RESEARCH ON THE USE OF BACTERIAL BIOPREPARATES IN TOMATO AND PEPPER CROPS IN ROMANIA

G. TOADER [4,1], Valentina FILIP[1], C. CHIURCIU [1], Viorica CHIURCIU [1], Valentina FILIP [1], N. MAIEREAN [1] , P. SEVCIUC [1], P. CHIȚONU [1], Elena-Violeta TOADER[5], Floarea BURNICHI [2], E. MIREA [2], Ana-Maria NECHITA[3], Nelly FÎNARU[3], L. ILIE [4]

[1]Romvac Company. Voluntari, Romania

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

[3] Bacău Research and Development Station in Vegetables. Bacău, Romania.

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

[5] Bucharest Academy of Economic Studies. Faculty of Agri-Food and Environmental Economics. Bucharest, Romania.

toadergeorge92@gmail.com / george.toader@romvac.ro

Abstract. *.The paper has the role to promote certain sustainable production systems, diversified and balanced, in order to prevent pollution of agricultural crops, the environment, the conversion of agri-food waste (whey) into veterinary medical products as well as the implementation of green, non-polluting technologies in the agricultural sector. The purpose of this work is to present and use the newest innovative technologies into the agricultural field and replace the traditional polluting products (chemical fertilizers), so that the farmers can reaches a much greater potential over the recorded productions. The alternative to the chemical fertilizers is fertilizer based on bacterial cultures. In agriculture, the main source of nutrition is the nutrients. These are the basis for the growth and development of cultural plants, and they are indispensable to any form of life. On the other hand, the amount of nutrients at some point in time may decrease or increase depending on certain pedoclimatic factors, soil typology, the ability of plants to return some of the nutrients consumed (by the decomposition of crop residues) and so on. Food safety, a normal objective for any man, farm and enterprise, is aimed to producing sufficient, varied and cheap food, corresponding to the physiological requirements and purchasing power of any person. In the developed countries, this objective has been achieved and often exceeded substantially by promoting intensive systems for both land cultivation, animal husbandry and veterinary medicine, as well as modern methods of processing and marketing agricultural products. Mechanization, chemization, animal husbandry technologies, technologies for applying treatments, growing plants and raising more and more productive animals, and modernizing processing and marketing systems have contributed, first and foremost, to improving human living conditions, as much as volume. and diversity of agri-food products, as well as accessible to buyers. In order to protect the environment and the agri-food health and safety of humans and animals, the company Romvac Company S.A has developed a series of innovative technologies with the help of the farmer have obtained certain ecological products for their use in the agro-zoo-veterinary field.*

Keywords: *biofertilisers, azospirillium lipoferum, azotobacter chroococcum, bacillus megaterium, Rom-Agrobiofertil NP, sustainable agriculture, Romvac Company S.A*

INTRODUCTION

There have always been some specific symbiosis processes between soil, bacteria, pedo-climatic factors and cultures. These processes take place both inside the root, near it and between soil-plant-bacteria. These processes can be endophytic as well as associative. Bacteria used in the production of biofertilizers or bioinsecticides are an innovation in modern agriculture. These bacteria have the role of providing the nutrients needed for plant growth and development (N, P, K, Mg, Cu, etc.). These products have emerged as a response to the overuse of conventional, chemical fertilizers. ((Sociedade Brasileira de Genética, 2015; AGGANI,, 2017).

For farmers, the main target is to increase their production and, implicitly, its profit. However, farmers do not place much emphasis on food safety, which must be taken into account by any farmer. The purpose of food safety is to produce healthy foods, with a wide range, but especially inexpensive and appropriate in terms of human needs and requirements. In some countries, food safety is placed first. Thus, the present objective was surpassed by the farmers placing a great emphasis on sustainable and sustainable agriculture, environmentally friendly. To maintain this contribution, farmers have started to use certain products based on live bacterial cultures. (Sociedade Brasileira de Genética, 2015; CARVAJAL-MUÑOZ, J.S, *et.al*, 2012).

The mechanization and fertilization systems of agriculture, the technologies of applying the treatments on agricultural crops have become more and more innovative. The modernization of these systems has led to the increase of agricultural production, the greening of the soil but especially a reduction of pollution in agriculture. The diversification of these technologies has led to obtaining agricultural by-products, increasing the profit for farmers but especially increasing the agri-food safety of the obtained products. (STEENHOUDT, O, 2000).

A first technology in the agricultural field is the use of biofertilizers. Biofertilizers are organic fertilizers, in which living microorganisms (fungi, bacteria, fungi, algae, etc.) are identified. These innovative products have the role of ensuring the elements necessary for the growth and development of plants through certain processes carried out in the soil structure or at the level of the plant roots. The role of these biofertilizers is to ecologize the soil, to decompose certain complex compounds in the soil structure, but especially to obtain high yields at lower costs. (Bio-fit.eu., 2019; Bhardwaj, Deepak Ansari., *et. al.* 2014; BAPAUME, L. *Et.al*, 2012).

Biofertilizers can be both liquid and lyophilized granules. Thus, these products can be applied both on the soil (directly) and on the plants (foliage) so that the microorganisms in their structure reach the soil. Once they have reached the soil structure, microorganisms contribute to soil processes, these bacteria providing a surplus of nutrients for plants and, implicitly, a greening of the soil, plants and products obtained (Bio-fit.eu., 2019; SBÎRCIOG, G. *et.al.* 2017; ATTILLA, H. I *et.al,* 2010).

A first biofertilizer produced and researched in Romania is the Rom-Agrobiofertil NP biofertilizer. This organic fertilizer represents an innovation in the agricultural field, being made up of three bacterial strains: Bacillus megaterium, Azospirillium lipoferum and Azotobacter chroococcum. Each bacterium in the structure of the biofertilizer has its role in soil processes, so grouped together, these bacteria lead to very good results from the point of view of agricultural production but especially of the greening and improvement of soil structure (TOADER, G, CHIURCIU, C et.al, 2019).

MATERIAL AND METHODS

Due to the continuation of the collaboration protocols between Romvac Company S.A and the research and development stations in agriculture in Romania, on 2018-2019, the experiments in the testing of the product Rom-Agrobiofertil NP were continued. Thus, like the previous year the biofertilizer Rom-Agrobiofertil NP was tested on on a wide range of crops.

Thus, along with colleagues from the research and development station, we were established three lots: the witness lot, the fertilized with chemical fertilizers lot and the fertilized with the biofertilizer Rom-Agrobiofertil NP lot. There was a delimitation between the lots so that the treatment applied to each lot did not influence the characteristics of the varieties, the production on the lot and, implicitly the morphological characteristics of the soil and plants. The first tranche of this biofertilizator was administered at a dose of 5 l/ha (3 types of bacterial cultures x 5 = 15 l bacterial cultures/ha – table 1) for each crop, as we can see into the table 1 (TOADER, G, CHIURCIU, C *et.al*, 2019).:

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 Rom-Agrobiofertil NP	*Azospirillum lipoferum*	5 l/ ha	5 l /ha
		Azotobacter chroococcum	5 l/ ha	5 l/ ha
		Bacillus megaterium	5 l/ ha	5 l/ ha

Source: Prospect Romvac Company S.A: Rom-Agrobiofertil NP product [5]

RESULTS AND DISCUSSIONS

Following the continuation of the collaboration protocol with the Research-Development Station in Vegetables Buzau and Research-Development Station in Bacău, on 2019, the experiments in the testing of the product Rom-Agrobiofertil NP were continued. Thus, like the previous year the biofertilizer Rom-Agrobiofertil NP was tested on on a wide range of crops such as tomatoes and sweet corn.

The bacterium *Azospirillium lipoferum* has the role of metabolizing the organic matter from the soil structure into elements that can be easily assimilated by plants. At the same time, the bacterium has the role of capturing atmospheric nitrogen through certain energy systems in order to fix it in the soil so as to provide the necessary elements for agricultural crops (Bio-fit.eu, 2019; IGIEHON, N. O; BABALOLA, O. O, 2018).).

Bacteria *Azotobacter chroococcum* is the role of a nitrogen cap in the atmosphere and fix it in the soil of the base where there are energy resources existing in the average plant-soil. (Bio-fit.eu, 2019; RAJA, N, 2013). The last bacterium *Bacillus megaterium* plays a role in the decomposition of certain elements that are difficult to assimilate by plants in the soil. Thus, this bacterium has the role of decomposing chelated compounds from the soil structure, converting insoluble phosphates into soluble phosphates, decomposition of nitrates and nitrites but especially the decomposition of plant residues into organic matter (Bio-fit.eu, 2019; TOADER, G, CHIURCIU, C *et.al*., 2019; BAPAUME, L, *et.al*, 2012).

To prove once again the efficacy of the biofertilizers against to the chemical fertilizers, at the Buzău Agriculture Research Station, the product Rom-Agrobiofertil NP, biological fertilizer based on bacterial cultures was tested again on the tomatoes. Following the measurements on the lots, numerous differences between the lots included in the experiment were identified. The first difference was identified on the plants, both in size and appearance (figure 1 and figure 2).

Figure 1. Plant control batch

Figure 2. Fertilized batch plant with Rom-Agrobiofertil NP

Three lots were set up at Buzau Research-Development Station in Vegetables: the control lot, the chemical fertilized lot and the biological fertilized lot with Rom-Agrobiofertil NP and the biological lot with Rom-Agrobiofertil NP lot near the . Three lots were set up within the resort: the control lot, the biological fertilized lot. According to the interaction of the treatment with the tomato variety, according to the pedo-climatic factors existing on each lot, as well as following the biometric determinations, the following results were obtained (table 2 and 3):

Table 2

Tomato biometric measurements Buzau 1600 – part 1

Lots	**Plant height (cm)**	**Shrub diameter (cm)**	**Nr. Leaves / plant**	**Nr. of Leaves under the first inflorescence**	**Leaf length (cm)**	**No. of inflorescents / plant**	**Nr. of Flowers / Inflorescences (t / ha)**
V1 - Witness	115.50	41.00	22.1	4.4	33	5.15	3.55
V2 Biol. Fert Rom-Agrobiofertil NP	111.00	41.35	22.1	4.4	39.65	4.05	3.25
V3 Biol.fert. Rom-Agr+Inocul bact trichoderma harzianum	117.00	46.95	21.35	4.45	38.2	4.25	3.3
Growth biol.fert. Vs Wt (%)	**-3.90**	**0.85**	**0.00**	**0.00**	**20.15**	**-21.36**	**-8.45**
Growth biol.fert.+inocul vs Mt (%)	**1.30**	**14.51**	**-3.39**	**1.14**	**15.76**	**-17.48**	**-7.04**
Growth fer.biol.fert +inocul vs biol.fert (%)	**5.41**	**13.54**	**-3.39**	**1.14**	**-3.66**	**4.94**	**1.54**

Table 3

Tomato biometric measurements Buzau 1600 – part 2

Lot	**Inflorescence distance (cm) / (g)**	**Fruit Abscission Area (cm)**	**Fruit height (cm)**	**Fruit diameter (cm)**	**Fruit Weight (g)**	**Insert diameter at the stem (cm)**	**Nr. Seminal Lodge**	**Thickness of the cap (cm)**
V1 - Witness	16.45	0.305	5.75	5.94	0.13	0.5	5.8	0.32
V2 Biol. Fert Rom-Agrobiofertil NP	19.4	0.3425	5.94	6.41	0.14	0.8	5.8	0.30
V3 Biol.fert. Rom-Agr+Inocul bact trichoderma harzianum	19.68	0.3275	5.90	6.12	0.14	0.6	5.95	0.29
Growth biol.fert. Vs Mt (%)	**17.93**	**12.30**	**3.31**	**7.87**	**5.31**	**43.98**	**0.00**	**-5.51**
Growth biol.fert.+inocul vs Mt (%)	**19.60**	**7.38**	**2.61**	**2.99**	**2.47**	**17.87**	**2.59**	**-10.24**
Growth fer.biol.fert +inocul vs biol.fert (%)	**1.42**	**-4.38**	**-0.67**	**-4.53**	**-2.70**	**-18.14**	**2.59**	**-5.00**

In the resort of Buzau Vegetable Research and Development, was conducted an experiment on tomato variety Buzau in 1600, with organic products from Romvac Company on a surface of 350SqM. The culture was established in the Ecological Polygon, in a solarium discovered. The experience was organized in 3 experimental variants on 4 repetitions, as follows: V1 - control version, untreated, V2 - variant with Romvac treatments (Rom-Agrobiofertil 5l / ha x 3 types), in which the seeds from which the seedlings with which the V2 variant was established were obtained were treated with a mixed bacterial inoculum provided by ICDPP Bucharest and V3 - variant with ROMVAC treatments (Rom-Agrobiofertil NP 5l / ha x 3 types), in which the leaves were treated with an biofungicide inoculum based on bacterial culture of Trichoderma, culture provided by ICDPP Bucharest.

You will find that this year there were few significant differences in the diameter of the bush, the distance between the inflorescences and the length of the leaves. This means that the plants were more vigorous and the production would have been better. The big problem (met) was the fruit. There were a lot of damage, and the ecological products used did not stop the depreciation of the fruit, so the registered productions were small. There were a lot of damage, and the ecological products used did not stop the depreciation of the fruit, so the registered productions were small.

Related to the present problem, the vegetal losses were due to the abundant rains of spring and then of the intensity of the solar radiation, these leading to the imbalance and the damage of the plants. This phenomenon was manifested in all the 3 solariums where we performed product tests with Romvac and two other partners, so it is an environmental problem, not a treatment.

The second experiment was carried out into Bacău Research and Development Station in Vegetable Culture. Together with the specialists of the station, the Rom-Agrobiofertil NP product was tested on a variety of vegetable crops. First crops that where tested Rom-Agrobiofertil NP biofertilizer were carried on tomatoes and pepper. There were two batches of this kind of tomatoes and pepper: one chemically fertilized and the other fertilized with the product Rom-Agrobiofertil NP (table 4).

Following the measurements on the lots, numerous differences between the lots included in the experiment were identified. The first difference was identified on the fruit, both in size and appearance (figure 3 and figure 4).

Figure 3.Variant fertilized with Rom-Agrobiofertil NP

Figure 4. Chemically fertilized variant

Table 4

Tomato biometric measurements ,,UniBac variety”

Lots	Fruit weight (g)	Production per ha (t)	Seed / fruit quantity (g)	Seed production / ha kg
Variant fertilized with Rom-Agrobioferil NP	91.9	28.4	0.54	44.3
Chemically fertilized variant	109.8	33.9	0.63	53
Growth% (chemical vs. biological)	**19.48**	**19.37**	**16.67**	**19.64**
Growth% (biological vs. chemical)	**-16.30**	**-16.22**	**-14.29**	**-16.42**

The second part of the experiments carried out within the S.C.D.L Bacău were carried out on the pepper crop, the variety "Ionel". Within this culture, a comparison was established on two batches: biometric determinations on the chemical batch and biological determinations on the batch fertilized with a biological fertilizer. Following the biometric determinations the following aspects were established: fruit length (mm), longitudinal diameter / transverse diameter (mm), pericarp thickness (mm), mass (g / fruit) of which: pulp (g), seeds (g) , production (t / ha) and seed quantity (kg / ha). Following these determinations, the following differences were identified between the two lots (table 5):

Table 5

Pepper biometric measurements ,,Ionel variety”

No. V/R	Fruit length (mm)	Longitudinal diameter (mm)	Transverse diameter (mm)	Pericarp thickness (mm)	Mass (g)	Pulp (g)	Seeds (g)	Production t / ha	Seed quantity kg / ha
Chemical batch (average x 3 samples)	139.1	40.5	34.6	3	60.9	48.5	1.1	36.1	75.2
Rom-Agrobiofertil NP biological lot (mean x 3 samples)	121.39	38.62	33.42	3.27	49.98	38.9	1.1	31.5	98.5
Growth% (chemical vs. biological)	**14.59**	**4.87**	**3.53**	**-8.26**	**21.85**	**24.68**	**0.00**	**14.60**	**-23.65**
Growth% (biological vs. chemical)	**-12.73**	**-4.64**	**-3.41**	**9**	**-17.93**	**-19.79**	**0.00**	**-12.74**	**30.98**

As we can see, the production of seed material of the biological fertilized lot was higher than the control lot, which demonstrates the effectiveness of the biofertilizer used. If within the chemically fertilized lot an amount of approx. 150 kg of chemical fertilizer per ha, the fertilized lot with the biofertilizer Rom-Agrobiofertil NP was fertilized with 30 liters. There were differences between the fruits of the two lots (figure 5 and figure 6).

Figure 5. Chemically fertilized variant

Figure 6. Biologically fertilized variant

CONCLUSIONS

The most important aspect in the use of biofertilizers is the ecological soil as well as the significant reduction of environmental pollution and the improvement of the conditions in the soil structure. These innovative technologies (biofertilizers) represent a contribution in obtaining large yields, in enriching the soil with the necessary elements for the growth and development of plants but especially in recolonizing the bacterial fauna of the soil.

Biofertilizers are organic products, they are environmentally friendly so that they do not affect the surrounding flora or fauna. Another important aspect regarding the use of these products is the provision of the important elements (nitrogen, phosphorus, potassium) necessary for the growth and development of plants. These products do not pose a threat to the health of humans and animals so biofertilizers can be applied in any culture.

Based on the present article, we can say that following the application of these organic products in relation to chemical fertilizers, the biometric yields and determinations were much higher compared to the chemical batch - biological batch vs. chemical batch. Following the analyzes carried out within the ICPA Bucharest in the lots where the biofertilizer was used Rom-Agrobiofertil NP was found a decrease of the complex compounds in the soil structure, an ecology of it but especially an increase of the soil fauna. Another important aspect is the increase of the soil loosening, loosening which allows a better aeration, a bigger water retention but especially a much faster facilitation of the energy exchange between soil-plant-environment.

The use of these products in the agricultural field represents a new innovation, a new technology and the fact that farmers are beginning to use these technologies proves that in the future agriculture will become sustainable, agriculture that will ensure large, healthy, products that will not affect human health and of animals.

BIBLIOGRAPHY

AGGANI, S. L.. Development of Biofertilizers and its Future Perspective. Sch. Acad. J. Pharm., 2013, 2 (4): 327-332

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 caracteristics 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

BAPAUME, L, REINHARDT, D. How membranes shape plant symbioses: signaling and transport in nodulation and arbuscular mycorrhiza. Front Plant Sci. 2012;3:223.

BHARDWAJ, Deepak Ansari; MOHAMMAD, Sahoo; RANJAN Tuteja, Narendra. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microbial cell factories. 13. 66. 10.1186/1475-2859-13-66.

Production managers in agriculture and forestry. LO9: Biofertilizers application for sustainable economic development - advantages and constraints. [Cited at 12.12.2019]. Avaible on the Internet at the address: https://www.bio-fit.eu/q9/lo9-bio-fertilizers-application-for-sustainable-economic-development-advantages-and-constraints.

CARVAJAL-MUÑOZ, J.S., CARMONA-GARCIA, C.E. Benefits and limitations of biofertilization in agricultural practices, Livestock Research for Rural Development 2012, 24 Article #43. Retrieved September 6, 2016, from http://www.lrrd.org/lrrd24/3/carv24043.htm

IGIEHON, N. O; BABALOLA, O. O. (2018). Rhizosphere Microbiome Modulators: Contributions of Nitrogen Fixing Bacteria towards Sustainable Agriculture. International journal of environmental research and public health, 15(4), 574. doi:10.3390/ijerph15040574

JING, R., KJELLERUP B. V. (2018). Biogeochemical cycling of metals impacting by microbial mobilization and immobilization. J. Environ. Sci. 66 146–154. 10.1016/j.jes.2017.04.035

LEVANDOVSCHI, N., CHIURCIU, C., CHITONU, P. (2017). Rom-Agrobiofertil NP - biological fertilizer for a sustainable agriculture. Sao Paulo, Brazil: 2nd World Biotechnology Congress, December 04-06, 2017 In: Journal of Biotechnology & Biomaterials, Vol. 7, Issue 6 (Suppl), pp. 69, ISSN 2155-952X, DOI: 10.4172/2155-952X-C1-086.

RAJA, N. Biopesticides and Biofertilizers: Ecofriendly Sources for Sustainable Agriculture. J Biofertil Biopestici, 2013, 4: e112. doi:10.4172/2155-6202.1000e112

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.

SELLAMUTHU, Gothandapani, SOUNDARAPANDIAN, Sekar, JASDEEP, C Padaria (2017) Azotobacter chroococcum: Utilization and potential use for agricultural crop production: An overview. Int. J. Adv. Res. Biol. Sci. 4(3): 35-42

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

STEENHOUDT, O., VANDERLEYDEN, J. (2000). Azospirillum, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiology Reviews, 24: 487-506.

TOADER, G, CHIURCIU, C, CHIURCIU, V, BURNICHI, F, PETRE, C, CHIȚONU, P, MAIEREAN, N, ILIE, L, BORA, C, PAUNESCU, G. (2019). Research on the action of fertilizers based on bacterial cultures on some agricultural crops in Romania. In: Lucrări Științifice, Seria I, Vol.XXI (2). AGROPRINT Timisoara, Romania - Lucrări Științifice Management Agricol. ISSN print 1453-1410. ISSN online 2069-2307.

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, 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.