e-ISSN 1983-4063 - *www.agro.ufg.br/pat* - Pesq. Agropec. Trop., Goiânia, v. 55, e81785, 2025

Research Article

Bioactive effect of cyanobacteria and calcareous algae mixture in bioassays and on potato growth and yield[1]

Juliana de Oliveira Amatussi[2], Gilda Mógor[2],
Gabriel Bocchetti de Lara[2], Harielly Marianne Costa Marques[2], Átila Francisco Mógor[2]

ABSTRACT

The growth-promoting effect of the micronized calcareous algae *Lithothamnion* sp. is related to the presence of humic acid and its bioactive auxin-like effect, characteristic of humic substances, whereas the plant growth-promoting effect of the microalgae (cyanobacteria) *Arthrospira platensis* (*Spirulina platensis*) has been attributed to the presence of L-amino acids and polyamines in its biomass. The present study aimed to identify the possible synergistic bioactive effect of a mixture of both sources (micronized *Lithothamnion* and *A. platensis* biomass) in bioassays and in the field, with foliar application, on organic potato (*Solanum tuberosum* L.) cultivation. Four bioassays were carried out to evaluate the root growth of *Vigna radiata*, as well as the expansion of *Solanum lycopersicum* hypocotyls, *Cucumis sativus* cotyledons and *V. radiata* hypocotyl-radicle axis. Subsequently, a field experiment was conducted in an organic system with foliar application to potato plants. The bioassays showed the bioactivity of the calcareous algae and of the cyanobacteria, and their combination, promoting biometric and metabolic changes, increasing the potato yield and tuber sugar content. Thus, the mixture of micronized *Lithothamnion* with *A. platensis* biomass can be a novel natural input to sustainably improve potato growth and yield.

KEYWORDS: *Lithothamnion* sp., *Arthrospira platensis*, *Solanum tuberosum* L.

RESUMO

Efeito bioativo da mistura de cianobactérias e algas calcárias em bioensaios e no crescimento e na produtividade da batata

O efeito promotor de crescimento da alga calcária micronizada *Lithothamnion* sp. está relacionado à presença de ácido húmico e seu efeito bioativo semelhante à auxina, característico das substâncias húmicas, enquanto o efeito promotor de crescimento vegetal da microalga (cianobactéria) *Arthrospira platensis* (*Spirulina platensis*) foi atribuído à presença de L-aminoácidos e poliaminas em sua biomassa. Objetivou-se identificar o possível efeito bioativo sinérgico da mistura de ambas as fontes (*Lithothamnion* micronizado e biomassa de *A. platensis*) em bioensaios e no campo, com aplicação foliar, no cultivo orgânico de batata (*Solanum tuberosum* L.). Quatro bioensaios foram conduzidos para avaliar o crescimento da raiz de *Vigna radiata* e a expansão dos hipocótilos de *Solanum lycopersicum*, dos cotilédones de *Cucumis sativus* e do eixo hipocótilo-radícula de *V. radiata*. Posteriormente, foi conduzido um experimento de campo, em sistema orgânico, com aplicação foliar, em plantas de batata. Os bioensaios mostraram a bioatividade das algas calcárias, das cianobactérias e de sua associação, promovendo mudanças biométricas e metabólicas, aumentando a produtividade da batata e o teor de açúcar nos tubérculos. Assim, o *Lithothamnion* micronizado associado à biomassa de *A. platensis* para o crescimento de batata pode ser um novo insumo natural para melhorar a sua produção de forma sustentável.

PALAVRAS-CHAVE: *Lithothamnion* sp., *Arthrospira platensis*, *Solanum tuberosum* L.

INTRODUCTION

The development of natural-based inputs can provide an efficient means of increasing crop yield. Potato is the world's third most consumed food, with a global production of 383 million tons (FAO 2023). Thus, developing novel nature-friendly agricultural practices is essential to ensuring food safety.

Biofertilizers, natural substances that act on plant metabolism, are bioactive, agrotoxin-free and capable of promoting increased yield (Brasil 2020). Recently, the Brazilian legislation classified them as bioinputs (Brasil 2024).

This reflects a growing demand, extending to large production areas, for more sustainable techniques and good practices that can reduce

[1] Received: Feb. 19, 2025. Accepted: Apr. 28, 2025. Published: June 05, 2025. DOI: 10.1590/1983-40632025v5581785.
[2] Universidade Federal do Paraná, Curitiba, PR, Brazil. *E-mail/ORCID*: julianaamatussi@gmail.com/0000-0002-8025-4638; gildamogor@gmail.com/0000-0002-4480-4487; gabrielbdelara@gmail.com/0000-0002-9090-6812; hariellymarques@hotmail.com/0000-0001-5906-5472; afmogor@gmail.com/0000-0003-4199-9079.
Editor: Luis Carlos Cunha Junior

production costs by decreasing the use of chemical inputs by conventional but innovative farmers (Vidal et al. 2021).

In addition, research on natural plant growth-promoting sources is increasingly relevant for improving the yield, quality and sustainability of agricultural production in a changing environment. Examples include the use of free L-amino acids to improve potato yield (Röder et al. 2018) and mitigate the effects of oxidative stress in soybean (Marques et al. 2021), microalgae associated with humic acid to enhance onion growth and yield (Gemin et al. 2019), algae extract to increase the growth of bean and maize under salt stress (Hussein et al. 2021), and chlorophyte microalga extracts to benefit common bean (Marques et al. 2023) and melon (Marques et al. 2025).

Macroalgae are classified into three major groups according to their pigmentation: Chlorophyta (green), Phaeophyta (brown) and Rhodophyta (red) (Freitas et al. 2022). *Lithothamnion* sp., a red alga, contains calcium (±31.93 %), magnesium (±3 %), humic substances (±10 %) and free amino acids (0.15 %), exhibiting proven bioactivity in several plants (Amatussi et al. 2020, Ramos et al. 2023).

While Brazilian regulations classify *Lithothamnion* sp. as a mineral fertilizer, based only on its mineral fraction (Brasil 2018), it can also be considered a biofertilizer (Brasil 2020) in the bioinputs class (Brasil 2024), if its bioactivity is demonstrated.

Lithothamnion sp. application has shown positive effects on root development, thereby increasing bell pepper (Evangelista et al. 2016) and melon yield (Negreiros et al. 2023), and inducing phytoalexin accumulation in bean and soybean (Faria et al. 2022). In onion, it increased plant growth, nutrient content and yield (Mógor et al. 2021).

The bioactivity of micronized *Lithothamnion* sp. (micrometer scale particles) has been attributed to its highly bioactive humic acid content (Amatussi et al. 2020). Humic substances combined with the microalgae chlorophyte *Scenedesmus subspicatus* have shown to increase plant growth and yield (Gemin et al. 2019), as well as to improve onion storage and post-harvest quality (Gemin et al. 2022). The foliar application of a mixture of *Lithothamnion* sp. and the cyanobacterium *Arthrospira platensis* (*Spirulina plantensis*) promoted onion growth, biochemical changes and yield gains (Amatussi et al. 2022).

Microalgae have demonstrated plant growth-promoting effects, including mitigating the harmful effects of water shortage in common bean (Marques et al. 2023) and oxidative stress under high salinity in melon (Marques et al. 2025). These effects are attributed to bioactive compounds such as proteins, polyamines, cytokinins and free L-amino acids (Mazepa et al. 2021, Cordeiro et al. 2022, Mógor et al. 2022a).

The cyanobacterium *Arthrospira platensis* has also shown a plant growth-promoting effect on various crops, including red beet (Mógor et al. 2018), lettuce (Mógor et al. 2017), red spinach (Mala et al. 2017), *Petunia x hybrida* (Plaza et al. 2018) and radish (Godlewska et al. 2019). Bioassays show that the cytokinin-like activity may be related to polyamines in *A. platensis* biomass (Mógor et al. 2017), and the presence of trans-zeatin and isopentenyl adenine, contributing to plant growth promotion in treated plants (Plaza et al. 2018).

However, the combination of different algae sources may exhibit synergistic biostimulatory action, thereby contributing to designing the next generation of plant growth-promoting sources for sustainable agriculture. In this context, the present study investigated the effect of the micronized calcareous algae *Lithothamnion* sp. combined with *A. platensis* in bioassays (to identify bioactivity) and in the field on potato growth and yield.

MATERIAL AND METHODS

As an initial step to identify possible bioactive properties of *Lithothamnion* sp. and *Arthrospira platensis* in plants, four bioassays were conducted: hypocotyl expansion of *Solanum lycopersicum* (Zhao et al. 1992, Stirk et al. 2002); cotyledon expansion of *Cucumber sativus* (Zhao et al. 1992, Stirk et al. 2002, Mógor et al. 2017); root emission with *Vigna radiata* (Tripepi & George 1991, Zhao et al. 1992, Amatussi et al. 2020); and hypocotyl-radicle expansion of *V. radiata*.

These bioassays were performed in March 2021 at the the Canguiri experimental farm of the Universidade Federal do Paraná (Paraná state, Brazil). The micronized calcareous algae *Lithothamnion* sp. sample was provided by NaturVita Bioagroindustria (Petrolina, Pernambuco state, Brazil) (Amatussi et al. 2020). *Arthrospira platensis* was cultivated in photobioreactors at the laboratory, then lyophilized

(Mógor et al. 2017) and suspended in distilled water as described below:

The hypocotyl expansion bioassay of *S. lycopersicum* was performed in plastic germination boxes (Gerbox®). Seeds were placed on pre-moistened germination paper (2.5 times the weight of the paper) in solutions with concentrations of micronized *Lithothamnion* sp. and *A. platensis*: control (distilled water only); *Lithothamnion* sp. (1.5 g L^{-1}); 1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*; 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*; and *A. platensis* (1.5 g L^{-1}). The bioassay was conducted in a growth chamber at 22 ºC, in the dark, for 9 days, in a completely randomized design, with four replications. The length (cm) and volume (cm³) of tomato hypocotyls were evaluated;

Cucumis sativus seeds were previously germinated in a vermiculite bioassay with cotyledons. Once developed, the cotyledons were placed in plastic Gerboxes on germination paper moistened (2.5 times the weight of the paper) with solutions containing concentrations of *Lithothamnion* sp. and *A. platensis*: control (distilled water only); *Lithothamnion* sp. (1.5 g L^{-1}); 1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*; 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*; and *A. platensis* (1.5 g L^{-1}). The bioassay was conducted in a growth chamber at 22 ºC, in the dark, for 9 days, in a completely randomized design with four replicates (n = 4). The length (cm) and volume (cm³) of the cucumber cotyledons were evaluated;

To evaluate the effect on root growth of *V. radiata*, seeds were sown in 144-cell plastic trays filled at a 1:1 ratio with a commercial substrate (Tropstrato®) and commercial organic compost (Provaso®) containing: C = 30.3 g kg^{-1}; N = 30.3 g kg^{-1}; P = 8.5 g kg^{-1}; K = 6.6 g kg^{-1}; Ca = 8.1 g kg^{-1}; and Mg = 4.1 g kg^{-1}. The trays were kept in a nursery with timed micro-sprinkler irrigation. At 10 days after germination, the seedling stems were excised and placed in microtubes containing the following solutions: control (water); *Lithothamnion* sp. (1.5 g L^{-1}); 1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*; 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*; and *A. platensis* (1.5 g L^{-1}). The bioassay was conducted in a growth chamber at 25 ºC, under a 12-hour photoperiod (photon flux intensity: 0.52-0.56 mmol m^{-2} s^{-1}) for 9 days, in a completely randomized design with four replicates (n = 8). The length (cm) and average diameter (mm) of *V. radiata* roots were evaluated;

The *V. radiata* seeds were soaked for 2 min in the following solutions: control (with water application); *Lithothamnion* sp. (1.5 g L^{-1}); 1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*; 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*; and *A. platensis* (1.5 g L^{-1}). The seeds were placed in plastic Gerboxes on germination paper previously moistened (2.5 times the weight of the paper) with distilled water. The bioassay was conducted in a growth chamber at 25 ºC, for 5 days, in a completely randomized design, with four replicates (n = 4).

The length (cm), volume (cm³) and mean diameter (mm) of *S. lycopersicum* hypocotyls, *C. sativus* cotyledons and *V. radiata* roots and hypocotyl-radicle were determined using the WinRhizo Pro® (Regent Instr® Canada) software and an Epson® (v700 PHOTO model) dual-lens scanner.

Based on the bioassay results, the potato growth and yield experiment was conducted in the organic horticulture area of the Canguiri experimental farm of the Universidade Federal do Paraná, in Pinhais, Paraná state, Brazil (25º25'S and 49º06'W), in August 2021. This region is on the first plateau of the Paraná state, at an altitude of 920 m. According to the Köppen classification, the regional climate is temperate (Cfb), with no defined dry season, cool summers, mild winters, average annual rainfall between 1,400 and 1,600 mm, and average annual minimum temperature between 17 and 18 ºC, with frequent and severe frosts (Embrapa 2013).

The chemical analysis of the 0-20 cm soil layer revealed the following average values: pH ($CaCl_2$) = 5.84; pH (H_2O) = 6.71; Al^{+3} = 0 $cmol_c$ dm^{-3}; H + Al^{+3} = 2.93 $cmol_c$ dm^{-3}; Ca^{2+} = 5.28 $cmol_c$ dm^{-3}; Mg^{2+} = 3.05 $cmol_c$ dm^{-3}; K^+ = 1.32 $cmol_c$ dm^{-3}; P (Mehlich) = 49.0 mg dm^{-3}; S = 33.49 mg dm^{-3}; C = 26 g dm^{-3}; V = 76.7 %; and CEC = 12.58 $cmol_c$ dm^{-3}.

After the soil preparation, planting furrows were made using a micro tractor and spread homogeneously with 12.5 t ha^{-1} of organic compost containing the following: C = 31.3 g kg^{-1}; N = 26.3 g kg^{-1}; P = 8.2 g kg^{-1}; K = 7.2 g kg^{-1}; Ca = 8.0 g kg^{-1}; Mg = 4.2 g kg^{-1}; and 50 kg ha^{-1} of magnesium thermophosphate.

The experimental area was irrigated using a sprinkler system to maintain the soil moisture at 80 % of its water holding capacity, as measured by a tensiometer. The design was completely randomized, with four replicates of the following foliar applications (treatments): control (water); *Lithothamnion* sp. (1.5 g L^{-1}); 1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*; 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*; and *A. platensis* (1.5 g L^{-1}). A total of six rows were established, with the first and last considered border rows, and 20 plots of eight plants each randomly distributed in the four central rows. Potato tubers of the 'BRS Clara' cultivar (Embrapa) were planted with spacing of 0.35 m between plants and 0.80 m between rows.

'BRS Clara', one of the most suitable cultivars for organic cultivation (Passos et al. 2022), is medium sized, with a semi-erect growth habit, moderately open leaves and average maturity of 100 days. The tubers have an elongated oval shape, smooth yellow skin and are moderately resistant to greening and resistant to powdery mildew (*Phytophthora infestans*), with average yield of 7.23 t ha^{-1} in the spring (Pereira et al. 2013) and average total soluble solids content of 3.2 ºBrix (Virmond et al. 2014). The hilling-up procedure was performed at 25 days after planting (DAP). Applications started at 32 DAP, and occurred weekly, totaling eight applications until leaf senescence (84 DAP). The application rate was 400 L ha^{-1} using a 10-L electronic sprayer (Kawashima®), with constant pressure (40 psi). To avoid drift, a plastic barrier was used in all applications.

The relative chlorophyll index was measured in the field with a chlorophyll meter (N-Tester®), which provided average values from 30 random readings taken on fully expanded leaves in the middle third of the plants (Mógor et al. 2013). The readings were taken on the three central plants of each plot (55 DAP).

Harvest was performed at 98 DAP, with the plants showing complete senescence. Three central plants from the plots were used for tuber mass quantification, and yield was calculated by extrapolating these values to a population of 40,000 plants ha^{-1}. Yield assessment followed the potato tuber size classification (Class I: > 70 mm; Class II-2: 50-70 mm; Class II-1: 42-50 mm; Class III: > 33-42 mm; Class IV: 28-33 mm; and Class V: < 28 mm). For calculations, only the commercial standard classes were used (I, II-1 and II-2). The total soluble solids content of the tubers was determined at harvest using a portable refractometer (EEQ9030), and the results were expressed in ºBrix.

For reducing and total sugars analysis, potato leaves and tubers were collected after the fourth and eighth application (55 and 98 DAP, respectively). Leaves and tubers (commercial standard classes at harvest) were collected, macerated in liquid nitrogen and stored (-20 ºC) for biochemical analysis. Leaf collections were performed between 9 and 10 a.m. (Mógor et al. 2022b).

The total sugars were quantified according to Maldonade et al. (2013), preceded by acid hydrolysis of the sample using 3,5-dinitro salicylic acid. The standard curve for reducing and total sugars was generated using glucose (5.5 mM). The values were expressed as µg of sugars per g of fresh plant material.

All data were evaluated for homogeneity and then submitted to analysis of variance (Anova). When significant differences were detected ($p < 0.5$), the Tukey test was applied. The statistical analyses used the Assistat® 7.7 Beta software (Silva & Azevedo 2016).

RESULTS AND DISCUSSION

The bioactivity of micronized *Lithothamnion* sp., lyophilized *Arthrospira platensis* biomass and their combinations was characterized as follows: 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis* concentration stimulated the expansion of tomato hypocotyls, resulting in a 15 % increase in length and 27 % in volume (Figures 1a and 1b). This increase may be due to the presence of bioactive substances in the algae, such as humic acid in *Lithothamnion* sp. (Amatussi et al. 2020), L-amino acids (Mógor et al. 2018) and polyamines in *A. platensis* (Mógor et al. 2017).

The length and volume of *S. lycopersicum* hypocotyls showed the bioactive effect of the *Lithothamnion* sp. and *A. platensis* combination at the highest concentration (1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*) (Figures 1a and 1b). The *S. lycopersicum* hypocotyls treated with 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis* showed a 15 % increase in length and 27 % in volume, when compared to the control.

Furthermore, the bioactivity of *Arthrospira platensis* was expressed in the *C. sativus* cotyledon bioassay, resulting in an 82.3 % increase in length (Figure 1c). A similar bioactivity was reported by

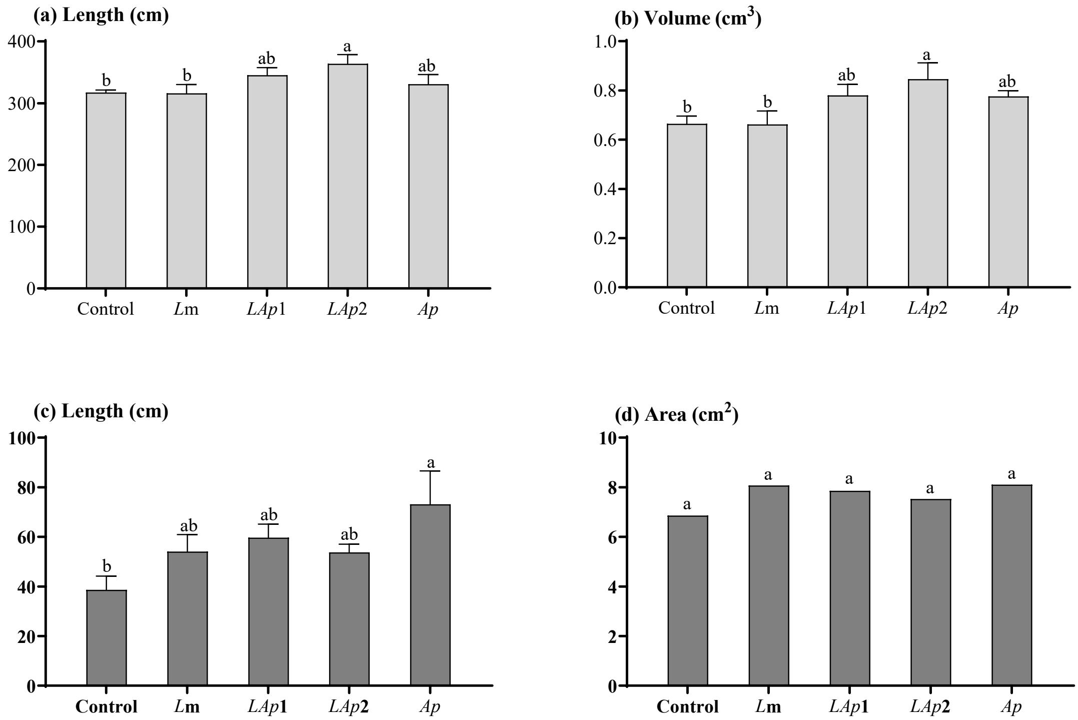

Figure 1. Length (a) and volume (b) of *Solanum lycopersicum* hypocotyl expansion; length (c) and area (d) of *Cucumis sativus* cotyledons, grown on germination paper treated with concentrations of micronized *Lithothamnion* sp. (*L*m) and *Arthrospira platensis* (*Ap*). Concentrations: control; *Lithothamnion* sp. (1.5 g L^{-1}); *LAp*1 (1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*); *LAp*2 (1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*); and *A. platensis* (1.5 g L^{-1}). Means followed by the same letter do not differ statistically ($p < 0.5$) according to the Tukey test. Bars indicate the standard deviation.

Mógor et al. (2017), attributed to an increase in polyamine spermine, and a similar result was found with chlorophyte microalga (*Scenedesmus obliquus*), which exhibited a cytokinin-like effect (Navarro-López et al. 2020). By contrast, the *Lithothamnion* sp. application showed no significant effect in the cucumber cotyledon expansion bioassay (Figure 1c).

The *V. radiata* bioassay serves as an indirect assessment of auxin-like substances by expressing bioactivity through rooting (Amatussi et al. 2020). In *V. radiata* plants treated with *Lithothamnion* sp., rooting was 84 % higher than in the control (Table 1). By contrast, plants treated with *A. platensis* alone or in combination with *Lithothamnion* sp. did not develop roots.

When algae extracts are applied to increase rooting, algae species with higher auxin-like activity are superior to extracts from species

Table 1. Average length (cm) and diameter (mm) of *Vigna radiata* roots and hypocotyl-radicle.

Treatments		Length (cm)	Average diameter (mm)
Root emission	Control	97.99 ± 3.70	0.97 ± 0.05
	Lithothamnion sp.	180.76 ± 20.52	0.68 ± 0.06
Hypocotyl-radicle emission	Control	267.98 a	1.034 ± 0.002 b
	Lithothamnion sp.	285.54 a	1.111 ± 0.084 ab
	*LAp*1	265.61 a	1.032 ± 0.017 b
	*LAp*2	255.36 a	1.163 ± 0.075 a
	Arthrospira platensis	272.46 a	1.104 ± 0.052 ab
	CV (%)	8.78	5.18

Concentrations: control; *Lithothamnion* sp. (1.5 g L^{-1}); *LAp*1 (1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*); *LAp*2 (1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*); and *A. platensis* (1.5 g L^{-1}). Treatments with *A. platensis* did not develop roots. Means followed by the same letter do not differ statistically ($p < 0.5$) according to the Tukey test. Values ± standard deviation. CV: coefficient of variation.

with lower activity (Lötze & Hoffman 2016), as observed in this study, when comparing the same concentration of *Lithothamnion* sp. and *A. platensis*. Solutions containing *A. platensis* did not promote root development in *V. radiata*. The effect of *Lithothamnion* sp. is attributed to its high bioactive humic acid content, which can exhibit auxin-like activity when applied to plants (Amatussi et al. 2020).

Treating *V. radiata* seeds with 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis* increased the mean diameter of the hypocotyl-radicle, showing the bioactive effect of micronized *Lithothamnion* sp. combined with *A. platensis* at the highest concentration (1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*) (Table 1). The hypocotyl-radicles of *V. radiata* in 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis* showed a 12.47 % increase, when compared to the control. However, the length did not differ between the treatments.

The results of these bioassays indicate that *Lithothamnion* sp. and *A. platensis* have different modes of action that may be complementary.

In the field experiment, the leaves of potato plants treated with *Lithothamnion* sp. and 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis* exhibited a higher relative chlorophyll index (Figure 2a), with 21 and 18 % increases, respectively, when compared to the control. Moreover, the higher chlorophyll content observed in the field resulted in higher tuber total soluble solids at harvest (Figure 2b), with 2 and 15 % increases in *Lithothamnion* sp. and 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*, respectively, when compared to the control. Total soluble solids (ºBrix) can be used as an indirect measure of sugar content (mainly sucrose). Factors such as photosynthetic rate, carbohydrate status and plant hormone balance are essential in sugar accumulation in storage organs (Ševčíková et al. 2017).

Consequently, the accumulation of sugars is related to the increase in leaf area (Figure 3c) and the chlorophyll index (Figure 2a), as observed with *Lithothamnion* sp. applications alone and combined with both concentrations of *A. platensis*, thereby increasing leaf area.

The biometric variables evaluated at the onset of tuberization (55 DAP) demonstrated the effectiveness of *Lithothamnion* sp. on the initial growth of plants, particularly regarding biomass accumulation in leaves and tubers (Figure 3) and leaf area, when applied alone or combined with *A. platensis* (1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis* and 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*). A 33 % increase in leaf dry mass was observed in plants treated with *Lithothamnion* sp. The leaf area of plants treated with *Lithothamnion* sp. alone and in combination (1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis* and 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*) increased by 52.7, 50.2 and 68.3 %, respectively. The tuber fresh mass (Figure 3c) increased by 132 % in the plants treated with *Lithothamnion* sp., when compared to the control.

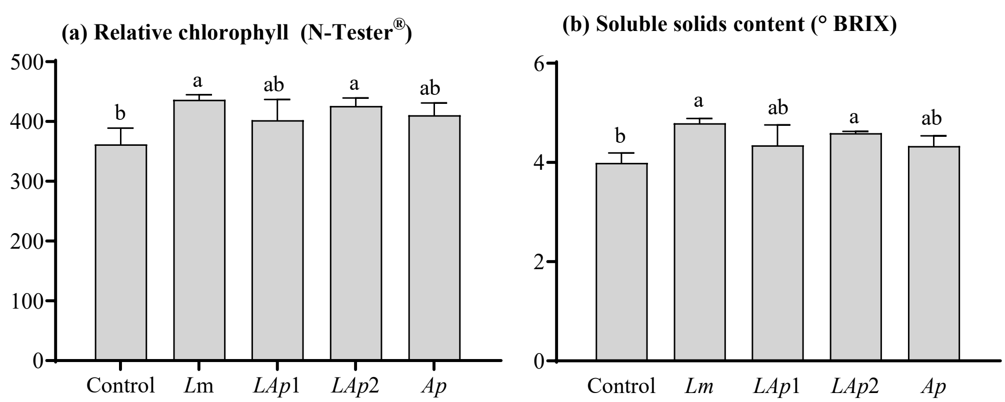

Figure 2. Relative chlorophyll index (a) of potato leaves (55 days after planting - DAP) and soluble solids content (b) of tubers (98 DAP) in plants treated with foliar applications of micronized *Lithothamnion* sp. (*L*m) and *Arthrospira platensis* (*Ap*). Concentrations: control; *Lithothamnion* sp. (1.5 g L^{-1}); *LAp*1 (1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*); *LAp*2 (1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*); and *A. platensis* (1.5 g L^{-1}). Means followed by the same letter do not differ statistically (p < 0.5) according to the Tukey test. Bars indicate the standard deviation.

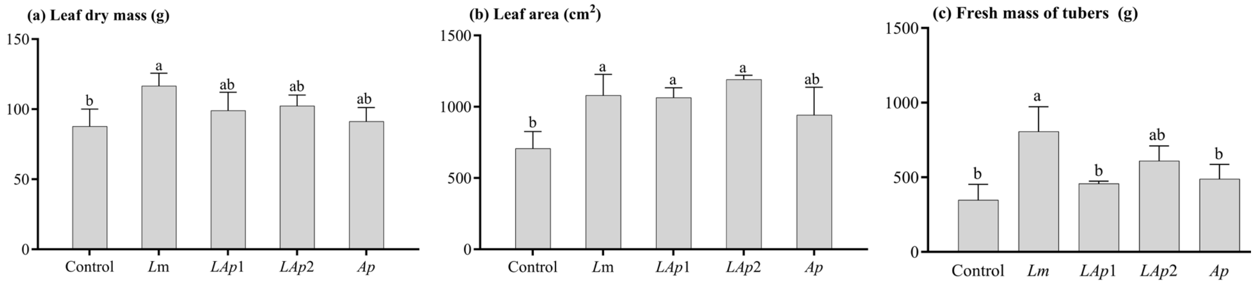

Figure 3. Leaf dry mass (a), leaf area (b) and tuber fresh mass (c) (55 days after planting) of plants treated with foliar applications of micronized *Lithothamnion* sp. (*L*m) and *Arthrospira platensis* (*Ap*). Concentrations: control; *Lithothamnion* sp. (1.5 g L^{-1}); *LAp*1 (1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*); *LAp*2 (1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*); and *A. platensis* (1.5 g L^{-1}). Means followed by the same letter do not differ statistically ($p < 0.5$) according to the Tukey test. Bars indicate the standard deviation.

These results corroborate those of Amatussi et al. (2020), who observed increased leaf dry mass (11.4 %) in tomato plants treated with *Lithothamnion* sp., and Amatussi et al. (2022), who reported a 43 % increase in onion. Gemin et al. (2019) demonstrated that the combination of humic acid with microalgae increased the initial growth of onion plants, as a result of enhanced rooting, characteristic of the auxin-like effect of bioactive humic substances.

The field results showed that the combination of *Lithothamnion* sp. with *A. platensis* increased the sugar content (Figure 4a). The 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis* combination stimulated the source-drain transport, leading to a higher potato yield. Sugars present in the leaves were translocated to the tubers (1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*), thus promoting an increased yield at harvest (Figure 5b).

Plants treated with 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis* and *A. platensis* (1.5 g L^{-1}) showed 50.2 and 38.8 % accumulation of total leaf sugars (Figure 4a). Reducing sugars (e.g., glucose and fructose) increased by 44.4 % in plants treated with the application of 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*. Moreover, non-reducing sugars (e.g., sucrose) in plants treated with *A. platensis* were 92.8 % higher than in the control. At harvest (Figure 4b), tubers from the 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis* treatment exhibited a 66.3 % increase in total sugars, when compared to the control.

In potato cultivation, auxin is important in the initiation, growth and sprouting of potato tubers

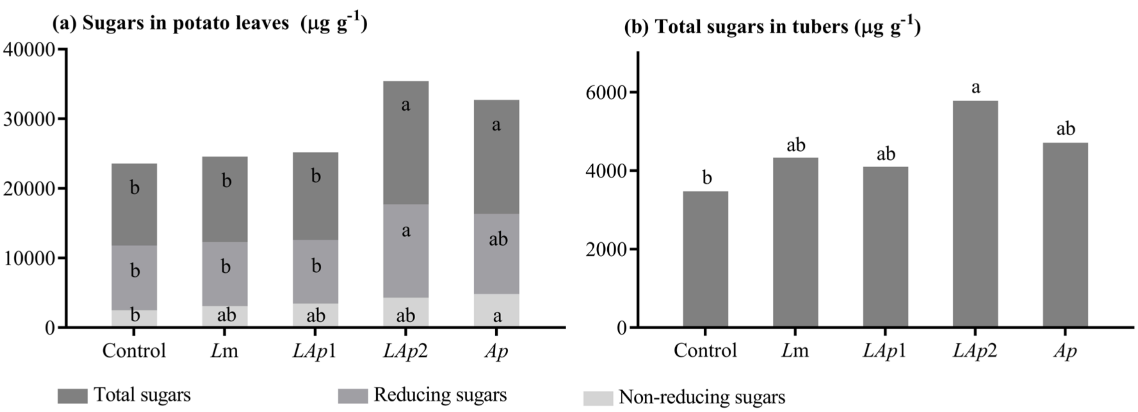

Figure 4. Total sugars, reducing sugars and non-reducing sugars (a) in potato leaves (55 days after planting - DAP); total sugars (b) in potato tubers (98 DAP) of plants treated with foliar applications of micronized *Lithothamnion* sp. (*L*m) and *Arthrospira platensis* (*Ap*). Concentrations: control; *Lithothamnion* sp. (1.5 g L^{-1}); *LAp*1 (1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*); *LAp*2 (1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*); and *A. platensis* (1.5 g L^{-1}). Means followed by the same letter do not differ statistically ($p < 0.5$) according to the Tukey test. Bars indicate the standard deviation.

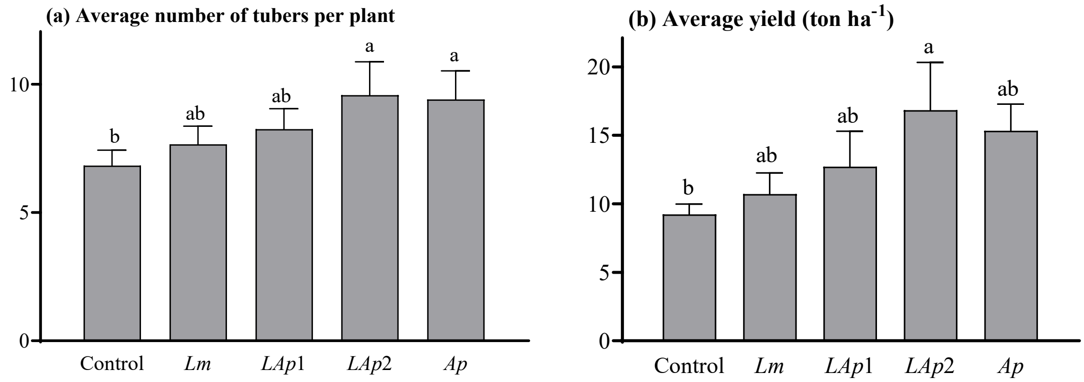

Figure 5. Average number of tubers per plant (98 days after planting - DAP; a) and average yield (t ha^{-1}; b) of potato tubers (98 DAP) in plants treated with foliar applications of micronized *Lithothamnion* sp. (*L*m) and *Arthrospira platensis* (*Ap*). Concentrations: control; *Lithothamnion* sp. (1.5 g L^{-1}); *LAp*1 (1.5 g L^{-1} of *Lithothamnion* sp. + 0.75 g L^{-1} of *A. platensis*); *LAp*2 (1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*); and *A. platensis* (1.5 g L^{-1}). Means followed by the same letter do not differ statistically ($p < 0.5$) according to the Tukey test. Bars indicate the standard deviation.

(Wadas & Dziugiel 2019), and is dependent on carbohydrate status (Kolachevskaya et al. 2019). In addition, the number of tubers per plant is a yield component in potato crops (Prajapati et al. 2016).

At harvest, 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis* and *A. platensis* (1.5 g L^{-1})-treated plants exhibited 40 and 38 % increases in number of tubers (Figure 5a), respectively. Potato yield (Figure 5b) at 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis* (1.5 g L^{-1})-treated plants was 82 % higher than in the control. Similarly, potato plants treated with foliar applications of the microalga *Asterarcys quadricellulare* obtained a higher sugar content, increased chlorophyll and greater potato yield (Cordeiro et al. 2022). These treatments act as stimuli for photoassimilate translocation from source (leaves) to sink (tubers) and showed an important role in increasing plant growth and yield.

The obtained results may be explained by a complex metabolic response enhanced by the algae combination. The synergistic effect of the calcareous algae and cyanobacteria mixture was demonstrated in 1.5 g L^{-1} of *Lithothamnion* sp. + 1.5 g L^{-1} of *A. platensis*, exhibiting an auxin-like effect (Table 1) due to the humic acid in *Lithothamnion* sp. (Amatussi et al. 2020, Ramos et al. 2023). In addition, the possible changes in polyamine metabolism, linked to increased plant growth and yield (Mógor et al. 2017, Chen et al. 2019), along with the cytokinin-like behavior observed following *A. platensis* biomass applications (Figure 1c), may have contributed to enhanced potato plant growth.

The positive effects on plant growth with the combined use of micronized *Lithothamnion* (Amatussi et al. 2020, Mógor et al. 2021) and *Arthrospira platensis* (Mógor et al. 2017) may be a synergistic blend of bioactive compounds that promotes growth and increases potato yield in an environmentally-friendly manner.

CONCLUSIONS

1. The bioactivity of micronized *Lithothamnion* L. and the *Arthrospira platensis* cyanobacteria was demonstrated in *Solanum lycopersicum*, *Cucumis sativus* and *Vigna radiata* bioassays. Foliar applications of the *Lithothamnion* sp. and *A. platensis* combination increased the growth of potato plants, stimulating their chlorophyll index, number of tubers, tuber sugar content and yield;
2. The beneficial effect of foliar application of micronized *Lithothamnion* (1.5 g L^{-1}) combined with lyophilized *A. platensis* biomass (1.5 g L^{-1}) on potato crops is a novel natural input to sustainably improve yield.

ACKNOWLEDGMENTS

The authors are grateful to Ely Cristina Negrelli Cordeiro (*in memoriam*), for her assistance with the study; and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for the doctoral scholarship awarded to the first author (Finance code 001).

REFERENCES

AMATUSSI, J. O.; MÓGOR, A. F.; MÓGOR, G.; LARA, G. B. Novel use of calcareous algae as a plant biostimulant. *Journal of Applied Phycology*, v. 32, n. 3, p. 2023-2030, 2020.

AMATUSSI, J. O.; MÓGOR, A. F.; CORDEIRO, E. C. N.; MÓGOR, G.; MARQUES, H. M. C.; LARA, G. B. Synergic combination of calcareous algae and cyanobacteria stimulate metabolic alterations improving plant growth and yield. *Journal of Applied Phycology*, v. 35, n. 1, p. 483-493, 2022.

BRASIL. Instrução normativa nº 39, de 8 de agosto de 2018. Estabelece as regras sobre definições, exigências, especificações, garantias, registro de produto, autorizações, embalagem, rotulagem, documentos fiscais, propaganda e tolerâncias dos fertilizantes minerais destinados à agricultura. *Diário Oficial da União*, Brasília, DF, seção 1, n. 154, p. 19, 10 ago. 2018.

BRASIL. Instrução normativa nº 61, de 8 de julho de 2020. Estabelece as regras sobre definições, exigências, especificações, garantias, tolerâncias, registro, embalagem e rotulagem dos fertilizantes orgânicos e dos biofertilizantes, destinados à agricultura. *Diário Oficial da União*, Brasília, DF, p. 5, 8 jul. de 2020.

BRASIL. Lei nº 15.070 de 23 de dezembro de 2024. Dispõe sobre a produção, a importação, a exportação, o registro, a comercialização, o uso, a inspeção, a fiscalização, a pesquisa, a experimentação, a embalagem, a rotulagem, a propaganda, o transporte, o armazenamento, as taxas, a prestação de serviços, a destinação de resíduos e embalagens e os incentivos à produção de bioinsumos para uso agrícola, pecuário, aquícola e florestal; e altera as Leis nº 14.785, de 27 de dezembro de 2023, 10.603, de 17 de dezembro de 2002, e 6.894, de 16 de dezembro de 1980. *Diário Oficial da União*, Brasília, DF, seção 1, n. 247, p. 3, 24 dez. 2024.

CHEN, D.; SHAO, Q.; YIN, L.; YOUNIS, A.; ZHENG, B. Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. *Frontiers in Plant Science*, v. 9, e1945, 2019.

CORDEIRO, E. C. N.; MÓGOR, Á. F.; AMATUSSI, J. O.; MÓGOR, G.; MARQUES, H. M. C.; LARA, G. B. Microalga biofertilizer improves potato growth and yield, stimulating amino acid metabolism. *Journal of Applied Phycology*, v. 34, n. 1, p. 385-394, 2022.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA (Embrapa). *Sistema brasileiro de classificação de solos*. 3. ed. Brasília, DF: Embrapa, 2013.

EVANGELISTA, A. W. P.; SÁ, A. R. M.; ALVES JÚNIOR, J.; CASAROLI, D.; LEANDRO, W. M.; SOUZA, J. L. M. Irrigation and *Lithothamnium* fertilization in bell pepper cultivated in organic system. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v. 20, n. 9, p. 830-835, 2016.

FARIA, V. de O.; RIBEIRO, L. L. O.; GODINHO, B. F.; SILVEIRA, B. B. de; SIMON, M. V.; STANGARLIN, J. R. Potencial indutor de fitoalexinas em feijão e soja por formulado a base de alecrim + *Lithothamnion*. *Revista Ambientale*, v. 14, n. 2, p. 1-9, 2022.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO). *Database 2023*. 2023. Available at: https://www.fao.org/faostat/en/#data/QCL. Access on: Jan. 30, 2025.

FREITAS, M. B.; PAULERT, R.; RIBEIRO, C. F.; BORBA, M. C.; ALINE, C.; VELHO, A. C.; STADNIK, M. J. Extratos de macroalgas marinhas como biofertilizantes. *In*: MÓGOR, A. F.; MÓGOR, G. (org.). *Aminoácidos, extratos de algas, extratos vegetais e substâncias húmicas como biofertilizantes*. Piracicaba: Pecege, 2022. p. 29-58.

GEMIN, L. G.; MÓGOR, Á. F.; AMATUSSI, J. O.; MÓGOR, G. Microalgae associated to humic acid as a novel biostimulant improving onion growth and yield. *Scientia Horticulturae*, v. 256, e108560, 2019.

GEMIN, L. G.; MÓGOR, Á. F.; AMATUSSI, J. O.; LARA, G. B.; MÓGOR, G. Organic onion growth, yield and storage improved by foliar sprays of microalgae and fulvic acid as a natural biofertilizer. *Bioscience Journal*, v. 38, e38045, 2022.

GODLEWSKA, K.; MICHALAK, I.; PACYGA, P.; BAŚLADYŃSKA, S.; CHOJNACKA, K. Potential applications of cyanobacteria: *Spirulina platensis* filtrates and homogenates in agriculture. *World Journal of Microbiology & Biotechnology*, v. 35, e80, 2019.

HUSSEIN, M. H.; ELTANAHY, E.; AL BAKRY, A. F.; ELSAFTY, N.; ELSHAMY, M. M. Seaweed extracts as prospective plant growth bio-stimulant and salinity stress alleviator for *Vigna sinensis* and *Zea mays*. *Journal of Applied Phycology*, v. 33, n. 2, p. 1273-1291, 2021.

KOLACHEVSKAYA, O. O.; LOMIN, S. N.; ARKHIPOV, D. V.; ROMANOV, G. A. Auxin in potato: molecular aspects and emerging roles in tuber formation and stress resistance. *Plant Cell Reports*, v. 38, n. 6, p. 681-698, 2019.

LÖTZE, E.; HOFFMAN, E. W. Nutrient composition and content of various biological active compounds of three South African-based commercial seaweed biostimulants. *Journal of Applied Phycology*, v. 28, n. 2, p.1379-1386, 2016.

MALA, R.; CELSIA, A. S. R.; MAHALAKSHMI, R.; RAJESWARI, S. Agronomic biofortification of *Amaranthus dubius* with macro nutrients and vitamin A.

IOP Conference Series: Materials Science and Engineering, v. 225, e012214, 2017.

MALDONADE, I. R.; CARVALHO, P. G. B. de; FERREIRA, N. A. *Protocolo para determinação de açúcares totais em hortaliças pelo método de DNS*. Brasília, DF: Embrapa Hortaliças, 2013.

MARQUES, H. M. C.; CORDEIRO, E. C. N.; AMATUSSI, J. O.; LARA, G. B.; MÓGOR, G.; NEDILHA, L. C. B. M.; MÓGOR, Á. F. Mitigation of water restriction effects on soybean with biofertilizer: metabolic and stomatal conductance changes. *Research, Society and Development*, v. 10, n. 11, e11101119377, 2021.

MARQUES, H. M. C.; MÓGOR, Á. F.; AMATUSSI, J. O.; LARA, G. B.; MÓGOR, G.; SANT'ANNA-SANTOS, B. F. Use of microalga *Asterarcys quadricellularis* in common bean. *Journal of Applied Phycology*, v. 35, n. 6, p. 2891-2905, 2023.

MARQUES, H. M. C.; MÓGOR, Á. F.; AMATUSSI, J. O.; LARA, G. B.; MÓGOR, G.; BRASILEIRO, B. P.; SANT'ANNA-SANTOS, B. F. Microalgal biomass application promotes growth and alleviates salt stress in melon plants stimulating antioxidant metabolism. *Journal of Applied Phycology*, v. 37, n. 1, p. 379-396, 2025.

MAZEPA, E.; MALBURG, B. V.; MÓGOR, G.; OLIVEIRA, A. C.; AMATUSSI, J. O.; CORRÊA, D. O.; LEMOS, J. S.; DUCATTI, D. R. B.; DUARTE, M. E. R.; MÓGOR, A. F.; NOSEDA, M. D. Plant growth biostimulant activity of the green microalga *Desmodesmus subspicatus*. *Algal Research*, v. 59, e102434, 2021.

MÓGOR, G.; MÓGOR, Á. F.; LIMA, G. P. P.; OLIVEIRA R. A.; BESPALHOK, J. C. F. Metabolic changes in sugarcane bud sprouting stimulated by microalga *Asterarcys quadricellulare*. *Sugar Tech*, v. 24, n. 8, p. 930-940, 2022a.

MÓGOR, A. F.; AMATUSSI, J. O.; MÓGOR, G.; GEMIN, L. G. Biostimulant action of *Lithothamnium* sp. promoting growth, yield, and biochemical and chemical changes on onion. *Journal of Applied Phycology*, v. 33, n. 3, p. 1905-1913, 2021.

MÓGOR, Á. F.; AMATUSSI, J. O.; MÓGOR, G.; LARA, G. B. Bioactivity of cyanobacterial biomass related to amino acids induces growth and metabolic changes on seedlings and yield gains of organic red beet. *American Journal of Plant Sciences*, v. 9, n. 5, p. 966-978, 2018.

MÓGOR, A. F.; BARBIZAN, T.; PAULETTI, V.; OLIVEIRA, J.; BETTONI, M. M. Teores de clorofila em cultivares de tomateiro submetidas a aplicações foliares de magnésio. *Pesquisa Agropecuária Tropical*, v. 43, n. 4, p. 363-369, 2013.

MÓGOR, A. F.; ÖRDÖG, V.; LIMA, G. P. P.; MOLNÁR, Z.; MÓGOR, G. Biostimulant properties of cyanobacterial hydrolysate related to polyamines. *Journal of Applied Phycology*, v. 30, n. 1, p. 453-460, 2017.

MÓGOR, G.; AMATUSSI, J. O.; CORDEIRO, E. C. N. Bioensaios e determinações bioquímicas na avaliação da bioatividade de biofertilizantes. *In*: MÓGOR, A. F.; MÓGOR, G. (org.). *Aminoácidos, extratos de algas, extratos vegetais e substâncias húmicas como biofertilizantes*. Piracicaba: Pecege, 2022b. p. 101-113.

NAVARRO-LÓPEZ, E.; RUÍZ-NIETO, A.; FERREIRA, A.; ACIÉN, F. G.; GOUVEIA, L. Biostimulant potential of *Scenedesmus obliquus* grown in brewery wastewater. *Molecules*, v. 25, n. 3, e664, 2020.

NEGREIROS, A. M. P.; MELO, N. J. A.; BARBOZA, H. S.; SALES JÚNIOR, R. Enhancing melon production and quality through the application of '*Lithothamnium*'. *Revista Caatinga*, v. 36, n. 3, e590, 2023.

PASSOS, S.; RECH, C.; KAWAKAMI, J.; NAZARENO, N. R. X.; BARBOSA, M. R.; NARDI, C. Performance of potato cultivars grown in the organic production system. *Horticultura Brasileira*, v. 40, n. 3, p. 268-274, 2022.

PEREIRA, A. S.; BERTONCINI, O.; SILVA, G. O.; CASTRO, C. M.; GOMES, C. B.; HIRANO, E.; BORTOLETTO, A. C. BRS Clara: cultivar de batata para mercado fresco, com resistência à requeima. *Horticultura Brasileira*, v. 31, n. 4, p. 664-668, 2013.

PLAZA, B. M.; GÓMEZ-SERRANO, C.; ACIÉN-FERNÁNDEZ, F. G.; JIMENEZ BECKER, S. Effect of microalgae hydrolysate foliar application (*Arthrospira platensis* and *Scenedesmus* sp.) on *Petunia x hybrida* growth. *Journal of Applied Phycology*, v. 30, n. 2, p. 2359-2365, 2018.

PRAJAPATI, A.; PATEL, C. K.; SINGH, N.; JAIN, S. K.; CHONGTHAM, S. K.; MAHESHWARI, M. N.; PATEL, C. R.; PATEL, R. N. Evaluation of seaweed extract on growth and yield of potato. *Environment & Ecology*, v. 34, n. 2, p. 605-608, 2016.

RAMOS, E. P.; FERREIRA, T. R.; AGUIAR, D. B. de.; ALVES, F. L.; DOUSSEAU-ARANTES, S. *Lithothamnion* sp. as biostimulant in plant cultivation. *Pesquisa Agropecuária Tropical*, v. 53, e76273, 2023.

RÖDER, C.; MÓGOR, Á. F.; SZILAGYI-ZECCHIN, V. J.; GEMIN, L. G.; MÓGOR, G. Potato yield and metabolic changes by use of biofertilizer containing L-glutamic acid. *Comunicata Scientiae*, v. 9, n. 2, p. 211-218, 2018.

ŠEVČÍKOVÁ, H.; MAŠKOVÁ, P.; TARKOWSKÁ, D.; MAŠEK, T.; LIPAVSKÁ, H. Carbohydrates and gibberellins relationship in potato tuberization. *Journal of Plant Physiology*, v. 214, n. 1, p. 53-63, 2017.

SILVA, F. A. S.; AZEVEDO, C. A. V. The Assistat software version 7.7 and its use in the analysis of experimental data. *African Journal of Agricultural Research*, v. 11, n. 39, p. 3733-3740, 2016.

STIRK, W. A.; ORDOG, V.; VAN STADEN, J.; JAGER, K. Cytokinin and auxin like activity in cyanophyta and microalgae. *Journal of Applied Phycology*, v. 14, n. 1, p. 215-221, 2002.

TRIPEPI, R. R.; GEORGE, M. W. Identification of bacteria infecting seedlings of mung bean used in rooting bioassays. *Journal of the American Society for Horticultural Science*, v. 116, n. 1, p. 80-84, 1991.

VIDAL, M. C.; AMARAL, D. F. S.; NOGUEIRA, J. D.; MAZZARO, M. A. T.; LIRA, V. M. C. Bioinsumos: a construção de um programa nacional pela sustentabilidade do agro brasileiro. *Economic Analysis of Law Review*, v. 12, n. 3, p. 557-574, 2021.

VIRMOND, E. P.; KAWAKAMI, J.; VONCIK, K. S.; CÓRDOVA, K. R. V.; SLOMPO, P. J. H. Características físico-químicas de cultivares de batata sob cultivo orgânico. *Ambiência*, v. 10, n. 1, p. 31-42, 2014.

WADAS, W.; DZIUGIEŁ, T. Growth and marketable potato (*Solanum tuberosum* L.) tuber yield in response to foliar application of seaweed extract and humic acids. *Applied Ecology and Environmental Research*, v. 17, n. 6, p. 13219-13230, 2019.

ZHAO, Z. R.; WU, Z. L.; HUANG, G. Q.; LI, G. R. An improved disk bioassay for determining activities of plant growth regulators. *Journal of Plant Growth Regulation*, v. 11, n. 1, p. 209-2013, 1992.