THE INFLUENCE OF CHEMICAL AND BIOFERTILIZERS ON THE YIELD, NITROGEN, PHOSPHORUS AND POTASSIUM CONTENT OF LETTUCE (LACTUCA SATIVA L.) IN A TWO-YEAR GREENHOUSE EXPERIMENT

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Keywords: lettuce, biofertilizer, nitrogen, phosphorus, potassium

Summary

The two-year greenhouse experiment on calcareous chernozem soil was performed to study the effects of biofertilizer in the presence of chemical fertilizer on the yield, nitrogen, phosphorus and potassium content of cabbage head lettuce (Lactuca sativa L.) and bioavailable nitrogen, phosphorus and potassium nutrients measured in 0.01 M $CaCl_2$, and AL soil extracts. In the experiment bi-factorial trials were arranged in a randomized complete block design with four replications, applying four levels of N as NH_4NO_3 with or without application of biofertilizer as Phylazonit MC. Inoculation of soil with biofertilizer was performed only in the first year, at the start of the experiment, the chemical fertilizers were applied in both experimental years.

The results obtained suggested: Nitrogen doses – in both years- markedly increased growth of lettuce and slight increasing trend was recorded for fresh weight as Phylazonit application appeared. Measured soil parameters are in good agreement with results of plant analysis. We measured higher N and K content of lettuce grown in the first experimental year (in 2006) and besides we could measure higher $CaCl_2$- NO_3-N and AL-K_2O in soil extracts in 2006 than that of in 2007. On the contrary, the P content of plants grown in the second year was higher and we could measure higher AL-P_2O_5 values in 2007, also.

Increased N fertilizer caused increased N content of plant and available nitrogen forms in soils in both year. Effect of biofertilizer on the N content of plant and mineral N content of soil also was the same.

Nitrogen fertilization did not caused statistically significant effect either P content of lettuce or AL-P_2O_5 values. With application of biofertilizer there was a tendency to increase P concentration of plant.

The increase of nitrogen doses did not resulted in significant exchange of K content of lettuce, however biofertilizer application had increasing effect on the K content of plant. This effect was not significant, but was expressed in the second experimental year.

INTRODUCTION

Nitrogen as a major constituent of all plants is one of the most important nutrients. It has got a unique position because relatively high amounts are required by most agricultural crops for optimal yields (Black, 1968, Stevenson and Cole, 1999). The use of nitrogen fertilizers is standard practice in vegetable production systems. Extensive use of chemical fertilizers may cause environmental pollution, ecological damage and increased production cost (Ghost and Bhat, 1998). Increasing concern over nitrate contamination of soils, nitrate leaching in groundwater and nitrate content in vegetable may require a reduction in N application in crop production, while maintaining optimal productivity (Gutezeit and Fink, 1999). For reducing application of chemical fertilizers, an alternative method must be developed. For this reason, environmental friendly product such as biofertilizer should be used. Applications of biofertilizers may help to avoid environmental hazards for plants, animals and human beings and may hold a great promise to improve yield trough better nutrient supply (Wu et al, 2005). Biofertilizers are products containing different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form (Hegde et al., 1999; Vessey, 2003, Vance, 1997). The major objective of our experiment was to evaluate the effects of nitrogen- and biofertilizer (Phylazonit MC) on the promotion of growth and nutrients uptake of lettuce *(Lactuca Sativa L.)* in greenhouse pot experiment, had been lasted for two years.

MATERIALS AND METHODS

The two-year greenhouse pot experiment was carried out using calcareous chernozem soil from region Debrecen-Látókép. Some properties of soil used are included in *Table 1*.

Table 1.

Properties of experimental soil

pH_{KCl}	pH_{H2O}	pH_{CaCl2}	Hu%	K_A
5.9	6.7	6.2	2.5	42

K_A: Plasticity index according to Arany

In the first experimental year 10 kg soil were weighed into Mitscherlich type pots. The experimental plant selected was cabbage head lettuce (*Lactuca sativa L.*). Four seeds of lettuce were sown per pot on 05.01.2006. Ion exchanged water was added to all pots to keep the soil at constant moisture (60% of the water-holdig capacity) using daily weighing.

The bi-factorial trials were arranged in a randomized complete block design with four replications, applying four levels of N with or without application of biofertilizer. The scheme of treatments applied can be seen in *Table 2.*. P and K doses applied were identical in all pots (0.2 g P_2O_5 pot^{-1} and 0.2 g K_2O pot^{-1}).

Table 2.

Scheme of treatments applied

Treatment	N doses g pot^{-1}	Phylazonit doses in sandy soil cm^3 pot^{-1} diluted one-thousandfold	Phylazonit doses in chern. soil cm^3 pot^{-1} diluted one-thousandfold	code
1.	0	0	0	control
2.	0	40	35	N_0+phyl
3.	0.05	0	0	N_1
4.	0.05	40	35	N_1+phyl
5.	0.1	0	0	N_2
6.	0.1	40	35	N_2+phyl
7.	0.2	0	0	N_3
8.	0.2	40	35	N_3+phyl

The nitrogen, phosphorus and potassium were added in solution of NH_4NO_3, KH_2PO_4 and KCl, respectively in both experimental years. The applied biofertilizer was Phylazonit MC (phyl), which contains carboxi-methyl-cellulose (CMC), microelements, *Azotobacter croococcum*, *Bacillus megatherium* soil bacteria, heteroauxin, gibberelin and vitamin B. The soil in each pot was stirred well to ensure uniform mixing of soil, Phylazonit MC and nutrients. Before application Phylazonit MC was diluted one-thousandfold and than 35 cm^3 pot^{-1} was added to the soil. The bacterial inoculation of soil was performed only in the first experimental year, at the beginning of experiment to study the long-lasting effect of this product in the second experimental year also.

At the sixth week after sowing leaf samples were collected and fresh weights were determined, soil samples were taken also from all pot and the other part of the soil were put back again into Mitscherlich type pots. Next summer four levels of nitrogen (according to Table. 2.), identical phosphorus and potassium (without application of Phylazonit MC) were added to each pot as a year before. Four seeds of lettuce were sown into all pots again. At the end of the growing season leaf samples were collected –as a year before- and fresh weights were determined.

Plant leaves -from both experimental years- after drying were digested by H_2SO_4-H_2O_2 method for P and K analysis. P was determined colorimetrically using the molybdenum blue colorimetric method, while potassium was quantified by atomic emission spectrophotometry. NO_3^--N of plant was measured from water extract by ion chromatography (Balláné, 2004) and the total nitrogen analysis was performed by dry combustion method (Nagy, 2000).

Soil samples were air dried and sieved (<2mm) for further analysis. Concentration of water soluble nitrogen forms were measured in 0.01 M $CaCl_2$ extracts with 1:10 soil:solution ratio (Jászberényi et al., 1994) by autoanalyser (SKALAR Segment Flow Analyser). Concentration of phosphorus and potassium in the soil taken up easily by plant were determined in ammonium lactate- acetic acid (AL) extract. P was measured colorimetrically, while potassium was quantified by atomic emission spectrophotometry.

Analysis of variance was carried out on the data in order to provide a statistical comparison between the treatment means. The least significant difference (LSD) test was used to detect differences between means at probability level $P \leq 0.05$.

RESULTS

Yield

The effects of bio- and nitrogen fertilization on fresh weight of lettuce cultivated in 2006, 2007 years are summarized in *Figure 1., in Table 3.*

Figure 1.: **Mean of lettuce fresh weight in 2006 and 2007 as influenced by NH_4NO_3 and Phylazonit MC applications**. **For interpretation of treatments see Table 2.**

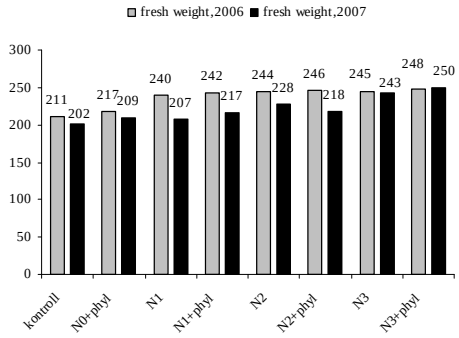

Table 3.

Summary of ANOVA (F-test) for different source of variance

Source of variation	fresh weight			
	2006		2007	
	significance	$LSD_{5\%}$	significance	$LSD_{5\%}$
NH_4NO_3	***	15.02	***	19.07
Phylazonit doses	n. s.	-	n. s.	-

n.s.:non-significant; ***:significant at P<0.1%;

The lettuce plant grown in two experimental years varied in total fresh matter. The fresh weight ranged 210.6 - 248.4 $g.pot^{-1}$ and 201 – 249,3 $g.pot^{-1}$ in 2006, 2007 respectively. In the second year, in 2007, the yield of lettuce in each pot was lower, than that of in 2006.

On the basis of ANOVA test it can be concluded that N fertilisation significantly increased fresh weights of lettuce. This effect was significant in both experimental years.

The application of biofertilizer was not as effective as N fertilizer. However, a slight increasing trend was recorded in both years for fresh weight as Phylazonit application appeared, but this effect was not statistically significant.

N, P and K content of lettuce

The effects of bio and nitrogen fertilization on nitrogen, phosphorus and potassium content of lettuce grown in 2006, 2007 are summarized in *Figure 2-4.*, in *Table 4.*

Figures 2., 3.: **Means of lettuce N % and P % harvested in 2006 and 2007 as influenced by NH_4NO_3 and Phylazonit MC applications. For interpretation of treatments see Table 2.**

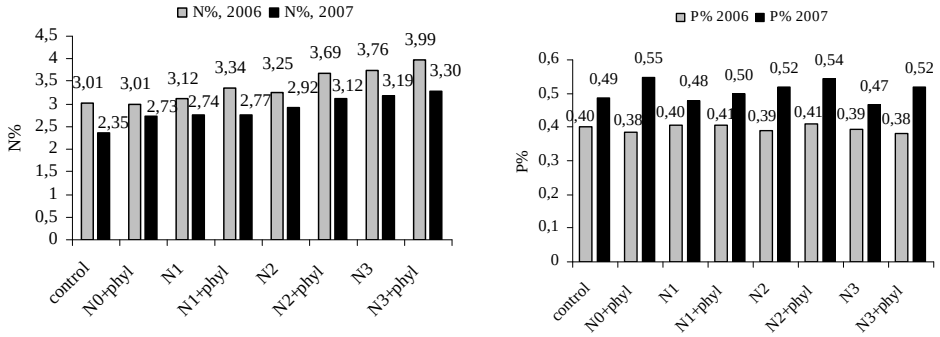

Figure 4. : **Mean of lettuce K % harvested in 2006 and 2007 as influenced by NH_4NO_3 and Phylazonit MC application. For interpretation of treatments see Table 2.**

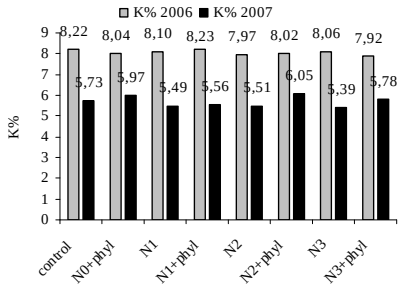

Table 4.

Summary of ANOVA (F-test) for different source of variance

Source of variation	2006		2007	
	significance	$LSD_{5\%}$	significance	$LSD_{5\%}$
	N %			
NH_4NO_3	***	0.354	***	0.255
Phylazonit	+	-	+	-
	P %			
NH_4NO_3	n.s.	-	n.s.	-
Phylazonit	n.s.	-	+	-
	K %			
NH_4NO_3	n.s.	-	n.s.	-
Phylazonit	n.s.	-	n.s.	-

n.s.:non-significant; ***:significant at P=0.1%; +:significant at P=10%;

Studying the nitrogen content of lettuce leaves we found, that these values in 2006 were a little bit higher than that of in 2007. The N % of plants ranged 3.00 % - 3.99 %, and 2.35 % - 3.24 % in 2006, 2007, respectively. On the basis of statistical analysis it can be concluded that there was highly significant relationship between N treatments and plant N content. This effect was significant at P = 0,1 % in both experimental years. Effect of biofertilizer on N content had not been so definite, but it is worth mentioning, that inoculation of soil with Phylazonit slightly increased this nutrient content of plants. This increasing effect on N % can be seen in both experimental years at P = 10 % significant level.

In contrast with N content, the phosphorus concentration of plant was higher in the second experimental year. The phosphorus content ranged from 0.385 % to 0.410 % and 0.466 % to 0.547 % in 2006 and 2007, respectively. Nitrogen fertilization did not cause statistically significant exchange on the P content of lettuce. With application of biofertilizer there was a tendency to increase P concentration of plant. The effect was stronger and significant at P = 10 % only in the second experimental year.

Similarly as nitrogen content of lettuce, the potassium content of plant was higher in the first experimental year. The K % of plants in 2006 ranged from 7.92 % to 8.23 %, and in 2007 ranged from 5.39 % to 5.97 %. The increase of nitrogen doses did not resulted in significant exchange of potassium content of lettuce, however biofertilizer application had a slight increasing effect on the K content of plant. This effect was not significant, but was stronger in the second experimental year.

Concentration of $CaCl_2$-N, $CaCl_2$-NO_3-N, AL-P_2O_5 and AL-K_2O

Concentrations of water soluble NO_3^--N and total N measured in 0.01 M $CaCl_2$ soil extracts and means of AL-P_2O_5, AL-K_2O in 2006, 2007 are presented in *Figures 5., 6., 7., 8.* Analysis of variance are summarised in *Table 5.*

Figures 5.,6.: **Means of total N and NO_3^--N (mg kg^{-1}) measured in $CaCl_2$ soil extract. For interpretation of treatments see Table 2.**

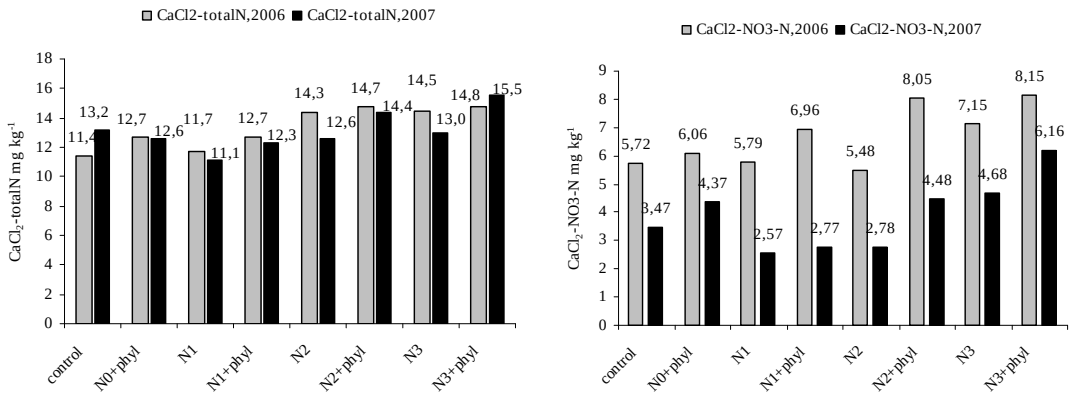

Figure 7.,8.: **Means of AL-P_2O_5 and AL-K_2O (mg.kg^{-1}) influenced by NH_4NO_3 and Phylazonit application rates. For interpretation of treatments see Table 2.**

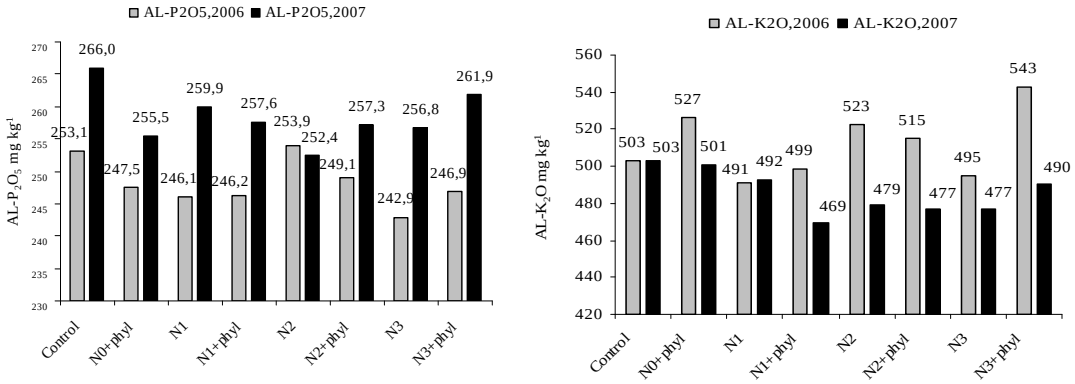

Table 5.

Summary of ANOVA (F-test) for different source of variance

Source of variation	2006		2007	
	significance	$LSD_{5\%}$	significance	$LSD_{5\%}$
	$CaCl_2$-total N			
NH_4NO_3	***	1,44	**	1,04
Phylazonit	+	-	+	-
	$CaCl_2$-NO_3-N			
NH_4NO_3	**	1,04	***	0,98
Phylazonit	***	0,73	**	0,69
	AL-P_2O_5			
NH_4NO_3	+	-	n.s.	-
Phylazonit	n.s.	-	n.s.	-
	AL-K_2O			
NH_4NO_3	***	11,67	n.s	-
Phylazonit	***	8,25	n.s.	-

n.s.:non-significant; ***:significant at P=0.1%; +:significant at P=10%;

The concentration of total N measured in 0.01M $CaCl_2$ soil extracts was almost the same in both experimental years. On the contrary, the $CaCl_2$-NO_3^--N was much lower in soil samples measured in 2007, than measured in the first year. This fact is in good aggreement with results of plant analysis, namely the N content of lettuce was also lower in the second experimental year.

Increase of N fertilizer doses had resulted in a significant enhancement in both N forms measured in 0.01 M $CaCl_2$ soil extracts.

Application of Phylazonit increased $CaCl_2$-total N at P = 10 % significance level, and increased $CaCl_2$-NO_3^--N at P = 5 % significance level. This increasing effect can be seen not only in the first but in the second year also.

In the first experimental year, in 2006, the measured AL-P_2O_5 values were lower by turns than that of in 2007. There was a weak relationship between N fertilization and measured AL-P_2O_5 values. The increased N fertilizer doses caused improvement in AL-P_2O_5 values, but this effect proved to be only in 2006. Biofertilizer did not cause any effects on these values.

Measured data show that similarly to $CaCl_2$-NO_3^- -N, the values of AL-K_2O were higher in the first experimental year by turns and the increased N fertilizer doses and appearance of biofertilizer caused significant improvement in these parameters. In the second year, in 2007, this effect can not be seen any longer.

Measured soil parameters are in good agreement with results of plant analysis. We measured higher N and K content of lettuce grown in the first experimental year and besides we could measure higher $CaCl_2$- NO_3-N and AL-K_2O in soil extracts than that of in 2007. On the contrary, the P content of plant was higher grown in the second year and we could measure higher AL-P_2O_5 values in 2007 also. Increased N fertilizer increased N content of plant and the NO_3^- -N, total N forms of $CaCl_2$ extracts. Effect of biofertilizer on the N content of plant and mineral N content of soil also was the same in both experimental years.

CONCLUSION

As a result of our experiment it can be concluded, that the effect of chemical fertilizer was stronger. With increasing of NH_4NO_3 significantly increased the fresh weight and the N content of lettuce and the N forms measured in 0.01 M $CaCl_2$ soil extracts. Biofertilizer application was not as effective as N fertilizer, but it can be underlined that when Phylazonit application appeared, slight increasing trends were recorded *in both years* for fresh weight, N, P and K content of lettuce and N, NO_3^- forms in 0.01 M $CaCl_2$ soil extracts. Biofertilizer did not cause any significant effects on AL-P_2O_5, but improved AL-K_2O values measured in the first year.

The effect of biofertilizers in practice, for examples in different type of soils has not achieved constant effect and the mechanisms, interactions, the time of its effect still are not well understood. To gain more, reliable applicability of Phylazonit MC, further studies are needed.

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A MŰTRÁGYÁZÁS ÉS A BIOTRÁGYA HATÁSA A FEJESSALÁTA (*LACTUCA SATIVA L.*) TERMÉSÉRE, NITROGÉN-, FOSZFOR- ÉS KÁLIUMTARTALMÁRA KÉTÉVES TENYÉSZEDÉNY KÍSÉRLETBEN