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Chlorella sorokiniana as a biostimulator of maize seed germination

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ABSTRACT: Microalgae exert several beneficial effects on plants. The present study aimed to evaluate the effect of *Chlorella sorokiniana* biomass as a biostimulator on the seed germination of the maize cultivar LG 36790. Germitest® paper germination tests were performed in a greenhouse. The treatments studied were:1) water (control), 2) 5% biomass of *Chlorella sorokiniana*; 3) 5% washed *Chlorella sorokiniana* biomass, and 4) micronutrient and urea solutions. On Germitest® paper, washed biomass treatment showed a higher seed germination (97%) but did not differ statistically from the control and treatment with 5% biomass. The 5% biomass treatment resulted in the highest values for root length and aerial part length at 5.96 and 3.82 cm, respectively, indicating better plant growth. In the greenhouse, treatment with micronutrients and urea showed the lowest values for seed germination, root size, and aerial part length and the washed biomass resulted in higher aerial part length at 11.76 cm. The treatment with biomass and washed biomass microalgae *Chlorella sorokiniana* contributes to an overall positive effect on germination, root size, and aerial parts growth.

Key words: bioactive compounds; growth promoter; microalgae; sustainable agriculture

Chlorella sorokiniana como bioestimuladora da germinação de sementes de milho

RESUMO: As microalgas trazem diversos efeitos benéficos para as plantas. O presente trabalho teve como objetivo avaliar o efeito da biomassa da microalga *Chlorella sorokiniana* como bioestimuladora da germinação de sementes de milho da cultivar LG 36790. Os testes de germinação foram realizados em papel Germitest® e em casa de vegetação. Os tratamentos estudados foram: 1) água (controle); 2) 5% de biomassa de *Chlorella sorokiniana*; 3) 5% de biomassa de *Chlorella sorokiniana* lavada e 4) solução de micronutrientes e ureia. No teste de germinação em papel Germitest®, as sementes com biomassa lavada apresentaram maior porcentagem de germinação (97%), mas não diferiu estatisticamente do controle e da biomassa a 5%. Para comprimento de raiz e parte aérea, a biomassa a 5% obteve os maiores valores com 5,96 e 3,82 cm, respectivamente, indicando um melhor crescimento de planta. Na casa de vegetação, o tratamento com micronutrientes e ureia demonstrou os menores valores para porcentagem de germinação, tamanho de raiz e parte aérea comparado aos tratamentos com microalga. A microalga *Chlorella sorokiniana* contribui para um efeito global positivo para a germinação, tamanho de raiz e parte aérea.

Palavras-chave: compostos bioativos; promotor de crescimento; microalga; agricultura sustentável

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Introduction

According to the Regulation (EU) 2019/1009 (European Commission, 2016), a biostimulant is any formulated substance of biological origin that, when applied to plants, seeds, or root systems, stimulates natural biological processes, improves plant productivity and tolerance to abiotic stress, and increases yield and seed performance in terms of vigor and emergence in the field. Microalgae can synthesize a variety of biologically active molecules, such as fatty acids, phytohormones (auxins, gibberellins, and cytokines), polysaccharides, and phenols (Ferreira et al., 2021; Cola & Rouphael, 2020). However, the modes of action of these compounds in plant development have not yet been fully elucidated (Costa et al., 2019).

Some studies have indicated that the possible mechanism of action of microalgae as biostimulants is through the constant supply of oxygen due to the photosynthesis processes carried out by these microorganisms (Barone et al., 2018) or through the production and excretion of hormones in the soil or surrounding environment (El-Arroussi et al., 2018). Barone et al. (2018) demonstrated the performance of microalgae as biostimulators of seed germination. In this article, beet seedlings treated with the microalgae *Chlorella vulgaris* and *Scenedesmus quadricauda* showed higher values for root features related to soil exploration and nutrient absorption, such as total root length, final root length, and number of root tips, suggesting a biostimulant effect in the expression of root traits and genes related to nutrient acquisition.

Martini et al. (2021) evaluated the biostimulant action of *C. reinhardtii* and *C. sorokiniana* on maize and observed better root system development with biostimulants than with the control treatment, such as an increase in the number of secondary roots and better accumulation of nutrients in the roots and aerial parts. In a recent literature review, González-Pérez et al. (2022) concluded that microalgae-based biostimulants can be used as alternatives for crop protection and plant growth regulation, and play an important role in increasing production levels, yields, and crop health. However, the authors stated that this is a field of research that still needs to be explored and should focus on investigating additional species of microalgae and their biologically active compounds.

The objective of the present study was to evaluate the effect of the microalgae *C. sorokiniana* biomass as a biostimulator of germination in the maize seed cultivar LG 36790.

Materials and Methods

The microalgae *C. sorokiniana* strain IPR 7104 of the, obtained from the microalgae collection of the Instituto de Desenvolvimento Rural do Paraná (IDR), Londrina, Paraná, was used in all experiments. The microalgae cultivation was carried out in 5 L transparent plastic packages, containing 10% (v/v) of initial inoculum of *C. sorokiniana* at 1 x 10^7 cells mL^{-1}, 10% (v/v) of solution of micronutrients (17% P_2O_5, 18% Ca, 7% Mg, 0.1% B, 0.05% Cu, 0.3% Mn, 10% Si, 0.55% Zn) and 1 g L^{-1} urea and 80% (v/v) of tap water. The packages were kept at 28 ºC with a photoperiod of 16 hours in the light and 8 hours in the dark at the Microalgae Cultivation Laboratory of the Anhanguera Pitágoras Unopar University, Campus Piza, Londrina, Paraná. After seven days of cultivation, the culture was centrifuged (Novatecnica centrifuge, NT820) at 3,500 rpm for 20 minutes and two fractions were formed: the sediment or pellet (biomass) and the supernatant. Both phases were collected separately and stored at 4 ºC.

The maize seed germination experiment was conducted Germitest® paper maize seeds cultivar LG 36790 supplied by a local rural producer and, according to the manufacturer, presented high productive potential, disease tolerance, precocity, and stability. The germination test followed the parameters established The Rules for Seed Analysis (RAS, 2009). The seeds were selected and placed on Germitest® paper moistened with a volume of 2.5 times the mass of the dry paper with the respective treatments. For each treatment, three replicate of 50 seeds were used, with a total of 150 seeds per treatment. Paper rolls were packed in plastic bags and kept in a germination chamber (BOD) for seven days at a temperature of 22 °C and a photoperiod of 12 hours. After seven days, the seeds were evaluated for germination percentage (%), root size (cm) and aerial part size (cm). A graduated ruler was used to measure the lengths of the roots and aerial parts.

The evaluated treatments as follows: 1) water (control); 2) 5% biomass diluted in water; 3) 5% biomass washed twice with water and 4) micronutrient and urea solutions. Washed biomass was included as a treatment to evaluate the effect of microalgae the micronutrient and urea residues.

Seed germination of maize cultivar LG 36790 was evaluated in a greenhouse and nine seeds were placed in plastic trays (54.7 cm long x 28.7 cm wide x 5 cm high) containing 128 cells filled with the commercial substrate Tropstrato HT Hortaliças (Vida Verde). Fifty seeds were used for each treatment: 1) water (control); 2) 5%biomass diluted in water; 3) 5% biomass washed twice with water and 4) micronutrient and urea solution. Sowing was carried out in August 2022 and after seven days; the first seedlings began to emerge. After 15 days, the seedlings were removed from the tray and the following features were analyzed: germination percentage (%), root length and aerial part (cm), as previously described. The temperature inside the greenhouse ranged from 25 to 32 °C.

The experiments were carried out in a completely randomized experimental design and the results obtained were subjected to analysis of variance and means compared by Tukey test ($p < 0.05$) using the Statistical Analysis System (SAS) program.

Results and Discussion

In the germination Germinest experiment, treatments with water, biomass and washed biomass showed a high seed germination rate (95 to 97%), but there was no statistical difference between them (Table 1). Treatment with micronutrients and urea resulted in the lowest germination percentage (70%). Bays et al. (2007) using the micronutrients Co, Mo and B and fungicide to treat soybean seeds, did not observe an increase in seed germination and physiological quality, indicating that the micronutrients associated with the biological fixation of nitrogen in soybean are important in stages after germination.

The treatment with microalgal biomass resulted in the greatest root length (5.96 cm), which was statistically different from the other treatments. For aerial part length, the treatment with biomass resulted in the greatest length (3.82 cm) but did not differ statistically from the control. These results suggest that microalgae have a positive effect on both variables, resulting in better initial plant growth. Ferreira et al. (2021) tested the biomass of the microalgae *Synechocystis* sp., *Tetradesmus obliquus*, *C. protothecoides*, and *C. vulgaris* to verify the biostimulant effect on germination, root size, and aerial parts of cucumber, barley, wheat, soybean, watercress, and tomato and observed that all microalgae showed a positive effect on the germination index of the tested seeds and a positive effect on root development compared to the control. However, the effect of the microalgae on the aerial parts of the plants was not statistically significant. Plaza et al. (2018) suggested that the foliar application of microalgae is necessary to promote better aerial part development.

Seed germination was measured in the greenhouse. As shown in Table 2, the lowest germination percentage (%) was observed in the micronutrient solution and urea treatments, whereas the other treatments showed germination rates between 82 and 90%. For root length, no statistical differences were found among the treatments; however, the treatments with microalgae resulted in the smallest root length. For aerial part length, the lowest value was obtained for the treatment with microalgae biomass, which corroborates the results of Plaza et al. (2018), who indicated the need for foliar application of microalgae extracts to improve aerial part performance.

The germination and initial development of maize seeds grown on paper and trays were different. Alves et al. (2015) reported different germination rates of guava seeds when placed on different substrates, such as paper rolls and sand, and at different temperatures. Germination on Germitest® paper and in a greenhouse were conducted at different temperatures, 22 °C and 25-32 °C, respectively. This factor can influence the percentage and speed of germination as it changes the speed of water absorption and the metabolic reactions of the reserves necessary for seedling survival (Alves et al., 2015; Borges et al., 2020).

The success of the initial seed emergence directly influences the establishment of crops in the field. The early emergence of seedlings from prepared seeds allows for the efficient and prolonged use of light and soil resources by plants during their growth and development (Damalas et al., 2019). Therefore, the application of biostimulators during sowing can result in more productive crops.

Although the beneficial effects of microalgae in agricultural practices are evident, their commercial use as biological products (biostimulants) has limitations, such as the high costs associated with the production of microalgae biomass and the extraction of its compounds. The autotrophic growth of microalgae can be expensive, and biomass productivity is still not high. In addition, biomass recovery after the production process can be costly and energy demanding (Gonçalves, 2021).

In the present study, the biomass of the microalga *C. sorokiniana* was used directly in the treatments on Germitest® paper and on the substrate; however, other studies using microalgae in agriculture have tested the application of microalgae extracts that require the extraction of the metabolites of interest, and these methods are also considered expensive (Renuka et al., 2018). The high costs associated with the production and processing of microalgal biomass result in an increase in biofertilizer/

Table 1. Germination (%), root size and aerial part (cm) of LG 36790 maize seeds grown on Germitest® paper for seven days.

Treatments	Germination (%)	Root length	Aerial part length
		(cm)	
Water (control)	95a	5.04b	3.50a
Biomass	96a	5.96a	3.82a
Washed biomass	97a	4.07c	2.93b
Micronutrient and urea solution	70b	2.02d	2.05c

**Average followed by the same lowercase letters in the column do not differ statistically by the Tukey Test (5%).

Table 2. Germination (%), root size and aerial part (cm) of LG 36790 maize seeds in trays containing commercial substrate in the greenhouse.

Tratamentos	Germination (%)	Root length	Aerial part length
		(cm)	
Water (control)	88a	7.79a	10.07ab
Biomass	82a	5.56a	8.62b
Washed biomass	90a	6.29a	11.76a
Micronutrient and urea solution	78b	7.20a	10.47b

**Average followed by the same lowercase letters in the column do not differ statistically by the Tukey Test (5%).

biostimulant costs that are not market-competitive compared with chemical-based products (Gonçalves, 2021) and then, microalgae-based products are not widely used in agriculture. Future studies are needed to improve the efficiency of large-scale production of microalgae and reduce costs to enable profitable commercial production. This present study deserves due attention, as suggest that the microalgae, and more specifically its biomass, present bioactive that directly contribute to the greater growth and development of the seed. Further studies are warranted to determine whether this effect is also observed at the field level. Furthermore, although sustainable production systems have considered the use of microalgae as biostimulants and biopesticides, little information is available regarding the interactions between microalgae (and their extracts), plants, and the environment (Ahmad et al., 2021). In this way, there is a vast area for studies and conducting scientific research and innovation in the use of microalgae in agriculture.

Conclusions

The biomass of the microalgae *Chlorella sorokiniana* contributed to a positive effect on the initial development of maize seedlings for the following variables: germination, root length and aerial part.

The treatment with biomass 5% has higher root length (5.96 cm) and aerial part (3.82 cm). These results demonstrate a potential biostimulant effect of this microalgae in agriculture.

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Compliance with Ethical Standards

Author contributions: Data curation: JST; Formal analysis: BA; Supervision: WM, HHS; Writing – original draft: JBS; Writing – review & editing: MMM.

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