Studies of the effects of N fertilizers and Microbion UNC biofertilizer on microelement content of horseradish (*Armoracia macrocarpa*)

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ABSTRACT

A field experiment on calcareous chernozem soil was performed to study the effects of different N and bacterial fertilizers on the nutrient content of horseradish (Armoracia macrocarpa). In the experiment the trials were arranged in a randomized block design with three replications, applying three levels of NH_4NO_3 and different N fertilizers, namely ammonium-nitrate, urea and calcium-nitrate, with or without application of Microbion UNC biofertilizer.

In the present paper the changes and distribution of manganese, zinc and copper contents of the horseradish plant are summarized by the effect of different treatments.

The Mn content of leaves were higher in all cases than those of roots, but Zn mainly accumulated in the roots. The distribution of copper within the horseradish plant was more equalized than that of Zn and Mn. Different N fertilizers and increasing doses of ammonium-nitrate had effects mainly on the microelement contents of leaves. The highest Mn contents of plant were measured in treatments of $Ca(NO_3)_2$ and $Ca(NO_3)_2$+Microbion. The lowest ammonium nitrate dose (N_1) decreased the Mn content of leaves compared to control, but further doses (N_2, N_3) did not alter these values any longer. Microbion UNC biofertilizer did not have any effect on the Mn content of roots, but we measured higher Mn in leaves in some combined treatments. $Ca(NO_3)_2$ increased the zinc content in leaves and roots in a noticable manner. With the increasing of NH_4NO_3 doses, the Zn content of leaves and roots augmented significantly. Neither N fertilizers (or the increasing doses of NH_4NO_3) nor the biofertilizer application influenced the Cu content of horseradish plant.

N fertilizers had higher effects on the microelement content of horseradish, the biofertilizer's effect was smaller and was not the same in every treatment.

INTRODUCTION

Nitrogen as a major constituent of all plants is one of the most important nutrient. It has got a unique position because relatively high amounts are required by most agricultural crops for optimal yields (Black, 1968). Nitrogen fertilizers may contain nitrogen in different chemical forms (nitrate, ammonium or amide). It has long been observed that ammonium and nitrate differ in their effects on the soil and on the growth and chemical compositions of plants (Maier et al., 2002). Plants take up nitrogen as nitrate (NO_3^-) and ammonium (NH_4^+) ions. NO_3^- and NH_4^+ uptakes induce a net release of OH^- and H^+ ions, respectively (Hinsinger et al, 2003). Hence, they will change the rhizosphere pH in different ways and pose the distinct influence on nutrient availability in soil (Jalloh et al., 2009). When urea is applied as a nitrogen fertilizer, it causes little pH change, but it can be hydrolyzed by microbially produced NH_4^+ and NO_3^- also.

It can be said that the application of different forms of N fertilizers may change the rhizosphere pH in different manners so they may influence the microbiological life of soil and the solubility and availability of nutrients, mainly micronutrients, differently.

Nevertheless, the overuse or not balanced application of N fertilizers may cause serious environmental damages. Nowadays the ratio of nitrogen fertilizers' application has been converted into autocrat that might cause environmental pollution and ecological damages, such as increasing nitrate accumulation of soils, acidification of soil, nitrate leaching in groundwater (Gutezeit and Fink, 1999; Sipos, 2009).

There has been a growing need for the realization of a well-balanced, soil test based, judicious nutrient management system (Saha et al., 2007). The main characteristics of the integrated nutrient management system are the reduced consumption of chemical fertilizers and the increased use of natural sources of plant nutrients such as manure, crop residues, or biofertilizers.

Soil is a natural habitat for a variety of agriculturally beneficial microorganisms. Certain soil microorganisms have an ability to absorb and convert atmospheric nitrogen into the readily available form to the plants, certain soil microorganisms solubilize a part of the bound phosphates of the soil and thereby make them available to the plants. Soil microorganisms are important components in the natural soil subecosystem because they cannot only contribute to nutrient availability in the soil, but also bind soil particles into stable aggregates, which improve soil structure and reduce the erosion potential (Shetty et al., 1994) These attributes make microorganisms important to be used as biofertilizers.

Biofertilizers are special products which contain different types of microorganisms (nitrogen fixing, phosphate solubilizing bacteria, cellulolytic decomposing microorganisms), have an ability to convert nutritionally important elements from unavailable to available form through biological processes (Hedge et al., 1999; Vessey, 2003).

In Hungary several studies were performed on the effects of different N fertilizer applications, but few studies have carried out researches on biofertilizer applications and especially on combined application of nitrogen and biofertilizers and their combined effects on nutrients availability and nutrient uptake by plants (Kincses et al., 2008).

The aim of our experiment was to evaluate the effects of different nitrogen fertilizers (ammonium-nitrate, urea and calcium-nitrate) and increasing doses of NH_4NO_3 in combination with the commercial bacterial fertilizer, Microbion UNC, on the nutrient uptake of horseradish *(Armoracia macrocarpa)* and the changes of available nutrient content of soil.

In this paper we summarized the effects of N fertilizers and Microbion UNC biofertilizer on microelement content (manganese, copper and zinc content) and their distribution in different parts of horseradish plant.

Horseradish is a perennial herb of the brassicaceae family. Horseradish with its high biological value contains many vitamins, mineral salts and flavour substances (Lihong et al., 2008; Géczi & Irinyiné, 2007). It is also a popular vegetable because of its spicy flavour (Hájas, 1976; Géczi, 1998). The roots of horseradish contain sinigrin compound having nitrogen and a sulphur content which is responsible for the hot flavour of plant. The nutrient content of horseradish –as well as manganese, copper and zinc contents- is important on the one hand, because of the life function of the plant, and on the other hand because of its effect on the human body.

MATERIALS AND METHODS

A field plot experiment was set up on the cultivation area of horseradish in Dombostanya in 2008. Dombostanya is located 15 km from Debrecen in Hungary. The soil of the experimental area was chernozem and had the following parameters: pH_{CaCl2}= 7.47; Hu%= 2.87; $CaCO_3$=18.1%; K_A (plasticity index according to Arany) = 43; AL-P_2O_5= 144.6 mg kg^{-1}; AL-K_2O= 141.4 mg kg^{-1}. The soil sufficiency levels of N and P were medium, K was poor.

The bi-factorial trials were arranged in a randomized complete block design with three replications, applying different nitrogen fertilizers (ammonium-nitrate, urea and calcium-nitrate) and increasing level of NH_4NO_3 with or without application of Microbion UNC biofertilizer. The scheme of treatments applied can be seen in *Table 1*.

Table 1

The scheme of treatments

Treatment codes	N fertilizers	N kg ha^{-1}	P_2O_5 kg ha^{-1} (as superphosphate)	K_2O kg ha^{-1} (as K_2SO_4)	Microbion UNC kg ha^{-1}
1. control (N_0)	0	0	75.6	242	0
2. U	urea	116	75.6	242	0
3. AN_1	NH_4NO_3	116	75.6	242	0
4. AN_2	NH_4NO_3	232	75.6	242	0
5. AN_3	NH_4NO_3	348	75.6	242	0
6. Ca-nitrate	$Ca(NO_3)_2$	116	75.6	242	0
7. N_0+Microbion UNC	0	0	75.6	242	2
8. U+Microbion UNC	urea	116	75.6	242	2
9. AN_1+Microbion UNC	NH_4NO_3	116	75.6	242	2
10. AN_2+Microbion UNC	NH_4NO_3	232	75.6	242	2
11. AN_3+Microbion UNC	NH_4NO_3	348	75.6	242	2
12. Ca-nitr.+Microb. UNC	$Ca(NO_3)_2$	116	75.6	242	2

Doses of N fertilizers were divided, the first half was applied on 10^{th} March before planting, the second half also was divided into two parts and were applied on 10^{th} June and on 25^{th} July. Phosphorous was ensured as superphosphate and potassium as potassium-sulphate, because according to Géczi (1998), KCl may hinder the growth of roots. P and K were equalized for all treatments and these fertilizers were applied without dividing.

The applied biofertilizer was Microbion UNC, which is a commercial product and contains different microorganisms, *Azotobacter vinelandii*-B 1795, *Bacillus megaterium*-B1091, *Clostridium pasteurianum*, *Azospirillum* sp., *Bacillus subtilis*, *Rhodobacter* sp., *Lactobacillus* sp., *Trichoderma reseei*, *Saccharomyces* *cerevisiae*, *Streptomyces* sp., agents, vitamins synthetized by microorganisms, GM-8 corncob milling product and dried brewer's yeast. The biofertilizer was mixed up and emitted with NPK fertilizers before planting.

The size of the plots was 15m^2 (3m x 5m). The date of planting of the horseradish was 11th April, and the picking time was 25th September 2008. We collected five plants per plots. The leaves and the roots were collected separately. The roots were washed with distilled water in order to remove soil residues.

After drying plant leaves and roots were digested by HNO_3-H_2O_2 methods, and the manganese, zinc and copper contents of the different parts of plant were quantified by atomic absorption 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

The effects of different N fertilizers and biofertilizer on Mn, Zn and Cu contents of horseradish

The effects of different treatments on Mn, Zn and Cu contents of horseradish are shown in *Table 2*.

Table 2

Means of manganese, zinc and copper contents of horseradish leaves and roots as influenced by different N fertilizers and Microbion UNC application

leaves of horseradish				roots of horseradish			
N treatments	Microbion UNC (kg ha^{-1})		$LSD_{5\%}$ (N)	N treatments	Microbion UNC (kg ha^{-1})		$LSD_{5\%}$ (N)
	0	2			0	2	
Mn (mg kg^{-1})							
N_0	39.8	34.1	5.39	N_0	10.57	11.83	n. s.
AN	30.4	33.1		AN	10.40	10.07	
U	31.4	30.8		U	11.03	11.10	
$Ca(NO_3)_2$	38.8	44.8		$Ca(NO_3)_2$	12.87	12.03	
means	*35.1*	*35.7*		*means*	11.22	*11.26*	
$LSD_{5\%}$ (Microbion UNC): 3.81				$LSD_{5\%}$ (Microbion UNC): n. s.			
Zn (mg kg^{-1})							
N_0	15.13	16.77	1.38	N_0	32.1	30.6	5.31
AN	14.97	17.37		AN	26.6	35.6	
U	15.57	15.93		U	27.3	37.3	
$Ca(NO_3)_2$	19.25	16.87		$Ca(NO_3)_2$	34.9	29.4	
means	*16.23*	*16.43*		*means*	*30.2*	*33.3*	
$LSD_{5\%}$ (Microbion UNC): 0.97				$LSD_{5\%}$ (Microbion UNC): 3.75			
Cu (mg kg^{-1})							
N_0	4.33	4.27	n. s.	N_0	4.87	5.10	n. s.
AN	4.30	4.90		AN	5.37	4.93	
U	4.13	4.47		U	4.77	5.27	
$Ca(NO_3)_2$	4.57	4.57		$Ca(NO_3)_2$	4.85	4.67	
means	*4.33*	*4.47*		*means*	*4.96*	*4.99*	
$LSD_{5\%}$ (Microbion UNC): n. s.				$LSD_{5\%}$ (Microbion UNC): n. s.			

n. s.: not significant

The Mn contents of leaves were higher in all cases than that of roots. The Mn content of leaves and roots ranged between 30.4-44.8 mg kg^{-1} and 10.07-12.87 mg kg^{-1}, respectively.

The N fertilizers and the biofertilizer mainly had effects on the Mn content of the leaves. In leaves we measured significantly lower Mn values in the ammonium-nitrate and urea treatments than in the control. These findings contradict to the observations of other studies (Kádár, 2005; Ragályi és Kádár, 2006) whereas ammonium-nitrate stimulates the Mn uptake of plants with decreasing the soil pH. In plots with $Ca(NO_3)_2$ treatment Mn values did not differ from the values of the control. Microbion UNC bacterial fertilizer decreased the Mn content of leaves compared to control, while in the parcel with combined, $Ca(NO_3)_2$+Microbion UNC treatment we measured significantly higher Mn content of leaves than in the N fertilizer ($Ca(NO_3)_2$) treatment.

The Mn content of roots has not been changed by either application of different N fertilizers, or Microbion UNC bacterial fertilizer.

Zn mainly accumulated in the roots. The Zn content of the leaves and roots ranged between 26.6-37.5 mg kg^{-1} and 14.97-19.25 mg kg^{-1}, respectively.

Among N fertilizers, only $Ca(NO_3)_2$ altered the zinc content of the leaves. We measured higher leaf Zn content in plots with $Ca(NO_3)_2$ treatment than in the control. The effect of Microbion UNC on Zn content of leaves was not the same in every case. In the plots with N_0+Microbion and AN+Microbion treatments an enhancement in the Zn content appeared compared to the appropriate values of the parcel not inoculated. On the contrary, the supplement of $Ca(NO_3)_2$ with biofertilizer resulted in a significant decrease of Zn content of leaves compared to the values of plots not inoculated.

We measured the lowest Zn content in roots in the parcels with ammonium nitrate treatment. In the plots with other fertilizers the Zn content of roots were the same. The supplement of ammonium-nitrate and urea with biofertilizer caused increased Zn values of roots compared to appropriated values of plots not inoculated. As we noticed in leaves Zn values, the supplement of $Ca(NO_3)_2$ with biofertilizer led to a significant decrease of root Zn content compared to the values of $Ca(NO_3)_2$ treatment.

The distribution of copper within the plant was more equalized than that of Zn. The copper contents of roots were a little bit higher than those of the leaves. The Cu contents of leaves were in the range of 4.13-4.90 mg kg^{-1} and in the roots ranged between 4.67-5.37 mg kg^{-1}. Neither N fertilizers nor biofertilizer application influenced the Cu content of the horseradish plant.

The effects of increasing NH_4NO_3 doses and Microbion UNC on Mn, Zn and Cu contents of horseradish

The effects of increasing doses of NH_4NO_3 and Microbion UNC biofertilizer on Mn, Zn and Cu contents of horseradish plant can be seen in *Table 3.*

Table 3

Means of manganese, zinc and copper contents of horseradish leaves and roots as influenced by increasing doses of NH_4NO_3 and Microbion UNC application

leaves of horseradish				roots of horseradish			
NH_4NO_3 doses	Microbion UNC ($kg\ ha^{-1}$) 0	2	$LSD_{5\%}$ (N)	NH_4NO_3 doses	Microbion UNC ($kg\ ha^{-1}$) 0	2	$LSD_{5\%}$ (N)
Mn ($mg\ kg^{-1}$)							
AN_0	39.8	34.1	3.25	AN_0	10.57	11.83	1.66
AN_1	30.4	33.1		AN_1	10.40	10.07	
AN_2	37.0	28.7		AN_2	9.93	10.17	
AN_3	32.2	28.8		AN_3	9.63	9.97	
means	*34.9*	*31.2*		*means*	*10.13*	*10.51*	
$LSD_{5\%}$ (Microbion UNC): 2.30				$LSD_{5\%}$ (Microbion UNC): 1.17			
Zn ($mg\ kg^{-1}$)							
AN_0	15.13	16.77	1.40	AN_0	32.1	30.6	5.64
AN_1	14.97	17.37		AN_1	26.6	35.6	
AN_2	20.37	15.60		AN_2	41.5	31.2	
AN_3	16.60	14.40		AN_3	42.3	31.5	
means	*16.77*	*16.03*		*means*	*35.6*	*32.2*	
$LSD_{5\%}$ (Microbion UNC): 0.99				$LSD_{5\%}$ (Microbion UNC): 3.99			
Cu ($mg\ kg^{-1}$)							
AN_0	4.33	4.37	n. .s.	AN_0	4.87	5.10	n. .s.
AN_1	4.30	4.90		AN_1	5.37	4.93	
AN_2	4.93	4.30		AN_2	5.37	4.67	
AN_3	4.37	4.63		AN_3	4.80	5.07	
means	*4.48*	*4.52*		*means*	*5.10*	*4.94*	
$LSD_{5\%}$ (Microbion UNC): n. s.				$LSD_{5\%}$ (Microbion UNC): n. .s.			

Compared to the control plant, the Mn contents of leaves and roots were lower in all parcel treated with NH_4NO_3 fertilizer, so we measured the highest plant Mn content in the control parcel. The decreasing effect of AN_1 dose was stronger in the case of values of leaves.

The further increasing NH_4NO_3 doses (AN_2, AN_3) did not alter the Mn contents any longer. In generally biofertilizer application did not influence the Mn content of roots, but there was a decreasing trend in the Mn values of leaves when Microbion UNC biofertilizer was applied compared to values measured in the parcel with no biofertilizer application.

The lower dose of ammonium nitrate, AN_1, did not changed the Zn content of horseradish, but with further increasing NH_4NO_3 doses the Zn contents of leaves and roots significantly increased. We measured the highest

Zn values of the leaves in the parcel with AN_2 treatment and in the roots with AN_3 treatment. These results meet our expectation, namely ammonium nitrate has influenced the soil pH, therefore the availability of Zn in soil expanded.

The effects of the bacterial fertilizer on the leaves' and roots' Zn contents depended on the doses of NH_4NO_3. When biofertilizer was applied with lower NH_4NO_3 doses (AN_1), the Zn values of the plants in the inoculated parcel were higher than without application of the biofertilizer. When higher N doses (AN_2, AN_3) were supplied with Microbion UNC, the Zn content of the plant decreased compared to the values of the parcel not inoculated.

The Cu contents of the leaves and the roots were not influenced by either the increasing doses of NH_4NO_3 or the application of biofertilizer.

CONCLUSIONS

The Mn content of leaves were higher in all cases than those of roots, but the Zn mainly accumulated in the roots. The distribution of copper within the horseradish plant was more equalized than Zn and Mn.

Different N fertilizers and the increasing doses of ammonium-nitrate had mainly effects on microelement contents of leaves. The highest Mn contents of plant was measured in $Ca(NO_3)_2$ and $Ca(NO_3)_2$+Microbion combined treatments. AN_1 dose decreased the Mn content of leaves compared to control, but further doses (AN_2 and AN_3) did not alter these values any longer. Microbion UNC biofertilizer did not have any effect on the Mn content of the roots, but we measured higher Mn contents of leaves in some combined treatments.

$Ca(NO_3)_2$ application increased the zinc content of the leaves and the roots to a remarkable extent. With increasing NH_4NO_3 doses, the Zn contents of leaves and roots -as expected- significantly augmented. The effect of the biofertilizer on Zn content of the plant was not uniform.

Neither N fertilizers (and increasing doses of NH_4NO_3) nor the biofertilizer application influenced the Cu content of the horseradish plant.

Finally, it can be concluded that N fertilizers had higher effects on microelement content of horseradish, while the biofertilizer's effect was smaller and was not the same in every treatment.

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