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# BIOLOGICAL PRODUCTIVITY OF VERTISOL CULTIVATED WITH FIELD PEA UNDER NITROGEN FERTILIZATION CONDITIONS

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## Abstract

This study evaluates the effect of different rates of mineral nitrogen fertilizer on ammonifier counts, protease activity and some chemical characteristics of vertisol (pH, phosphorus and potassium levels) under field pea cultivation. Increasing rates of mineral nitrogen fertilizers (20, 40 and 60 kg ha$^{-1}$) combined with standard rates of phosphorus and potassium (75 and 60 kg ha$^{-1}$, respectively) were incorporated prior to seeding, with their effect on the above biological parameters being monitored during two stages of field pea development (pod formation and wax-ripe stage), and their effect on soil chemical characteristics after field pea harvest. The results show that the use of these fertilizers led to a significant decrease in ammonifier counts in the first stage of the study (particularly the 60 kg ha$^{-1}$ rate), and a non-significant effect in the wax-ripe stage. Conversely, protease activity was significantly increased by lower fertilization rates (20 and 40 kg ha$^{-1}$) during the first period of the study, and by all three nitrogen fertilization rates at the milk-wax ripeness stage. The use of these fertilizers did not affect soil pH, unlike readily available phosphorus and potassium levels.

**Keywords:** *ammonifiers, nitrogen fertilizers, proteolytic activity, vertisol, field pea.*

## Introduction

As a legume having a marked ability to establish an effective symbiosis with active nitrogen-fixing bacterial strains, field pea does not require significant amounts of mineral nitrogen fertilizers for its growth and development (Đukić et al., 2009). However, this fact is often neglected in intensive production systems which use inadequate (higher) rates of these fertilizers, thereby risking having unfavourable physicochemical and biological characteristics of the soil and, eventually, crop yield and quality. Therefore, the judicious efficient use of mineral fertilizers (notably nitrogen fertilizers, in these plants) can be possible only if a complex approach involving microbiological research is adopted. The importance of microbiological research lies in the fact that microorganisms, as the most active component of biogeocenosis in biogeochemical terms, are a vital factor of soil biological productivity, environmental status and quality of crop production (Milošević et al., 2004; Essah and Delgado, 2009). Moreover, the count and activity of soil microorganisms can serve as an indication of the economic justifiability of the use of different types, combinations and rates of fertilizers (Černy et al., 2003; Stark et al., 2007). As a rule, mineral fertilization, used as a radical method for improving soil nutrient balance, intensifies microbial processes (Fauci and Dick, 1994). However, long-term fertilization, particularly with high rates of nitrogen fertilizers, can have deleterious effects, leading to increased gaseous nitrogen losses, deteriorated physicochemical and biological properties of the soil and, eventually, diminished safety of the crop produced (Barabasz et al., 2002; Ayoola and Adeniyan, 2006). This is particularly the case under long-term fertilization which results in failure to obtain expected crop yields. The dual nature of mineral fertilizers, once they provide nutrient supply to the plant, allows them to ensure yield increases, while at the same time deteriorating soil properties and acting as a hidden cause of soil degradation (Mandić et al., 2004). Changes in the count of certain groups of soil microorganisms under the effect of different types of mineral fertilizers suggest their justifiable use as an indicator of soil fertility and crop yield (Đukić and Mandić, 2000). Hole et al. (2005) showed that a change in microbial biomass is a parameter that provides a clearer faster response to the incorporation of mineral fertilizers into the soil, which eventually affects its potential and effective productivity.

The objective of this study was to evaluate the effect of different rates of mineral nitrogen and constant rates of phosphorus and potassium on ammonifier counts, protease activity and some chemical characteristics of vertisol under field pea cultivation.

## Material and methods

The experiment was carried out at the Faculty of Agronomy in Čačak (2006) on vertisol (acid in reaction - $pH_{KCl}$ 5.01, having a moderate supply of humus - 2.68% and nitrogen - 0.134%, a good supply of readily available potassium - 0.264 mg $g^{-1}$ and a low supply of readily available phosphorus - 0.029 mg $g^{-1}$), in a randomized block design in four replications. The study involved the following treatments (factor A): unfertilized control; $N_1$PK (20: 75: 60 kg $ha^{-1}$); $N_2$PK (40:75:60 kg $ha^{-1}$) and $N_3$PK (60: 75: 60 kg $ha^{-1}$). The fertilizers were applied before sowing in the form of urea (nitrogen fertilizer), superphosphate (phosphorus fertilizer) and KCl (potassium fertilizer). The experimental plot was 10 $m^2$ in size, with a spacing of 0.5 m between plots. Field pea cv. Junior was sown by a mechanical seed drill at a rate of 40 kg $ha^{-1}$. During the growing season, common crop care practices were employed, including chemical weed control and mechanical preparation of the soil between the plots. Soil samples were collected for microbiological analysis aseptically up to 25 cm depth during two periods of field pea development (factor B): period I: onset of pod formation; period II – full wax ripeness.

Microbiological analyses involved determination of ammonifier counts, by the indirect method of growth on MPA medium (Pochon and Tardieux, 1962), and protease activity (Romejko, 1969). Soil chemical analysis, across treatments, was carried out after harvest, involving determination of readily available phosphorus and potassium using the Egner Riehm AL method ($P_2O_5$ – spectrophotometry, $K_2O$ – flame photometry and pH (potentiometric method).

The results obtained were subjected to a two-factor analysis of variance (fertilization, growing season). Testing of the significance of differences was performed by LSD test (Statistica SPSS 5).

## Results and discussion

The analysis of variance of ammonifier counts suggests a significant effect of nitrogen fertilizers and growing season on the presence of this physiological group of microorganisms.

The results obtained show that all nitrogen application rates, excepting the lowest rate (20 kg $ha^{-1}$), induced a decrease in ammonifier count during the first period of the study (Table 1), with the most depressive effect being observed in the treatment with 60 kg $ha^{-1}$.

This effect is often associated with changes in soil physicochemical characteristics (Stark et al., 2007) or, more likely in this case, alterations in the structure of soil microbial cenosis expressed through the predominance of toxinogenic and phytopathogenic fungi, as induced by excess nitrogen remaining unused by the plant. Their predominance, along with the increase in ammonifier count, can cause a decline in the count of Gram-negative bacteria, in particular, and other poorly competing types of soil microorganisms (Barabasz et al., 2002). The same authors underline that inadequate nitrogen fertilization can result in the production of toxic metabolites (nitrosamines, nitrosamides, etc.) which can, apart from their depressing effect on most soil microorganisms, cause teratogenic, carcinogenic and allergic effects in higher organisms (plants, animals and humans) through the food chain. A somewhat lower depressive effect of nitrogen fertilizers at the first stage of the study was observed in treatments with 40 kg ha$^{-1}$. During the second period of the study, the three nitrogen rates had a non-significant effect on ammonifier count. This can be attributed to the decrease in free nitrogen levels in the soil and, hence, their lower impact on this group of microorganisms in the final vegetation stages of field pea development (Đukić, 1984).

Overall, the significantly lower count of ammonifiers in the second sampling period can be due to the effect of environmental factors (low soil moisture and high temperature) and reduced root metabolism of the crop (Bolton et al., 1992).

Table 1. Average ammonifier count in the soil ($10^6$ g$^{-1}$ absolutely dry soil) and protease activity (number of gelatine-like units g$^{-1}$ air-dry soil) as dependent upon the fertilizers used

| | | Ammonifier count | Proteolytic activity |
|---|---|---|---|
| Fertilization (A) | | | |
| Control | | 40,7 a | 34,83 b |
| $N_1PK$ | | 39,7 ab | 58,75 a |
| $N_2PK$ | | 35,0 b | 57,50 a |
| $N_3PK$ | | 21,0c | 55,3 a |
| Periods (B) | | | |
| | I | 65,3 a | 44.87 b |
| | II | 2,63 b | 57,92 a |
| | | | |
| Control | I | 78,0 a | 41,5 de |
| | II | 3,5 d | 26,7 f |
| $N_1PK$ | I | 76,5 a | 45,0 cd |
| | II | 3,0 d | 72,5 a |
| $N_2PK$ | I | 68,0 b | 47,5 b |
| | II | 2,0 d | 67,5 b |
| $N_3PK$ | I | 40,0 c | 45,5 c |
| | II | 2.0 d | 65,0 b |
| Anova | | | |
| | A | ** | ** |
| | B | ** | ** |
| | AB | ** | ** |

Values followed by different small letters within columns are significantly different (P≥0.05) according to LSD test

As opposed to ammonifiers, soil protease activity was significantly increased by mineral fertilization (Table 1), primarily by lower nitrogen application rates (20 and 40 kg ha$^{-1}$) in the first and most notably in the second period of the study. The higher nitrogen rate (60 kg ha$^{-1}$) is not economically justified, since it did not have a significant effect on the activity of proteolytic enzymes, as compared to the lower nitrogen rates. Similar results were reported by other authors who stressed the positive effect of lower rates of mineral fertilizers on most soil hydrolytic enzymes (Sarić et al., 1986, Blecharczyk et al., 1993, Koper and Piotrowska, 2003).

In all treatments, excepting the untreated control, protease activity was higher in the second period of the study, showing an opposite tendency relative to the count of ammonifiers. This finding complies with the results of other authors who found that soil protease activity is dependent more upon the metabolic activity of the root than upon weather factors during the plant growing season (Kandeler et al., 1999).

The test fertilizers also had an effect, although somewhat lower, on soil chemical characteristics (Table 2).

Table 2. Major chemical characteristics of vertisol as dependent upon mineral fertilizer after field pea harvest

| | pH (nKCl) | $P_2O_5$ (mg $g^{-1}$ soil) | $K_2O$ (mg $g^{-1}$ soil) |
|---|---|---|---|
| Control | 4.97 | 0.018 | 0.267 |
| $N_1PK$ | 4.88 | 0.019 | 0.293 |
| $N_2PK$ | 5.00 | 0.028 | 0.299 |
| $N_3PK$ | 4.94 | 0.025 | 0.268 |

The smallest changes were observed in soil pH. The lower values for readily available phosphorus, recorded after pea harvest in the untreated control and in the treatments with the lowest nitrogen fertilizer rate, suggest marked plant requirements for this nutrient as well as its removal from the soil by the crop. Similar results were obtained by other authors (Ćirović and Jocić, 1992, Bošković, 1993). Higher nitrogen rates incorporated into the soil ($N_2$ and $N_3$) can largely prevent the uptake of phosphorus by the crop, which can result in higher levels of phosphorus in these treatments as compared to the untreated control and treatment with the lowest nitrogen rate. Higher nitrogen rates had a similar effect on the level of readily available phosphorus in the soil. Since substantial changes in soil chemical characteristics can be expected only after the long-term use of mineral fertilizers (Lebedeva and Zagumennikov, 1977, Jelić, 1997), the changes observed in this study can be considered fully expected.

## Conclusion

The nitrogen fertilizers used for field pea production induced a change in the count of ammonifiers and protease activity, whereas their effect on soil chemical parameters was lower.

The decline in ammonifier count was observed in the initial stage of pod formation in treatments with 40 and 60 kg $ha^{-1}$ nitrogen, whereas their effect in the milk wax ripeness stage, in all fertilization treatments, was statistically non-significant. Overall, throughout the pea growing season, differences in ammonifier counts in the treatments with 20 kg $ha^{-1}$ and 40 kg $ha^{-1}$ were statistically non-significant, whereas the count in the treatment with 60 kg $ha^{-1}$ was statistically significantly lower as compared to the two treatments and the control.

In both periods of the study, soil protease activity was particularly stimulated by lower nitrogen fertilization rates (20 and 40 kg $ha^{-1}$ N). The non-significant effect of the highest nitrogen rate (60 kg $ha^{-1}$) on the activity of proteolytic enzymes as compared to the two lower rates suggests that the highest nitrogen rate is not economically justified in field pea production.

The fertilizers applied did not have a significant effect on soil pH. However, all fertilizers, particularly the higher nitrogen rates, induced a certain increase in the levels of readily available phosphorus and potassium in the soil under field pea.

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