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REVOLUTIONARY METHODS IN FERTILIZATION PROCESSES OF AGRICULTURE OF ROMANIA: BACTERIAL BIOPREPARATIONS – A `GREEN REVOLUTION`

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Abstract

The paper aims to present the use of new fertilization technologies in agricultural crops in Romania. At the same time, the paper presents the results obtained after testing these new fertilizer products (ecological fertilizers based on bacterial cultures) in parallel with the fertilization of crops with chemical fertilizers. These researches were carried out in two prestigious research institutions, the first being the Suceava Research-Development Station for Agriculture and the second being the Buzau Research-Development Station for Vegetable Culture. Within the two research institutions were established the crops to which both chemical and biological fertilizers were applied, during the experiments analyzing a series of biological parameters of the plant, soil and agricultural production. Following the completion of the experiments, it was observed that the physiology and anatomy of the plants showed changes as well as the production of the crops themselves, the yields showing an increase of up to 57% (in lots fertilized with organic fertilizer, compared to lots that were fertilized with a chemical fertilizer).

***Key words**: plant growth-promoting, biofertilizers, phytohormones, sustainable agriculture, nitrogen fixation, bacterial inoculants, nutrient solubilisation.*

INTRODUCTION

Bacteria in the soil structure have a role in the processes carried out at the soil level as well as in the growth and development of plants. Bacterial cultures can be used as biological/organic fertilizers, bioinsecticides, biofungicides in agriculture so as to ensure an increase in crop yield and maximization of their production (Khalid et al., 2009).

The use of these bacteria as biological fertilizers in agricultural crops leads to better plant growth through various mechanisms that these bacteria produce, mechanisms such as the synthesis of nutrients and phytohormones, (which are absorbed and used by plants), the mobilization of compounds in soil structure (decomposition of complex soil compounds into soluble compounds that are used in the basic processes of plants-photosynthesis and chemosynthesis), role in plant protection (stress conditions, pest attack, disease, etc.) (Malusá & Vassilev, 2014).

The use of these revolutionary processes and methods of fertilization in agricultural crops in Romania have proven a great potential in growing, developing and maximizing plant production, as well as in restoring the soil structure and its beneficial fauna. The use of these biofertilizers based on live bacterial cultures has the role of greening the soil, opening a new way for farmers to approach a sustainable, sustainable agriculture, and obtaining much healthier products rich in nutrients.

Thus, the use of PGPR mechanisms in agricultural crops in Romania brought with it the obtaining of additional productions for farmers, productions which, for them, represented a substantial profit. Also, the PGPR mechanism is a simple one, but based on which plants receive a much higher amount of nutrients as well as a certain resistance to certain factors (Figure 1).

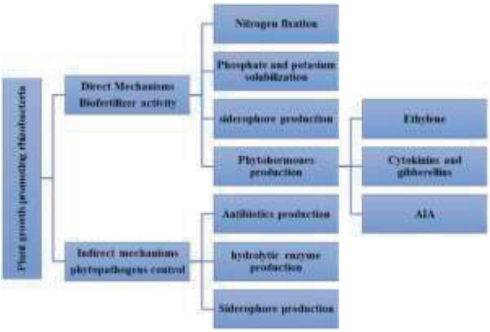

Figure 1. Mechanisms of Plant Growth-Promoting Rhizobacteria (Sansinenea, 2019)

Nitrogen is the basic element in the processes carried out inside the plants and in the soil structure. Nitrogen is needed in the formation of amino acids and proteins, it is the main element for plant growth and development.

Atmospheric nitrogen is combined into organic forms, forms that can be assimilated by plants (Tejera et al., 2005).

The most representative bacteria that act as atmospheric nitrogen fixers are those of the genera *Azospirillum*, *Azotobacter* and *Bacillus* (symbiotic bacteria associated with cereals and legumes), *Frankia* (actinorizal plants) (Ravikumar et al., 2007; Ininbergs et al., 2011).

Through this process of hydrolysis, ammonia and α-ketobutyrate will be obtained. These obtained sources can be used as carbon and nitrogen sources in plant growth and development processes (Yang & Hoffman, 1984).

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The use of these biological fertilization technologies, based on bacterial cultures, was an important step in Romanian agriculture. Thus, these technologies were an alternative to classical chemical fertilization, the bacteria in the composition of fertilizers having a role in the growth and development of plants, the restoration of beneficial soil fauna and an increase in plants and agricultural production.

MATERIALS AND METHODS

In order to prove the effectiveness of a biological fertilization product, in relation to a chemical fertilization product, into Buzău Research-Development Station for Vegetable Growing and Suceava Research-Development Station for Agriculture, a series of experiments were performed, as follows:

SCDA Suceava - corn crop, millennium variety:

V1 lot - unfertilized lot,

V2 lot - chemically fertilized NPK 20: 20: 0,

V3 lot - biological fertilizer lot Rom-Agrobiofertil NP.

SCDL Buzău - culture of pepper, superior yellow variety:

V1 lot - Biological fertilizer Rom-Agrobiofertil NP (based on live cultures of *Azotobacter chrooccum, Azospirillium lipoferum* and *Bacillus megaterium*),

V2 lot - Biological fertilizer Azoter (based on *Azotobacter chroococcum, Azospirillum brasilense, Bacillus megaterium* bacteria).

V3 lot - Chemical fertilizer NPK 16:16:16.

The study was carried out in 2019-2020 period. The data, collected from those two research-development stations, have been statistically processed and interpreted, based on the obtained results and interpretations proving the effectiveness of the biological fertilizer.

RESULTS AND DISCUSSIONS

In SCDA Suceava, the chemical fertilizer Complex 20: 20: 0 (V2-300 kg/ha - control lot) was tested in parallel with the organic fertilizer Rom-Agrobiofertil NP (V3-15 liters x 2 applications/ha), referring to an unfertilized lot (V1), for the cultivation of corn, the Millennium variety. In carrying out the experiments (Table 1), a series of parameters were followed, with the following results obtained into the three groups (Figure 2), as follows (Table 1, Table 2):

Table 1. Corn crop production data

Variant	No. of grains / row	MMB/ g	Grain yield%	Hectolitre mass /kg	Production / kg / ha	Humidity/ %
V1- unfertilized lot	31	251	81.7	72.7	4587	16.6
V2-chemical fertilizer 300 kg / ha 20: 20: 0	36	274	89.0	78.7	6478	16.4
V3- organic fertilizer Rom-Agrobiofertil NP	37	303	98.9	80.4	7315	16.6

Table 2. Corn crop production data-Anova test

Anova: Single Factor						
SUMMARY						
Groups	**Count**	**Sum**	**Average**	**Variance**		
V1-unfertilized lot	6	5040	840	3376633		
V2-chemical fertilizer 300 kg / ha 20: 20: 0	6	6972.1	1162.02	6790709		
V3-organic fertilizer Rom-Agrobiofertil NP	6	7850.9	1308.48	8669230		
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	689247.7	2	344624	0.05489	0.947	3.682
Within Groups	94182862	15	6278857			
Total	94872110	17				

Figure 2. Corn crops (V1 - batch not fertilized, V2 - batch chemically fertilized, V3 - batch biologically fertilized)

Between the three groups, a series of differences were observed in the predators noticed during the experiments (Figure 3).

Among the most important differences are the production of seed material (production per hectare), plant height and the number of plants per lot. Following the experiments, it was found that the plants in the group fertilized with the biological product Rom-Agrobiofertil NP showed a higher growth in height, compared to the two groups (chemically and non-fertilized). The fructification as well as the other parameters followed gave a higher economic yield than the other two lots (Table 1 and Figure 3).

Figure 3. Corn plants (V1 - unfertilized lot, V2 - chemically fertilized lot, V3 - biologically fertilized lot)

Biometric determinations were performed using the Anova test to highlight the effects of organic fertilizers relative to chemical fertilizer product (Table 2).

The second experiment was carried out at SCDL Buzău, on superior yellow variety of pepper culture. Following the experiments on the pepper culture, a series of parameters were settled such as plant height, number of fruits per plant, total production, etc., as well as aspects related to chlorophyllmetry or dry matter mass. Biometric determinations were performed using the Anova test to highlight the effects of organic fertilizers relative to chemical fertilizer product (Table 3).

Table 3. Pepper crop production data

Objectives	V1-Rom-Agro	V2-Azoter	V3-NPK
Plant height (cm)	46.33	49.33	45.22
Plant diameter (cm)	44.89	42.67	39.89
Number of leaves	142.00	120.11	108.11
Leaf length (cm)	17.68	17.48	16.58
Leaf width (cm)	6.88	7.08	6.89
Number buds	25.56	19.22	18.56
Number of fruits	6.89	5.44	6.56
Number of flowers	7.78	5.44	6.33
Stem diameter (cm)	1.11	1.11	1.16
Total production (t/ha)	20.5	18.7	12.3

The production per biologically lots showed an increase of up to 66.67% (V1 lot compared to V3 lot) and 52.03% (V2 lot compared to V3 lot). This experiment showed that the production of biologically fertilized lots was much higher than the chemically fertilized lot, due to bacterial activity of the organic fertilizers (Table 4).

Table 4. Pepper crop production data-Anova test

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
46.33	9	273.29	30.365556	1927.9028		
49.33	9	237.25	26.361111	1391.0202		
45.22	9	216.38	24.042222	1122.4135		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	184.1921	2	92.096048	0.0622083	0.9398381	3.4028261
Within Groups	35530.693	24	1480.4455			
Total	35714.885	26				

Figure 4. Differences between pepper plants, the upper yellow variety: a. Rom-Agrofertil NP; b. Azoter; c. NPK chemical fertilized plant

Some differences were identified between the three lots, the most visible being the biologically fertilized lots. Thus, in the two biologically fertilized groups there was a much higher plant growth than the control group, chemically fertilized. At the same time, the number of fruits per plant also showed a variation of growth between the three groups (Figure 5).
From Figure 4 we can deduce that the effect of organic fertilizers applied in pepper culture (Rom-Agrobiofertil NP and Azoter) had a beneficial effect on plant growth, shoot grow and, implicitly, agricultural production.
Experiments performed with *Azospirillum lipoferum* inoculated seeds, revealed the bacterium ability to produce antioxidant enzymes such as catalases, glutathione, and peroxidases, which have different roles (establishing plant protection in their stress phases, protection of plants from reactive oxygen- superoxide, hydroxyl ion, hydrogen peroxide) (Mittler, 2002).

Figure 5. Superior yellow bell pepper plant (organic fertilized lot produced Rom-Agrobiofertil NP)

From the research performed with *Azospirillum lipoferum* bacterium, it was found that an action of bacterial colonies on the soil surface, lower the level of ethylene in order to increase plant growth in stress conditions (Glick et al., 2007).
The role of these bacteria in the content of the two biological fertilizers, in addition to the processes they carry out in the soil, also have the role of producing certain antioxidant enzymes to allow the plant optimal growth (both plant and production) under stress (pedo-climatic) (Fu et al., 2010).
The application of organic fertilizers in agricultural crops has had the effect of a substantial increase in the plants themselves, an increase in the amount of nutrients in the soil and bacterial recolonization of soil structure. The use of organic fertilizers was an important step in Romanian agriculture as well as globally. The fact that chemical fertilizers can be substituted with bacterial biopreparations was an important factor for farmers to move towards sustainable, organic agriculture and to obtain much healthier products, with a large amount of nutrients, proteins necessary for human and animal food.

CONCLUSIONS

Recently, many emerging technologies have been implemented in the agricultural field in Romania, such as the use of drones, the use of robots to measure the degree of pest attack, the occurrence of diseases, the use of precision sensors and the use of bacterial biopreparations (bacterial based biofertilizers).

The replacement of chemical fertilizers with bacterial biopreparations was a great success in Romanian agriculture because plant-beneficial bacteria applied on the soil surface ensure a recolonization of the soil, a restoration of its texture and pH and an increase in agricultural production, these aspects representing an alternative to chemical fertilization technologies. At the same time, the growth and maximization of agricultural production has brought with it an additional profit for farmers as well as a growing demand for agricultural products obtained from these crops (Revillas et al., 2000).

The use of excess chemical fertilizers, the use of plant protection products in increasing doses has led to lower soil fertility, acidification and poor productivity of crops but especially to pollution of soil, groundwater and of the environment. The use of biological fertilizers based on live bacterial cultures have the role of reducing these effects given by chemical fertilizers as well as restoring nutrients from the soil, recolonizing the soil with beneficial bacteria and obtaining healthier production for humans and animals (Mahanty et al., 2017) .

The use of plant beneficial bacteria in agriculture have had the effect of stimulating the seed material and protecting it from soil pests, stimulating plant growth, growth and development of agricultural crops and obtaining a high yield of agricultural production. Bacteria play a role in the production of enzymes, proteins, acids and even the solubilization of insoluble compounds in the soil into soluble compounds. At the same time, the use of biopreparations in agriculture is a significant reduction in costs for farmers.

The microorganisms in the composition of organic fertilizers have the role of balancing the processes inside the soil, these bacteria through certain physico-chemical processes, provide nutrients for the growth, development and production of plants. The decomposition of insoluble elements into soluble elements will lead to an increase in soil nutrients, increased soil fertility and obtaining healthy crops, with a higher production than conventionally fertilized lots.

At the same time, on the research that was carried out on the corn and sunflower seeds (inoculation of seeds in bacterial cultures) it was found that the activity of bacteria from the Rom-Agrobiofertil NP product has a beneficial effect in the processes of stopping and creating a plant defence against *Tanymecus dilaticollis* attack.

Another important aspect in using bacterial cultures in Romanian agriculture it is the beneficial effect that they have on the ground. The use of the decomposer *Azospirillum lipoferum* aims to decompose insoluble compounds in the soil. The decomposition of these compounds will lead to the release of soluble compounds necessary for plant growth and development.

The soil becomes richer in the elements necessary for the plants (nitrogen, phosphorus, potassium, etc.). A large amount of elements will lead to a better assimilation of them by plants.

Soil microorganisms are an important component of agricultural ecosystems. These microorganisms play an important role in soil fertilization, plant growth, development and production, as well as maintaining a much healthier ecosystem.

The population of soil microorganisms includes bacteria, cyanobacteria, acetomycetes, fungi and a certain category of viruses. The most important microorganisms are rhizobacteria. These bacteria have the role of promoting plant growth (PGPR) as well as an effect of protecting plants against diseases and pests.

The use of chemicals (fertilizers, pesticides, herbicides, insecticides, etc.) has led to a decrease in the soil microbial community. As such, the entire soil ecosystem has been disrupted. This disorder has led to a low yield of agricultural production as well as favoring pest attack and disease.

An important aspect of chin is that certain bacteria (*Pseudomonas syringae*) have the ability to protect plants from frost by producing proteins (INA proteins, produced by INA genes). The use of these proteins in the plant cycle has the role of forming films called "antifreeze proteins" that have the role of protecting the plant from low soil temperatures and the environment (Sajid et al., 2016).

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