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CORRELATION BETWEEN FERTILIZER TYPE AND RATE, UREASE ACTIVITY AND TOTAL NITROGEN AND AMMONIUM NITROGEN CONTENTS IN A CHERNOZEM SOIL UNDER WHEAT

SUMMARY

Research was conducted on the effects of mineral fertilizers and manure on urease activity and total nitrogen and ammonium nitrogen contents, and their correlation in a chernozem soil during four developmental stages of wheat.

The study involved the following treatments: unfertilized control; N2 (100 kg/ha N); P2 (100 kg/ha P); K2 (100 kg/ha K); N2P2; N2K2; P2K2; N1P1K1 (50:50:50 kg/ha); N2P2K2; N3P3K3 (150:150:150 kg/ha), and N1P1K1 + solid manure (20 t/ha). Urease activity was determined by the method of Hofmann, and total nitrogen and ammonium nitrogen by the Kjeldahl method.

The combined application of low NPK fertilizer rates and manure resulted in increased urease activity. All treatments with three-component mineral fertilizers, two-component P2K2 and phosphorus P2 applied during the first two phenophases of wheat inhibited the soil urease activity, whereas at other developmental stages their effect and the effect of other mineral fertilizers on the activity of the enzyme were non-significant. The results indicate differing effects of mineral fertilizers on total N in the soil regardless of developmental stages. Of the single-component fertilizers, the highest total N level was recorded under K treatment. The use of two-component fertilizers produced a higher total N content compared to the single-component ones. Of the NPK treatments, total N content was highest under N2P2K2 treatment throughout the growing season. The N1P1K1 + manure treatment gave the highest amount of total N. Significant differences were found in ammonium N levels between the control and most of the other treatments during the growing season, but no definite pattern could be established.

There were no correlations either between the mineral fertilizers used and urease activity or between urease activity and total nitrogen and ammonium nitrogen contents.

Key words: soil, mineral fertilizers, manure, urease activity, total nitrogen, ammonium nitrogen

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INTRODUCTION

The enzyme urease (3.5.1.5.) belongs to the group of hydrolytic enzymes and is characterized by the specific activity – it hydrolyzes only urea, without affecting its products. The optimum pH of urease is near 7.0, although its activity in the soil may take place within a rather wide pH range. The urease activity is calculated by means of the determination of ammonia formed during urea decomposition.

Urease activity in the soil is dependent on soil type, environmental conditions and cultural practices. In this regard, special importance is given to the type, rate and combination of fertilizers as the pillar of effective soil fertility. Zakarauskaitė *et al.* (2008) found that low rates of NPK mineral fertilizers have no significant effect on urease activity, in contrast to high rates, particularly of P and K, which lead to a significant reduction in the activity of the enzyme in cambisols. However, Leinweber *et al.* (2008) reported that low NPK fertilizer rates (60 kg ha⁻¹), during the first month after treatment, increase urease activity up to 35 days following application, with their stimulating effect fading away thereafter. Moreover, the use of organic substrates (solid manure, sewage sludge etc.) commonly stimulates the soil enzymatic activity (Crecchio *et al.*, 2001; Madejon *et al.*, 2001; Balezentiene and Klimas, 2009). High urease activity may also lead to certain adverse effects due to ammonia volatilization or denitrification losses, particularly in slightly alkaline and overly irrigated soils (Zaman *et al.*, 2008; Gioacchini *et al.* 2002; Parfitt *et al.* 2006). This fact must be considered when determining the application rates and types of fertilizers for different types of soils and under specified agroenvironmental conditions.

Apart from urease activity, the amount of available nitrogen assimilates is also governed by the activity of other soil enzymes, such as proteolytic enzymes. The intensity and direction of these processes are also affected by soil and anthropogenic factors. Fertilization systems play an important role in this process as well. Narrowing the C : N ratio through the separate or combined incorporation of nitrogenous fertilizers and well-rotted manure accelerates mineralization, thus favorably affecting the soil proteolytic activity and, hence, the amount of available nitrogenous compounds (Kaur *et al.*, 2005; Chu *et al.*, 2007).

These facts justify the assertion that soil enzymatic activity can be used as an important criterion in assessing the quality and productivity of soils (Yao *et al.*, 2006).

The objective of this study was to evaluate the effect of different types and application rates of fertilizers on urease activity, total nitrogen and ammonium nitrogen in a chernozem soil, as well as their correlations during the wheat growing season.

MATERIAL AND METHODS

The experiment was conducted in Rimski Šančevi (latitude 45° 45' N; longitude 19° 51' E; altitude 84 m a.s.l.), Republic of Serbia, on a chernozem soil (pH_{KCl} 7.02, 2.5% humus, 0.14% N, 0.16 mg/g P₂O₅, 0.22 mg/g K₂O) in a

randomized block design with four replications and plot size 12 m² (4×3 m). Winter wheat cultivar 'Evropa 90' was used as the test plant. The following fertilization treatments (factor A) were employed: N2 (100 kg/ha N); P2 (100 kg/ha P); K2 (100 kg/ha K); N2P2; N2K2; P2K2; N1P1K1 (50:50:50 kg/ha); N2P2K2; N3P3K3 (150:150:150 kg/ha), and N1P1K1 + solid manure (20 t/ha). Nitrogenous, phosphorus and potassium mineral fertilizers were applied as urea, superphosphate and KCl, respectively. The total phosphorus and potassium fertilizer amounts and 1/3 of nitrogenous fertilizer were used prior to seeding in autumn, and the remaining 2/3 of the nitrogenous fertilizer as a soil dressing in March at the tillering stage. Solid manure (dry matter - 17.4%, organic matter - 13.3%, nitrogen - 0.37%, phosphorus - 0.23%, potassium - 0.48%) was plowed under in autumn 10 days before seeding. Soil samples for the analysis of urease activity, total nitrogen and ammonium nitrogen were taken aseptically down to a depth of 25 cm in four phenophases of wheat (factor B): I period – full stem elongation; II period – beginning of flowering; III period – milk stage; IV period – full maturity.

The amount and distribution of rainfall and soil temperatures during the growing season are presented in Table 1.

Table 1. Amount and distribution of rainfall (mm) and temperatures of soil (°C) during the growing season of wheat

Month	Mean monthly temperatures of soil (°C)			Rainfall (mm)
	2 cm	10 cm	30 cm	
IV	12.1	11.5	10.6	26
V	21.5	19.4	17.2	24
VI	26.2	24.3	22.9	90
VII	22.6	21.6	20.9	67

Soil urease activity was assayed by the method of Hofmann (Hofmann, 1963) and expressed in mg NH₃ per 1 g of soil for 24 hours (1 ml 0.1 n H₂SO₄ corresponds to 1.7 mg NH₃). Total nitrogen and ammonium nitrogen were determined by the Kjeldahl method.

The results were subjected to a two-factor analysis of variance (fertilization, wheat phenophase), and the significance of differences was computed by the Lsd test, using SPSS software (StatSoft.Inc, 1995). The correlation between fertilizer application rates, urease activity and total nitrogen and ammonium nitrogen contents was expressed by the simple correlation coefficient.

RESULTS AND DISCUSSION

The analysis of variance showed a significant effect of fertilizers on soil urease activity, total nitrogen and ammonium nitrogen contents during the growing season of wheat. Their effect was found to depend on the type, rate and combination of fertilizers, as well as on wheat phenophase.

The highest increase in urease activity compared to the control was under the combined application of low NPK fertilizers rates and manure, particularly at initial developmental stages of wheat. At the end of the growing season (full maturity), the effect was non-significant (Table 2). The results are in agreement with the findings of Plaza *et al.* (2004) and Kaur *et al.* (2005) who stressed the importance of separate or combined applications of manure and low mineral fertilizer rates in increasing the numbers of soil microorganisms *i.e.* their enzymatic activity. In this research, during the first two phenophases, all three-component mineral fertilizers, two-component fertilizers (P2K2) and the single application of phosphorus (P2) resulted in reduced urease activity in the soil. During the other wheat phenophases, their effect was non-significant, as was the effect of the other types, rates and combinations of mineral fertilizers throughout the growing season. The results of the research conducted over several years by Zakaruskaitė *et al.* (2008) also suggest that low rates of mineral fertilizers have no significant effect on urease activity, whereas application rates above 150 kg/ha significantly reduce the activity of the enzyme in cambisols. Yao *et al.* (2006) and Balezientienė and Klimas (2009) observed that the decline in urease activity during the wheat growing season may be due to lowered mineralization substrate availability and increased soil temperature.

Table 2. Urease activity (mg NH₃/g soil per 24 hours) in chernozem during the four phenophases of wheat (I – full stem elongation; II – beginning of flowering; III – milk stage; IV – full maturity) depending on the applied fertilizers

Fertilization (A)	Phenophase (B)			
	I	II	III	IV
Without fertilization	0.361 c	0.282 cde	0.215 e-j	0.193 g-k
N2*	0.346 cd	0.223 e-h	0.204 e-k	0.177 g-k
P2	0.278 def	0.194 g-k	0.153 h-k	0.164 g-k
K2	0.346 cd	0.215 e-j	0.176 g-k	0.175 g-k
N2P2	0.334 cd	0.202 e-k	0.170 g-k	0.166 g-k
N2K2	0.340 cd	0.214 e-j	0.175 g-k	0.170 g-k
P2K2	0.198 f-k	0.153 h-k	0.130 k	0.136 jk
N1P1K1	0.244 efk	0.175 g-k	0.147 h-k	0.158 h-k
N2K2P2	0.218 e-j	0,154 h-k	0.141 ijk	0.153 h-k
N3P3K3	0.204 e-k	0,144 h-k	0.136 jk	0.147 h-k
N1P1K1 + manure	1.130 a	0,511 b	0.342 cd	0.204 e-k

*N2–100 kg/ha N; P2–100 kg/ha P; K2–100 kg/ha K; N1P1K1–50:50:50 kg/ha; N3P3K3–150:150:150 kg/ha, solid manure–20 t/ha.

Values followed by different small letters within the table are significantly different ($P \leq 0.05$) according to the LSD test;

The results obtained (Table 3) indicate a more or less opposite effect of mineral fertilizers in relation to the total N in the soil, which was in agreement with the results of Stanojković (2010). Regardless of developmental stages, of the single fertilizers applied, the highest total N level was recorded in the variant with potassium. The variants with two-component fertilizers produced a higher total N content compared to the one-component ones. The highest N content was obtained with the variant N2P2, which is in agreement with the results of Polesko (1970). Of NPK variants, total N content was highest with the variant N2P2K2 throughout the vegetation period. However, the introduction of manure (N1P1K1 + manure) increased the total N content throughout the vegetation, so that this variant produced the highest amount of total nitrogen, as confirmed by the results of other authors (Kaur et al., 2005; Chu et al., 2007).

The tendency for the increase in the total N during the vegetation of wheat can be supported by the fact that the plants grown on chernozem secrete larger amounts of organic matter at the phase of heading (in small grains) and that root parts die out (Jakovljević, 1979).

Table 3. Content of total nitrogen (mg N/kg soil) in chernozem during the four phenophases of wheat (I – full stem elongation; II – beginning of flowering; III – milk stage; IV – full maturity) depending on the applied fertilizers

Fertilization (A)	Phenophase (B)			
	I	II	III	IV
Without fertilization	1314 r	1325 qr	1344 pqr	1379 op
N2*	1358 pq	1365 pq	1435 n	1451 mn
P2	1339 pqr	1360 pq	1414 no	1449 mn
K2	1484 lm	1433 n	1442 n	1454 mn
N2P2	1587 e-h	1591 d-g	1594 def	1681 a
N2K2	1505 kl	1517 jkl	1533 jk	1538 ijk
P2K2	1517 jkl	1531 jk	1535 ijk	1540 ijk
N1P1K1	1533 jk	1552 g-j	1549 hi	1582 fgh
N2K2P2	1631 bcd	1596 def	1626 b-e	1664 abc
N3P3K3	1556 f-i	1575 f-i	1589 efg	1591 d-g
N1P1K1 + manure	1654 abc	1624 cde	1659 abc	1666 ab

*N2–100 kg/ha N; P2–100 kg/ha P; K2–100 kg/ha K; N1P1K1–50:50:50 kg/ha; N3P3K3–150:150:150 kg/ha, solid manure–20 t/ha.

Values followed by different small letters within the table are significantly different ($P \leq 0.05$) according to the LSD test;

Under N2, P2, medium (N2P2K2) and high rates (N3P3K3) of the three-component fertilizers and the combined treatment with mineral and organic fertilizers, ammonium nitrogen levels significantly decreased at all phenophases, except the first one, compared to the same phenophases in the control (Table 4). Such variability in the value of this form of nitrogen may be associated with its

conversion into nitrate or other nitrogenous forms, as well as with partial volatilization, particularly in fertilized treatments under increased soil temperature and moisture conditions, as confirmed by other authors (Kaur *et al.*, 2005; Zaman *et al.*, 2008). Generally, the highest levels of ammonium nitrogen, in single-component mineral fertilization treatments, were recorded for the N2 treatment and, except in the first period, they were significantly higher than for P2 and K2 treatments which resulted in approximately the same amount of this form of nitrogen throughout the growing season. Except the third phenophase under N2P2 treatment, the amounts of ammonium nitrogen following two-component fertilizer applications were also similar. As for the three-component fertilizers, the amount of ammonium nitrogen was the highest under N3P3K3 treatment and combined N1P1K1 + manure treatment. In the other two combinations of three-component fertilizers, ammonium nitrogen levels were approximately the same throughout the growing season.

A correlation was found to exist between the content of ammonium nitrogen and the application rate of NPK during the vegetation of wheat, which is in agreement with the results of Stanojkovic, (2010).

Table 4. Content of N-NH₄ (mg N-NH₄/kg soil) in chernozem during the four phenophases of wheat (I – full stem elongation; II – beginning of flowering; III – milk stage; IV – full maturity) depending on the applied fertilizers

Fertilization (A)	Phenophase (B)			
	I	II	III	IV
Without fertilization	6.42 fgh	10.5 abc	9.92 bcd	11.25 ab
N2*	5.80 ghi	7.00 fg	8.75 de	7.00 fg
P2	5.25 h-k	4.08 kl	5.25 h-k	5.25 h-k
K2	5.25 h-k	3.50 l	5.83 ghi	5.25 h-k
N2P2	4.08 kl	3.50 l	9.33 cd	5.25 h-k
N2K2	3.50 l	3.50 l	5.60 hij	4.08 kl
P2K2	3.50 l	3.50 l	6.30 fgh	5.25 h-k
N1P1K1	4.66 ijk	5.25 h-k	6.42 fgh	3.50 l
N2K2P2	7.58 ef	5.25 h-k	6.65 fgh	4.20 jkl
N3P3K3	6.42 fgh	9.30 cd	11.50 a	3.50 l
N1P1K1 + manure	8.75 de	7.58 ef	8.75 de	5.25 h-k

*N2–100 kg/ha N; P2–100 kg/ha P; K2–100 kg/ha K; N1P1K1–50:50:50 kg/ha; N3P3K3–150:150:150 kg/ha, solid manure–20 t/ha.

Values followed by different small letters within the table are significantly different ($P \leq 0.05$) according to the LSD test;

On the basis of the results obtained (Table 5) it can be concluded that there is no correlation either between the mineral fertilizers applied and the activity of ureases, or between urease and total and ammonium nitrogen.

Table. 5. The correlation coefficient between single fertilizers (N2, P2, K2 – 100 kg/ha N, P, K respectively), the urease activity and the content of total and ammonium nitrogen in chernozem during the four phenophases of wheat (I – full stem elongation; II – beginning of flowering; III – milk stage; IV – full maturity)

Phenophase	Fertilizer			Phenophase											
	N2	P2	K2	I			II			III			IV		
				Urease activity	Ammonium N	Total N	Urease activity	Ammonium N	Total N	Urease activity	Ammonium N	Total N	Urease activity	Ammonium N	Total N
IV	.593	.479	.063	.301	.952****	.057	-.140	.956****	.111	.153	.990****	-.216	-.679*		
	-.441	-.573	.447	.167	-.651*	.435	.539	-.684*	.321	.053	-.695**	.540			
	-.117	-.418	.878****	.500	-.178	.896****	.395	-.271	.887****	-.055	-.217				
III	.619*	.521	.069	.250	.962****	.064	-.154	.979****	.128	.195					
	.581	.423	.064	.330	.127	.051	.711	.193	.028						
	.010	-.289	-.202	.963****	.606	.127	.975****	.379	.051						
II	.551	.566	.024	.143	.977****	.013	-.187								
	.213	-.001	-.044	.469	.634	-.206	.455								
	-.091	-.272	-.212	.998****	.615	.084									
I	.497	.485	.091	.240											
	.215	.153	.088	.615*											
	-.108	-.245	-.197												
K2	.315														
P2	.315														

*, **, ****, significant at $P < 0.05$; $P < 0.02$ and $P < 0.001$, respectively

CONCLUSIONS

The fertilizers used had a significant effect on urease activity and total nitrogen and ammonium nitrogen contents in the soil during the growing season of wheat. Their effect differed, depending on cultivar, fertilizer rates and combinations, and wheat phenophase. The combined application of low NPK fertilizer rates and manure resulted in increased urease activity. During the first two phenophases, all three-component mineral fertilizers, two-component fertilizers (P2K2) and the single application of phosphorus (P2) inhibited the soil urease activity, whereas at other phenophases their effect and the effect of the other mineral fertilizers on the activity of the enzyme was non-significant. All fertilizers led to a significant increase in total nitrogen content in the soil, particularly under N2P2K2 and combined N1P1K1 + solid manure treatment. Of the two-component fertilizers, N2P2 gave the highest total nitrogen content. Under N2, P2, medium and high rates of the three-component fertilizers (N2P2K2 and N3P3K3, respectively) and the combined treatment with mineral and organic fertilizers, ammonium nitrogen levels significantly decreased at all phenophases, except the first one, compared to the control. No positive correlation was observed between the fertilizers applied and urease activity, nor between urease activity and total nitrogen and ammonium nitrogen contents in the soil.

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