

UDK: 631.462

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**THE INFLUENCE OF MINERAL FERTILIZERS ON THE
ACTIVITY OF PHOSPHOMONOESTERASE IN THE SOIL
GROWN WITH MAIZE**

**UTICAJ MINERALNIH DUBRIVA NA AKTIVNOST
FOSFOMONOESTERAZE U ZEMLJIŠTU ZASADENOM KUKURUZOM**

Abstract

The aim of this study was to establish the influence of mineral fertilizers on the activity of both acidic and alkali phosphomonoesterase at the soil which was used for maize growing. The investigation was performed at the experimental area of Maize Research Institute in Zemun Polje, grown with maize hybrid ZPSC-704 (FAO 700) under continual monoculture at the low carbonated chernozem. The following fertilizing systems (treatments) were observed: 1) Control (no mineral or organic fertilizers), 2) 332 kg/ha NPK, and 3) 664 kg/ha NPK. The activity of both acidic and alkali phosphomonoesterase depended on the amount of applied NPK fertilizer, maize growth stage and the year in which the investigation was conducted. The NPK fertilizers inhibited the activity of the alkali phosphomonoesterase, while the activity of the acidic phosphomonoesterase was at the control level.

Larger amount of the NPK fertilizer (664 kg/ha) reduced the activity of the alkali phosphomonoesterase by approximately 20% in comparison with the control treatment (no fertilizer applied).

Key words: fertilizer, enzyme, maize, soil.

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Izvod

Cilj ovog proučavanja bio je da se ustanovi uticaj mineralnih đubriva na aktivnost kako kiselih, tako i alkalnih fosfomonoesteraza u zemljištu koje je upotrebljeno za uzgoj kukuruza. Istraživanje je obavljeno na eksperimentalnom polju Instituta za kukuruz u Zemun Polju, zasadenom hibridom kukuruza ZPSC-704 (FAO 700) pod stalnom monokulturom na nisko karbonatnom černozevu. Razmatrani su sledeći sistemi đubrenja (tretmani): 1) kontrola (bez mineralnih ili organskih đubriva), 2) 332 kg/ha NPK, i 3) 664 kg/ha NPK. Aktivnost kisele i alkalne fosfomonoesteraze zavisila je od količine primenjenog NPK đubriva, faze razvoja kukuruza i godine u kojoj je istraživanje sprovedeno. NPK đubriva inhibirala su aktivnost alkalne fosfomonoesteraze, dok je aktivnost kisele fosfomonoesteraze bila na nivou kontrole.

Veća količina NPK đubriva (664 kg/ha) smanjila je aktivnost alkalne fosfomonoesteraze otprilike 20% u poređenju sa kontrolom (bez primenjenog đubriva).

Ključne riječi: đubrivo, enzim, kukuruz, zemljište

INTRODUCTION

Phosphomonoesterases play an important role in the cycle of phosphorus due to their catalyzing hydrolysis of orthophosphorous acid esters and, thus, converting organic phosphorous compounds into more available forms. Microorganisms (bacteria, fungi, algae, protozoans) are the most important source of both acidic and alkali phosphatase in soil. Namely, they create extra cellular phosphatase either as a constitutive or as a inducible enzyme (Dick and Tabatabai, 1984). Another important source is a plant root which synthesize acidic phosphatase only (Gould et al., 1979). Production of this particular enzyme differs, depending on the plant growth stage, and is induced by the lack of available phosphorus in soil (James et al., 1990). Therefore, fertilization, generally known as indispensable cultivation measure, can either stimulate or inhibit the phosphomonoesterase activity, depending on fertilizer type, application rate or time. Haynes and Williams (1992) emphasize that the phosphatase activity has been inhibited due to a long term application of phosphorous fertilizers. According to Speir and McGill (1979), an increase in available phosphorus content is consistent with an increase in microbiological synthesis of the phosphatases. On the other hand, nitrogen fertilizers induce the synthesis of both plant and microorganism phosphatase, because they mobilize organic N and P compounds, changing N : P ratio towards lack of phosphorus (Haziev, 1982). Moreover, plant root is known to produce acidic phosphatase in unfavourable conditions of the lack of available phosphorus in soil.

Taking all this into account, the aim of this study was to investigate the influence of various application rates of NPK fertilizers on the activity of phosphomonoesterases at the rhizosphere and the surrounding soil during different growth stages of maize.

MATERIAL AND METHODS

The trial was set on the experimental field of The Maize Research Institute, Zemun Polje, where the ZPSC-704 (FAO 700) maize hybrid has been grown in long term monoculture. The soil (low carbonated chernozem type, <5% CaCO₃) exhibited favourable physical, chemical, and biological properties with respect to agricultural production (humus content 2.64%, total Nitrogen content 0.18%, pH (H₂O) 8.02, P₂O₅ content 11.0 mg/100g, K₂O content 18.4 mg/100g, texture class - light clay) (Vesković, 1988; Đorđević, 1993).

The study included the following fertilizing systems of maize:

1. Control (no mineral or organic fertilizers).
2. 332 kg/ha of NPK fertilizer, ratio 1 : 0.9 : 0.55, consequently 135 kg N, 123 kg P₂O₅, and 74 kg K₂O.
3. 664 kg/ha NPK fertilizer, ratio 1 : 0.9 : 0.55, consequently 270 kg N, 246 kg P₂O₅, and 148 kg K₂O.

The phosphatase activity was being observed in 1994 and 1995, during the three growth stages: 5-7 leaves, male flowering, and milk grain maturity. The samples were taken both from the rhizosphere (0.5 mm from the root surface) and from the surrounding soil. The activity of both acidic and alkali phosphatase was determined according to Tabatabai (1982), by means of universal modified buffer, pH 6.5 and 11. The experiment was performed in three replications. Statistical analysis consisted of standard factorial analysis of variance, while the significance of differences among the averages was estimated by F-test or LSD intervals ($P=0.05$).

RESULTS AND DISCUSSION

The activity of both alkali and acidic phosphomonoesterase in soil grown with maize depended on the NPK application rate, maize growth stage, and the year in which the experiment was performed.

Mineral fertilizers expressed different influence on the activity of alkali or acidic phosphomonoesterase. Thus, the NPK fertilizers inhibited alkali phosphomonoesterase, whereas the activity of the acidic one reached the level in the control treatment (no fertilizers present). Tadano and Sakai, (1991) emphasized that the plants secreted acidic phosphatase in case of the root being exposed to the lack of available phosphorus in soil, while the level of secretion depended on the plant species. In addition, according to Tadano et al. (1993),

application of the phosphorous fertilizers had caused an increase in the level of phosphatase secretion in sugar beet root. The NPK fertilizers did not significantly inhibited the activity of acidic phosphomonoesterase (in comparison with the alkali one), probably due to an increased level of acidic phosphatase secretion by the maize root.

Larger application rate of the NPK fertilizers (664 kg/ha) reduced the activity of alkali phosphomonoesterase by approximately 20%, comparing to the control (no fertilizers applied). The level of this particular decrease depended on the year in which the experiment was conducted, and the maize growth stage. During 1994 and 1995, both application rates of fertilizers in question reduced the enzyme activity (table 1)

The inhibition of the alkali phosphomonoesterase by the NPK fertilizers was particularly expressed during the male flowering of maize, in which case higher application rate of the NPK fertilizers caused a decrease in the activity of the alkali phosphomonoesterase by approximately 16% (Table 2). However, during the milk grain maturity stage, there was an increase in the activity of this enzyme in the treatment of 332 kg/ha, by 12% comparing to the control treatment (no fertilization). Haynes and Williams (1992) concluded that the four year period of high application rates of phosphorous fertilizers reduced the phosphatase activity. Orthophosphate inhibits the phosphomonoesterase activity according to the competitive inhibitory model (Pang and Kolenko, 1986), being both competitive inhibitor and the regulator of the enzyme synthesis. Spiers and McGill (1979) claimed that the inhibition of the acidic phosphatase activity and a decrease in the intensity of mineralization of organic phosphorous compounds occurs when orthophosphate was added to the incubation mixture in concentration of 0.55 mM phosphatase. The microbiological phosphomonoesterase are repressible enzymes (Nannipieri, 1994) whose both production and the activity decrease when the microorganisms are transplanted from the medium lacking in phosphates to the medium containing favourable phosphate content. However, the relationship between phosphatase and the phosphate concentration is more complex and dependable on the presence and the activity of the enzymes, immobilized on colloides. Nannipieri et al. (1978) reported that the level of the phosphatase activity in soil in which orthophosphates were present, was caused by extracellular stabilized enzyme components, which, in contrast to the intracellular ones, were not inhibited. These results corroborated the assumption that the phosphatase activity was exclusively repressible (Nannipieri, 1994). By intake of inorganic phosphorus into soil in ratio of C : P 20 : 1, microbiological phosphatase synthesis is suppressed (Speirs and McGill, 1977), while the enzyme activity is represented by the activity of the immobilized enzymes on colloides.

Table 1 The Influence of the Application rate of the NPK Fertilizers, Maize Growth Stage, and the Year of the Experiment on The Alkali Phosphomonoesterase Activity in Soil

Tabela 1. Uticaj doze primjene NPK dubriva, faze razvoja kukuruza i godine kada je izvršen eksperiment u vezi aktivnosti alkalne fosfomonoesteraze u zemljištu

Fertilizer	1994						1995																													
	I		II		III		I		II		III																									
	5-7 leaves		male flowering		milk grain maturity		5-7 leaves		male flowering		milk grain maturity																									
D	C	R	Z	R	Z	R	Z	R	Z	R	Z	R	Z	D																						
1.	360.9	292.5	302.3	241.1	184.7	193.6	404.9	324.9	348.5	350.1	282.1	297.1	282.1	298.5																						
2.	266.1	293.8	190.2	219.1	200.6	166.3	438.9	288.5	286.4	249.6	340.8	289.5	340.8	269.1																						
3.	350.8	275.3	297.3	194.5	194.1	152.5	455.5	330.5	239.0	292.8	300.8	318.2	300.8	283.4																						
C	325.9	287.2	263.3	218.2	193.1	170.8	434.8	314.6	291.3	297.5	301.6	301.6	307.9																							
B	306.5						240.7						181.9						374.7						294.4						304.7					

L.sd:

0,05 9,1 3,5 3,1 7,5
0,01 21,0 5,4 34,0 9,9

B- Plant Growth Stage

C - faza razvoja biljke

D-Fertilizers

D - dubriva

C / Rhizosphere/Soil

C - rizosfera/zemljište

I. Control

2. 332 kg/ha NPK

3. 664 kg/ha NPK

Table 2. The Influence of the Application rate of the NPK Fertilizers, Maize Growth Stage, and the Year of the Experiment on The Acidic Phosphomonoesterase Activity in Soil
 Tabla 2. Uticaj doze primjene NPK đubriva, faze razvoja kulture i godine kada je izvršen eksperiment u vezi aktivnosti alkalne fosfomonoesteraze u zemljištu

Fertilizer	1994						1995						
	I		II		III		I		II		III		
	5-7 leaves	male flowering	5-7 leaves	male flowering	milk grain maturity	5-7 leaves	male flowering	milk grain maturity	5-7 leaves	male flowering	milk grain maturity		
D	C	R	Z	R	Z	R	Z	R	Z	R	Z	R	Z
1.	113.0	92.93	114.3	99.9	163.2	100.5	75.9	87.59	84.48	69.00	85.10	83.78	97.45
2.	180.0	82.02	90.8	91.1	217.0	91.4	96.6	88.02	77.82	91.61	74.40	69.80	104.2
3.	132.1	63.4	128.7	84.6	163.7	78.5	67.4	104.2	88.44	81.00	66.98	71.63	94.22
C	141.7	79.4	111.3	91.9	181.3	90.1	79.9	93.27	125.37	80.54	75.49	75.07	
B	110.6		101.59		135.7		86.63		102.95		75.28		

Lsd: A B C D
 0.05 11.0 6.2 2.6 6.5
 0.01 25.4 9.4 3.5 8.5

B- Plant Growth Stage C / Rhyzosphere/Soil
 B - faza razvoja biljke C - rizosferu zemljište

D-Fertilizers I, Control 2. 332 kg/ha NPK 3. 664 kg/ha NPK
 D - đubriva

The inhibition of the activity of alkali phosphomonoesterase is, also, due to its depressing influence on the microorganisms populations. Smaller amount of the NPK fertilizers increased the total number of microorganisms in soil, the number of aminoheterotrophs, oligonitrophyls, phosphomobilizers, and phosphomineralisators. The application rate of the NPK fertilizers of 664 kg/ha decreased the number of all groups of microorganisms, except actinomycetes (Đorđević, 1998). Sparling et al. (1986) used regression analysis to establish that the activity of phosphatase is negligible in the absence of microorganisms population. This leads to a conclusion that the high level of phosphatase activity in soil also depends on the microbiological activity.

CONCLUSIONS

On the basis of these results, following conclusions can be drawn:

1. The activity of both acidic and alkali phosphomonoesterase in soil grown with maize depends on the NPK application rate, maize growth stage and the year in which the experiment is performed.
2. The NPK fertilizers inhibited the activity of alkali phosphomonoesterase, while the activity of the acidic phosphomonoesterase was similar to the control treatment (no fertilization).
3. Higher application rate of the NPK fertilizers (664 kg/ha) decreased the level of activity of alkali phosphomonoesterase by approximately 20% comparing to the control.

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FOSFOMONOESTERAZE U ZEMLJIŠTU ZASADENOM KUKURUZOM**

by

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Sažetak

Cilj ovog proučavanja bio je da se ustanovi uticaj mineralnih đubriva na aktivnost kiselih i alkalnih fosfomonoesteraza u zemljištu koje je upotrebljeno za uzgoj kukuruza.

Istraživanje je obavljeno na eksperimentalnom polju Instituta za kukuruz u Zemun Polju, zasadenom hibridom kukuruza ZPSC-704 (FAO 700) pod stalnom monokulturom na nisko karbonatnom černozemu.

Proučavanjem su obuhvaćeni sledeći sistemi đubrenja kukuruza:

- 1) kontrola (bez mineralnih ili organskih đubriva).
- 2) 332 kg/ha NPK, odnos 1:0,9:0,55 i
- 3) 664 kg/ha NPK, odnos 1:0,9:0,55.

Na bazi dobijenih rezultata, može se zaključiti da:

- aktivnost i kisele i alkalne fosfomonoesteraze u zemljištu zasadenom kukuruzom zavisi od doze primjene NPK, faze razvoja kukuruza i godine kada je obavljen eksperiment
- NPK đubriva inhibirala su aktivnost fosfomonoesteraze, dok je aktivnost kisele fosfomonoesteraze bila slična kao i kod kontrole (bez đubrenja)
- Veća doza primene NPK đubriva (664 kg/ha) smanjila je nivo aktivnosti alkalne fosfomonoesteraze oko 20% u poređenju sa kontrolom.