

RESEARCH ON THE ACTION OF BACTERIA BIOPREPARATES ON AGRICULTURAL CULTURES AND SOIL STRUCTURE

G. TOADER^{4,1}, Viorica CHIURCIU¹, Valentina FILIP¹, C.-I. FLOAREA¹, N. MAIEREAN¹, Floarea BURNICHI², E. MIREA², P. CONSTANTIN², Elena-Violeta TOADER³, L. ILIE⁴

¹Romvac Company. Voluntari, Romania

²Buzău Research and Development Station in Vegetables. Buzău, Romania.

³Bucharest Academy of Economic Studies. Faculty of Agri-Food and Environmental Economics. Bucharest, Romania.

⁴University of Agricultural Sciences and Veterinary Medicine Bucharest. Faculty of Agriculture. Bucharest, Romania.

Corresponding author: toadergeorge92@gmail.com / george.toader@romvac.ro

Abstract. Reducing the impact of chemicalization and harmful organisms in agricultural crops presents many approaches: microbiological, genetic, ecological, etc. Selecting and improving the genetic resources of both seed material, varieties and hybrids of agricultural crops but especially of plant protection and fertilization products (organic products) is the biggest concern of farmers in the last period. The elaboration and use of means of biological control of both the excessive chemicalization and of the pests in the agricultural crops represent the foundation for the application of microbiology as a science in the agricultural field. The paper aimed to present the action of organic fertilizers on the soil and implicitly of agricultural crops (crop plants and agricultural production). The use of chemical fertilization and plant protection products in agricultural crops led to the emergence of the phenomenon of soil supersaturation with chemical elements. The pollution of the soil with chemical elements has led to the increase of the pH from a basic / neutral one to an acid one. The acidification of the soil results in the decrease of the agricultural production, the decrease of the resistance of the plants to certain pests of the structure of the soil but especially the pollution of the soil, of the groundwater with certain chemical elements found in the structure of the fertilizers. The increase of the productions (in the organic fertilized lots) compared to a chemical fertilizer proves that the action of the bacteria in the content of the organic fertilizer has a positive effect on the soil and the crops. The strategy proposed by the researchers from Romvac Company SA, in the field of microbiology, is that of the formulation and use of bacterial biopreparations in the protection of agricultural crops that are of great economic interest, in the elaboration and extension of the range of ecological plant protection products as well as in the processes. to try to replace the conventional chemical fertilizers (pollutants) with organic fertilizer products based on bacterial cultures. The advantages of these strategies proposed by the researchers are represented by the decrease of the attacks of pests in agricultural crops, the soil ecology with the help of bacteria, the favorable evolution of agricultural ecosystems but especially obtaining a high yield of agricultural crops, implicitly the agricultural production and ensuring superior qualities to the products derived from agricultural production.

Keywords: bacterial biopreparations, biological protection and fertilization, ecology, ecological fertilization technologies

INTRODUCTION

In order to support the growth and development of crop plants, the soil must provide the plants with the mineral elements they need in their growth and development, as well as to obtain a large agricultural production. In the soil structure there are many bacteria, fungi or fungi that live in symbiosis. The use of microbiology in agriculture has been a huge leap forward because the production of fertilizers with the help of certain bacteria has been an innovation in agriculture. The role of these bacteria is to ensure the nutrients necessary for growth, plant development but especially, through the interaction of bacteria with the environment, soil and plants, obtaining high yields, significantly higher than the production of

chemically fertilized lots (Sociedade Brasileira de Genética, 2015; AGGANI , 2017). An important aspect in the use of biopreparations in the agricultural field is the (relatively) high impact of the degree of pest attack in agricultural ecosystems. These massive pest attacks lead to losses of both the crops themselves and their related agricultural production. The main pests of crops of great economic interest are represented by insects. Due to the excessive chemicalization of both the soil (fertilization) and the numerous chemical treatments in order to protect the plants, the pests have acquired a high resistance on their effect. This has led to the ineffectiveness of protection products as well as to a pollution of the soil and the environment. A massive pest attack in crops can lead to the loss of approx. 60-70% of the respective culture (CHANDLER, D et.al, 2010; LARKIN, HANSEN ANNE, 2010).

In parallel with the beneficial effects of chemical fertilizers and the application of plant protection products (increasing agricultural production, removing pests from agricultural crops, etc.), these products of any chemical have the effect of creating an ecological imbalance in the agricultural ecosystem. This imbalance is explained by the decrease in the activity of bacterial fauna (useful soil fauna), the application of these products for as long as possible causing adverse effects on soil and pests (soil acidification, decreased fertility, pest resistance, etc.). Consumption of products from these ecosystems by humans and animals has irreversible side effects for the body: decreased immunity, decreased life expectancy, morbidity and others. As a result, researchers in the field of agriculture have launched an appeal to farmers so that they organize (carefully) plant protection measures and fertilization processes of agricultural crops (TONCEA, I., et.al, 2012; VOLOȘCIUC, L , 2009a).

Based on research on fertilization and plant protection processes, researchers based on the dynamics of pest attacks and bacterial processes in the soil structure identified the needs and requirements of agricultural ecosystems in relation to the environment. These bases have allowed the development of innovative technologies for fertilization and plant protection using microbiology - bacterial biopreparations. These technologies (based on the dynamics previously studied by researchers) have led to the development of organic fertilization systems for both fertilization of agricultural crops and plant protection. The appearance of these systems led to the appearance of the first ecological products that were tested and used in agriculture: biofertilizers, bioinsecticides and biofungicides (VOLOȘCIUC, L., 2009b).

Referring to the experience gained in the field of microbiology, bacteriology, virology and vaccine production, the need to obtain organic products used as substitutes for chemical fertilizers and plant protection products, researchers from Romvac Company SA have a special role in the development, research, testing and production of organic products for agriculture. Thus, following these elaborate processes, two ecological products were implemented and approved (for Romanian agriculture) which represented a slope for the launch of Romvac on the Romanian agricultural market: the biofertilizer Rom-Agrobiofertil NP and a bioinsecticide based on bacterial cultures of *Bacillus thuringiensis* (TOADER, G, et al., 2019). The two bacterial biopreparations represented a lever for reducing costs in agriculture, for greening the soil structure but especially for combating pests that have acquired resistance to the effect of plant protection products (TOADER, G; CHIURCIU, C et.al., 2019).

MATERIAL AND METHODS

The following species of bacteria were used as research materials: *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus megaterium* and *Bacillus thuringiensis*. All of these bacteria were purchased from world-renowned gene banks. During the production of the Rom-Agrobiofertil NP biofertilizer, the testing of the product was performed within the Research-Development Stations in Agriculture / Vegetable Cultivation in Romania. Within

these stations, a series of methods and techniques have been developed so as to observe and prove the effectiveness of bacterial biopreparations against chemical fertilizers. On the batches established by mutual agreement with the researchers within the resorts, the biopreparation based on Rom-Agrobiofertil NP bacteria was applied according to the manufacturer's recommendations (Table 1). After the application of bacterial biopreparation and chemical fertilizers in the established batches, methods for determining the growth and development of plants, observation methods as well as statistical methods were used.

Table 1.

Treatment scheme for Rom-Agrobiofertil NP biofertilizer

Treatment scheme for the use of the Rom-Agrobiofertil NP biofertilizer				
Nr.of treatment	The type of product applied	Composition of the fertilizer	Recommended dose / ha	Used dose/ ha
1	Biological fertilizer	<i>Azospirillum lipoferum</i>	5 l/ ha	5 l/ha
	Rom-Agrobiofertil NP	<i>Azotobacter chroococcum</i>	5 l/ ha	5 l/ ha
		<i>Bacillus megaterium</i>	5 l/ ha	5 l/ ha

RESULTS AND DISCUSSIONS

In order to research the effect of Rom-Agrobiofertil NP organic fertilizer on soil and agricultural crops, within the Buzau Vegetable Research and Development Station on the cabbage crop, this organic fertilizer was applied. Thus, following the biometric determinations on the seed cabbage crop, the “de Buzau” variety, the following results were obtained (Table 2):

Table 2

Biometric data - “De Buzău” seed cabbage

Lots	No shoots	Silicon length	No medium / plant siliceous	No seeds / silica	Average seed / plant	
					No seeds / plant	g/pl
V1- Witness	21	6,25	209	24	2021	8,31
V2 - Rom-Agrobiofertil NP 5l/ha x 3 = 15 l/ ha	23	7,80	424	29	6564	44,4
Growth (%)	9.52 %	24.8 %	102.87 %	20.83 %	224.79 %	434.3 %

The semi-waxy cabbage crop reacted very well to the biofertilizer Rom-Agrobiofertil NP, a biofertilizer produced and marketed by Romvac Company S.A. It was also a growth promoter for cabbage cultivation both as a vegetable mass and as a production. During the experiment with the cabbage crop we took a series of pictures to distinguish between the control group and the group treated with Rom-Agrobiofertil NP. At the same time, there was an increase in the total production of seed production, as follows (Table 3):

Table 3

Total production of cabbage seed crop „by Buzau”

Lots	Average seed production kg / ha	Sale price / kg	Total value of seeds / lei	MMB g	No seeds / 1 g
V1 - Witness	422.46	500	211.23	4.11	243.0

V2 - Rom-Agrobiofertil NP	117.76	500	585.88	6.76	147.8
Growth (%)	177.4%	0%	177.36%	64.5%	-39.17%

Following the application of the treatment with Rom-Agrobiofertil NP, differences were identified regarding the germination of the seed material of the cabbage culture as well as on the flowering period. Thus, it was found that, on the treated group, the seed material sprouted about 9 days earlier than the seed material in the control group. The plants in the group



fertilized with the biofertilizer Rom-Agrobiofertil NP had a flowering period of approx. 6 days earlier than the plants in the control group (see following figures).



Figure 1. Control batch, cabbage seed „by Buzau”

Figure 2. Organic fertilized lot, cabbage „by Buzău” (Rom-Agrobiofertil NP)



Figure 3. Differences between the control group and the organic fertilized group

The Rom-Agrobiofertil NP product, a product supplied by the manufacturer Romvac Company S.A., was tested at the Buzău Vegetable Research and Development Station. This product consists of three bacterial cultures: *Azospirillum lipoferum*, *Azotobacter chroococcum* and *Bacillus megaterium*. Following its testing on the lots established by mutual agreement with the researchers from S.C.D.L Buzău, it was decided to take some soil samples from the tested lots, samples related to a lot. The analytical results were elaborated from the interpretation of the indicators. Following the statistical determinations performed, the following results were obtained (Table 4):

Table 4

Analytical results main mineral elements

Identification	pH	Humus	Nt	P _{AL}	P _{AL} ¹	K _{AL}
Test 1-lot witness	8.04	2.59	0.191	562	275	460
Test 1-lot treated	8.01	2.59	0.185	570	287	404
Growth (sample 1) T vs Mt	-0.37 %	0.00 %	-3.14 %	1.42 %	4.36 %	-12.17 %
Test 2-lot witness	8.09	2.53	0.186	590	275	484
Test 2-lot treated	8.14	2.53	0.182	611	270	424
Growth (sample 2) T vs Mt	0.62 %	0.00 %	-2.15 %	3.56 %	-1.82 %	-12.40 %
Test 3-lot witness	6.61	2.53	0.365	317	302	569
Test 3-lot treated	6.54	2.47	0.359	310	297	563
Growth (sample 3) T vs Mt	-1.06 %	-2.37 %	-1.64 %	-2.21 %	-1.66 %	-1.05 %

From table 4 the following aspects can be observed: **decreasing soil pH**. In the treated lots the pH of the soil decreased. The decrease in soil pH indicates that the activity of bacteria in the content of the biofertilizer Rom-Agrobiofertil NP led to a decrease in pH from an acidic pH to a slightly neutral, slightly basic one. Thus, the decrease of the pH from an acid to a neutral one proves that these chelated compounds from the soil structure were subjected to the solubilization / decomposition process by bacteria.

Decrease of the main element: nitrogen. Following the pedological analyzes performed, in the batches treated with organic fertilizer, a decrease of the nitrogen element was found. The decrease of this element was proved by the fact that the bacteria in the soil structure, based on the processes carried out by them, solubilized the nitrogen, transforming it into mineral elements easily assimilated by plants. **Phosphorus fluctuation**: compared to

nitrogen, phosphorus is an element that cannot be fixed in the Earth's atmosphere (phosphorus is found in the soil). Following the analyzes performed, there was a fluctuation of the amount of phosphorus in the structure of the samples taken. Thus, in the first two samples the amount of phosphorus increased. This was based on the decomposition of certain complex compounds in the soil structure, compounds in which phosphorus was also found. **Decreased potassium in the soil (K):** together with nitrogen and phosphorus, potassium is one of the organic elements that plants need in their growth and development. The results of pedological analyzes proved that the decomposition processes of complex compounds under the influence of soil bacterial fauna resulted in microelements easily assimilated by plants. Thus, the potassium in those complex compounds was solubilized, this process leading to its assimilation in plant tissues.

CONCLUSIONS

The foundation of ecological systems of fertilization and biological protection of plants is an integrated system of regulation and balancing of the soil and ecosystems of agricultural crops, these systems ensuring (at the same time) the maintenance of crops of economic interest in optimal parameters, ensuring an increase in agricultural production and maximum economic and ecological efficiency. The researchers from Romvac Company S.A., in ensuring these systems of fertilization and ecological protection of agricultural crops were based on the creation of biotechnological systems, systems that have as components the following items: bacterial preparations (actinomycetic bacteria, virulent bacteria, fungi, fungi, etc.), ancillary components of bacterial biopreparations (organic and environmentally friendly substances and components), manufacturing technology (production line, fermenters, etc.).

The prospects for the application of bacterial preparations in modern agriculture was an innovation. The combination of bacteria suitable for agriculture has led to the phenomenon of preventing pest attack in agricultural crops but, more importantly, preventing soil acidification, preventing damage to bacterial fauna in the soil structure and greening the entire agricultural ecosystem. The bacterial preparations produced by the Romvac Company have the role of combating the attack of pests from agricultural crops as well as of providing a nutritional supplement by decomposing the complex elements in the soil by the bacteria from the soil fauna.

From the tests performed within the Research-Development Station for vegetable growing in Buzau, it was shown that the application of a mix of bacterial biopreparation leads to obtaining very good results. The fact that the total production increased by 177.4% (compared to the control group), the number of siliceous by 102.87%, the number of seeds per plant by 224.79% was a huge advantage in favor of organic fertilizer based on bacterial preparations. The product Rom-Agrobiofertil NP represents a mix of three bacterial preparations: *Azotobacter chroococum*, *Azospirillum lipoferum* and *Bacillus megaterium*. This mix of bacterial preparations, applied according to the manufacturer's recommendation, led to impressive results compared to a control batch.

The current agricultural trend requires farmers to accept and use organic systems for fertilizing crops and plant protection as well as opening a new niche market in agriculture, that of organic products at affordable prices for any farmer. Fertilization and protection systems have many operating mechanisms that bring many advantages. Romvac's proposal for farmers is to expand the production processes of bacterial preparations for agriculture and to expand the range of products for agriculture in order to green agricultural land, to control pests and diseases of crops, the most important aspect being the restoration of soil fauna. and obtaining much higher, ecological productions compared to chemically fertilized lots.

Romvac Company requires (imperatively) the continuation of research in order to obtain new bacterial premixes, new mixtures of organic bacterial preparations, their production in complex form and the implementation of new structures and technological processes on research, testing and production of new generations of bacterial biopreparations intended for agriculture.

BIBLIOGRAPHY

- AGGANI, S. L.. Development of Biofertilizers and its Future Perspective. Sch. Acad. J. Pharm., 2013, 2 (4): 327-332,
- AHMAD F, AHMAD I, KHAN MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163(2):173–181,
- AHN TS, KA JO, LEE GH, SONG HG (2007) Microcosm study for revegetation of barren land with wild plants by some plant growth-promoting rhizobacteria. J Microbiol Biotechnol 17(1):52–57,
- ATTILLA, H. I., ALHASIN, A. M., NASIB, M. A., GHAZALI, A. H., ZAKARIA, L., JAIS, H. M., BALAL, I. A. A, SALLEH, B. (2010) Occurrence and microbiological characteristics of Azospirillum strain associated with leguminous and non- leguminous plants in Al Jabal Al Akhdar Eco region, Lybia. American –Eurasian J. Agric. & Environmental Sciences 8 (6): 617-625,
- CHANDLER D., GREAVES J., PRINCE G., TATCHELL M., BAILEY A. Biopesticides: Pest Management and Regulation. CABI, 2010. 256 p.,
- LARKIN Hansen Anne. The Organic Farming Manual. 2010. 437 p.,
- LĂCĂTUȘU, Radu. Agrochimie. Iași: Editura Terra Nostra, 2016,
- MADJAR, Roxana; DAVIDESCU, Velicica. Agrochimie. București: Universitatea de Științe Agronomice și Medicină Veterinară București, Facultatea de Horticultură, Specializarea Horticultură- Învățământ la distanță, 2009,
- SBÎRCIOG, G., ȘOVĂREL, G., COSTACHE, M. (2017). Stimulating effect of Rom-Agrobiofertil NP fertiliser on tomato, cucumber and cauliflower crops. In: Agriculture & Food, Vol. 5,
- SOCIEDADE BRASILEIRA DE GENÉTICA. Genetics and Molecular Biology, 38, 4, 401-419 (2015) Copyright © 2015.. Printed in Brazil DOI: <http://dx.doi.org/10.1590/S1415-475738420150053> Review Article,
- Tasarea solului. Accesat Noiembrie 04, 2020 la adresa: <https://agrointel.ro/51823/tasarea-solului-fenomenul-care-scade-productiile-cu-pana-la-35-ce-solutii-au-fermierii-pentru-a-sparge-hardpan-ul/> ,
- TOADER G, CHIURCIU C, CHIURCIU V, BURNICHI F, PETRE C, CHIȚONU P, MAIEREAN, ILIE L. (2019) Research on the action of rom-agrobiofertil np biofertilizer on agricultural crops. In: Scientific Papers. Series A. Agronomy, Vol. LXII, No. 1, 2019. ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785.,
- TOADER G, CHIURCIU C, CHIURCIU V, BURNICHI F, PETRE C, CHIȚONU, P, MAIEREAN N, ILIE L, BORA C, PĂUNESCU G. (2019) Research on the action of fertilizers based on bacterial cultures on some agricultural crops in Romania. In: Agroprint timișoara: Lucrări Științifice, seria I, VOL.XXI (2). ISSN print 1453-1410; ISSN online 2069-2307.
- TONCEA I., ENUȚĂ S., IONIȚĂ Nițu GEORGETA, ALEXANDRESCU Daniela, TONCEA V. A. Manual de Agricultură Ecologică. Cluj-Napoca, 2012. 360 p.,
- VOLOȘCIUC L. Biotehnologia producerii și aplicării preparatelor baculovirale în agricultura ecologică. Chișinău: Mediul ambiant, 2009a, 262 p.,
- VOLOȘCIUC L. T. Probleme ecologice în agricultură. Chișinău. 2009b, 264 p.