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PHOTOSYNTHETIC INOCULANTS FOR PROMOTING SEED GERMINATION AND DEVELOPMENT OF TOMATO PLANTS

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Abstract

Microalgae and cyanobacteria are a potentially sustainable alternative for the improvement and protection of agricultural crops. Mineral fertilizers raise environmental and health issues. and current agricultural practices depend to a large extent on the application of fertilizers and pesticides.

In this study. we examined the effect of cyanobacteria and microalgae on tomato seed germination. seedling growth. green and dry biomass. gas exchange from leaves (plant photosynthesis and transpiration). and the chlorophyll content of leaves (chlorophyll a. b and carotene). as well as on the soil respiration process.

The obtained results highlight higher values of green and dry biomass in the variants inoculated with cyanobacteria and microalgae compared to the control variant and comparable to the values obtained in the technologically fertilized variants. Tomato seedlings showed higher photosynthesis values compared to the control and compared to the technologically fertilized variant in the soil of the pea plot (7.06 µmol $CO_2 / m^2 / s$ compared to 6.33µmol $CO_2 / m^2 / s$ in the control variant and 7.06 µmol $CO_2 / m^2 / s$ in the technologically fertilized version). And in the case of the soil in the museum plot, the values of photosynthesis in the version with the inoculum of cyanobacteria and microalgae were statistically assured compared to the control.

Keywords: biofertilizers, germination, the inoculum.

1. INTRODUCTION

Society faces a challenge, not only to increase agricultural production amid threatening global climate change, to reduce crops in many parts of the world, but also to develop innovative technologies that increase agricultural yields, minimize incomes and discourage further environmental pollution (Tilman et al., 2002; Foley et al., 2011).

Excessive use of synthetic agrochemicals has led to massive ecological degradation worldwide, leading to dead ocean areas, eutrophication, soil infertility and biodiversity loss (Köhler & Triebskorn, 2013; Chagnon et al., 2014; Hallmann et al., 2014; Van der Sluijs et al., 2014).

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The use of microalgae and cyanobacteria as biofertilizers offers a possible solution. The use of algae in agriculture dates back thousands of years (Dmytryk & Chojnacka, 2018; Górka et al., 2018): for example, in coastal areas of Europe, farmers applied algae harvested near the shore in their crops, both directly and indirectly, after composting, observing positive effects in soil fertilization. From this period, algae biomass was widely used in agriculture, but in the twentieth century, products obtained from algae extracts attracted the attention of farmers around the world (Dmytryk & Chojnacka, 2018).

In search of more sustainable and environmentally friendly solutions to improve agricultural productivity, researchers have focused on organic products. with microalgae and cyanobacteria emerging as a valuable resource for crop production and protection due to their potential for biofertilization and biostimulation (Pathak et al., 2018; Chaiaese et al., 2018; Dmytryk & Chojnacka, 2018; Pan et al., 2019; Gorka et al., 2018).

Biofertilizers are considered to be an ecological, cost-effective and sustainable alternative to synthetic fertilizers, because they not only increase agricultural production, but also reduce environmental pollution (Kawalekar, 2013). Biofertilizers are products that contain living microorganisms or natural compounds derived from organisms such as bacteria, fungi and algae that improve the chemical and biological properties of the soil. stimulate plant growth and restore soil fertility (Abdel-Raouf et al., 2012).

Given that microalgae and cyanobacteria contain high levels of micronutrients and macronutrients essential for plant growth, they have a potential application as biofertilizers. Several studies have established an association between higher nutrient uptake, higher biomass accumulation, and higher crop yields when incorporating microalgae biofertilizers (Shaaban, 2001; Faheed & Abd-El Fattah, 2008).

Following studies that have investigated the effects of algae extracts on seed germination have shown faster germination and higher growth of rice seeds (Shukla & Gupta, 1967). Other more recent studies have obtained similar results using seaweed extracts on tomato and wheat seeds, although they have also observed growth inhibition with increasing extract concentrations (Kumar & Sahoo, 2011; Kumari et al., 2011; Hernández-Herrera et al., 2013).

Microalgae and cyanobacteria are an important source of biologically active compounds, such as phenolic compounds, polysaccharides, hormone-like substances and proteins, known for their benefits as antioxidants, plant growth promoters, among others. Moreover, living organisms, both prokaryotes (e.g., nitrogen-fixing cyanobacteria) and eukaryotes (e.g., microalgae and macroalgae / seaweeds), are widely recognized for their role in soil fertilization and plant growth stimulation (Chaiaese et al., 2018).

It has been shown that cell extracts and the growth environment of several species of microalgae contain phytohormones (gibberellins, auxins and cytokinins), which are known to play a crucial role in plant development (Tarakhovskaya et al., 2007).

In the context of the depreciation of the quality of agricultural areas, but also of their irrecoverable losses, it is necessary to establish new strategies for preserving agricultural land. Among these, the

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most important should present the following research directions: evaluation of the quality of soil biological resources and the state of agricultural land degradation, evaluation of anthropogenic action on soil fertility assessed with the help of biological indicators. establishment of improvement technologies on preservation of soil fertility, etc. (Agud, 2012).

The use of microalgae / cyanobacteria biomass (or their extracts) can be a sustainable and viable alternative to chemical fertilizers, pesticides and growth stimulants. In addition, the use of these biologically based substances / organisms is an important step towards improving agricultural productivity, which is essential for meeting the growing food needs of the world's population.

The aim of this paper is to continue our research on the use of biocrust formation with selected consortia of cyanobacteria and green micralgae (Sfîrloagă et al., 2020) to stimulate tomate growth and to introduce for the first time in our scientific work, following pioneering national work (Eliade et al., 1975) in agreement with international trends (Sandor, 2010) the quantification of endogenous soil respiration.

2. MATERIALS AND METHODS

Previous research has shown that cyanobacteria and microalgae have been used as biological fertilizer, and may be an increasingly viable alternative to the problems caused by climate change (Aladhali, 2021).

Aladhali, 2021 showed that cyanobacteria and microalgae have contributed to the accumulation of nutrients and organic matter in the soil, but at the same time, help to fix them in the plant.

At RDSPCS Dăbuleni, a study was initiated on the influence of the application of cyanobacteria and microalgae in the soil improvement process, as well as their role in the germination of *Romec 554j* tomato seeds.

The experience was bifactorial, with the following factors:

Factor A - soil type;

A1- soil from the pea plot (improved soil type);

A 2- soil from the museum plot (type of sandy soil in natural state).

Factor B- Treatments performed:

V1- Soil from the pea plot Control;

V2 - Soil from the pea plot fertilized according to technology (N₈₀ P₁₅₀ K₁₀₀);

V3- Soil from the pea plot treated with the inoculum of cyanobacteria and microalgae;

V4- Soil from the museum plot Control;

V5- Soil from the museum plot fertilized according to technology (N₈₀ P₁₅₀ K₁₀₀);

V6- Soil from the museum treated with the inoculum of cyanobacteria and microalgae.

The experiment was placed inside the Biochemistry Laboratory. in rectangular plastic containers. with a volume of 4 l, which were filled with the 2 types of soil and the treatments from the established variants were performed. Tomatoes of the *Romec 554j* variety were sown in pots.

During the period of seedling growth, the following observations and determinations were made:

- for plants - biometric measurements on plants (seedling length in cm);

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- determination of wet biomass by weighing on the KERN type electronic balance;

- determination of dry biomass by gravimetric method (drying at 105 0 C), using a *BINDER* type thermoregulation oven;

- in leaves - determination of chlorophyll a + b and carotenoid pigments by spectrophotometric method using a *Metertek SP-830 Spectrophotometer*;

- determination of the physiological processes of photosynthesis and perspiration with the portable device *LC pro SD*;

Endogenous soil respiration measurement was done following (Gupta & Singh.1977). After the titration, it is possible to calculate the released CO_2 with the next equation:

$$RCO2 = 2.2 (Vb - Vp)/m_s w_{sd}$$

Where:

- RCO₂ is the rate of CO₂ evolution on a dry soil (mgCO₂ g -1);
- Vb is the volume of HCl consumed in the control (ml);
- Vp is the volume of HCl consumed in the test sample (ml);
- ms is the mass of the soil sample (g);
- 2.2 is a factor (1 ml of 0.1 molar HCl corresponds to 2.2 mg of CO₂) (mg ml-1).

3. RESULTS AND DISCUSSIONS

Microalgae and cyanobacteria are an important source of a wide variety of bioactive compounds that can regulate several plant response mechanisms: improving soil quality and plant nutrition protecting plants against biotic and abiotic factors and stimulating growth.

The biocrust of microorganisms has a unique potential to increase productivity in a variety of agricultural and ecological situations and plays an important role in the development of soil fertility, thus increasing yield. The use of cyanobacteria and microalgae as a soil additive has been shown to significantly improve germination, nitrate reduction potential, root volume, chlorophyll formation, carotenoid accumulation, plant yields, dry shoot weight and plant height (Tripathi et al., 2008 ; Lin et al., 2013; Odjadjare et al., 2017).

In the soil variants from the pea parcel, the best results regarding the green and dry biomass were obtained in the version with cyanobacteria and microalgae (0.73 g respectively 0.05 g)(Table 1). In the case of the soil in the museum plot (sandy soil in natural state) the values of green and dry biomass presented statistically assured values as significant in the technologically fertilized variant (1.11 g respectively 0.10g compared to the control variant 0.66 g respectively 0.05 g).

Tomato seedlings (seedlings) showed higher values of photosynthesis compared to the control variant and the technologically fertilized variant in the soil of the pea plot (7.06 μ mol CO₂ / m² / s compared to 6.33 μ mol CO₂ / m² / s in the control variant and 5.52 μ mol CO₂ / m² / s in the technologically fertilized variant). In the case of the soil in the museum plot, the values of photosynthesis in the version with the inoculum of cyanobacteria and microalgae, as well as in the technologically fertilized version were ensured statistically compared to the control version.

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The higher the vegetative mass and the more intense photosynthesis of the seedlings, the higher the transpiration values of the leaves. Between the two physiological processes a significant polynomial correlation was established (r = 0.99 **), the perspiration of the leaves intensifies with the increase of the photosynthesis (Figure 1).

The factor A - Soil type	The factor B - Treatments performed	Green biomass (g)	Dry biomass (g)	Photosynthesis rate (µmol CO ₂ /m ² /s)	Transpiration rate (mmoli H ₂ O /m ² /s)
Soil from the pea plot	V ₁ - Control	0.55	0.04	6.33	1.65
	V_2 - soil fertilized according to technology $N_{80} P_{150} \; K_{\ 100}$	0.39	0.02	5.52	1.71
	V ₃ - soil treated with the inoculum of cyanobacteria and microalgae.	0.73	0.05	7.06	1.53
Soil from museum plot	V ₄ - Control	0.66	0.05	5.35	1.83
	$V_{5}\text{-}$ soil fertilized according to technology $N_{80}P_{150}K_{100}$	1.11^{*}	0.10^{*}	9.69*	3.30
	V ₆ - soil treated with the inoculum of cyanobacteria and microalgae.	0.69	0.06	8.96*	2.84
DL 5%=0.45		b=0.45	DL 5%=0.04	DL 5%=3.33	DL 5%=2.07

Table 1. The influence of treatments with cyanobacteria and the inoculation of microalgae in the soil, onsome physiological properties of tomato seedlings

DL 1%=0.66DL 1%=0.06DL 1%=4.84DL 1%=3.02DL 0.1%=0.99DL 0.1%=0.09DL 0.1%=7.27DL 0.1%=4.52

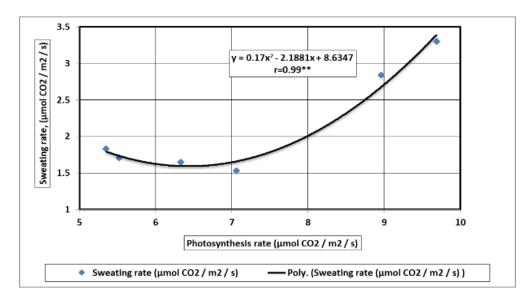


Figure 1. Correlation between the rate of photosynthesis and transpiration in the variants with the inoculum of cyanobacteria and microalgae on the two soil types

Also a negative polynomial correlation was established between the amount of chlorophyll and the intensity of photosynthesis in the leaves, photosynthesis increases with the increase of the amount of chlorophyll up to values of 10.5-11 mg / g fresh substance (Figure 2).

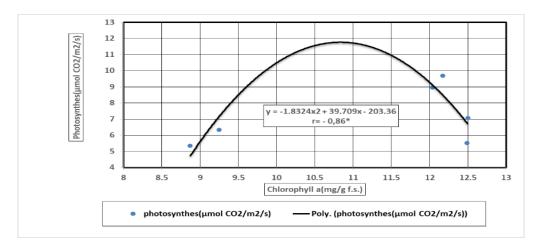


Figure 2. Correlation between the amount of chlorophyll a in the leaves and photosynthesis

Treatment of the two soil types with the inoculation of cyanobacteria and algae positively influenced the accumulation of chlorophyll a + b and carotene in the leaves of tomato seedlings (Table 2). The highest values were determined in the variants with soil from the technologically fertilized pea plot and with cyanobacteria, and in the case of the soil from the museum plot in the technologically fertilized version, followed by the variant with inoculum with cyanobacteria and microalgae.

and carolene in the leaves of tomato seedlings						
The factor A	The factor B	Chlorophyll a + b (mg / g f.s*)	Carotenoid (mg / g f.s*)			
	V ₁ - Control	20.63	6.41			
Soil from the pea plot	V_2 - soil fertilized according to technology $N_{80}P_{150}$ K $_{100}$	26.25	11.54			
	V ₃ - soil treated with the inoculum of cyanobacteria and microalgae.	26.20	11.92			
	V ₄ - Control	17.62	8.10			
Soil from	V_{5} - soil fertilized according to technology $N_{80}P_{150}$ K $_{100}$	26.93	11.88			
museum plot	V ₆ . soil treated with the inoculum of cyanobacteria and microalgae.	25.11	11.37			

Table 2. The influence of inoculum of cyanobacteria and microalgae in the soil. on the content of chlorophyll a + band carotene in the leaves of tomato seedlings

f.s*- fresh substance

The amount of carotene in the leaves of tomato seedlings accumulates with increasing amount of chlorophyll a + b, after a polynomial correlation, with a significant correlation factor (r = 0.94 **) (Figure 3).

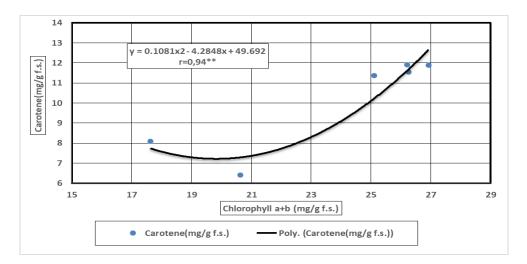


Figure 3. Correlation between the amount of chlorophyll a + b in the leaves and the amount of carotene

In the next figure (Figure 4) there are presented the time evolution of endogenous soil aerobic repiration in the six variants of soil treatment (sea materials and methods).

Endogenous soil respiration is influenced by the biocrust at short term levels 10 days. There is a decrease in the endogenous respiration in time, probably as an effect of substrate consumption in time.

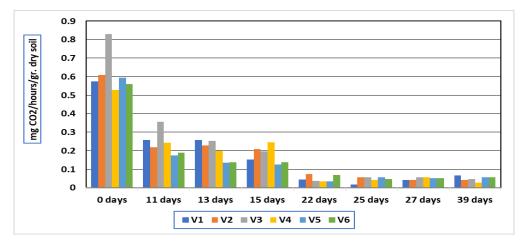


Figure 4. Soil respiration expressed in mg CO₂/24 hours/g dry soil

At the beginning in variant with soil treated with the inoculum of cyanobacteria and microalgae, in soil from the pea plot, has a higher respiration activity, probably because of the contribution of

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phototrophic microorganisms to organic carbon budget in the soil, more pronounced in this variant than in soil treated with the inoculum of cyanobacteria and microalgae, in soil from museum plot. The soil harvested from the pea plot has a higher amount of organic matter, which comes from the biomass of the microbial communities in the soil, as well as from the roots of the plants (peas has a pivoting root which penetrates 30-90 cm, well branched and which develops nodules populated by bacteria - *Rhizobium leguminosarum*, atmospheric nitrogen fixers). The amount of activated carbon, which can serve as a source of food and energy readily available for the microbial flora of the soil, was higher in the variant, with the inoculation of cyanobacteria and microalgae. By determining soil respiration, which is an indicator of carbon dioxide (CO₂) released as a result of the decomposition of organic matter by soil microorganisms and root respiration, the role of the addition of the cyanobacterial and microalgae inoculum in the accumulation of organic carbon in degraded soils such as sandy soils.

4. CONCLUSIONS

The values of green and dry biomass from the variants inoculated with cyanobacteria and microalgae were higher compared to the control variant and comparable to the values obtained in the technologically fertilized variants, regardless of the type of soil studied.

In the case of the soil in the museum plot, the values of photosynthesis in the variant with the inoculum of cyanobacteria and microalgae were statistically ensured compared to the control variant.

The photosynthesis of the leaves increased significantly with the increase of the amount of chlorophyll in the leaves to its values of 10.5-11 mg / g fresh substance.

The treatment of the two soil types with the inoculation of cyanobacteria and algae positively influenced the accumulation of chlorophyll a + b and carotene in the leaves of tomato seedlings. The highest values were determined in the variants with soil from the technologically fertilized pea plot and with cyanobacteria, and in the case of the soil from the museum plot in the technologically fertilized version, followed by the variant with inoculum with cyanobacteria and microalgae. Endogenous soil respiration is influenced by the biocrust at short term levels 10 days.

There is a decrease in the endogenous respiration in time, probably as an effect of substrate consumption in time.

At the beginning in variant with soil treated with the inoculum of cyanobacteria and microalgae, in soil from the pea plot, has a higher respiration activity, probably because of the contribution of phototrophic microorganisms to organic carbon budget in the soil, more pronounced in this variant than in soil treated with the inoculum of cyanobacteria and microalgae, in soil from museum plot.

By determining soil respiration, which is an indicator of carbon dioxide (CO₂) released as a result of the decomposition of organic matter by soil microorganisms and root respiration, the role of the addition of the cyanobacterial and microalgae inoculum in the accumulation of organic carbon in degraded soils such as sandy soils.

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