

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

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ABSTRACT

Field experiment on calcareous chernosem soil was performed to study the effects of different N fertilizers and bacterial fertilizer on nutrient content of horseradish (*Armoracia macrocarpa*). In experiment 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 present paper the changes and distribution of manganese, zinc and copper content of horseradish plant are summarized by the effect of different treatments.

The leaves Mn were higher in all cases than that of values of roots, but Zn is accumulated mainly in roots. The distribution of copper within horseradish plant was more equalized than Zn and Mn. Different N fertilizers and increasing doses of ammonium-nitrate had mainly effects on leaves microelement content. The highest Mn contents of plant were measured in $\text{Ca}(\text{NO}_3)_2$ and $\text{Ca}(\text{NO}_3)_2$ +Microbion combined treatments. The lowest ammonium nitrate dose (N_1) decreased leaves Mn 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 Mn content of roots, but we measured higher Mn of leaves in some combined treatments. $\text{Ca}(\text{NO}_3)_2$ increased the zinc content of leaves and roots in noticeable manner. With increasing NH_4NO_3 doses, the Zn content of leaves and roots significantly augmented. Either N fertilizers (and increasing doses of NH_4NO_3) or biofertilizer application did not influence the Cu content of horseradish plant.

N fertilizers had higher effects on 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 nutrients. It has got a unique position because of 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 composition of plants (Maier et al., 2002). Plants take up nitrogen as nitrate (NO_3^-) and ammonium (NH_4^+) ions. NO_3^- and NH_4^+ uptake by plant induce a net release of OH^- and H^+ ions, respectively (Hinsinger et al, 2003). Hence they will change the rhizosphere pH in different way and pose the distinct influence on nutrient availability in soil (Jalloh et al., 2009). When urea is applied as 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 application of different forms of N fertilizers may change the rhizosphere pH in different manner so they may effect differently on the microbiological life of soil and on the solubility and availability of nutrients, mainly micronutrients.

Nevertheless 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 damage, 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 realization of a well-balanced, soil test based, judicious nutrient management system (Saha et al., 2007). The main characteristic of the integrated nutrient management system is a reduced consumption of chemical fertilizers and increased use of natural sources of plant nutrients such as manure, crop residues, or biofertilizers.

Soil is a natural habitat of variety of agriculturally beneficial microorganisms. Certain soil microorganisms have an ability to absorb and convert atmospheric nitrogen to the readily available form to the plants, certain soil microorganisms solubilize 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 can not only contribute to nutrient availability in the soil, but also bind soil particles into stable aggregates, which

improve soil structure and reduce erosion potential (Shetty et al., 1994) These attributes make microorganisms important to be used as biofertilizers.

Biofertilizers are products containing different types of microorganisms (nitrogen fixing, phosphate solubilizing bacteria, cellulolytic microorganisms), which 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 been performed research on biofertilizer application 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 nutrient uptake of horseradish (*Armoracia macrocarpa*) and changes of available nutrient content of soil.

In present 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 high biological value is containing many vitamins, mineral salts and flavour substances (Lihong et al., 2008; Géczi & Irinyiné, 2007). Horseradish is popular vegetable because of its spicy flavour also (Hájas, 1976; Géczi, 1998). Roots of horseradish contain sinigrin compound having nitrogen and a sulphur content which is responsible for hot flavour of plant. The nutrient content of horseradish –also manganese, copper and zinc content- is important on the one hand because of the life function of the plant, and on the other hand may be significant 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 chernosem and had the following parameters: $\text{pH}_{\text{CaCl}_2} = 7,47$; $\text{Hu}\% = 2,87$; $\text{CaCO}_3 = 18,1\%$; K_A (plasticity index according to Arany) = 43; $\text{AL-P}_2\text{O}_5 = 144,6 \text{ mg kg}^{-1}$; $\text{AL-K}_2\text{O} = 141,4 \text{ mg kg}^{-1}$. 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	$\text{Ca}(\text{NO}_3)_2$	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	$\text{Ca}(\text{NO}_3)_2$	116	75.6	242	2

Doses of N fertilizers were divided, the first half one was emitted on the 10th of March before planting, the second half one also was divided into two parts and were emitted on 10th of June, and on 25th of July next to the plant near the roots. Phosphorous was ensured as superphosphate and potassium as potassium-sulphate, because according to Géczi (1998) KCl may delay the growth of roots. P and K were equalized for all treatments and these fertilizers were emitted 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 reesei*, *Saccharomyces cerevisiae*, *Streptomyces* sp., agents, vitamins synthesized by microorganisms, GM-8 corn cob milling product and dried brewer's yeast. Biofertilizer was mixed up and emitted with NPK fertilizers before planting.

The experiment size of plots was 15m² (3m x 5m). Time of planting of horseradish was 11th of April, and the picking time was 25th of September 2008. We collected five plants per plots. Leaves and roots were collected separately. Roots were washed with distilled water in order to remove soil residues.

Plant leaves and roots after drying were digested by HNO₃-H₂O₂ methods and manganese, zinc and copper contents of 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

Effects of different N fertilizers and biofertilizer on Mn, Zn and Cu content of horseradish

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

Table 2.

Means of manganese, zinc and copper content 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 values of roots. The Mn of leaves and roots ranged between 30.4-44.8 mg kg⁻¹ and 10.07-12.87 mg kg⁻¹, respectively.

N fertilizers and biofertilizer mainly had effects on leaves Mn. In leaves we measured significantly lower Mn values with ammonium-nitrate and urea treatments compared to control. This findings are opposite of observation 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(NO₃)₂ treatment Mn values did not differed from values of control. Microbion UNC bacterial fertilizer decreased the leaves Mn compared to control, on the contrary in the parcel with combined, Ca(NO₃)₂+Microbion UNC treatment we measured significantly higher Mn content of leaves compared to values having only appropriate N fertilizer (Ca(NO₃)₂) treatment.

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

Zn is accumulated mainly in roots. The Zn of 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(NO₃)₂ altered the zinc content of leaves. We measured higher leave Zn in plots with Ca(NO₃)₂ treatment compared to control. Effect of Microbion UNC on leaves Zn was not the same in every cases. In plots with N₀+Microbion and AN+Microbion treatments an enhancement in the Zn content were appeared compared to appropriate values of parcel being not inoculated. On the contrary, supplement of Ca(NO₃)₂ with biofertilizer resulted in a significant decrease of Zn content of leaves compared to values of plots being not inoculated.

We measured lowest roots Zn in parcels with ammonium nitrate treatment. In plots with other different fertilizers the Zn content of roots were the same. Supplement of ammonium-nitrate and urea with biofertilizer caused increased Zn values of roots compared to appropriated values of plots being not inoculated. As we noticed in leaves Zn values, supplement of Ca(NO₃)₂ with biofertilizer led to a significant decrease of root Zn compared to values having only Ca(NO₃)₂ treatment.

The distribution of copper within plant was more equalized than that of Zn. The copper content of roots maybe was a little bit higher than that of values of leaves. Cu content of leaves were in the range of 4.13-4.90 mg kg⁻¹ and in the roots ranged in 4.67-5.37 mg kg⁻¹. Either N fertilizers or biofertilizer application did not influence the Cu content of horseradish plant.

Effects of increasing NH₄NO₃ doses and Microbion UNC on Mn, Zn and Cu content of horseradish

The effects of increasing doses of NH₄NO₃ and Microbion UNC biofertilizer on Mn, Zn and Cu content of horseradish plant can be seen in *Table 3*.

Table 3.

Means of manganese, zinc and copper content of horseradish leaves and roots as influenced by increasing doses of NH₄NO₃ and Microbion UNC application

leaves of horseradish			roots of horseradish				
NH ₄ NO ₃ doses	Microbion UNC (kg ha ⁻¹)		LSD _{5%} (N)	NH ₄ NO ₃ doses	Microbion UNC (kg ha ⁻¹)		LSD _{5%} (N)
	0	2			0	2	
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 control plant, the Mn content of leaves and roots were lower in all parcel treated with NH₄NO₃ fertilizer, so we measured the highest plant Mn in control parcel. Decreasing effect of AN₁ dose was higher in the case of leave's values.

Further increasing NH₄NO₃ doses (AN₂, AN₃) did not alter the Mn content any longer. In generally biofertilizer application did not influence the Mn content of roots, but there was a decreasing trend of leaves Mn values when Microbion UNC biofertilizer was applied compared to values measured in parcel with no biofertilizer application.

The lower dose of ammonium nitrate, AN₁ did not changed the Zn content of horseradish, but with further increasing NH₄NO₃ doses the Zn content of leaves and roots significantly increased. We measured highest Zn

values of leaves in parcel with AN₂ treatment and in roots with AN₃ treatment. These results are in good agreement with our expectation, namely ammonium nitrate has influenced the soil pH, therefore the availability of Zn in soil expanded.

Effects of bacterial fertilizer on the leaves and roots Zn content was depended on the doses of NH₄NO₃. When biofertilizer was applied with lower NH₄NO₃ doses (AN₁), the Zn values of plant in inoculated parcel were higher than that of values without application of biofertilizer. When higher N doses (AN₂, AN₃) was supplied with Microbion UNC the Zn content of plant decreased compared to values of parcel being not inoculated.

Cu content of leaves and roots were not influenced by either increasing doses of NH₄NO₃ or by the application of biofertilizer.

CONCLUSIONS

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

Different N fertilizers and increasing doses of ammonium-nitrate had mainly effects on leaves microelement content. We measured the highest Mn contents of plant in Ca(NO₃)₂ and Ca(NO₃)₂+Microbion combined treatments. AN₁ dose decreased leaves Mn compared to control, but further doses (AN₂ and AN₃) did not alter these values any longer. Microbion UNC biofertilizer did not have any effect on Mn content of roots, but we measured higher Mn of leaves in some combined treatments.

Ca(NO₃)₂ application increased the zinc content of leaves and roots in noticeable manner. With increasing NH₄NO₃ doses, the Zn content of leaves and roots -as expected- significantly augmented. Effect of biofertilizer on Zn content of plant was not uniform.

Either N fertilizers (and increasing doses of NH₄NO₃) or biofertilizer application did not influence the Cu content of horseradish plant.

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

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