers could be expected allowing for their remarkably lower value and amount compared with the year of their introducing. Accordingly, Š ć e r b a k o v a (1983) pointed out that over the third year upon manure introducing, the enzyme processes proceeded rather slowly, which was clear-cut only in the variant with manure applied in large amounts (above 80 t · ha^{-1}).

Tab. 6. The average proteolytic activity (gel. units g^{-1} of soil) during 1998

A		Control		Azoto-bacter		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	35.0	36.0	35.5	37.0	40.0	42.5	47.3	48.0	38.0	41.3	39.0	43.6	33.0	36.3	39.41
	II	41.0	43.6	39.8	47.0	52.3	52.0	50.2	51.5	40.2	43.3	40.6	44.0	48.6	43.6	45.58
	III	57.0	56.5	55.0	57.3	60.0	61.5	57.5	58.5	50.0	55.5	58.3	58.6	51.0	56.3	56.63
$\overline{X}$		44.86		45.22		51.33		52.08		44.67		47.50		44.77		
$\overline{X}$	Edaphosphere									46.14						
	Rhizosphere									48.28						

L s d

Lsd	A	B	C	A×B	A×C	B×C	A×B×C
0.05	1.98	0.99	1.31	2.81	3.46	1.84	4.89
0.01	2.62	1.31	1.73	3.72	4.58	2.44	6.47

Ed. — Edaphosphere; Rh — Rhizosphere
A — fertilizers applied; B — sampling zone; C — vegetation period

Statistically highly significant effect of the fertiliser types and amounts applied was manifested on the corn yield, too (Tab. 7).

Tab. 7. Maize corn yield (t·ha^{-1}) as affected by the fertilizers applied (1996—1998)

Fertilizers (A)	Year (B)			$\overline{X}$
	1996	1997	1998	
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

A — fertilizers applied; B — sampling zone; C — vegetation period

The high amounts of N fertilisers used during 1996 brought about a pronounced increase in the corn yield compared with the control variant, as well as with that of 90 kg · ha^{-1} N, with no statistically significant differences found between N_2 and N_3. Also, in the same year and on the comparatively same level was exhibited the statistically highly higher yield on the variants with organic fertilisers. The achieved yields approximated that on the variants with mineral fertlisers.

Similarly to the previous year, during 1997, the highest yield was achieved on the N_3 variant, with no statistically significant differences found between the two nearly identical N concentrations, or none of them manifested among organic fertilisers, the use of which barely increased yield in relation to the control variant.

No significant impact of organic fertilisers was prolonged over 1998 just as being with their lower rates being utilised. Fairly high N amounts applied to this year, characteristic of the dry year conditions.

In general, the increase in N amounts up to the level of 150 kg · ha^{-1} cannot be considered to be purposeful economically and environmentally, particularly with respect to the microbiological characterization of soil (S t e v o v i ć, 2001). The impact of organic fertilisers on the attained corn yield was fully expected, as corroborated by the results of numerous authors (K a r l e n and C a m p, 1985).

In contrast to the treatments we have been concerned above, the pre-sowing inoculation of the corn seed with the strain 84 *Azotobacter chroococcum* was found to scarcely statistically exert corn yield increase all the three research years round unlike the results obtained by some other authors (G o v e d a r i c a et al., 1997). Such an effect can be attributed to the acid soil reaction, which inhibited Azotobacter growth to a lesser degree, but significantly reduced its energetic metabolism and nitrogenous activity, thereby affecting the corn green matter yield (W e r n e r, 1995).

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ПРОТЕОЛИТСКА АКТИВНОСТ ЗЕМЉИШТА И ОРГАНСКА ПРОДУКЦИЈА КУКУРУЗА У УСЛОВИМА ПРИМЕНЕ РАЗЛИЧИТИХ СИСТЕМА ИСХРАНЕ

Лека Г. Мандић, Драгутин А. Ђукић, Владета И. Стевовић
Агрономски факултет Чачак, Цара Душана 34,
32000 Чачак, Србија и Црна Гора

Резиме

Током трогодишњих испитивања праћен је утицај растућих доза минералног азота (90; 120; 150 kg · ha^{-1}), течног (80 t · ha^{-1}) и чврстог стајњака (45 t · ha^{-1}) и инокулације са *Azotobacter chroococcum* (соj 84) на протеолитску активност земљишта и принос зрна кукуруза гајеног у монокултури.

Резултати истраживања показују да су протеолитска активност и принос зрна кукуруза значајно зависили од примењених врста и доза ђубрива, као и од године истраживања.

Највећа активност протеаза, током прве године истраживања, забележена је на варијантама са органским ђубривима, док се у друге две године стимулативни ефекат ових ђубрива значајно снижава, што се посебно односи на течни стајњак. Високу стимулацију наведених биолошких показатеља остварила су и минерална ђубрива, посебно средња доза азота (120 kg · ha^{-1}). У свим годинама истраживања, најзначајније повећање приноса кукуруза добијено је применом 120 kg N · ha^{-1}. Инокулација семена кукуруза са *Azotobacter chroococcum* соj 84, није значајно утицала на промену протеолитске активности земљишта и принос зрна кукуруза.