ECOLOGICAL PRODUCTS FOR AGRICULTURE

Nicoleta RADU¹, Ana Aurelia CHIRVASE¹, Nela ZAMBILĂ²

¹Biotechnology Department, I.N.C.D.C.P. Bucharest, Splaiul Independentei street, 202, Bucharest, Romania; e-mail: nicolbiotec@yahoo.com.com

Corresponding author email: nicolbiotec@yahoo.com

Abstract

This paper aims to reduce the environmental pollution caused by fertilizer use in agriculture by chemical synthesis, by identifying suitable technologies and biological products to be used to fertilize soil in Romania. An alternative to limiting pollution from synthetic fertilizers and pesticides is to use in agriculture some products based on bacteria, bacterial consortia or biomass with plant or animal origin. The microbial fertilisers shall be characterized in terms of agrochemical efficiency.

Key words: bacterial fertilisers, agrochemical efficiency

INTRODUCTION

Fertile soil contains several hundred million microorganisms/gram (table 1). The most numerous are bacteria (unicellular), followed in decreasing order of actinomycetes (specialized groups there are species of bacteria that produce antibiotics), fungi, soil algae, cyanobacteria (blue-green algae that are actually photosynthetic microorganisms that can add small amounts of carbon in soil and can cause trouble on the golf lawn adorned with lawn areas) and soil protozoa (unicellular organisms in the soil with organic material role in the decomposition)

Table 1. Types of microorganisms from the soil [1]

Group of	Number of germs / gram
microorganism	soil
Bacteria	100.000.000 -
	1.000.000.000
Fungi	100.000-1.000.000
Algae and cyanobacteria	1.000- protozoa 1.000.000
Protozoa	1000-100.000

One of macronutrients – nitrogen, is essential for all living organisms for the synthesis of proteins, nucleic acids and other compounds that contain nitrogen. Relative independence of leguminous plants from the soil available nitrogen has great ecological importance

because it reduces the required nitrogen mineral fertilizers in crop vegetables prevents accumulation of nitrogen compounds in groundwater and ensures the accumulation of high concentrations of protein in beans. Symbiotic association of bacteria of the genera Rhizobium and Bradyrhizobium leguminous plants, soil building each year with 90 x 10⁶ metric tons of nitrogen fixed amount is double that obtained by industrial processes [1-5]. According to studies undertaken by several researchers, symbiotic nitrogen fixation productivity in grain and leguminous plants and the feed is presented in Table 2 and Table 3

Table 2. Symbiotic fixation in grain [1]

Vegeta-	Average level	Maximum level,
bles type	kg•ha⁻¹ •year⁻¹	kg•ha⁻¹•year⁻¹
beans	210	552
peas	65	80
soy	60	100
lentil	100	114

The relationship between vegetables and bacteria (*Rhizobium* or *Bradyrhizobium*) can be presented as a symbiotic process that takes place for the benefit of both parties that:

- bacteria receive nutrients from the plant (carbohydrate);
- plant receives its nitrogen needs from bacteria.

²Biotechnology Department S.C. ECOAGRICOLA SRL, Fantanica street, 7, District 2 Bucharest

Nodule-forming bacteria have specific hosts, such as specific types of plants are node specific species of bacteria (Table 4).

Table 3. Symbiotic fixation in fodder vegetables [1]

Vegetables type	Average level kg•	Maximum level
	ha ⁻¹ •year-1	kg• ha ⁻¹ •year ⁻¹
Lucerne	184	465
Melilotus alba	183	-
Clover	183	673
Lotus corniculatus	116	-
Vetch	73	93

Table 4. Types of plant which make nodules with

bacteria		
Bacteria which form nodules	Types of plants	
Rhizobium meliloti	lucerne, clover	
Rhizobium leguminosarum	peas	
Rhizobium trifolii	white or red clover	
Rhizobium phaseoli	dried beans	
Rhizobium viceae	vetch	
Rhizobium loti	clover	
Bradyrhizobium japonicum	soybeans, peanuts	

MATERIALS AND METHODS

The influence of microbial fertilizers on the germination of crop plants

Experiments were conducted in green house at SC ECOAGRICOLA SRL in 3 repetitions, each repetition using the 40 seeds each. Seeds of *Cucumis sativus*, *Daucus carota, Beta vulgaris*, *Solanum lycopersicum*, *Capsicum annum*, *Triticum aestivum*, *Pisum sativum* and *Vicia faba* were immersed for 10 minutes in:

- a) solution of Rhizobium inocula, or in
- b) solution of compost, or in
- c) water (as witness)

After immersion, the seeds were dried in the shade, and then were planted in plastic pots. The substrate used in experiment was washed sand. Culture vessels were kept in the greenhouse and were watered as often as was necessary. Seedlings obtained were collected,

were washed under running water and were dried with filter paper were then weighed with a Sartorius balance type digital. Counting seedlings were made every day.

The influence of microbial fertilizers on the development of crop plants

Experiments were conducted in randomized blocks in three repetitions in period: autumn 2 until next summer. Soil plots had pH = 5.64 and initial nutrient content of soil is shown in table 5.

Table 5. Initial nutrient content of the soil

C_{total}	$N_{total} \over {}^{0}\!\!/_{0}$	P _{total}	$K_{total}, 0/0$
1.03	0.108	0.00158	1.85

Experimental variants included soil fertilization with fertilizer mixed and/or with mixed fertilization+bacterial inocula or organic fertilizer (compost)+bacterial inocula, according to Table 6, and as test plant was used *Glycine max* (soybean))

Table 6. Experimental fertilization variants for soil cultivated with *Glycine max*

	cultivated with Glycine max		
Ferti- liza-tion vari-ant	Quantity of active ingredient per hectare (fertilization of basis) (NPK)	Crop	Quantity of active ingredient per hectare (spring fertilization) (NPK)
V1	80:60:30	Glycine max.	100:60:30
V2	80:60:30	Glycine max.	60:60:30
V3	80:60:30	Glycine max.	30:60:30
V4	80:60:30	Glycine max.	0:60:30
V5	80:60:30	Glycine max.	Inocula*+ 60:60:30
V6	80:60:30	Glycine max.	Inocula+ 30:30:30
V7	80:60:30	Glycine max	Compost+ 60:60:30
V8	80:60:30	Glycine max.	Compost+ 30:60:30
V9	80:60:30	Glycine max	Inocula+ 0:0:0
V10	80:60:30	Glycine max.	Compost +0:0:30
V11	80:60:30	Glycine max.	Compost + inocula + 30:60:30

^{*} Inocula:=strains of *Rhyzobium sp.* + *Bradyhrzobium sp.*

Bio fertilizer (inocula) was incorporated into the soil with seeding; time of use was 1:10 (1 gram to 10 grams of inoculated seeds). One third of the total amount of nitrogen and all phosphorus and potassium were applied in autumn. The remainder of nitrogen (2/3) was applied after 20 days and 35 days respectively after sowing. For soybeans has adopted an area of 40x20 cm, corresponding to a population of about 375.000 plants•hectare⁻¹. Plant height, number of nodules, nodule mass was determined by a range between (14÷60) days from sowing.

RESULTS AND DISCUSSIONS

INFLUENCE OF MICROBIAL FERTILISERS REGARDING SEED GERMINATION

Significant differences were obtained between the two treatments in terms of percentage of germination for most species (figure1-8) treatment with bio fertilizer solution indicated a large number of seeds germinated. Inoculated microbial seed treatment with *Rhyzobium* type can increase the percentage of germination of leguminous plants type *Cucumis sativus*, *Daucus carota*, *Beta vulgaris*, *Solanum lycopersicum*, *Capsicum annum*, *Tritium aestivum*, *Pisum sativum*, and *Vicia faba*, and furthers the plant normally developed.

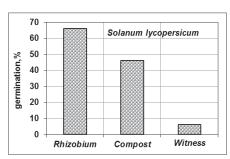


Fig. 1. Experimental variants influence on seed germination of *Solanum lycopersicum*

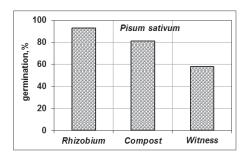


Fig. 2. Experimental variants influence on seed germination of *Pisum sativum*

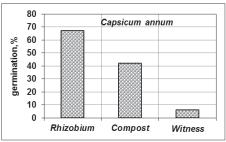


Fig. 3. Experimental variants influence on seed germination of *Capsicum annum*

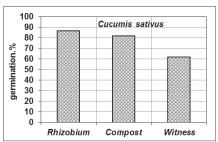


Fig. 4. Experimental variants influence on seed germination of *Cucumis sativus*

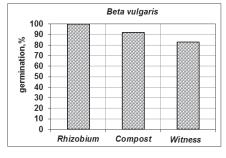


Fig. 5. Experimental variants influence on seed germination of *Beta vulgaris*

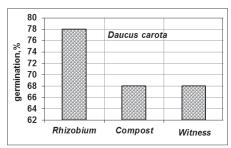


Fig. 6. Experimental variants influence on seed germination of *Daucus carota*

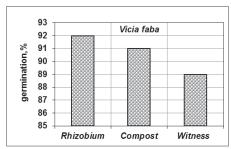


Fig. 7. Experimental variants influence on seed germination of *Vicia faba*

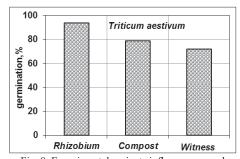


Fig. 8. Experimental variants influence on seed germination of *Triticum aestivum*

INFLUENCE OF MICROBIAL FERTILISERS REGARDING THE DEVELOPMENT OF CROP PLANTS

After two weeks of sowing, the average height of *Glycine max* plants ranged from 12 to 15.7 cm. Highest plants were obtained for variant V1 (100:60:30). After 28 and 42 days after sowing, after harvest values are obtained for both large and variant V1 to V11 version (Fig. 9-12)

Influence of fertilization variant to the number of nodules formed

After 28 days of seeding, the numbers of nodules on *Glycine max* ranging from 15.3 to

35.3. Higher values are obtained in variant V6 and V5, as a result of nitrogen fixing capacity by microorganisms (figure 13).

After six weeks after sowing, the average number of nodes varies from 19.3 to 50, in this case the largest number of nodes is recorded for variant V6 (figure 14)

After 56 days from sowing, significant differences occur in cases where inorganic fertilizers are applied without inoculated or in the presence of inocula and compost (figure 15). These differences can be attributed to development of nitrogen fixing ability by microorganisms.

Fertilizer type influence regarding the mass of nodules formed

After 28 days, nodules formed mass range from 93.3 to 283.3 mg, the highest value being obtained for the V6 version, probably because nitrogen-fixing microorganisms used as inocula (figure 16).

After six weeks of sowing, the average mass formed nodules ranging from 121.7 to 400 mg, the highest values being obtained for variant V6 and V5. These values show that under the influence of inocula, nodules formed mass increases significantly compared to the version that uses high doses of nitrogen fertilization with inorganic sources (figure 17), obtaining similar results after 56 days (figure 18).

Fertilization type influence regarding biomass production of *Glycine max*

After 28 days, the amount of biomass obtained range from 4782 to 9375 t•ha⁻¹, and in this case the highest values are obtained for fertilizer variant V1 (100:60:30) or V5 (I +60:60:30) where I=inocula) and V7 (C +60:60:30) where C=compost) (figure 19). The results show that small doses of fertilizer applied with inoculated compost can get the same biomass production and variant V1 (100:60:30).

After 42 days from sowing, most production is obtained in the fertilization variant V5 (I+60:60:30) production that exceeds that obtained in the variant V1 (100:60:30) (figure 20), similar results were obtained 56 days after sowing (Fig. 21).

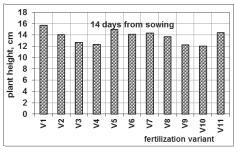


Fig. 9. Influence the types of fertilization regarding *Glycine max* plant height after 14 days from sowing.

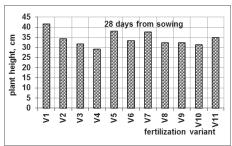


Fig. 10. Influence the types of fertilization regarding *Glycine max* plant height after 28 days from sowing.

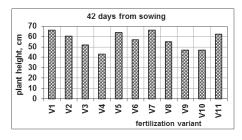


Fig. 11. Influence the types of fertilization regarding *Glycine max* plant height after 42 days from sowing.

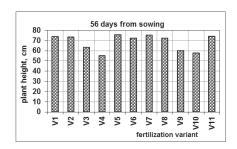


Fig. 12. Influence the types of fertilization regarding *Glycine max* plant height after 56 days from sowing.

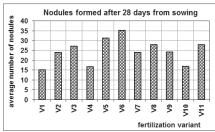


Fig. 13. Influence of fertilization variants on the number of nodules formed on roots of *Glycine max*. after 28 days from sowing.

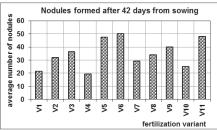


Fig. 14. Influence of fertilization variants on the number of nodules formed on roots of *Glycine max*. after 42 days from sowing.

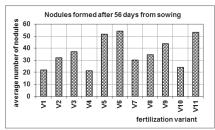


Fig. 15. Influence of fertilization variants on the number of nodules formed on roots of *Glycine max*. after 56 days from sowing

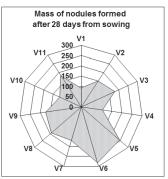


Fig. 16. Influence of fertilization type regarding the mass of nodules formed on roots of *Glycine max* after 28 days from sowing

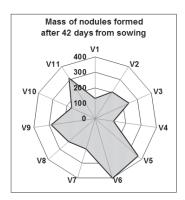


Fig. 17. Influence of fertilization type regarding the mass of nodules formed on roots of *Glycine max*. after 42 days from sowing.

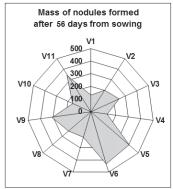


Fig. 18. Influence of fertilization type regarding the mass of nodules formed on roots of *Glycine max*. after 56 days from sowing.

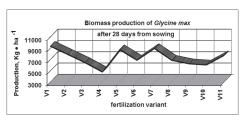


Fig. 19. Influence of fertilization types regarding biomass production of *Glycine max* after 28 days from

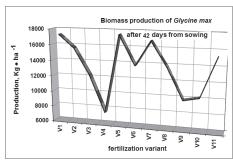


Fig. 20. Influence of fertilization types regarding biomass production of *Glycine max* after 42 days from sowing

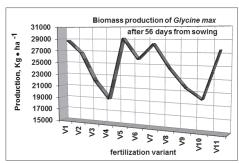


Fig. 21. I Influence of fertilization types regarding biomass production of *Glycine max* after 56 days from sowing

CONCLUSIONS

- Experiments performed in order to establish the effects of microbial inocula regarding germination of crop plants such as *Cucumis sativus*, *Daucus carota*, *Beta vulgaris*, *Solanum lycopersicum*, *Capsicum annum*, *Triticum aestivum*, *Pisum sativum and Vicia faba* have shown that treating seeds with Rhyzobium sp. inocula can increase germination percentages, favouring obtaining the normally developed plants.
- Monitoring crop demonstration of *Glycine max* (soybean) made from a complex pattern of fertilization has been used as fertilizer material combinations inoculated with Rhizobium, compost and NPK fertilizer type, demonstrated that:
- one after 42 and that after 56 days from sowing height of plants fertilized spring mix inoculated microbial + compost + NPK with the low concentration of nitrogen was compared with the control group (which was made only chemical fertilization and the

amount of active substance (kg / hectare) was 100:60:30);

- average number of nodules formed on roots of *Glycine max* is maximum after 42 and 56 days from sowing for the version microbial inocula + NPK of fertilization with nitrogen and phosphorus in low concentrations. This conclusion is valid in terms of mass of nodules.
- after 42 or 52 days after sowing, the highest biomass production per hectare of Glycine max is obtained for fertilization variant which include bacterial inocula variant + NPK with an average amount of nitrogen;

In conclusion, it can be stated that microbial organic fertilizer to replace chemical fertilizers at least some of the following reasons:

- the rising cost of fertilizers decreases their accessibility for small farmers;
- in 2020 the estimated world production of 321 million tons of grain•year⁻¹, nutrient requirements would be at about 28.8 million tons•year⁻¹, chemical synthesis is possible only to a quantity of about 21.6 million tons thus creating a deficit of about 7.2 million tons of soil nutrients [6-7].

ACKNOWLEDGEMENTS

This paper was carried out in the Project No. PN 09.09.01.04/2012 financed by National Authority for Scientific Research, Romania.

REFERENCES

- [1]. Burns R.C., *Nitrogen fixation in bacteria and higher plants*. Mol Biol Biochem Biophys.1975, (21):1–189.
- [2]. Albrecht S.L., Maier R.J., Hanus F.J., Russell S.A., *Hydrogenase in Rhizobium japonicum Increases Nitrogen Fixation by Nodulated Soybeans*. Science, 1979, **203**(4386):1255–1257.
- [3]. Bethlenfalvay G.J., Phillips D.A. *Variation in nitrogenase and hydrogenase activity of alaska pea root nodules*. Plant Physiol. 1979, **63**(5):816–820.
- [4]. Ruiz-Argüeso T, Maier R.J, Evans H.J. Hydrogen Evolution from Alfalfa and Clover Nodules and Hydrogen Uptake by Free-Living Rhizobium meliloti. Appl Environ Microbiol.1979, 37(3):582–587.
- [5]. Schubert K.R., Evans H.J. *Hydrogen* evolution: A major factor affecting the efficiency of nitrogen fixation in nodulated symbionts. Proc Natl Acad Sci U S A.1976, **73**(4):1207–1211
- [6]. Nicoleta Radu, Ana Aurelia Chirvase, Narcisa Babeanu, Ovidiu Popa, Misu Moscovici, Nela Zambila, Niculae Dinca, 2008. Feasibility of biotechnological products with bio stimulatory, fertilizers and microbial antagonism effect applied in agriculture. Edited by PRINTECH, ISBN 978-606-521-059-2.
- [7]. Ana Aurelia Chirvase, Nela Zambila, Nicoleta Radu, 2006. *Control methodology used to obtaining bio products with symbiotic nitrogen fixing bacteria*. Edited by PRINTECH, ISBN 973-718-466-1/978-973-718-466-5