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TESTING BIOLOGICAL FERTILIZERS USEFUL FOR SUSTAINABLE AGRICULTURE

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Abstract

Biofertilizers represent an ideal alternative for sustainable agriculture, ensuring, in addition to increasing crop productivity, low costs and easy handling. The aim of this work was to test commercialized biofertilizers (Symbiomyco Grow - organic product based on mycorrhizal fungi), as well as potential biofertilizers (algae culture - Chlorella sorokiniana UTEX 1230 - with Symbiomyco Grow) to highlight the possible beneficial influence on some physiological and biochemical parameters in cucumber, Cucumis sativus L. Symbiomyco Grow was tested in two concentrations (1 and 0.1 g L^{-1} respectively) and 4 variants (with and without algae culture) and applied to the seeds before germination (experiment 1) and at the seedling stage (experiment 2). At the end of the experiments, the root and stem lengths were measured, and the fresh weight, total phenolic content, and concentration of the assimilatory pigments were determined. Among the parameters significantly increased compared with the control, after applying the algae culture with Symbiomyco Grow, we mention: axial organ length, fresh weight, and chlorophyll b.

Key words*: biofertilizers, cucumber, growth, assimilatory pigments, polyphenols.*

INTRODUCTION

The challenges of providing food under conditions of food and nutritional security for a population of 7.8 billion that is growing at a fast pace seem impossible to manage.

According to Alabdallah and Hasan (2021), agricultural crop demand will increase by up to 70-100% by 2050.

At first sight, there are two options to resolve these challenges: using additional land to be cultivated - but the world's area used for cultivation is 1.5 billion ha and is affected by various factors (climate change, pollution, etc.) or increasing the productivity of land already cultivated by using large-scale chemical fertilizers.

However, the use of chemical fertilizers has negative effects on the environment by: reducing the water retention capacity and fertility of soil, increasing soil acidity, and reducing the number of microorganisms, resulting in nutritional imbalances in the soil (Nosheen et al., 2021).

To meet the 2030 UN Sustainable Growth Goal of "Zero Hunger", the development should not compromise the ability of future generations to meet their own needs (Calabi-Floody et al., 2018).

The main problem in agriculture, especially in developing countries, is the inaccessibility of essential plant nutrients, either due to their insufficient quantity or due to the use of fertilizers (Itelima et al., 2018).

In this regard, biofertilizers (microorganisms or their products) have been shown to be an essential alternative for increasing crop productivity while being environmentally friendly, economical, and easy to use, and they have long-lasting benefits. Ancient Romans and classical Greeks used the term "biofertilizer" for various agricultural techniques intended to increase plant yield (Zafar et al., 2024).

Zhao et al. (2024) analyzed 12,880 journal articles to highlight the history of biofertilizers over 4 decades: 1980-2022. They divided biofertilizer research into three stages:

– the first stage (1980-2005) - nitrogen fixation;
– the second stage (2006-2015) focused on mechanisms for increasing plant yield;
– the third stage (2016-2022) - the application of biofertilizers to improve soil characteristics.

The aim of this work was to test commercialized biofertilizers (Symbiomyco Grow - organic product based on mycorrhizal fungi), as well as potential biofertilizers (algae culture - *Chlorella sorokiniana* UTEX 1230 - with Symbiomyco Grow) to highlight the possible beneficial influence on some physiological and biochemical parameters in cucumber, *Cucumis sativus* L.

Symbiomyco Grow is a biostimulator (organic product based on mycorrhizal fungi) which increases the nutrient supply capacity of plant roots, ensures the development of a well-branched root system capable of assimilating water much more easily, induces increased resistance to stress factors (drought, excessive humidity, sudden temperature changes), and protects plants against attacks by diseases and pests.

Algae culture was obtained by inoculating the BG 11 (Blue Green Medium 11) culture medium with *Chlorella sorokiniana* UTEX 1230 (Culture Collection of Algae at the University of Texas at Austin), a green alga (Chlorophyceae) capable of mixotrophic growth in the presence of carbon or nitrogen sources (Ramanna et al., 2014; Bohutskyi et al., 2016; Lizzul et al., 2018).

MATERIALS AND METHODS

For the experiments, cucumber seeds (*Cucumis sativus* L.) were hydrated for 1 h in distilled water. Two types of experiments were carried out. Within each experiment, 6 variants were performed (Table 1) with 10 seeds each, and the experiments had 3 repetitions.

Table 1. Experimental variants

Variants	Content
C (control)	distilled water
S_1	0.1 g Symbiomyco Grow L^{-1}
S_2	1 g Symbiomyco Grow L^{-1}
S_1A	0.1 g Symbiomyco Grow L^{-1}+ algae culture
S_2A	1 g Symbiomyco Grow L^{-1}+ algae culture
A	algae culture

1st Experiment: After hydrating in distilled water, the seeds were immersed in test solutions for 1 h (Table 1). We placed them in Petri dishes with filter paper and watered them periodically with distilled water for 4 days until the roots, stems, and fresh weight were measured.

2nd Experiment: The hydrated seeds were allowed to germinate for 3 days, after which they were immersed in the test solutions (Table 1) for 5 min. The seeds were distributed in Petri dishes on filter paper and watered periodically for 5 days using the test solutions. At the end of the experiment, the root and stem lengths were measured, and the fresh weight was determined. Also, the total phenol content (Orţan et al., 2015) and concentration of the assimilatory pigments (Popoviciu, 2018) were determined.

The results obtained after 3 repetitions were statistically analyzed using IBM SPSS Statistics 23 software. The mean and standard error were calculated, and the averages were compared using Duncan's test (a, b, c, etc).

RESULTS AND DISCUSSIONS

The influence of biofertilizers on seedlings growth and biochemical parameters in Cucumis sativus L. in 1st Experiment

The results obtained after 5 days of starting the experiment indicate significant differences between the control variant (Figure 1) and those in which algae (A) or algae mixed with Symbiomyco Grow (S_1A, S_2A) were used (Figures 2-4). Thus, a slight inhibition of root growth was observed in S_1A, whereas in S_2A and A, the roots had a significant increase in length, these variants recording the best increase in root length. A slight stimulation of root growth was also observed in variants S_1 and S_2 exposed to 0.1 and 1g L^{-1} Symbiomyco Grow (Figure 5).

Figure 1. Control

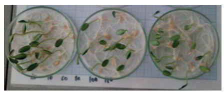

Figure 2. A variant

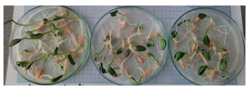

Figure 3. S_1A variant

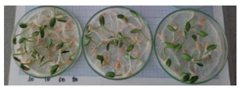

Figure 4. S_2A variant

The growth of the seedling stem, with the exception of variant S_1, which was exposed to 0.1g L^{-1} Symbiomyco Grow, was significantly influenced, with the best results obtained in variants S_2A and A (Figure 6). An analysis of the results obtained for the growth of the two axial organs showed that the most intense growth was determined by variants S_2A and A.

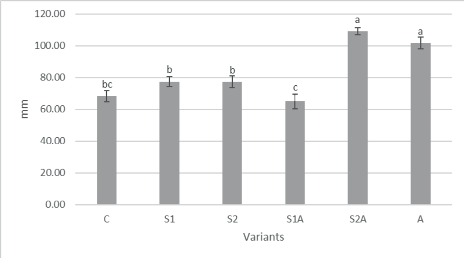

Figure 5. Influence of Symbiomyco Grow and algae culture on root growth in *Cucumis sativus* L. seedlings

The results for fresh biomass were correlated with those obtained for the growth of the seedling: all variants exposed to the tested fertilizer solutions, except for S_1, showed significant differences compared with the control (Figure 7).

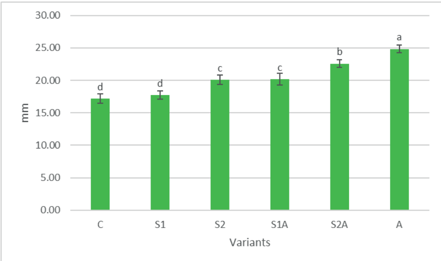

Figure 6. Influence of Symbiomyco Grow and algae culture on shoot growth in *Cucumis sativus* L. seedlings

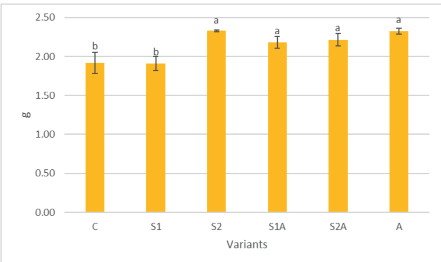

Figure 7. Influence of Symbiomyco Grow and algae culture on fresh weight in *Cucumis sativus* L. seedlings

Phenols represent a class of important compounds that act as biomarkers of stress induced by different biotic and abiotic factors. Analysis of the results indicates a significant decrease in the amount of total phenols (Figure 8), which can be interpreted as a lack of stress in the case of variants exposed to the tested fertilizer formulations.

Regarding the amount of pigments (Figure 9), only S_2A and A variants showed significant increases for chlorophyll *b* compared with the control, whereas in the rest of the variants, the decrease or increases were statistically insignificant.

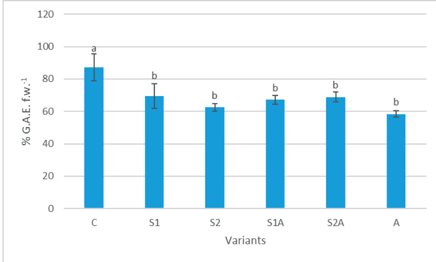

Figure 8. Influence of Symbiomyco Grow and algae culture on total phenolic content in *Cucumis sativus* L. seedlings

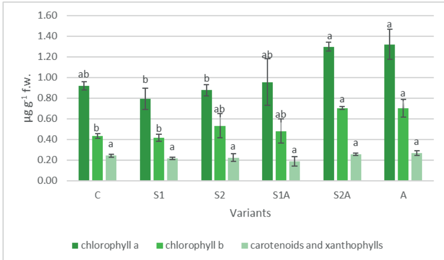

Figure 9. Influence of Symbiomyco Grow and algae culture on pigments content in *Cucumis sativus* L. seedlings

The influence of biofertilizers on seedlings growth and biochemical parameters in Cucumis sativus L. in 2nd Experiment

For the second experiment, the results indicate significant differences only between the control variant and the variant exposed to algae (A) variants in terms of the inhibition of root length growth (Figures 10-11). A slight stimulation of root growth was observed in S_1A, but the difference was not significant (Figure 14).

In the case of the stem and fresh weight (Figures 15-16), the results were similar to those obtained in 1st experiment, recording a more intense growth in length, with significant differences for variants S_2, S_1A, S_2A, and A, respectively, in weight in variants S_1A (Figure 12) and S_2A (Figure 13).

The total phenol content in the seedlings exposed to the fertilizer solutions did not vary significantly compared with the control variant (Figure 17), with slightly lower values recorded in the S_2, S_2A, and A variants.

The content of chlorophyll *a*, chlorophyll *b*, carotenoids, and xanthophylls did not significantly change. Only the S_2 and S_2A variants showed an increase above the value recorded in the control, but no increase was observed in chlorophyll *a* (Figure 18).

Figure 10. Control

Figure 11. A variant

Figure 12. S_1A variant

Figure 13. S_2A variant

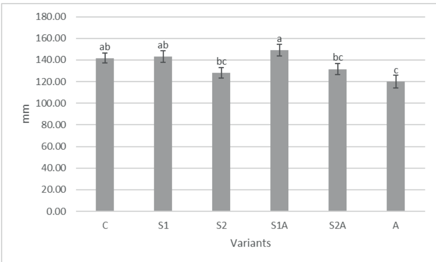

Figure 14. Influence of Symbiomyco Grow and algae culture on root growth in *Cucumis sativus* L. seedlings

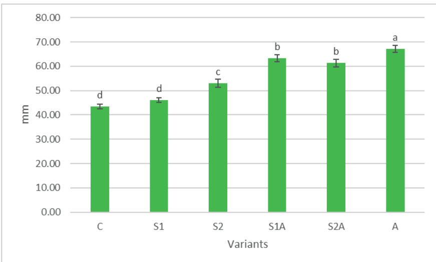

Figure 15. Influence of Symbiomyco Grow and algae culture on shoot growth in *Cucumis sativus* L. seedlings

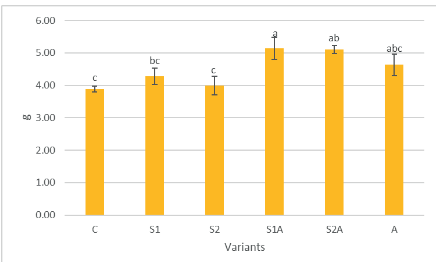

Figure 16. Influence of Symbiomyco Grow and algae culture on fresh weight in *Cucumis sativus* L. seedlings

Biofertilizers improve the physiological processes and health of plants, ultimately leading to increased productivity.

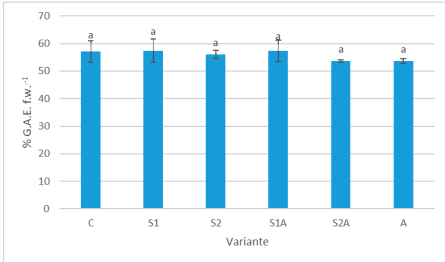

Figure 17. Influence of Symbiomyco Grow and algae culture on total phenolic content in *Cucumis sativus* L. seedlings

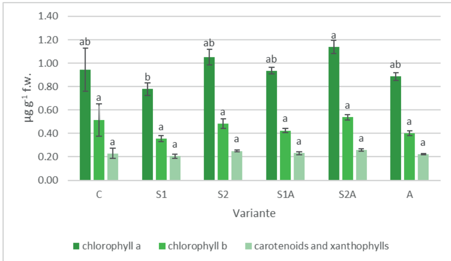

Figure 18. Influence of Symbiomyco Grow and algae culture on the assimilatory pigments content in *Cucumis sativus* L. seedlings

Biofertilizers currently represent an eco-friendly alternative for increasing productivity, as shown by various researches. Certain species of microorganisms (algae, fungi) or combinations thereof may determine superior results, similar to those reported by us for *Cucumis sativus*, exposed to certain concentrations of Symbiomyco Grow, *Chlorella sorokiniana* or combinations of the two.

So, the study conducted by Muscalu et al. (2020) regarding the effect of organic fertilizers in cucumber cultivation showed that the best results were obtained when using Rom-Agrobiofertil, which contains a mix of microorganisms (*Azospirillum lipoferum*, *Azotobacter chroococcum*, and *Bacillus megaterium*).

A series of research has indicated that algae stimulate plant growth, a fact also found for some of the variants tested in the two experiments (A, S_1A, S_2A). Thus, Lv et al. (2020) found that microalgae biomass significantly stimulated growth in height, the number of leaves and flower buds, and the diameter of cucumber stems.

Algae suspensions (*Chlorella* sp.) of 0.17 and 0.25 g L^{-1} can stimulate root and stem length growth in cucumbers (Bumandalai & Tserennadmid, 2019), as we also obtained when using the algae *Chlorella sorokiniana* (A) or the algae and Symbiomyco Grow mixture (S_2A) for root growth, respectively A, S_1A, S_2A for stem growth

The presence of *Chlorella* species in the culture medium promotes cucumber seed germination (Abd Elhafiz et al., 2015).

Gitau et al. (2021) mention that the stimulation of plant growth in the presence of microalgae is due to the auxins and cytokines produced by them.Regarding the content of assimilatory pigments, various studies have indicated the beneficial effects of algal biostimulators (Gitau et al. 2021; Guedes et al., 2018; Faheed & Fattah, 2008; El-Naggar et al., 2005), as observed in our experiments, in some of the variants (A and S_2A). The importance of this aspect arises from the fact that studies are known for many species useful for food, agriculture, or medicinal purposes in the context of sustainable agriculture. Thus, in a study conducted by Gitau et al. (2021), the effects of two *Chlorella* species (MACC-360 and MACC-38) and the *Chlamydomonas reinhardtii* (cc124) on *Medicago truncatula* were monitored under greenhouse conditions. The application of algal cells influenced the pigment content of the plant; thus, in the variants with *C. reinhardtii*, the chlorophyll content increased remarkably (32% in the case of chlorophyll a, 35% chlorophyll b, 32% total chlorophyll). In contrast, the application of *Chlorella* MACC-38 cells had a negligible effect on chlorophyll a (0.3% increase), a moderate effect on chlorophyll b (15% increase), and a reduced effect on total chlorophyll (3.7% increase). In the treatment with *Chlorella* MACC-360, both chlorophyll a and total chlorophyll decreased by 5% and 4%, respectively, whereas chlorophyll b content increased by 1%.

The use of micro- and macroalgae extracts increases pigment content (Guedes et al., 2018). Thus, by applying *Chlorella vulgaris* powder, the dry and wet weight of *Lactuca sativa* shoots increased, as did the pigment content (Faheed & Fattah, 2008).

Chlorella kessleri extracts rich in phytohormones (auxins and gibberellins) stimulated seed germination and increased leaf area and pigment content in *Vicia faba* (El-Naggar et al., 2005).

Variant S_2 (1g Symbiomyco Grow L^{-1}) showed an increase in chlorophyll *b* levels above the value recorded in the control group in the first experiment and in the second experiment for chlorophyll *a*.

A similar situation was observed in *Arabidopsis thaliana* inoculated with *Azospirillum brasilense* (Cohen et al., 2015).

Also, *Pseudomonas* sp., *Bacillus lentus*, and *Azospirillum brasilense* strains alleviated water stress in basil plants by increasing the levels of photosynthetic pigments and antioxidant activity (Heidari & Golpayegani, 2012).

The present study demonstrated the efficiency of using the green algae *Chlorella* in a mixture with an organic product based on mycorrhizal fungi (variants S_1A and S_2A) in terms of improving fresh weight and chlorophyll content in *Cucumis sativus.*

Increases in weight and carotenoid content were observed in lettuce following the application of *Chlorella vulgaris*, *Bacillus* sp., *Azotobacter* sp. and *Azospirillum* sp. (Kopta et al., 2018).

Algae extracts (1%), phosphate-solubilizing bacteria biofertilizers (4%) and their combinations were tested by Azizi et al. (2024) under drought conditions in *Calendula officinalis*. The combination of the two types of biofertilizers led to the best results: the highest increase in relative water content (39.80%), chlorophyll a (45.19%), chlorophyll b (46.62%), total chlorophyll (45.49%), flavonoids (13.33%), flavonols (4.35%), proline (6.87%), and catalase (127.27%) compared with the control. In addition, plant photosynthesis is stimulated by mycorrhizae because they enhance the transport of inorganic elements from the soil to plants, causing an increase in chlorophyll content and, implicitly, a higher photosynthetic rate (Feng et al., 2002; Hend et al., 2007).

Maize (Zhu et al., 2010), sunflower (Abdallah et al., 2013), and wheat (Allah et al., 2015) had an increased chlorophyll content as a result of mycorrhizae inoculation. Other plant parameters positively influenced by the presence of mycorrhizal fungi are: increased water content in leaves, turgor potential, increased root length, accumulation of nutrients in aerial parts (Ngwene et al., 2010), protection from pathogens (Elsen et al., 2008).

Dasgan et al. (2022) studied the effects of biofertilizers on the antioxidant content of basil, *Ocimum basilicum*. The highest phenol content was observed in the variants with bacteria-based biofertilizers, whereas the lowest content was observed in those with microalgae, which is similar to our results: the algae variant (A) exhibited the lowest concentration of polyphenol content in both experiments.

Babu et al. (2015) concluded that the presence of *Bacillus subtilis* and *B. cereus* promotes the accumulation of enzymes (peroxidase and polyphenol oxidase) involved in the metabolism of phenols and flavonoids.

CONCLUSIONS

Considering the results obtained for the influence of Symbiomyco Grow and algae culture on *Cucumis sativus* seedlings applied at the seed stage, the significant changes recorded (+ and -) indicate that the variants in which the seeds were immersed in the algae culture (A) and in the mixture of Symbiomyco Grow (1%) + algae (S_2A) had the most significantly increased parameters, compared to the control, respectively: growth in length of the axial organs of the seedling, wet biomass, and chlorophyll b. The summary of the influence of Symbiomyco Grow and algae culture on *Cucumis sativus* seedlings applied at the seedling stage indicates significant changes recorded (+ and -) in the variants in which the seedlings were immersed in the mixture of Symbiomyco Grow (0.1%) + algae (S_1A) and Symbiomyco Grow (1%) + algae (S_2A). The most significantly increased parameters compared to the control were the growth in stem length and wet biomass. The research carried out must be continued to evaluate the influence of biological fertilizers for a longer period of time and for more parameters.

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