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MICROALGAL BIOSTIMULANT IN BARLEY CULTIVATION

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ABSTRACT

The aim of this work was to evaluate the application of a microalgal biostimulant based on *Spirulina platensis* biomass in seed treatment and foliar application in barley cultivation. Five treatments were carried out, varying between seed treatment and foliar application of microalgal extracts in different concentrations. It was observed increase in length (T3 and T5) and dry root mass (T1 and T5) and length (T2, T3, T4 and T5) and dry shoot mass (T4 and T5) in the treatments with the bioproduct of microalgae in relation to the control test ($p<0.05$). Yield also increased by up to 607 kg ha^{-1} in seed treatment (T3) with microalgae compared to the control trial. Thus, the microalgal biostimulant is a potential biofertilizer for improving the physical characteristics and productivity of the barley crop.

Keywords: Microalgae. Seed treatment. Foliar application.

1 INTRODUCTION

One of the most significant consequences of the world's growing population is the need to increase food production. Agricultural crops are required to produce more per cultivated area, and this production must be of high quality (Sánchez-Quintero; Fernandes; Beigbeder, 2023). In addition to the challenges related to consumer demand, climate change and adverse weather phenomena have caused droughts and intense rainfall in recent years, negatively impacting food quality and production (IPCC, 2019). Synthetic fertilizers have been used to improve the yield and quality of food crops, but their excessive use directly contributes to water, soil and air pollution (Kumar; Kumar; Prakash, 2019). In addition, these environmentally hazardous consequences do not only occur at the time of their application, but start from their production cycle (Wu et al., 2023), where toxic chemical gases (NH_4^+, CO_2, CH_4, etc.) are released, directly polluting the atmosphere. Thus, it is vital to move towards a sustainable and regenerative agricultural system, reducing environmental impact, enabling increased crop yields and quality products (Kapoore et al., 2021). The agribusiness sector is already changing, both by reducing the use of chemical products and by adding biofertilizers, biostimulants or the use of microorganisms (fungi, bacteria and yeasts) as a means of solubilizing nutrients or as a disease control agent (Ajeng et al., 2022).

Among microorganisms, microalgae have been gaining ground. These are photosynthetic beings that can be cultivated using waste with nitrogen and phosphorus concentrations and do not require arable land for their cultivation (Xu et al., 2023). The biomass obtained from microalgae can be used as a biofertilizer when planting different crops or applied as a biostimulant by foliar application. Microalgae biomass contain bioactive molecules such as phytohormones, polysaccharides and phenolic compounds. These molecules promote a series of benefits for crops, such as aid in disease control, greater development of the root and shoot system, greater availability of nutrients and productivity gains (Alvarez et al., 2021; Gonzálezs-Gloria, 2021). Thus, the use of microalgae as a biofertilizer allows crops to be enhanced, healthier food to be produced and environmental impacts to be reduced.

The aim of this work was to evaluate the application of a microalgal biostimulant based on *Spirulina platensis* biomass in seed treatment and foliar application in barley cultivation.

2 MATERIAL & METHODS

The trial was conducted in Coxilha, Brazil, in an experimental area belonging to Ambev. The experimental design adopted was a randomized block design with four replications in 6 m^2 plots. The plots consisted of 6 rows spaced 17 cm apart with a density of 250 plants m^{-2}. Sowing took place on 20/06/2023. Crop management during this period was carried out in accordance with the standardized protocols of the management recommendations for growing barley (Embrapa, 2022).

Five treatments were carried out to evaluate the use of the microalgal biostimulant produced from *Spirulina patensis* biomass extracts, as well as a control trial, varying combinations of seed treatment and foliar application (Table 1). The seed treatment (ST) was carried out using *Spirulina platensis* dry biomass extract at a concentration of 5% (m/v) and an application rate of 1.5 L of product per 100 kg of seeds. Foliar application varied in two concentrations of microalgal extract (0.1 and 0.5% m/v), with an application volume of 120 L ha^{-1} of product. To obtain the extracts, the dry biomass of *S. platensis* was dissolved in distilled water and sonicated in an ultrasonic probe (Desruptor, Ultronique) in 5 cycles of 1 min, in order to rupture the cells and provide the extraction of bioactive compounds (Rempel et al., 2018). The seed treatment took place on the day of planting, and the foliar applications were made on 19/07/23 and 21/08/23, corresponding to 29 days and 61 days after planting.

Table 1 Treatments used in the study.

Treatment	ST – Microalgal biostimulant	Foliar application
Control	No	0
T1	No	0.1%
T2	No	0.5%
T3	Yes	0
T4	Yes	0.1%
T5	Yes	0.5%

Three weeks after the second foliar application, barley samples were collected to evaluate the following parameters: length and dry biomass of the root and aerial part of the plant. The length was measured using a graduated ruler and the dry mass was measured using an analytical balance after drying in an oven at 65°C for 72 hours (Meneguzzo et al., 2021). The plots were harvested on 07/11/23 by specific harvesters and the parameters of productivity, grain classification according to Class I (diameter greater than 2.5 mm) and protein content were analyzed.

The differences between the means of the analyses were evaluated by analysis of variance with a 95% confidence level, and then compared using the Fischer test in the Statsoft software. The results were expressed as mean ± standard deviation.

3 RESULTS & DISCUSSION

Analysis of the morphology of the barley plants made it possible to verify the influence of the treatments with *Spirulina platensis* microalgal biostimulant on the increase in the roots and shoot part of the plant (Figure 1).

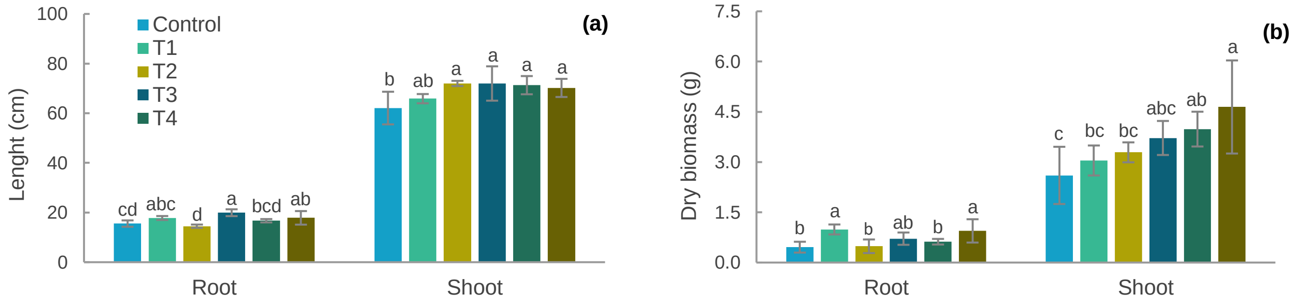

Figure 1 Length (a) and dry biomass (b) of root and shoot of barley plant at 82 days of cultivation, using different treatments of microalgal biostimulant. Note: equal letters show no statistical difference (p>0.05). T1 = 0.1% foliar application; T2 = 0.5% foliar application; T3 = TS; T4 = TS + 0.1% foliar application; T5 = TS + 0.5% foliar application.

Treating the seeds with the microalgal biostimulant resulted in an increase mainly in the roots of the barley. It can be seen that treatments T3 and T5 (TS only and TS with 0.5% foliar application) showed a statistical difference with the control ($p<0.05$), indicating the influence of the microalgae on root development. With regard to the increase in root dry biomass, T1 and T5 showed the best results compared to the control.

The best results in terms of increasing the length of the shoot of the plant were also recorded in the treatments with the biostimulant, with all the treatments being greater than the control ($p<0.05$). With regard to the dry biomass of the shoot of the barley, the trials that differed most were the treatments that used seed treatment with foliar application at the two concentrations studied (T4 and T5). This shows that the use of microalgal biostimulants alongside conventional barley treatment can improve the physical characteristics of the plant, helping it to develop better. However, there were no significant differences between the two concentrations of foliar application tested, indicating the use and study of lower concentrations, reducing the cost of using this bioproduct.

The biomass of the microalga *S. platensis* stands out as a source of nutrients for the development of crops and its extracts have been identified as a potential biostimulant for different crops (Braun; Colla, 2023). Different compounds in this bioproduct have been identified as plant development agents, such as proteins and amino acids (Muys et al., 2019, Matos; da Silva; Sant'Anna, 2021). These compounds can make nitrogen available for the plant to use in its metabolic processes, increasing levels of chlorophyll and phenolic compounds, and consequently favoring its growth (González et al., 2020). In addition, microalgae are known to have other compounds such as phytohormones and exopolysaccharides (Capek et al., 2023). Phytohormones, such as gibberellins, auxins and cytokinins, may be present in the extract or act in a similar way to favor plant growth. These act mainly on root growth and plant elongation, thus corroborating the results found in the present study (Alvarez et al., 2021, Zapata et al., 2021). Exopolysaccharides, in turn, act mainly in plant defense against abiotic stresses and pathogens (Ranglová et al., 2021, El Arroussi et al., 2018).

With regard to the final grain analysis (Table 2), carried out after harvest, there was a significant difference in the amount of starch in the grain (T1 compared to the control), and also in the area's productivity, with an increase of up to 607 kg ha^{-1} in treatment T3 compared to the control. The protein parameter showed no significant difference between the treatments and the control, so the bioproduct did not interfere with protein quality for the brewing industry. There was also no statistical difference between the treatments and the control. However, it is thought that the atypical year with a lot of rain from September onwards may have affected the area and the expression of better results from the effect of using algal biostimulants. As previously reported, all the benefits that biostimulants bring to plant development, improving its physiological characteristics, can result in increased crop productivity (Silambarasan et al., 2021).

Table 2 Analysis and grain yield of barley in different treatments with microalgal biostimulant.

Treatment	Starch (%)	Protein (%)	1st quality grains	Grain yield (kg ha^{-1})
Control	60.20 ± 0.35 [b]	11.85 ± 0.75 [a]	76.0 ± 4.3 [a]	2969 ± 541 [b]
T1	61.60 ± 1.51 [a]	11.97 ± 0.21 [a]	75.3 ± 0.6 [a]	3296 ± 130 [ab]
T2	61.30 ± 0.62 [ab]	11.68 ± 0.49 [a]	78.3 ± 2.6 [a]	3242 ± 596 [ab]
T3	60.65 ± 0.66 [ab]	12.13 ± 0.35 [a]	77.0 ± 0.8 [a]	3576 ± 373 [a]
T4	61.58 ± 1.34 [ab]	11.70 ± 0.16 [a]	77.0 ± 3.5 [a]	3333 ± 173 [ab]
T5	61.50 ± 0.53 [ab]	12.05 ± 0.45 [a]	78.0 ± 1.6 [a]	3389 ± 324 [ab]

Means followed by equal letters in the same column do not show statistical difference between treatments using Fisher's test ($p>0.05$) (mean ± standard deviation).

4 CONCLUSION

The biostimulant produced from extracts of *Spirulina platensis* biomass was able to improve the development of the root and shoot of the barley plant. In addition, a higher production yield was recorded in a trial with microalgal seed treatment compared to the control. Thus, the microalgal biostimulant showed potential for use in barley fields and could improve the physical characteristics and productivity of crops. In addition, with these results we can continue to study the dosage of foliar application and also better verify the effects on barley grain in years when climatic conditions will be less stressful for crops.

REFERENCES

1 SÁNCHEZ-QUINTERO, Á., FERNANDES, S. C. M., BEIGBEDER, J. B. 2023. Overview of microalgae and cyanobacteria-based biostimulants produced from wastewater and CO2 streams towards sustainable agriculture: A review. Microbiol. Res. 127505.

2 IPCC. 2019. IPCC-Global Warming of 1.5 oC.

3 KUMAR, R., KUMAR, R., PRAKASH, O. 2019. Chapter-5 the impact of chemical fertilizers on our environment and ecosystem. Chief Ed, 35, p. 69.

4 WU, W., TAN, L., CHANG, H., ZHANG, C., TAN, X., LIAO, Q., ZHONG, N., ZHANG, X., ZHANG, Y., HO, S. H. 2023. Advancements on process regulation for microalgae-based carbon neutrality and biodiesel production. Renew. Sustain. Energy Rev. 171. 112969.

5 KAPOORE, R. V., WOOD, E. E., LLEWELLYN, C. A. 2021. Algae biostimulants: A critical look at microalgal biostimulants for sustainable agricultural practices. Biotechnol. Adv. 49. 107754.

6 AJENG, A. A., ROSLI, N. S. M., ABDULLAH, R., YAACOB, J. S., QI, N. C., LOKE, S. P. 2022. Resource recovery from hydroponic wastewaters using microalgae-based biorefineries: A circular bioeconomy perspective. J. Biotechnol. 360. 11-22.

7 XU, P., LI, J., QIAN, J., WANG, B., LIU, J., XU, R., CHEN, P., ZHOU, W. 2023. Recent advances in CO2 fixation by microalgae and its potential contribution to carbon neutrality. Chemosphere. 137987.

8 ALVAREZ, A. L., WEYERS, S. L., GOEMANN, H. M., PEYTON, B. M., GARDNER, R. D. 2021. Microalgae, soil and plants: A critical review of microalgae as renewable resources for agriculture. Algal Res. 54, 102200.

9 GONZÁLEZ-GLORIA, K. D., RODRÍGUEZ-JASSO, R. M., APARICIO, E., GONZÁLEZ, M. L. C., KOSTAS, E. T., RUIZ, H. A. 2021. Macroalgal biomass in terms of third-generation biorefinery concept: Current status and techno-economic analysis–A review. Bioresour. Technol. Rep. 16. 100863.

10 EMBRAPA. 2022. Reunião Nacional de Pesquisa na Cevada. Indicações Técnicas para a Produção de Cevada Cervejeira nas Safras 2023 e 2024. Passo Fundo. 88p.

11 REMPEL, A., MACHADO, T., TREICHEL, H., COLLA, E., MARGARITES, A. C., COLLA, L. M. 2018. Saccharification of Spirulina platensis biomass using free and immobilized amylolytic enzymes. Bioresour. Technol. 263. 163-171.

12 MENEGUZZO, M. R. R., MENEGHELLO, G. E., NADAL, A. P., XAVIER, F. D. M., DELLAGOSTIN, S. M., CARVALHO, I. R., GONÇALVES, V. P., LAUTENCHLEGER, F., LANGARO, N. C. 2021. Comprimento de plântulas e vigor de sementes de soja. Ciência Rural, 51.

13 BRAUN, J. C. A., COLLA, L. M. 2023. Use of Microalgae for the Development of Biofertilizers and Biostimulants. Bioenergy Res. 16 (1). 289-310.

14 MUYS, M., SUI, Y., SCHWAIGER, B., LESUEUR, C., VANDENHEUVEL, D., VERMEIR, P., VLAEMINCK, S. E. 2019. High variability in nutritional value and safety of commercially available Chlorella and Spirulina biomass indicates the need for smart production strategies. Bioresour. Technol. 275. 247-257.

15 MATOS, A. P., SILVA, T., SANT'ANNA, E S. 2021. The feasibility of using inland desalination concentrate (DC) as an alternative substrate for Spirulina platensis mass cultivation. Waste Biomass Valor. 12. 3193-3203.

16 GONZÁLEZ, I., EKELHOF, A., HERRERO, N., SILES, J. Á., PODOLA, B., CHICA, A. F., MARTÍN, M. A., MELKONIAN, M., IZQUIERDO, C. G., GÓMEZ, J. M. 2020. Wastewater nutrient recovery using twin-layer microalgae technology for biofertilizer production. Water Sci Technol. 82 (6). 1044-1061.

17 CAPEK, L., UHLIARIKOVÁ, I., KOŠŤÁLOVÁ, Z., HINDÁKOVÁ, A., CAPEK, P. 2023. Structural properties of the extracellular biopolymer (β-D-xylo-α-D-mannan) produced by the green microalga Gloeocystis vesiculosa Nägeli. Carbohydr. Res. 525. 108766.

18 ZAPATA, D., ARROYAVE, C., CARDONA, L., ARISTIZÁBAL, A., POSCHENRIEDER, C., LLUGANY, M. 2021. Phytohormone production and morphology of Spirulina platensis grown in dairy wastewaters. Algal Res., 59. 102469.

19 RANGLOVÁ, K., LAKATOS, G. E., MANOEL, J. A. C., GRIVALSKÝ, T., ESTRELLA, F. S., FERNÁNDEZ, F. G. A., MOLNAR, Z., ORDOG, V., MASOJÍDEK, J. 2021. Growth, biostimulant and biopesticide activity of the MACC-1 Chlorella strain cultivated outdoors in inorganic medium and wastewater. Algal Res. 53. 102136.

20 EL ARROUSSI, H., BENHIMA, R., ELBAOUCHI, A., SIJILMASSI, B., EL MERNISSI, N., AAFSAR, A., MEFTAH-KADMIRI. I., BENDAOU, N., SMOUNI, A. Dunaliella salina exopolysaccharides: a promising biostimulant for salt stress tolerance in tomato (Solanum lycopersicum). Journal of Applied Phycology, v. 30, p. 2929-2941, 2018.

21 SILAMBARASAN, S., LOGESWARI, P., SIVARAMAKRISHNAN, R., INCHAROENSAKDI, A., CORNEJO, P., KAMARAJ, B., CHI, N. T. L. 2021. Removal of nutrients from domestic wastewater by microalgae coupled to lipid augmentation for biodiesel production and influence of deoiled algal biomass as biofertilizer for Solanum lycopersicum cultivation. Chemosphere. 268. 129323.

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