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INCREASING THE CHEMICAL PARAMETERS OF SOIL AND PHYSIOLOGICAL CHARACTERISTICS OF THE TOMATOES *ROMEC 554j* VARIETY BY EXTRARADICULAR TREATMENTS WITH THE INOCULUM OF CYANOBACTERIA AND MICROALGES

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Abstract. The results obtained regarding the use of inoculations with cyanobacteria and microalgae for soil enrichment in nitrogen and organic carbon in tomato culture have highlighted values that could recommend this method to increase soil fertility. Both in the control variant, and in the technologically fertilized version, the nitrogen content, as well as the other chemical components in the soil decreased, and in the variant with the cyanobacteria and microalgae inoculum, the amount of nitrogen, organic carbon and soil pH remained at almost constant values. The production results showed, in comparison with the control variant, production increases both in the technologically fertilized variant (603.66 g) and in the variant with the cyanobacteria and microalgae inoculum (532 g). The data on the quality of the fruit revealed that the fruits of the variant with the cyanobacteria and microalgae inoculum are sweeter, have a smaller amount of water, but also the acidity is lower, both compared to the control variant and to the chemically fertilized variant. The best bacterial detachment protocol for the following chemical treatment 0.5 % v/v Tween 20 + 3mM sodium pyrophosphate is 30 minutes of sonication.

Keywords: phototrophic, microorganisms, sandy soil, quality.

Rezumat. Creşterea parametrilor chimici ai solului şi a trăsăturilor fiziologice ale tomatelor, soiul *Romec 554j* prin tratamente extraradiculare cu inocul de cianobacterii şi microalge. Rezultatele obținute cu privire la folosirea inoculilor cu cianobacterii și microalge pentru îmbogățirea solului în azot și carbon organic, la cultura de tomate, au evidențiat valori care pot recomanda această metodă în creșterea fertilității solului. Atât în varianta martor, cât și în varianta fertilizată tehnologic, conținutul de azot, cât și celelalte componente chimice din sol au scăzut, iar în varianta cu inocul de cianobacterii și microalge, atât cantitatea de azot, carbonul organic și pH-ul solului au rămas la valori aproape constante. Rezultatele de producție au arătat, comparativ cu varianta martor, creșteri ale producției atât în varianta fertilizată tehnologic (603,66 g), cât și în varianta cu inocul de cianobacterii și microalge (532 g). Datele cu privire la calitatea fructelor au scos în evidență că, fructele din varianta cu inocul de cianobacterii și microalge sunt mai dulci, au o cantitate mai mică de apă, dar și aciditatea este mai scăzută, atât față de varianta martor, cât și față de varianta fertilizată chimic. 30 de minute este timpul optimizat de ultrasonicare corelat cu puterea generată de baia de sonicare (30 minute la 90% putere), pentru următorul tratament chimic 0,5% v / v Tween 20 + 3m M pirofosfat de sodiu.

Cuvinte cheie: fototrofe, microorganisme, sol nisipos, calitate.

INTRODUCTION

Climate change and land use are the main components of ongoing global environmental changes, which also act as major desertification factors, as defined by the United Nations Convention to Combat Desertification as Land Degradation (i.e. reduction or the loss of biological or economic productivity of the land) in the subhumid arid, semi-arid and dry areas (dry lands) resulting from various factors, including climatic variations and human activities (***. FAO, 2015).

In the context of the deterioration of the quality of agricultural areas, but also of their irrecoverable losses, it is necessary to establish new strategies for the preservation of agricultural land. Among them, the most important ones should present the following research directions: evaluation of the quality of the biological resources of the soils and of the state of degradation of the agricultural lands, evaluation of the anthropic action on the state of fertility of the soils appreciated with the help of biological indicators, establishment of improvement technologies regarding soil fertility conservation, etc. (AGUD, 2012).

The sandy soils of the southwest of Oltenia are poorly supplied with organic matter, nitrogen, phosphorus, potassium and have very high permeability, even excessive for water and air and a reduced water retention capacity. In order to obtain the most economically efficient productions, large quantities of chemical fertilizers were used which led to soil pollution (increase of nitrate content on soil profile and water from groundwater), as well as decrease of organic matter from soil. The tendency is to reduce soil and environment pollution, and to find methods of increasing soil fertility "as naturally as possible". The use of phototrophs (cyanobacteria and microalgae) can be considered an alternative way of increasing soil fertility and yields in sustainable agriculture.

Cyanobacteria are phototrophic prokaryotes encountered along with microalgae usually in a wide range of habitats on Earth, in temperate and extreme climates, in fresh and fresh waters and from soil to rocks (ROSSI & DE PHILIPPIS, 2015). Several authors have already described their ability to promote the stability of soil aggregates against moisture and physical force breakdown (ROGERS & BURNS, 1994; MALAM-ISSA et al., 2007). Also, the presence of cyanobacteria positively affects soil organic carbon level and influences water distribution (COLICA et al., 2014; SINGH et al., 2016; CHAMIZO et al., 2018; MUÑOZ-ROJAS et al., 2018).

Cyanobacteria together with microalgae aggregate the sand to form a dense and sticky network consisting of filaments and exopolysaccharides (EPS) that bond between the sand particles, forming cyanobacterial biocrusts (or algal biocrusts). The role of cyanobacteria from soil biocrusts in restoring soil functions of degraded land has been highlighted in recent years. These organisms are capable of improving soil structure and promoting N and C soil fixation.

Soil biological crusts (biocrusts) are communities of microscopic and macroscopic organisms that include cyanobacteria, algae, fungi, lichens and muscles that live in the upper layers of the soil (ELDRIDGE & GREENE, 1994; BOWKER et al., 2010; CHILTON et al., 2017). Soil biocrusts are present in most environments, but they are particularly important in dry areas, where they comprise much of the living (covering) of soils (BELNAP et al., 2003; CONCOSTRINA-ZUBIRI et al., 2014). Cyanobacteria are the oldest group of biocrusts and contribute to multiple ecosystem functions, including improvement soil structure, stability, fertility, surface hydrology and soil C and N cycles (CHAMIZO et al., 2012; ZHAO et al. 2014). Moreover, cyanobacteria can excrete exopolysaccharides (EPS) – specialized compounds that facilitate soil aggregation and stability and improve soil water infiltration (ROSSI et al., 2017; ADESSI et al., 2018).

The benefits of applying cyanobacteria together with microalgae as bio-tools to improve soil fertility and initiate the recovery of soil functions in infertile substrates can be substantial: reducing the costs associated with the contribution of external fertilizers, modifying the soil by reducing pollution, etc. (SINGH et al., 2016; MAESTRE et al., 2017). Moreover, cyanobacteria from soil biocrusts occur naturally in arid landscapes and, although in a dry and inactive state for several years, have the ability to react after rain (BELNAP et al., 2013; WILLIAMS et al., 2014; BÜDEL et al., 2018). Worldwide, the use of photosynthetic microorganisms (cyanobacteria and microalgae) in agriculture has been reported since 1936-1939 by the researcher De, in India, where rice crops recorded a high level through the use of these fertilizers. In Japan and China microalgae-based preparations have been used in rice cultivation (WATANABE 1959; MUZAFAROV 1967). Research has been reported in 2013-2014 in Egypt, where there have been increases in production of rice crops, and in recent years they have registered results in both India and Iran where semi-arid conditions have influenced the production of rice, which could be improved by using the inoculum of cyanobacteria and microalgae.

In our country, rigorous experiments were performed at the Research Institute for Cereals and Technical Plants Fundulea, where the results were not conclusive (NEGREANU et al., 1959). ELIADE G. et al., 1975, following research on tomatoes, showed an increase in productivity due to the use of a mixture of microorganisms (ammonifiers, nitrifiers, denitrifiers, cellulolytics, nitrogen fixers), which contributed to the increase of soil pH.

MATERIAL AND METHODS

At RDSPCS Dăbuleni, a study was initiated on the influence of the application of Cyanobacteria in soil, compared to technological fertilization in tomato culture *Solanum lycopersicum* L., *Romec 554j* variety. The experience was established using the randomized blocks method, in three repetitions. The surface of each experimental variant was 6.3 m^2 and a number of 9 plants were planted on each experimental variant. The tomatoes were sown in alveolar cubes filled with peat on 02.04.2019. Tomatoes were planted in the experimental field on 29.05.2019 at distances of 30 cm between plants per row and 70 cm between rows. Three experimental variants were taken into study, with the species *Solanum lycopersicum* L. (tomatoes). *Romec 554j* variety as cultivation plant:

- V1- Control;
- V2- Fertilization according to technology (N_{80} P_{150} K_{100});
- V3-Treated with cyanobacteria and microalgae inoculum.

The inoculum contains a mixture of photosynthetic microorganisms (both cyanobacteria and microalgae) together with their associated non-photosynthetic microorganisms, previously used in experiments concerning purification of artificial water from recirculation-based aquaculture systems (ARDELEAN et al., 2019) together with photosynthetic microorganisms grown from terrestrial habitats.

Phototrophic microorganisms (cyanobacteria and microalgae) were obtained in the Microbiology Laboratory of the Bucharest Institute of Biology and were inoculated in the soil as follows:

- the mixture of phototrophic microorganisms was filtered through coarse filter paper, previously weighed, and the wet weight was determined by weighing with a laboratory balance KERN EG type, three decimals (error +/- 0.01g), and 2 g of phototrophic microorganisms were inoculated into the soil for each plant.

- the amount of wet biomass was equally divided for each experimental variant.

The following observations and determinations were made:

- soil - the chemical composition of the soil at the beginning of the experiment. as well as at the end of the study;

The samples were collected at a depth of 0-50 cm, were recorded and conditioned in the laboratory, from which the following determinations were made:

1.- total nitrogen - Kjeldahl method;

2.- extractable phosphorus (P-AL) - Egner - Riem Domingo method, by which the phosphates are extracted from the soil sample with a solution of acetate - ammonium lactate at pH - 5.75, and the extracted phosphate anion is determined colorimetrically as blue molybdenum;

3.- exchangeable potassium (K-AL) - Egner - Riem Domingo method by which the hydrogen and ammonium ions of the extraction solution replace by exchange the exchangeable potassium ions in the soil sample which are thus passed into the solution. The potassium is determined in the solution thus obtained by flame emission photometry.

4.- organic carbon - wet oxidation and titrimetric dosing method (after Walkley-Blak in the Gogoașă modification);

5.- soil pH, potentiometric method

- to plants - biometric measurements on plants and fruits (plant height. number of shoots/plant number of flowers/plant. height of the fruit, diameter of the fruit, weight of the fruit);
- fruit production by variant - by weighing fruits by variant;
- the nutritional quality of the fruits:

1. water and total dry matter (TDM) (%) - gravimetric method;
2. soluble dry matter (SDM) (%) - refractometric method;
3. soluble carbohydrates (%) - Fehling Soxhlet method;
4. C vitamin (mg / 100g fresh substance) - iodometric method;
5. titratable acidity (tartaric acid per 100g of fresh substance) - titrimetric method.

The detachment of microorganisms from soil particles was carried out as follows: wet soil was mixed and diluted 100 times with sterile phosphate saline buffer (mL of buffer/g of wet soil) supplemented with 0.5 % v/v Tween 20 + 3 mM sodium pyrophosphate (EICHORST et al., 2015; MORONO et al., 2013) and vortexed for 1 minute (VELP SCIENTIFICA) fixed at 30 Hz.

This suspension was sonicated (SONOTEX DIGITAL 10 P BANDELIN) at 90% power for different periods of time (10 minutes, 30 minutes, 50 minutes and 70 minutes). After each period of sonication 2mL were transferred under axenic conditions and centrifugated (5 minutes at 310g). The resulting supernatant was used to prepare decimal dilutions in PB, 8 dilutions for each treatment.

For the determination of the number of colony forming units, the mini-drop method was used (HOBEN et al., 1982; MILES & MISRA, 1938); shortly, each Petri dish was divided into 4 zones, each one for a different decimal dilution (2 plates for each sample). Each of the 8 zones was inoculated (in triplicate) with 10µL of microbial suspension. The plates were incubated at 28^{o} C on a specific medium for aerobic heterotrophic nitrogen fixers (BOSTRÖM et al., 2007).

The colony-forming units were quantified based on the number of CFUs that can be appropriately counted using lens (10x magnification), and taking into account the decimal dilutions (HOBEN et al., 1982; MILES & MISRA, 1938). The recorded data were analysed and statistically processed. The results are preliminary and will continue in 2020.

RESULTS AND DISCUSSIONS

Under the conditions in our country, the soil is subjected to a strong anthropogenic action (use of fertilizers and pesticides, etc.), so that it is important to use new methods of increasing soil fertility and recent research has shown that the use of inoculants with cyanobacteria and microalgae for enrichment of soil in nitrogen and organic carbon could be one of these directions.

The application of chemical fertilizers and inoculation with cyanobacteria and microalgae influenced the chemical composition of the soil. Determinations were made at the establishment of the experience (May) and after fruit harvesting (September). The experiment was located on a soil with low nitrogen supply (0.12-0.13%) (Table 1). The extractable phosphorus showed values between 107 ppm and 114 ppm, values that characterize the soil as being well supplied with phosphorus according to the data in scientific literature. Good phosphorus nutrition leads to the development of a rich root system, having a positive influence on plant growth.

The exchangeable potassium content was between 25 ppm and 38 ppm. The values obtained characterize the soil with a very low supply state. The efficiency of potassium fertilizers is stronger than the one of nitrogen and phosphorus fertilizers. Organic carbon showed values between 1.10% - 1.22%, the state of supply of soil in organic matter being medium to normal supply. The pH of the soil on which the experiment was located ranged between 6.75 and 7.69 values showing a neutral reaction.

Table 1. Soil chemical composition of tomato crop at the beginning and end of the experiment 2019.

Variant	Total nitrogen (%)		Phosphorus extractable (ppm)		Potassium exchangeable (ppm)		Organic carbon (%)		pH in the water	
	May	September	May	September	May	September	May	September	May	September
Control	0.12	0.09	107	85	25	20	1.10	0.97	7.69	7.35
Technologically fertilized	0.13	0.11	114	92	38	35	1.22	1.12	6.75	6.30
Treated with cyanobacteria and microalgae inoculum	0.12	0.12	112	86	38	36	1.15	1.14	7.52	7.50

After fruit harvesting, the determinations highlight the effectiveness of the treatments with cyanobacteria and microalgae. Both in the untreated variant and in the technologically fertilized version., the nitrogen content as well as the other chemical components in the soil decreased and in the variant with the cyanobacteria and microalgae inoculum, both the amount of nitrogen, organic carbon and soil pH remained at almost constant values. The research will continue on another, much poorer sandy soil in organic matter and macroelements.

In the pedo-climatic conditions of 2019, the obtained results regarding the growth and fructification of the plants, as well as production, in the *Romec 554j* tomato variety highlighted differences between the variants taken in the study.

The results regarding the morphological investigations during the vegetation period are presented in table 2.

The values obtained regarding the average height of the plant showed values between 29.76 cm in the untreated variant and 35.36 cm in the technologically fertilized variant, according to the technology specific to tomato crops. Following the biometric measurements, the average height of the plant in the variant with the phototrophic inoculum (cyanobacteria and microalgae) registered a value of 30.23 cm compared to the untreated variant, with a value of 29.76 cm. It was noted that, for the technologically fertilized variant, the average height of the plant in the *Romec 554j* tomato variety has a distinctly significant difference of 5.6 cm compared to the average height of the plant in the untreated variant.

Table 2. Biometric measurements during the vegetation period in *Romec 554j* tomato variety.

Variant	Average height of the plant (cm)	The average number of shoots / plant
Control	29.76	2.1
Technologically fertilized	35.36^{xx}	3.3
Treated with cyanobacteria and microalgae inoculum	30.23	3.6
DL 5% =	2,97	6,36
DL 1% =	4,92	10,53
DL 0,1% =	9,21	19,71

From the point of view of the average number of shoots per plant, there were no significant differences between the technologically fertilized variant and the variant with the phototrophic inoculum (cyanobacteria and microalgae), corresponding to 2 g of wet living biomass/ variant compared to the untreated variant.

Plants in the variant with cyanobacteria and microalgae inoculum showed a faster growth in height, a higher number of shoots per plant than in the untreated variant. The amount of phototrophs (cyanobacteria and microalgae) in the soil influenced the number of shoots per plant.

Analysing the estimated production of the *Romec 554j* tomato variety (Table 3), differences were obtained between the variants used in the study, but which were not statistically relevant. Compared to the untreated variant, production increases were obtained both in the technologically fertilized variant (603.66 g) and in the variant with the cyanobacteria and microalgae inoculum (532 g). The presence of phototrophic microorganisms (cyanobacteria and microalgae) in the variant with inoculum is seen both by modifying the chemical composition of the soil and by increasing production. Phototrophic microorganisms (cyanobacteria and microalgae) may be a way of increasing soil fertility, a biological substitute for chemical fertilizers with nitrogen-based formulas.

Table 3. Results regarding the estimated production on variant in the *Romec 554j* tomato variety.

Variant	Estimated output per variant (g)
Control	324.66
Technologically fertilized	603.66
Treated with cyanobacteria and microalgae inoculum	532.00
DL 5% =	651,88
DL 1% =	1078,65
DL 0,1 % =	2018,95

Analysing the main physical properties of tomato fruit, respectively the average height of the fruit, the average diameter of the fruit and the average weight of the fruit, we could notice significant differences between the variant with the inoculum of phototrophic microorganisms (cyanobacteria and microalgae), having values of 5.58 cm, 50.32 mm, 79.8 g compared to the untreated variant, respectively the technologically fertilized variant with $N_{80}P_{150}K_{100}$ having values of 4.57 cm, 40.68 mm and 43.6 g (Fig. 1).

Although there were no statistically significant differences between the variants, inoculation with phototrophic microorganisms (cyanobacteria and microalgae) in the soil in tomato culture had a positive effect in increasing the size of tomato fruits.

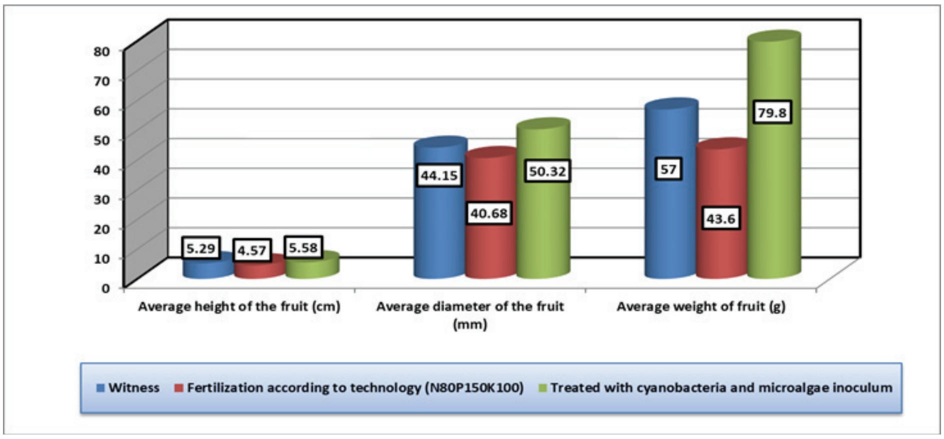

Figure 1. The physical properties of tomato fruits.

The results obtained regarding the nutritional quality of the fruits, based on the variants used for the study are presented in fig. 2. In the unfertilized variant, due to the earlier maturation of the fruits, the process of accumulating assimilates is more intense compared to the fertilized variants with chemical fertilizers and cyanobacteria and microalgae. The best results regarding the quality of the fruits were obtained in the chemically fertilized version with the dose of N_{80} P_{150} K_{100}, as well as in the variant in which cyanobacteria and microalgae were applied (7.75% total dry matter, 5.50% carbohydrates and 32.56 mg / 100 g fresh vitamin C).

It has been found that the fruits of the variant with the cyanobacteria and microalgae inoculum are sweeter, have a smaller amount of water, but also the acidity is lower, both compared to the untreated variant and to the chemically fertilized version.

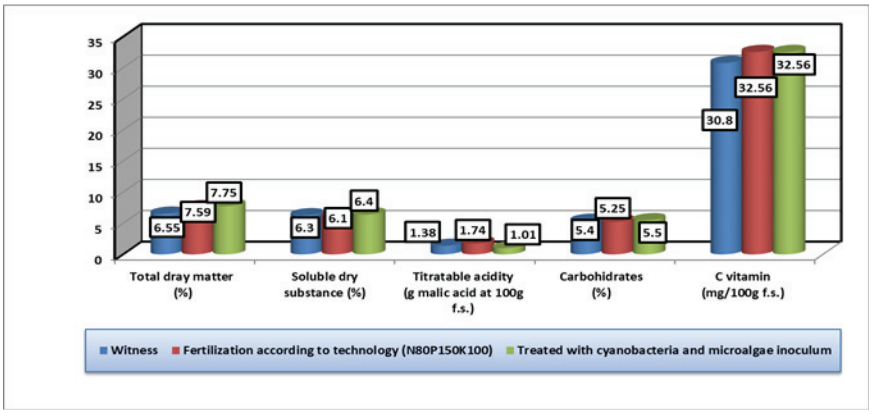

Figure 2. Chemical composition of tomato fruits according to the studied variants.

There is a close connection between living organisms, living conditions in the soil and plant nutrition. In the absence of proper microbiological activity, the soil does not provide the plants with enough nutrients, so low fertility occurs. The fact that the production results and the quality of the fruits are similar between the chemically fertilized variant and the one treated with the cyanobacteria and microalgae inoculum shows us the need to continue and deepen research on the use of cyanobacteria and microalgae in agriculture. Our results are in agreement with the already known role of microalgae and cyanobacteria in maintaining the soil health and fertility required for sustained agriculture (ABINANDAN et al., 2019).

As the occurrence of microbial cells in the soil is a very important biological parameter, their quantification should be done by appropriate and verified protocols, one essential step being the detachment of microbial cells from their substrate (MORONO et al., 2013).

Figure 3 presents results concerning experimentally determined CFU of heterotrophic aerobic nitrogen fixing microorganisms on the soil sample subjected to ultrasonication for different periods of time (see material and methods). The results are presented using the highest number of CFU as 100%, in order to comparatively present the effect of ultrasonication period on the detachment of viable cells (able to grow and multiply).

As one can see for the technologically fertilized soil sample from the culture of *Pisum sativum* suspended in phosphate buffer saline supplemented with 0.5 % v/v Tween 20 + 3m M sodium pyrophosphate, the highest number of CFU was obtained at 30 and 50 minutes of ultrasonication. The CFU counts are lower at shorter treatments (10 minutes) as an expression of inefficient microbial detachment. The results for longer treatments (70 minutes of ultrasonication) suggest that some cells, which are too vigorous, are killed (MORONO et al., 2013).

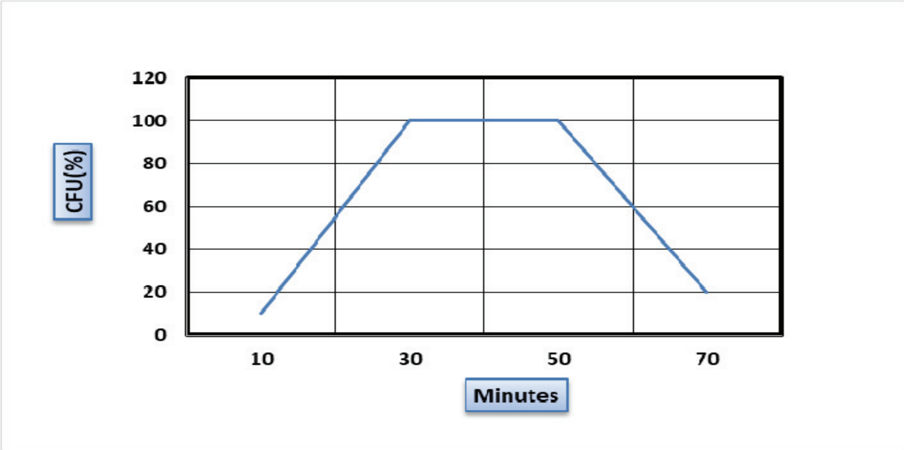

Figure 3. The effect of ultrasonication time on the detachment of microbial cells from the technologically fertilized soil from the culture of *Pisum sativum*.

These results represent a first step in establishing, for the concerned soil, a reliable protocol for cell detachment, a primary requirement for many methods in soil microbiology. These results suggest the need for further optimization of the detachment procedures, mainly with respect to chemical treatment, as well as the need to combine the determination of colony forming units with other (functional) methods in soil microbiology (PHILIPPOT et al., 2012).

CONCLUSIONS

- Both in the control variant, and in the technologically fertilized version, the nitrogen content, as well as the other chemical components in the soil decreased, and in the variant with the cyanobacteria and microalgae inoculum, both the amount of nitrogen, organic carbon and soil pH remained at almost constant values.
- The production results showed, in comparison with the control variant, increases in production both in the technologically fertilized variant (603.66 g) and in the variant with the cyanobacteria and microalgae inoculum (532 g).
- The data on the quality of the fruits revealed that the fruits of the variant with the cyanobacteria and microalgae inoculum are sweeter, have a smaller amount of water, but also the acidity is lower, both compared to the control variant and to the chemically fertilized variant.
- The results obtained regarding the use of inoculations with cyanobacteria and microalgae for soil enrichment in nitrogen and organic carbon in tomato culture have highlighted values that could recommend this method to increase soil fertility.
- The best bacterial detachment protocol, for the following chemical treatment 0.5 % v/v Tween 20 + 3m M sodium pyrophosphate, is 30 minutes of sonication.

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