NPK-FERTILIZATION INFLUENCES ON PROTEINASE ACTIVITY IN ALLUVIAL SOIL

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Abstract: During the period of 2003-2005 the effect of different NPK fertiliser rates on the proteinase activity of alluvium planted to plum was examined. The trial was carried out in a trial plum plantation of the Fruit Research Institute and at the Microbiology Department of the Faculty of Agronomy in Cacak. The soil was treated with mineral fertilisers having a 8:16:24 + 3 % MgO formulation, as follows: variant 1 (control) – non-fertilised soil; variant 2 – 400 kg ha^{-1}; variant 3 – 600 kg ha^{-1}; variant 4 – 800 kg ha^{-1}; variant 5 – 1000 kg ha^{-1}.The fertiliser effect was determined three times during the growing season and was observed by identifying the proteinase activity. Determination of the enzyme activity was made using standard microbiological methods.The investigation results showed that the soil proteinase activity was affected by the fertilisation variants employed, sampling period and research years. High mineral fertiliser rates used caused a reduction in the soil proteinase activity. Of all the fertiliser variants examined the most pronounced inhibitory effect was exhibited by the variant 5 (the variant with the highest nitrogen rate used). Conversely, lower mineral nitrogen rates had a stimulatory effect on the activity of this enzyme.The fertiliser effects mentioned were most pronounced in the third sampling period. Furthermore, a more marked effect was recorded in 2003.

Key words: plum plantation, NPK-fertilization, soil proteinase

Introduction

As a highly important component of each soil type microorganisms are the most accountable for the majority of soil biological processes. During their lifetime they synthetise different extracellular enzymes catalysing degradation of complex organic compounds making them available for plants and microorganisms (Acosta-Martinez and Tabatabai, 2000).

The processes of transformation of organic forms of nitrogen are enabled by the effect of microbiological proteases (E.E.3.4. proteases) contained in a high number of bacteria, fungi and actinomycetes. Peptides and amino acids develop in the process to be further mineralised to the ammonium form and used in plant and microorganism nutrition. The process level in modern agricultural production is, among other things, dependent on both the type and rate of mineral fertilisers used (Sims and Wander, 2002).

Narrowing of the C:N ratio through nitrogen fertiliser incorporation brings about facilitation of mineralisation processes, favourably affecting the soil proteinase activity and consequently the amount of nitrogen compounds available (Solovova et al., 2001; Ragályi and Kádár, 2006; Kádár, 2007). However, increased hydrolytic capacity of the soil can lead to weakening of its physical and chemical and biological properties and to other serious environmental effects (Gostowska et al., 1998), indicating that this aspect of using mineral fertilisers should be given specific attention. Researches defining the limits of permitted soil load with these chemical compounds are of extremely high significance.

This determined the objective of the research – examination of the effect of different mineral fertiliser rates on soil proteinase activity.

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Materials and methods

The field experiment and soil characteristics

The research was carried out during the period of 2003-2005 in a trial plum plantation (alluvial soil) of the Fruit Research Institute and at the Microbiology Department of the Faculty of Agronomy in Cacak. Mineral fertilization (NPK 8:16:24 + 3 % MgO; product of "Zorka" Sabac, was made in autumn of 2002 in the amounts as follows (kg ha^{-1}): a = 0 (the control); b = 400; c = 600; d = 800; e = 1000. In the next two years, residual effects of applied fertilization were tested and plum plantation was not fertilized. The trial was set up according to a randomised block design in three replications (A = fertilization = five steps ; B = sampling period = three steps, C = year = three steps). The elementary plot size was 68 m^2. Common cropping practices were used during the growing season. The soil samples were taken in the terms as follows: May 12, September 17 and November 11 of each testing year, for the first, the second and the third sampling, respectively. The proteinase activity (number of gelatinolytic units per 1.0 g air-dry soil) was determined by the method described by Romejko (1969). The data obtained were processed by the analysis of variance and the significance of differences between the variants examined was analysed by the LSD-test. The experimental soil was slighty acid reaction (pH in 1nKCl = 5.9), moderate supplied with organic matter (2.65% humus) and well supplied with plant available P and K (AL-method: mg 100 g^{-1} of soil = 15.0 P_2O_5 and 20.4 K_2O).

Weather characteristics

Drought and the excessive warmth were main characteristics of the 2003 growing season. For example, in the7-month period May-November precipitation was for one third lower than long-term mean (LTM). At the same time, air-temperature was for 2.9°C higher (Table 1). The remaining two years were a bit warmer and regarding precipitation near to LTM.

Table 1. Weather characteristics (Čačak Weather Bureau) and long-terms means (LTM)

The period	Precipitation and mean air-temperatures in Čačak*								Total	Mean
		May	June	July	Aug.	Sept.	Oct.	Nov.		
2003	mm	62	51	69	6	34	77	27	326	
	°C	19.8	25.1	24.2	26.4	17.3	10.2	8.9		18.8
2004	mm	66	121	82	58	35	27	98	487	
	°C	16	21.7	23.5	22.8	18.1	14.3	6.2		17.5
2005	mm	72	84	100	66	91	23	83	519	
	°C	17.2	21	23.7	20.3	18.2	11.8	5.2		16.8
LTM	mm	89	98	76	60	56	48	59	486	
(1965-1994)	°C	16.2	19.5	20.9	20.5	16.9	11.8	5.8		15.9

* air-distance from the experiment about 5 km in E direction

Results and discussion

NPK-fertilization (the factor A) significantly influenced proteinase activity in the soil. For example, relative values of its activity (the control = 100) were as follows: 127, 162, 78, and 53, for 400, 600, 800 and 1000 kg ha^{-1} of NPK fertiliser. Regarding term of soil sampling influences (the factor B), the highest proteinase activity was found in the second term of sampling (for 28% higher compared to the control), while in the

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third term of sampling it was on the same level as the control (non-significant difference). The year of testing (the factor C was) also an important factor of proteinase activity because in the third year of study (2005) it was for 28% (2003) and 26% (2004) lower, while difference between 2003 and 2004 were non-significant. Proteinase activity differences among terms of soil sampling were non-significant for the control, but for the applied fertilization it was significantly higher in the second sampling compared both with the first and third terms of the treatments 600, 800 and 1000 kg ha^{-1} of fertilizer (Table 2).

Table 2. Influences NPK-fertilization (the factor A), sampling period (the factor B) and the growing season ("year" = the factor C) on proteolytic activity (number of gelatinolytic units/1.0 g air-dry soil)

Proteolytic activity (number of gelatinolytic units/1.0 g air-dry soil)							
The factor		The factor A: Fertilization (NPK 8:16:24 + 3% MgO) kg ha^{-1}					
Sampling (B)	Year (C)	a A1 = 0	b A2 = 400	c A3 = 600	d A4 = 800	e A5 = 1000	
		Interaction AB (fertilization x term of soil sampling)					Mean B
B1-first term		11.861	14.153	19.500	8.320	6.097	11.986
B2-second t.		13.958	18.056	23.958	12.570	8.616	15.432
B3-third term		12.709	16.790	19.097	9.375	5.707	12.736
Mean A		12.843	16.333	20.852	10.088	6.807	
		Interaction AC (fertilization x year)					Mean C
	C1 = 2003	15.209	18.125	23.541	11.111	7.033	15.004
	C2 = 2004	12.917	16.235	20.695	11.805	9.563	14.243
	C3 = 2005	10.403	14.638	18.319	7.348	3.823	10.905
		Interaction ABC (fertilization x term of soil sampling x year)					BC
B1	C1	13.542	17.499	23.749	6.250	5.626	13.333
	C2	11.875	13.334	19.376	10.625	8.957	12.833
	C3	10.167	11.625	15.375	8.084	3.709	9.792
B2	C1	17.709	21.876	29.166	15.417	8.763	18.586
	C2	13.542	16.042	22.292	13.125	11.459	15.292
	C3	10.625	16.250	20.417	9.167	5.625	12.417
B3	C1	14.376	15.000	17.709	11.667	6.710	13.092
	C2	13.333	19.330	20.417	11.666	8.275	14.604
	C3	10.417	16.040	19.167	4.7925	2.137	10.510
	Analysis of variance (LSD – test)						
	A	B	C	AB	AC	BC	ABC
LSD 5%	1.247	0.966	0.966	2.160	2.160	1.673	3.741
LSD 1%	1.652	1.280	1.280	2.861	2.861	2.216	4.956

The highest total proteinase activity was recorded in the second sampling period (15.432 gelatinolytic units). A decline in the activity in final vegetative development stages of plum resulted from decreased soil moisture, which significantly induced a reduction in the number of microorganisms accountable for the production of many enzymes including the proteolytic ones (Jemcev and Đukić, 2000; Kátai et al., 2005).
The variants with lower fertiliser rates had a stimulatory effect on proportionally equal level on the proteinase activity in all three research periods. Increased proteinase activity triggered by the effect of mineral fertilisers could be attributed to soil enrichment with mineral elements required for the development of proteolytic microorganisms (Mandic et al., 2001; Németh, 2006). These fertilisers affected the mobility of soil organic matter which was subjected to hydrolysis immediately after

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their incorporation into the soil, prior to activation of biological processes in the soil. Furthermore, a great number of authors reported that the proteinase activity increase induced by nitrogen incorporation into the soil occurred because hydrolytic processes were initiated much faster, so microorganisms started to use even the organic compounds which had previously been unavailable to them (Solovova et al., 2001).
An analysis of the results obtained determined that all variants with higher fertiliser rates used had an inhibitory effect on the activity of the enzyme mentioned (Table 2). The using of the highest NPK-rate in all of the three sampling periods had produced the highest inhibitory effect on this enzyme. The tendency resulted from a change in the established soil biological equilibrium following the incorporation of high mineral fertiliser rates in the soil and activation of non-specific toxinogenic groups of microorganisms (Kandeler et al., 1999).

Conclusion

Based on the research results on the effect of mineral fertilisers on the proteinase activity of soil under plum plantation the following can be concluded: a) high rates (800 and 1000 kg ha^{-1}) of the applied fertilisers and lower ones (400 and 600 kg ha^{-1}) induced a reduction and increase in the proteinase activity, respectively; b) the most pronounced inhibitory effect was expressed by the variant using the highest nitrogen rate (variant 5), and the most marked stimulatory effect was exerted by the variant 3; c) the soil proteinase activity was the most pronounced in the second sampling period, during the entire research period (2003-2005).

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