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THE EFFECT OF CHLORELLA SOROKINIANA UTEX 1230 AS A BIOFERTILIZER ON TOMATO CULTURE ROMEC 554J VARIETY CULTIVATED ON SANDY SOILS AT RDSPCS DĂBULENI

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Current Trends in Natural Sciences

Abstract
In the context of climate change, the use of microalgae is an alternative to meet the requirements of agriculture, food and renewable energy. A major area of application is the use of microalgae as biofertilizers in agriculture.
The aim of this paper is to study the influence of the application of Chlorella sorokiniana UTEX 1230 inoculum on the evolution of soil chemical composition, as well as the ability of microalgae, as a biofertilizer, on the growth and development of tomato plants, including the influence on plant physiological indices. Ground determinations, at 60 days after application of Chlorella sorokiniana UTEX 1230 inoculum indicated higher values of nitrogen, phosphorus, potassium and organic carbon content in the soil, The results regarding the growth and development of the plants obtained in the protected area (solar greenhouse), for the Romec 554j tomato variety showed differences between the variants studied. The Romec 554j tomato variety recorded a higher rate of photosynthesis in the version treated with the inoculum of Chlorella sorokiniana UTEX 1230, compared to the control version and the technologically fertilized version (12,66 µmol CO_2 / m^2 / s, compared to 11,82 µmol CO_2 / m^2 / s in the control version and 12,48 µmol CO_2 / m^2 / s in the technologically fertilized version), but the values were not statistically assured.

Keywords: biofertilizer, Chlorella sorokiniana UTEX 1230, soil sandy.

1. INTRODUCTION

From the study of climate change it is estimated that higher temperatures and increasing their frequencies, as well as the duration of drought during the current century, will have a negative impact on agricultural production (Edgerton, 2009). In the context of climate change, higher temperatures associated with water deficit will affect plant productivity (Dima et al., 2020). In order to promote a sustainable agriculture system on sandy soils, the choice of species and varieties with great adaptability to climatic and soil conditions is necessary to obtain a high, safe and stable production in areas affected by drought (Draghici et al., 2016).
Crops are not adapted to extreme climatic conditions, and applied management cannot meet current requirements. Thus, other ways must be found to be able to increase the resistance of plants to

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stressors and to increase production to meet the food demand of the population. Microalgae extracts represent a major opportunity in plant growth and development and for future crop production.

A potential way to reduce the negative impact on the environment resulting from the continued use of chemical fertilizers is to promote inoculation with microorganisms to improve its fertility (Choudhury & Kennedy, 2005; Rai, 2006; Renuka et al., 2018; Guo et al., 2020; Nosheen et al., 2021), and microalgae may be useful in this regard. Thus, in order for agriculture to fully exploit the biological benefits of microalgae extract/ biocrusts, a major interdisciplinary research effort will be needed to elucidate their complex modes of action and applications in different crops and in different production environments.

Microalgae play a vital role in fixing carbon and nitrogen, stabilizing the soil and altering the hydrological properties (e.g., water retention) of biocrust-coated soils in arid and semi-arid environments (Evans & Johansen, 1999; Belnap & Lange, 2003). Therefore, the activity of microalgae that improve soil functions and properties could be developed and exploited through applications of biofertilizers containing live algae biomass. In addition to their soil health-enhancing functions, soil applications of microalgae can also be used to attenuate or sequester atmospheric carbon dioxide.

Microalgae-based biostimulators have a high efficiency, complete biodegradability, without phytotoxicity, which makes them promising products for agricultural plants (Kim & Chojnacka, 2015). These can increase seed germination, plant growth and development, flowering and fruit production, and shelf life after harvest (Khan & Rayirath, 2009; Joana Gil-Chavez et al., 2013; Calvo et al., 2014). Examples of commercially available based on microalgae products for agriculture have already been reported (Michalak & Chojnacka, 2016).

The use of microalgae can be a component of organic agriculture, and can contribute to increasing soil fertility over a longer period of time and achieving high yields (Kumar et al., 2015; Grzzesik & Romanowska-Duda, 2015; Ronga et al., 2019; Rumin et al., 2020; Martínez-Dalmau et al., 2021; Gonçalves, 2021).

Soil microalgae eliminate bioregulators that favor plant growth and production (Osman et al., 2010; Maqubela et al., 2012; Awale et al., 2017).

Of all the microalgae, the genus *Chlorella* has been the most widely used for biofertilization to date and was the first microalgae to be cultivated (Wijffels, 2013*). Chlorella sp.* provides large amounts of macro and micronutrients, constituents or metabolites, such as carbohydrates and proteins (Elarroussia et al., 2016) and growth-promoting factors, such as cytokines (Kholssi et al., 2018).

Research conducted by Ozdemir et al., (2016), showed that the use of microalgae as biofertilizers in the cultivation of tomatoes in greenhouses, led to a better growth of plants and fruit production. Also in the fruit was determined a higher content of dry matter, C vitamin and organic acids.

Grzzesik & Romanowska-Duda, (2015), showed that the faster development of plants and their resistance to diseases and pests, following treatments with *Chlorella* may be due to bioactive compounds left in the soil, which activate plant metabolism.

The influence of microalgae on seed germination and plant growth may be due to the positive effect of *Chlorella* on nutrient absorption, which influences physiological reactions (photosynthesis and transpiration) in plants (Grzzesik & Romanowska-Duda, 2014; Ghiloufi et al., 2016; Borchhardt et al., 2017).

According to international research, the application of inoculum amounts of *Chlorella sorokiniana* has positive effects on the growth of crop plants. Biofertilizers from these microalgae can be an alternative to mineral fertilization, and organic farming can become a major segment in achieving

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healthier crops, ensuring food security and environmental protection. These statements are supported by numerous recent research: Kholssi et al., 2019; Ronga et al., 2019; Flavio Martini et al., 2020; Supraja and Behera 2020; Kumbhar et al., 2020; Thi Cam Van Do et al., 2020; Rachidi et al., 2021.

In order for agriculture to benefit from the use of microalgae extracts in the future, well-developed techniques (technologies) must be established, based on rigorous research.

The research focused on the influence of the application of *Chlorella sorokiniana UTEX 1230* inoculum as a biocrust on the evolution of soil chemical composition, as well as the ability of microalgae, as a biofertilizer, on the growth and development of tomato plants, including the influence on plant physiological indices.

2. MATERIALS AND METHODS

At RDSPCS Dăbuleni, a study was initiated on the influence of the application of *Chlorella sorokiniana UTEX 1230* biocrust in the soil improvement process, as well as the role of microalgae in the growth and development of tomato plants *Solanum lycopersicum L*., *Romec 554j* variety**.** The experiment was set up in a protected space (solar greenhouse) according to the method of randomized blocks, in three repetitions. The surface of each experimental variant was 6,3 m^2 and a number of 12 plants were planted on each experimental variant**.** The tomatoes were sown in alveolar cubes filled with peat on June 15. 2021 and were planted in the experimental field on August 17. 2021, at a distance between plants, 30 cm in a row and 70 cm between rows. Three experimental variants were studied, having as culture plant the species *Solanum lycopersicum* L., (tomato), *Romec 554j* variety:

-V1- control;

-V2- treated with the inoculum of *Chlorella sorokiniana UTEX 1230;*

-V3- fertilization according to tomato cultivation technology with $N_{80}P_{150}K_{100}$.

In the Microbiology Laboratory of the Bucharest Institute of Biology, a protocol was developed in order to obtain biocrust based on the green microalga *Chlorella sorokiniana UTEX 1230* (Figura 1), already cited in the literature for biotech crust biotechnology (Kholssi et al., 2019), The algal biomass of *Chlorella sorokiniana UTEX 1230* was inoculated into the soil on 02.07.2021. The amount of wet biomass was divided equally into the three repetitions.

The components of such a product can influence the development processes of cultivated plants, but also serve as indirect factors as fertilizers, having an effect on soil fertility or the development of soil microorganisms, involved in humidification processes.

Thus, microalgal biomass is considered an efficient source of bio-fertilizer to improve the physico-chemical characteristics of the soil, such as water retention capacity and nutrient mineral composition in degraded arable lands.

The following observations and determinations were made:

- in the soil - the chemical composition of the soil 60 days after the application of the inoculum.

The depth of sampling was 0-30 cm, and after harvesting the samples were recorded and dried in laboratory conditions. The determinations performed on the ground were the following (after Rauta and Chiriac, 1980):

- the nitrogen content of the soil, which was determined by the Kjeldahl method;
- the content of extractable phosphorus (P-AL) and exchangeable potassium (K-AL) which was determined by the Egner - Riem Domingo method;

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- the content of organic matter (organic carbon) in the soil, which was achieved by the method of wet oxidation and titrimetric dosing (after Walkley-Blak in the modification Gogoaşă);
- soil reaction (pH in water) was determined by the potentiometric method.

-to plants:
- biometric measurements on plants (plant height, number of shoots and inflorescences / plant);
- determination of the physiological processes of photosynthesis and perspiration with the portable device LC pro SD.

The recorded data were analyzed and statistically processed after Saulescu and Saulescu (1967).

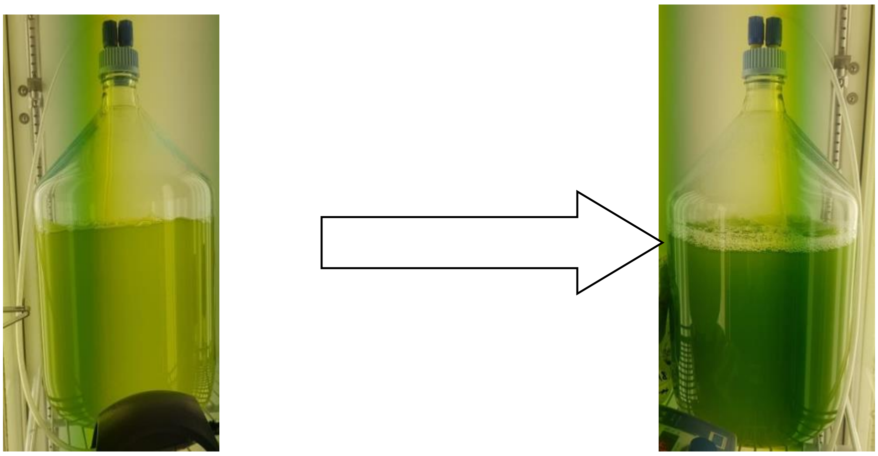

Figure 1. Aspects regarding the inoculum of Chlorella sorokiniana UTEX 1230

3. RESULTS AND DISCUSSIONS

In the current conditions, in which the climate undergoes dramatic changes by increasing air and soil temperature, increasing CO_2 emissions, soil and water pollution with fertilizer and pesticide residues, etc., the use of microalgae as biofertilizers in agriculture offers a solution to deal with these challenges (Chatzissavvidis and Therios, 2014). Research conducted in this field is very numerous and highlights the positive role of microalgae on increasing the fertility of degraded soils, as well as on plant growth (Li et al., 2017; Renuka et al., 2018; Guo et al., 2020; Nosheen et al., 2021).

These statements support the study conducted at RDSPCS Dăbuleni, on sandy soils, which in the current conditions of agricultural practices, need new sources of nutrients and organic carbon.

The determinations performed on the soil, 60 days after the application of the inoculum of *Chlorella sorokiniana UTEX 1230* and the dose of technological fertilization, indicated higher values of nutrient content and organic matter in the soil.The results obtained are presented in table 1.

The nitrogen content was between 0.12% and 0.17%, values that indicate a medium to well-supplied soil supply status according to the data in the literature (table 2). The highest value was obtained in the version treated with the inoculum of *Chlorella sorokiniana UTEX 1230* (0.17%), 0.05% higher than the control version and 0.03% higher compared to the technologically fertilized version $N_{80}P_{150}K_{100}$.

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Table 1. Influence of the application of Chlorella sorokiniana UTEX 1230 inoculum on the chemical composition of the soil in tomato culture

Variant	Total nitrogen (%)	Phosphorus extractable (P-AL) (ppm)	Potassium exchangeable (K-AL) (ppm)	Organic carbon (%)	pH in the water
Control	0.12	77	25	2.12	7.34
Treated with the inoculum of *Chlorella sorokiniana UTEX 1230*	0.17	81	39	3.04	7.20
Fertilization according to technology ($N_{80}P_{150}K_{100}$)	0.14	96	45	2.41	6.41

The much higher value of the nitrogen content in the version with the inoculum of *Chlorella sorokiniana UTEX 1230* may be due to the fact that microalgae can synthesize the enzyme nitrogenase and can fix nitrogen.

Chen et al., (2014) showed that microalgae can develop a category of undivided cells, called heterocysts and that would contain the enzyme dehydrogenase, with a role in nitrogen fixation.

Fixing nitrogen in the soil, without mineral fertilization, is a major lever for agriculture, which can lead to a decrease in the amount of chemical fertilizers applied in the soil. In this way, leachate losses on the soil profile will be avoided. These losses can be both economical and also reduce environmental pollution (Martínez-Dalmau et al., 2021).

Table 2. Characterization of the state of soil supply with the main macroelements and organic matter (Davidescu, 1981; Răuță and Chiriac, 1980)

Supply status	Total nitrogen (%)	Phosphorus extractable P-AL (ppm)	Potassium exchangeable K-AL (ppm)	Organic carbon (%)
low	< 0.10	8.1 - 18	< 66	< 0.58
medium	0.11 - 0.15	18.1 - 36	66.1 - 132	0.59 - 1.16
normal	0.16 - 0.20	36.1 - 72	132.1 - 200	1.17 - 2.32
very good	0.21 - 0.30	72.1 - 144	200.1 - 400	2.33 - 4.64
very high	>0.30	>144	>400	-

After, more research conducted by Nabti et al., (2017), Renuka et al., (2018), Martínez-Dalmau et al., (2021), the ability to fix nitrogen in the inorganic form in the air and the possibility of transforms into organic form in the soil, which can be used by plants, it was estimated that it would reduce by 25-40% the amount of chemical fertilizers based on nitrogen, and according to Veluci et al., (2006) the natural fixation of nitrogen with the help of microalgae can limit the impact of nitrogen on the environment and human health.

Regarding the content of extractable phosphorus and exchangeable potassium, the values obtained indicate a higher content both in the version treated with the inoculum of *Chlorella sorokiniana UTEX 1230* and in the technologically fertilized version, compared to the control variant (table 1).

Organic carbon showed values in the range of 2.12% in the control version and 3.04% in the version treated with the inoculum of *Chlorella sorokiniana UTEX 1230*, the state of soil supply in organic matter being much better in the version with microalgae.

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One of the important factors for the establishment and development of plants in the soil is their content of organic matter. Manure, green manure or various types of sludge are usually used to grow organic matter in the soil, which is an economic problem for farmers. Singh and Ahluwalia, (2013) highlighted the role of seaweed and microalgae in the accumulation of carbon in the soil, which can then be used by plants. Yilmaz and Sönmez, (2017), Gonçalves, (2021) obtained similar results.

Sustainable agriculture can only be achieved by increasing soil fertility with renewable resources and reducing the amount of chemical fertilizers.

The pH of the soil on which the experiment was placed ranged from 6.41 in the technologically treated version to 7.20 in the *Chlorella sorokiniana UTEX 1230* inoculated version, values that show a neutral reaction. There is a tendency to decrease the pH values, in the version technologically treated with mineral fertilizers ($N_{80}P_{150}K_{100}$).

Microalgae activate in the upper crust of the soil, and the amount of exopolysaccharides in their biomass (about 25%) can act as a real gluing agent on soil particles. This process leads to soil aggregation, increasing the amount of organic carbon as well as water retention capacity (Malamlssa et al., 2001).

The results regarding the morphological investigations, during the vegetation period are presented in table 3. The values obtained regarding the average height of the plant, showed values between 57.54 cm in the control variant and 61.75 cm in the variant treated with inoculum of *Chlorella sorokiniana UTEX 1230*. Following the biometric measurements, the average height of the plant from the technologically fertilized variant according to the specific technology for tomatoes recorded a value of 59.72 cm. The variant treated with the inoculum of *Chlorella sorokiniana UTEX 1230* was noticed, in which the average height of the plant is 61.75 cm, higher than the control variant (57.54 cm), with a difference of 4.21 cm, difference not assured from the point statistically.

Table 3.Influence of the application of Chlorella sorokiniana UTEX 1230 inoculum on biometric measurements during the period of intense plant growth in Romec 554j tomato varieties

Variant	Average plant height (cm)	Average number of shoots / plant	Average number of inflorescences / plant
Control	57.54	8.33	11.25
Treated with the inoculum of *Chlorella sorokiniana UTEX 1230*	61.75	9.83	14.42
Fertilization according to technology ($N_{80}P_{150}K_{100}$)	59.72	9.21	13.92
DL5% =	7.70	4.70	11.07
DL 1%=	12.75	7.77	18.31
DL 0.1%=	23.86	14.55	34.28

Various researches have shown that microalgae are a source of natural nutrients, able to achieve plant stimulation and/or biocontrol effects of pests and pathogens. Among these we list the research conducted by Nabti et al., (2017), Righini et al., (2019), Ronga et al., (2019) Stirk et al., (2020), etc. Polysaccharides produced by microalgae have biostimulating activities for plant growth, nutrient absorption and tolerance to biotic and abiotic stress (Zou et al., 2019).

From the point of view of the number of shoots and inflorescences per plant, there were no statistically significant differences between the three variants studied, but the highest values were

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obtained in the variant treated with inoculum *Chlorella sorokiniana UTEX 1230* (9.83 average number of shoots and 14.42 average number of inflorescences). Aspects from the experiment are shown in Figure 2. Intense plant growth can be observed in the inoculum treated with *Chlorella sorokiniana UTEX 1230* inoculated shortly after planting (figure 2.a).

a ***b*** ***c***

Figure 2. Aspects of the experience, a- tomato plants 10 days after planting, b and c -tomato plants in the treated version with the inoculum of Chlorella sorokiniana UTEX 1230

Garcia-Gonzalez and Sommerfeld, (2016) following a study, which tested cell extracts and dried seaweed biomass as foliar fertilizer to observe plant growth and fruit production in tomatoes. Aqueous extracts applied as foliar fertilizers at 3.75 g/ml led to an increase in plant height and a greater number of flowers and branches per plant.

International researchers say that of all the microalgae in the genus *Chlorella*, *Chlorella sorokiniana* has been the most widely used so far for soil biofertilization and was the first cultivated microalgae. This species has been used as a biofertilizer in the production of organically grown tomatoes in a protected area (solar). Tomato plants grew much faster and were resistant to diseases and pests, which showed that the genus *Chlorella* is a very good source of bioactive compounds that can influence plant metabolism by controlling growth, development (Grzzesik & Romanowska-Duda, 2015), as well as the improvement of some physiological indices.

The *Romec 554j* tomato variety recorded a higher rate of photosynthesis in the version treated with the inoculum of *Chlorella sorokiniana UTEX 1230*, compared to the control version and the technologically fertilized version (12.66 µmol CO_2 / m^2 / s, compared to 11.82 µmol CO_2 / m^2 / s in the control version and 12.48 µmol CO_2/ m^2 / s in the technologically fertilized version), but the values obtained are not ensured from a statistical point of view (table 4). In the case of soil inoculated with *Chlorella sorokiniana UTEX 1230*, the value of the transpiration rate was 2.20 mmol H_2O / m^2 / s being statistically significant as negatively significant.

Plant photosynthesis and transpiration were higher after flowering phenophase in tomato plants, compared to the values of physiological processes in seedling phenophase (Sfîrloagă et al., 2021). Numerous researches have shown that by applying mycalgae extracts on the ground, or foliar to different plant species, higher values of physiological indices in plants have been obtained.

Exogenous application of microalgae extract has led to an increase in chlorophyll content, photosynthesis and sweating rates in apple leaves (Spinelli et al., 2009). The increase in chlorophyll content in the leaves could be caused by the higher amount of nitrogen assimilated by microalgae

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and delivered to plant tissues (Spiller and Gunasekaran, 1990; Nilsson, 2005; Karthikeyanb et al., 2007).

Table 4. Influence of soil Chlorella sorokiniana inoculum on some physiological properties of tomato plants

Variant	Rate of photosynthesis ($\mu mol\ CO_2/m^2/s$)	Rata of transpiration ($mmoli\ H_2O/m^2/s$)
Control	11.82	2.93
Treated with the inoculum of *Chlorella sorokiniana UTEX 1230*	12.66	2.20^0
Fertilization according to technology ($N_{80}P_{150}K_{100}$)	12.48	2.32
DL 5%=	6.29	0.70
DL 1%=	10.41	1.16
DL 0,1% =	19.48	2.16

This biofertilization increased the stability of the cytomembranes, the chlorophyll content, the intensity of net photosynthesis, transpiration and the decrease of the intercellular concentration of CO_2.
A positive polynomial correlation was established between the average plant height and the plant photosynthesis rate, given by a second-degree equation, with a significantly positive correlation factor r = 0.97 ** (figure 3). Plant photosynthesis intensifies with increasing plant height to 61-62 cm.

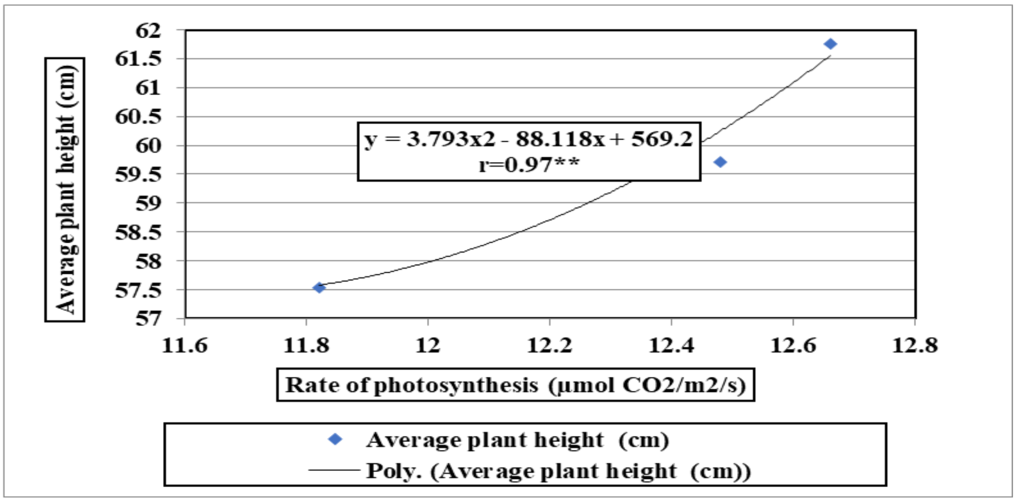

Figure 3. Correlation between average plant height and rate plant photosynthesis

Fernández-Calleja M. et al., (2020) showed that microalgae treatments induced a more efficient heat dissipation compared to the control variant. It has been previously reported that the difference in temperature of the leaves is directly related to the intensity of the physiological indices of the plant. The increase of the formation of the roots, as well as of the plants in the variants treated with microalgae cultures allows thus a more intense photosynthesis, a more intense opening of the stomata, as well as a more efficient perspiration.

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4. CONCLUSIONS

1.Determinations carried out in the soil 60 days after application of the inoculum of *Chlorella sorokiniana UTEX 1230* indicated higher values of the content nitrogen, phosphorus, potassium and organic carbon in the soil.

2.The highest value of total nitrogen in the soil was obtained in the inoculated version of *Chlorella sorokiniana UTEX 1230* (0.17%), 0.05% higher than the control variant and 0.03% higher compared to the technologically fertilized version $N_{80}P_{150}K_{100}$.

3.With regard to the extractable phosphorus and exchangeable potassium content, the values obtained indicate a higher content both in the version treated with *Chlorella sorokiniana UTEX 1230* inoculum and in the technologically fertilized version, compared to the control version.

4.The organic carbon showed values in the range of 2.12% in the control version and 3.04% in the version treated with the inoculum of *Chlorella sorokiniana UTEX 1230*, the state of soil supply in organic matter being much better in the version with microalgae.

5.The results regarding the growth and development of the plants obtained in the protected area (solar greenhouse), for the *Romec 554j* tomato variety showed differences between the variants studied.

6.The values obtained regarding the average height of the plant showed values between 57.54 cm in the control variant and 61.75 cm in the one treated with the inoculum of *Chlorella sorokiniana* with a difference of 4.21 cm, difference not assured from the point of view statistical.

7.In terms of the number of shoots and inflorescences per plant, there were no statistically significant differences between the three variants studied, but the highest values were obtained in the treated version. with the inoculum of *Chlorella sorokiniana UTEX 1230* (9.83 average number of shoots and 14.42 average number of inflorescences).

8.The *Romec 554j* tomato variety had a higher rate of photosynthesis in the version treated with the inoculum of *Chlorella sorokiniana UTEX 1230*, compared to the control version and the technologically fertilized version (12.66 µmol CO_2 / m^2 / s, compared to 11. 82 µmol CO_2 / m^2 / s in the control version and 12.48 µmol CO_2 / m^2 / s in the technologically fertilized version), but the values were not statistically assured.

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