COMPARATIVE EXAMINATION OF A BACTERIUM PREPARATION (BACTOFIL® A10) AND AN ARTIFICIAL FERTILIZER [CA(NO$_3$)$_2$] ON HUMIC SANDY SOIL

Magdolna Tállai - Anita Jakab – Kovács Zsuzsa - János Kátai

University of Debrecen, Centre for Agricultural and Applied Economic Sciences, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Agrochemistry and Soil Science, Debrecen, tallaim@agr.unideb.hu

Abstract

In the Institute of Agricultural Chemistry and Soil Science, University of Debrecen, we carried out a pot experiment (2008-2010) comparing the effects of different doses of a natural (Bactofil® A10) and an artificial fertilizer [Ca(NO$_3$)$_2$] on certain chemical and microbiological parameters of the soil and the quantity of test plant biomass. Our test plant was the perennial rye-grass (Lolium perenne L.).

The artificial fertilizer increased the easily accessible nutrient content of humic sandy soil to a 21% greater extent than the bacterial fertilizer treatment. The microbiological activity of humic sandy soil increased by 24% following bacterial fertilization—independently of the dose applied—and by 48% due to Ca(NO$_3$)$_2$ treatment. The Bactofil A10 preparation also increased the total number of bacteria and cellulose decomposing bacteria, and the extent of soil respiration. A double dose of Ca(NO$_3$)$_2$ preparation stimulated all the microbiological parameters of the soil examined. The Bactofil preparation increased test plant dry mass by 7%, independently of the dose applied. The Ca(NO$_3$)$_2$ treatment increased the dry mass to a significantly greater extent. We concluded that an adequate dose of Bactofil A10 preparation can increase the nutrient content of the soil and stimulate its biological activity, and the combined application of artificial and bacterial preparations could be an environmentally friendly way of nutrient supply.

Keywords: bacterial fertilizer, artificial fertilizer, humic sandy soil

INTRODUCTION

Organic and green manuring are among the oldest and most widely used methods for the enrichment of the organic and inorganic colloid content and nutrient managements of soils with unfavourable properties (Müller, 1991; Blaskó, 2005). With the development of intensive cultivation the use of artificial fertilizers has also spread, and there have been several studies on the adequate dose, their effects on different types of soils and plants, and their economical application (Németh, 2006; Vágó et al., 2007). Within integrated cultivation there are several alternatives for improving the fertility of soils using natural substances. Plant production is positively influenced, in addition to several inorganic properties of the soil, by living, bionic factors (Biró, 2007). These facts taken into consideration, another form of nutrient supply has become widely used, the application of bio-based preparations, with which environmental impact can be reduced, the fertility of the soil can be improved and soils' natural microorganic composition activated. The positive effects of bio-based or bacterial soil inoculation is supported by the studies of Biró, 2004; Mahfouz & Sharaf-Eldin, 2007; Kaewchai et al., 2009; Ogbo, 2010.

Taking the above into consideration, we set up a model experiment with the purpose of comparing the effects of a bacterial preparation and an artificial fertilizer on humic sandy soil, under controlled conditions in a greenhouse. We compared the effects of a natural bacterial preparation, Bactofil® A10, and an artificial one, the $Ca(NO_3)_2$ on certain chemical and microbiological properties of the soil and on the quantity of testplant biomass.

MATERIALS AND METHODS

Our pot experiment was set up in the greenhouse of the Institute of Agricultural Chemistry and Soil Science, UD, Faculty of Agricultural and Food Sciences and Environmental Management between 2008-2010. The soil types examined were humic sandy soil ($pH_{(H2O)}$ 5,7) from Pallag. Our test plant was the perennial rye-grass *(Lolium perenne L.)*.

In each pot perforated at the bottom 1-1 kg air dried soil was placed. The experiment was carried out in three randomized iterances. The moisture content of the soil was adjusted to 60% of maximum water capacity. Each year there were two plant and soil samplings per growing season, in the 4^{th} and 8^{th} week from the shooting of the test plant. Soil samples were taken in three iterances per treatment both times, and were blended with thorough homogenization. Plant samples were obtained by cutting the plant 2 cm above the soil. They were air-dried for one week, and then dried in a laboratory at $50^{\circ}C$. Dry mass was measured in g kg^{-1}, all in one, pertaining to one growing season. The experiments were carried out in the soil chemical and microbiological laboratory of the Institute of Agricultural Chemistry and Soil Science.

As a basic treatment, each pot received 100 mg P_2O_5 and 100 mg K_2O in the form of a solution of potassium dihydrogen phosphate (KH_2PO_4) and potassium sulphate (K_2SO_4) blended. Nitrogen was added in the soil in the form of $Ca(NO_3)_2$ solution (100 and 200 mg kg^{-1} N), while bacteria were ingested after a multi-stage dilution of a concentrate preparation, also in solution (1xBactofil A10: 10.75 $x10^9$ cell kg^{-1}; 2xBactofil A10: 21.50 $x10^9$ cell kg^{-1}). The doses of the bacterial preparations applied in the experiment were 2.5 and 5 times that of field recommendation. The details of the treatments are represented in *Table 1.*

Table 1

The treatments applied $Ca(NO_3)_2$-fertilizer and Bactofil® A10 biofertilizer between 2008-2010 (Pot-experiment, Debrecen)

Number of treatments	Dose	Treatment
1	Control	
2	1x	*Bactofil A10®
3	2x	**Bactofil A10®
4	1x	100 mg kg^{-1} N [$Ca(NO_3)_2$]
5	2x	200 mg kg^{-1} N [$Ca(NO_3)_2$]
** 2.5 times the field dosage (10.75*10^5 bacteria kg^{-1})*		
*** 5 times the field dosage (21.50*10^5 bacteria kg^{-1})*		

During the experiment we determined the accessible nutrient content and thus the NO_3^-N content of the soil on the basis of Felföldy (1987), as well as its ammonium lactate soluble phosphorous and potassium content (Egnér et al., 1960; Buzás et al., 1988). We also determined certain microbiological parameters of the soil, the total number of bacteria (in broth agar), and of microscopic fungi (in peptone-glucose agar) from ground-water suspension using plate diluting method (Szegi, 1979). The number of cellulose decomposing bacteria was determined with the most probable bacteria number method of Pochon and Tardieux (1962), while the amount of carbon dioxide was determined with the help of Witkamp's (1966) method. We measured the microbial biomass carbon content of the soil according to Jenkinson & Powlson's (1976) fumigation extraction method. The statistical analysis of the results was carried out with the help of SPSS13.0 program (Huzsvai, 2004), where we calculated the average values of samples dispersion, and the significant difference values at 5% probability levels generally accepted in agriculture. To explore the relations between the soil properties examined we carried out correlation analysis with both forms of nutrient supply.

RESULTS AND DISCUSSION

We evaluated the results based on three years' average (between 2008-2010).

Bacterial fertilizer treatments did not lead significant changes in the *easily accessible nutrient content* of humic sandy soil compared to the control, aside from the average values of AL-soluble potassium *(Fig. 2)*. All the three nutrients examined showed higher values under a double dose. The NO_3^-N *(Fig. 1)* and easily accessible AL-P_2O_5 content *(Fig. 2)* of the soil increased to a small extent, while its AL-K_2O content to a significant extent in Bactofil treatments. We did not experience significant differences between the effects of different doses. The nutrient content of humic sandy soil also grew significantly due to *artificial fertilization*. Between the effects of different doses there were no significant changes, but we experienced that with an increase of the doses the easily accessible nutrient capital of the soil also increased.

Fig. 1: The impact of nutrient supply methods on the NO_3^--N content of soil (Debrecen, 2008-2010, average values)

Fig. 2: The impact of nutrient supply methods on the AL-P_2O_5 and the AL-K_2O content of soil (Debrecen, 2008-2010, average values)

Among the *microbiological properties* of the soil we examined five parameters, highlighting some elements of the soil's microbiological carbon cycle as well *(Table 2.)*. In humic sandy soil the *bacterial treatments* had only ad hoc statistically justifiable effects on the soil's microbiological parameters examined. In 1 kg pots, the single dose we applied did not have a stimulating effect on the soil's CO_2-production. The other soil microbiological properties examined showed a slight increase under a single dose, while a double dose brought up a significant increase in the total number of bacteria, the quantity of cellulose decomposing bacteria and the extent of soil respiration.

$Ca(NO_3)_2$-artificial fertilization proved to be more stimulating regarding the microbiological properties of humic sandy soil. With the application of a single dose, the number of bacteria and microscopic fungi, the extent of soil

respiration showed a tendency towards growth. A single dose was sufficient to increase significantly the number of cellulose decomposing bacteria—which showed a 2.5-fold increase—the microbial biomass-C content. In humic sandy soil all the microbiological parameters of the soil examined were stimulated significantly by a double dose of artificial fertilizer.

Table 2

The impact of nutrient supply methods on some microbiological parameters of soil (Debrecen, 2008-2010, average values)

Treatments number	Total number of bacteria (*10^6 g^{-1} soil)	Microscopic fungi (*10^3 g^{-1} soil)	Cellulose decomposing bacteria (*10^3 g^{-1} soil)	CO_2-production (CO_2 mg $100g^{-1}$ 10 $days^{-1}$)	Biomass-C (µg g^{-1})
1	4.00 a	58.55 a	2.56 a	5.15 a	127.70 a
2	4.59 a	59.61 a	3.81 a	5.01 a	128.40 a
3	**7.00** b	57.50 a	**5.59** ab	**5.49** b	128.36 a
4	4.99 a	64.17 a	**6.38** ab	5.40 ab	**130.78** b
5	**6.15** ab	**65.61** ab	**8.64** c	**6.28** c	**131.83** ab
$LSD_{5\%}$	1.22	6.82	1.71	0.34	2.16

The *quantity of plant biomass* also grew in the case of both Bactofil and artificial treatments *(Fig. 3)*. Under *Bactofil treatments* there were no statistically significant differences between the effects of a single and double dose, we experienced weight growth under the bigger dose. Both a single and double dose of *$Ca(NO_3)_2$-fertilizer* increased the quantity of plant dry mass significantly. The application of a double dose resulted in a significantly higher dry matter production values than that of a single dose.

Fig. 3: The impact of nutrient supply methods on the testplant biomass (Debrecen, 2008-2010, average values)

We carried out further statistical analysis in order to find connections between the soil properties examined and the changes in the quantity of plant biomass.

We calculated the *correlation values* also for bacterial (Bactofil® A10) and $Ca(NO_3)_2$ fertilizer treatments. In the present article we represent those correlation values that were statistically justifiable with the allocation of the two forms of nutrient supply.

We experienced some cases of medium correlation both in the case of bacterial and of artificial treatments *(Table 3.)*. As regards the easily accessible nutrient content of the soil, a medium positive correlation can be evinced between the easily accessible potassium content and microbial biomass-C content of the soil (Bactofil A10-treatment r=0.528; $Ca(NO_3)_2$-treatment r=0.634) and the quantity of plant dry matter (Bactofil A10-treatment r=0.556; $Ca(NO_3)_2$-treatment r=0.714). As for microbiological properties, there is a medium correlation between the total number of bacteria and on the one hand, the microbial biomass-C content of the soil (Bactofil A10-treatment r=0.521; $Ca(NO_3)_2$-treatment r=0.704). In humic sandy soil one tight correlation was attested: the one between the microbial biomass-C content of the soil and the quantity of plant biomass under $Ca(NO_3)_2$ artificial treatment ($Ca(NO_3)_2$-treatment r=0.838).

Table 3

Comparison study between soil parameters examined and plant biomass with $Ca(NO_3)_2$-fertilizer and Bactofil® A10 biofertilizer on humic sandy soil
(Debrecen, Pot experiment, between 2008-2010)

Humic sandy soil (Pearson-correlation) (n=18)		r-value	
Soil parameters examined		Bactofil A10	$Ca(NO_3)_2$
		Medium correlation	
AL-K_2O	Biomass-C	0.528**	0.634**
	Plant biomass	0.556**	0.714**
Total number of bacteria	Biomass-C	0.521**	0.704**
		Tight correlation	
Biomass-C	Plant biomass	-	0.838**
* Correlation is significant at the 0.05 level (2-tailed)			
** Correlation is significant at the 0.01 level (2-tailed)			

CONCLUSION

Summarizing our findings, we can conclude that all the preparations and doses applied increased the *nutrient content* of humic sandy soil. On three year's average, bacterial treatments increased the easily accessible nutrient content of the soil by 17%, while artificial fertilization did so by an average 38%. Under controlled conditions we measured the highest values of nutritional parameters under double doses.

We examined five *microbiological parameters* of soil under different treatments, with special regard to certain elements of the carbon-cycle.

The *bacterial treatments* – independently of the doses – increased the microbiological activity of the soil by 24%. Double doses were found more stimulating, as they brought up a significant – statistically justifiable – increase in the total number of bacteria, the number of cellulose decomposing bacteria, the extent of soil respiration. *Artificial treatments* also proved effective, having a stimulating effect of 48%. However, in the case of $Ca(NO_3)_2$-artificial treatments we experienced an increase in activity values parallel with the doses. Under a double dose all the parameters examined increased. In humic sandy soil, among the microbiological parameters, the number of cellulose decomposing bacteria, the total number of bacteria showed sensitive to both bacterial and artificial preparations. The number of cellulose decomposing bacteria doubled under bacterial treatments, and trebled under artificial fertilizer treatments.

The application of two forms of nutrient supply also resulted in different *quantities of plant biomass*. Due to bacterial treatments the quantity of plant biomass grew to a nearly equal — not significantly — extent. The Bactofil-preparation increased the quantity of test plant dry mass by 7%, independently of doses. $Ca(NO_3)_2$-artificial treatment increased the quantity of dry mass to a significantly greater extent, by 38% compared to the control treatment.

The results showed that the $Ca(NO_3)_2$ treatment was more effective than the bacterial fertilizer, but we concluded that an adequate dose of Bactofil A10 preparation can increase the nutrient content of the humic sandy soil and stimulate its biological activity.

REFERENCES

1. Biró B. 2007, „Fitotechnológiák a fenntartható földhasználat és az élelmiszerbiztonság szolgálatában”. „Arbuszkuláris mikorrhiza termékek és alkalmazásuk a mezőgazdaságban” (A COST 8. 59. és 8. 70. számú akciói). Agrokémia és Talajtan, Vol. 56. No. 1 pp 191-192. p.
2. Biró B. 2004, A környezeti állapot megőrzésének, indikálásának és helyreállításának mikrobiológiai eszközei a növény-talaj rendszerben. Akadémiai doktori értekezés MTA Talajtani és Agrokémiai Kutatóintézet Budapest, 65-66, 73.
3. Blaskó L. 2005, A talajjavítás jelene és jövője. In: A talajok jelentősége a 21. században. Szerk: Stefanovits P. – Michéli E. Bp. MTA Társadalomkutató Központ. 267-289.
4. Buzás I., Murányi A. & Rédly L.-né, 1988, A talaj kémhatásának vizsgálata. In: Talaj-és agrokémiai vizsgálati módszerkönyv 2. (Szerk: Buzás I.) Mezőgazdasági Kiadó. Budapest. 87–102.
5. Egnér, H. – Riehm, H. & Domingo, W. R. 1960, Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. K. Lantbr. Högsk. Ann. 26. 199-215.

6. Felföldy L., 1987, A biológiai vízminősítés. (4. javított és bővített kiadás). Budapest. Gerei L. (szerk.), 1970. Talajtani és agrokémiai vizsgálati módszerek. OMMI. Bpest.
7. Huzsvai L., 2004, Biometriai módszerek az SPSS-ben, SPSS alkalmazások, DE MTK, Debrecen. (Kézirat). 46-50 p.
8. Jenkinson, D. S. & Powlson, D. S., 1976, The effects of boidical treatments on metabolism in soils. A method for measuring soil biomass. Soil Biol. Biochem. 27/8, 209-213.
9. Kaewchai, S., Soytong, K. & Hyde, K. D., 2009, Mycrofungicides and fungal biofertilizers. Fungal Diversity. 38. 25–50.
10. Mahfouz, S. A. & Sharaf-Eldin, M. A., 2007, Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel (Foeniculum vulgare Mill.) International Agrophysics. 21. 361–366.
11. Müller G. 1991, Az agroökológia talajmikrobiológiai kérdései és az intenzív mezőgazdasági termelés. Agrokémia és Talajtan. 40/3-4, 263-272.
12. Németh, T. 2006, Nitrogen in the soil-plant system, nitrogen balances. Cereal Research Communications, Proceedings of the V. Alps-Adria Workshop Opatija, Croatia, 6-11 March, 2006, Vol. 34. No. 1. Akadémiai Kiadó, 61-65.
13. Ogbo, F. C., 2010, Conversion of cassava wastes for biofertilizer production using phosphate solubizing fungi. Bioresource Technology. 101. 4120–4124.
14. Pochon, J. & Tardieaux, P., 1962, Tecniques D' Analyse en Micobiologie du Sol. Collection „Technivues de Base". 102.
15. Szegi J., 1979, Talajmikrobiológiai vizsgálati módszerek. Mezőgazd. Kiadó. Budapest.
16. Vágó, I. – Kovács Balla, A. & Nagy P. T., 2007, Effect of boron, calcium and magnesium foliar fertilization on apple (Malus domestica) yields. Cereal Research Communications, Vol. 35. No. 2. pp. 1261-1264.
17. Witkamp, M., 1966, Decomposition of leaf litter in relation to environment microflora and microbial respiration. Ecology. **47.** 194–201.