

Biostimulant effect of microalgae *Chlorella sorokiniana* on growth of tomato (*Solanum lycopersicum* L.)

Efecto bioestimulante de la microalga *Chlorella sorokiniana* en cultivo de tomate (*Solanum lycopersicum* L.)

Padilla-Valle, Y. K. , Ulloa-Mercado, G. , Gutiérrez-Coronado, M. A. ,
Rentería-Mexía, A. , Gortáres-Moroyoqui, P. , Díaz- Quiroz, C. A. 

¹ Departamento de Biotecnología y Ciencias Alimentarias. Instituto Tecnológico de Sonora. 5 de Febrero 818 Sur, 85000, Ciudad Obregón, Sonora, México.



Please cite this article as/Como citar este artículo: Padilla-Valle, Y. K., Ulloa-Mercado, G., Gutiérrez-Coronado, M. A., Rentería-Mexía, A., Gortáres-Moroyoqui, P., Díaz- Quiroz, C. A. (2025). Biostimulant effect of microalgae *Chlorella sorokiniana* on growth of tomato (*Solanum lycopersicum* L.). *Revista Bio Ciencias*, 13, e1922.
<https://doi.org/10.15741/revbio.13.e1922>

Article Info/Información del artículo

Received/Recibido: March 13th 2025.

Accepted/Aceptado: September 30th 2025.

Available on line/Publicado: October 15th 2025.

ABSTRACT

Microalgae are rich in bioactive compounds with biostimulant effects that, when applied to plants, stimulate their physiological processes. This study aimed to evaluate the biostimulant effect of *Chlorella sorokiniana* on tomato crops. Concentrations of 0.1, 0.5, and 1 % microalgal biomass were tested with three application methods: irrigation, foliar spray, and combined (irrigation and foliar). The main results showed that plant height and chlorophyll content were higher under soil application at 0.1 %. The greatest fruit weight (67 ± 0.08 g) and yield (13.01 ± 2.17 t·ha⁻¹) were obtained with soil application at 0.5 %. Likewise, the highest number of fruits was observed with soil application at 1 % (331 ± 7.95), which was significantly higher ($p \leq 0.05$) than the control (123 ± 2.77). Leaf and root dry weights under foliar and combined applications at 0.1 and 0.5 % were significantly greater ($p \leq 0.05$) than the control. Foliar nutrient analysis further showed that macronutrient levels remained within the “sufficient” range, while in the control, they were “low.” These findings demonstrate that low concentrations of microalgal extract stimulate plant growth, nutrient uptake, and crop yield.

KEY WORDS: Intracellular extracts, Crops, Microorganisms, Bioactive compounds, Physiological variables.

*Corresponding Author:

Gabriela Ulloa-Mercado. Departamento de Biotecnología y Ciencias Alimentarias. Instituto Tecnológico de Sonora. 5 de Febrero, 818 Sur, 85000, Ciudad Obregón, Sonora, México. Teléfono: +52 (644) 4109000. E-mail: ruth.ulloa@itson.edu.mx

RESUMEN

Las microalgas son ricas en compuestos bioactivos con efecto bioestimulante que, al ser aplicados a plantas, estimulan sus procesos fisiológicos. El objetivo fue evaluar el efecto bioestimulante de *Chlorella sorokiniana* en el cultivo de tomate. Se evaluó a concentración de 0.1, 0.5 y 1 % de biomasa de microalga, con aplicaciones en 3 formas: riego, foliar y mixto (riego y foliar). En los principales resultados se observó que la altura y clorofila fueron mayores en la aplicación al suelo a 0.1 %. El mayor peso del fruto (67 ± 0.08 g) y rendimiento (13.01 ± 2.17 t·ha⁻¹) se tuvo en la aplicación al suelo a 0.5 %. Así mismo, se obtuvo mayor número de frutos en la aplicación al suelo al 1 % (331 ± 7.95), siendo significativamente mayor ($p \leq 0.05$) al testigo (123 ± 2.77). El peso seco foliar y radicular en las aplicaciones foliares y mixtas a concentraciones de 0.1 y 0.5 % fueron mayores ($p \leq 0.05$) al testigo. Así mismo, se observó en el análisis foliar que los macronutrientes permanecieron en el rango “suficientes”, mientras que el testigo fueron “bajos”. Con ello se demuestra que bajas concentraciones de extracto de microalga, estimula tanto el crecimiento de la planta, el aprovechamiento de nutrientes y el rendimiento del cultivo.

PALABRAS CLAVE: Extractos intracelulares, cultivos agrícolas, microorganismos, compuestos bioactivos, variables fisiológicas.

Introduction

Currently, the increasing demand for food poses a challenge in the search for new technologies that aim to improve crop performance while also addressing objectives such as conserving agroecosystems and producing food free of toxic residues for consumers (Ronga *et al.*, 2019). This challenge has led to the development of innovative agricultural products, including biofertilizers and plant growth biostimulants (Supraja *et al.*, 2020). According to the European Union regulation on fertilizer products, biostimulants are defined as “products containing mixtures of substances or microorganisms that can be applied to plants or the rhizosphere to stimulate natural processes, nutrient absorption, nutrient use efficiency, tolerance to stress (biotic and abiotic), and, consequently, crop quality.” This regulation establishes that plant biostimulant products must comply with fertilizer marketing legislation, as they are considered complementary to fertilizers under Regulation (EU) 2019/1009.

Biostimulants may contain bioactive compounds such as polysaccharides (Farid *et al.*, 2019), phytohormones, vitamins, pigments, amino acids, and antimicrobial compounds with biopesticidal activity (Mutale-joan *et al.*, 2021). Among the substances considered biostimulants are protein hydrolysates, humic acids, and microorganisms such as bacteria, fungi, and microalgae.

Microalgae are photosynthetic microorganisms, some marine and others freshwater, with varying nutrient compositions. They are biotechnologically exploited in the food, agricultural, pharmaceutical, and biofuel industries (Ranglová *et al.*, 2021). Plant growth-promoting substances (phytohormones) such as auxins, cytokinins, gibberellins, and abscisic acid have been identified in microalgae. Stirk *et al.* (2013) studied phytohormone content in microalgal strains and demonstrated that total auxin concentrations exceeded those of cytokinins in all strains analyzed. Auxins such as indole-3-acetic acid (IAA) and indole-3-acetamide (IAM) were present in all strains, with IAA concentrations consistently higher than IAM. No correlation was found between phytohormone concentration and the growth phase of the microalga or with biomass accumulation in culture. Phytohormones have been detected in the families Charophyceae, Chlorophyceae, Trebouxiophyceae, and Ulvophyceae, with predominant microalgal species including *Chlorella vulgaris*, *Chlorella ellipsoidea*, *Chlorella infusionum*, *Chlorella sp.*, *Dunaliella salina*, *Chlorella sorokiniana*, and *Spirulina máxima* (Mutale-Joan *et al.*, 2020).

The biostimulant activity of microalgae has been linked to the content of primary metabolites, including carbohydrates, proteins, and lipids (Mutale-joan *et al.*, 2020). Furthermore, some microalgae can be cultivated in wastewater and assimilate nitrogen, phosphorus, and carbon present in it (Ferreira *et al.*, 2021), thereby reducing nutrient input costs and minimizing the environmental impact of wastewater discharge (Navarro-López *et al.*, 2020). This, however, requires compliance with safety criteria to ensure no health risks are associated with food agriculture. In this regard, when determining the presence of total and fecal coliforms in the biostimulant, their concentration was below the limits established by NOM-003-ECOL-1997.

Several authors have suggested that combining microalgal extracts with inorganic and organic fertilizers could enhance sustainable agricultural productivity. There are three common methods of applying biostimulants to crops: foliar application, seed treatment, and soil inoculation, all of which have shown positive responses such as increased germination, root system development, yield (Arioli *et al.*, 2024), higher chlorophyll content and leaf area, as well as improved fruit quality, vigor, and greater resistance to biotic and abiotic stress (Shukla *et al.*, 2019).

Tomato (*Solanum lycopersicum* L.) is one of the most important crops worldwide. It can be cultivated in different soil types and throughout the year, depending on irrigation practices (Campobenedetto *et al.*, 2021). Tomato is an affordable and globally available vegetable, thereby contributing to food security. For this reason, and due to its short harvest cycles, it has been widely used as a model crop in numerous studies (Krid *et al.*, 2023).

Given this background, the objective of the present study was to evaluate the agronomic effects of applying a biostimulant derived from the microalga *Chlorella sorokiniana* to *Solanum lycopersicum* L. Furthermore, the following hypothesis was proposed: the application of a *Chlorella sorokiniana*-based biostimulant will significantly increase agronomic yield and improve fruit quality in tomato cultivation.

Material and Methods

Microalgal biomass production

A *Chlorella sorokiniana* strain, previously isolated in southern Sonora, Mexico, and characterized by the research group, was used. A thin-layer cascade photobioreactor was operated in continuous mode (renewal rate of 30 % d⁻¹) with a working volume of 20 L, using swine wastewater as the nutrient source. The wastewater was characterized by determining soluble chemical oxygen demand (COD), N-NO₃⁻, N-NH₄⁺, and P-PO₄³⁻, according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005), at the beginning and throughout a five-day culture period. The photobioreactor was inoculated with 10 % v/v microalgae. The culture was carried out under ambient conditions with minimum temperatures of 17 ± 2 °C and maximum temperatures of 25 ± 2 °C during the day, and a pH of 8.5 (recorded three times daily at 09:00, 13:00, and 19:00 h). Aeration was applied at a rate of 66.4 L·min⁻¹ (Gracida-Valdepeña *et al.*, 2020). Once steady state was reached, daily harvests were performed, centrifuged to obtain biomass, which was subsequently used for the preparation of biostimulant suspensions and for biochemical composition analysis.

Preparation of the biostimulant

After harvesting, the dry biomass weight was determined as the basis for preparing the different concentrations of the biostimulant, either by diluting or concentrating (by centrifugation) the culture to obtain 0.1, 0.5, and 1.0 % w/v based on dry biomass. Once the required volume of microalgal suspension was adjusted to the desired concentration, it was subjected to five freeze-thaw cycles at -120 °C and ambient temperature, respectively, to disrupt the cells and release intracellular compounds prior to application in tomato cultivation.

Experimental design

A completely randomized design with a 3² factorial arrangement plus a control was used. For the application method factor, three treatments were evaluated: soil (directly applied to the substrate), foliar (via sprayer), and combined (soil + foliar), at a dose of 150 mL per tomato (*Solanum lycopersicum* L.) plant. The second factor was dry biomass concentration in the biostimulant (w/v): 0.1, 0.5, and 1.0 %. The experiment consisted of 10 treatments with 10 replicates each, defined as: T1: foliar 0.1 %; T2: foliar 0.5 %; T3: foliar 1 %; T4: soil 0.1 %; T5: soil 0.5 %; T6: soil 1 %; T7: combined 0.1 %; T8: combined 0.5 %; T9: combined 1 %; T10: control.

Agronomic management

Saladette-type tomato seeds (Kristen Seed®, “Río Grande” variety, determinate growth habit) were sown in 10 L pots containing “PRO-MIX®” substrate (70 % peat moss, 30 % perlite) under greenhouse conditions at 24 ± 2 °C and 72 ± 3 % relative humidity. A density of 100 plants was established in a 6 x 8 m area, with 30 cm spacing between pots (one plant per pot) and 90 cm

between rows. Applications of the microalgal treatments were performed during the phenological stages of vegetative growth, flowering, and fruit ripening.

Conventional fertilization was applied at rates of 250-150-250 N-P-K kg·ha⁻¹. Urea (46 %) and potassium nitrate (Ultra N-K-S) were used as nitrogen and potassium sources in five applications at 20, 40, 50, 60, and 80 days after emergence (dae). Diammonium phosphate (MAP, technical grade) was used as the phosphorus source in three applications: at emergence, and at 40 and 60 dae. In addition, calcium (Ca(NO₃)₂) and magnesium (MgSO₄) were applied three times at 2 kg·ha⁻¹ each, along with micronutrients (Bluefeed micros[®]) at manufacturer-recommended rates, applied every 10 days starting from the first floral buds.

Physiological measurements

Throughout the tomato growth cycle, plant height (cm) and chlorophyll content (SPAD units) were measured. Chlorophyll content was determined using the Soil Plant Analysis Development method (SPAD-502, Minolta Co., Osaka, Japan). Measurements were taken weekly between 11:00 and 14:00 h. Three readings were taken per leaf from each replicate per treatment, and the mean value was recorded. Leaf and root dry weights were determined gravimetrically after drying leaves, stems, and roots at 60 °C for 48 h.

Nutritional analysis of foliar tissue

Macro- and micronutrient contents of plant tissues were determined during early flowering and fruit maturation stages (Jones *et al.*, 1991). For nutritional analyses, the fourth leaf of each plant was dried at 70 °C for 12 h and ground in a mortar. Acid digestion was performed with 4 mL H₂SO₄ using a preheated HACH digester at 440 °C for 1 min, followed by the addition of 7 mL 50 % H₂O₂ for 4 min. Macro (%) and micro (ppm) nutrient contents were quantified using HACH reagent kits (Alcántar & Sandoval, 1999).

Statistical analysis

A completely randomized design with a 3 x 3 factorial arrangement plus a control was applied. The factors were application method and biomass concentration of the biostimulant (% w/v). Data were analyzed using factorial ANOVA followed by Tukey's multiple mean comparison test at a significance level of $p \leq 0.05$. Main effects and factor interactions were determined using IBM SPSS Statistics software.

Results and Discussion

The microalga was cultivated in swine wastewater. After four days of culture, removal rates above 90 % were achieved for CODs and N-NO₃⁻, and around 35 % for N-NH₄⁺ and P-PO₄³⁻ (Table 1). Once the culture reached steady state, a cell concentration of $138 \pm 9.2 \times 10^6$ cells·mL⁻¹ was obtained, equivalent to 1.9 g·L⁻¹ of dry biomass. This dry weight served as the basis for

biostimulant preparation, and its biochemical composition was subsequently determined (Table 2), showing protein levels of up to $200 \mu\text{g}\cdot\text{mL}^{-1}$, lipids of $2700 \mu\text{g}\cdot\text{mL}^{-1}$, and carbohydrates of up to $700 \mu\text{g}\cdot\text{mL}^{-1}$.

This pattern of nutrient removal from wastewater by microalgae has been well documented. The results obtained in the present study were consistent with those reported by Gracida-Valdepeña *et al.* (2020), who used the same wastewater source, strain, and reactor. Moreover, the cultivation of microalgae in wastewater makes them protein-rich due to the nitrogen content in the medium. This also suggests that they are a promising source of essential amino acids, such as tryptophan and arginine, which plants utilize to synthesize key hormones. In addition, polysaccharides have been systematically shown to promote plant growth and enhance stress tolerance (Ferreira *et al.*, 2021), the lipid content of microalgae also exerts a positive impact on plants; even small amounts of certain sterols, such as campesterol, are crucial. These sterols act as precursors of oxidized steroids, collectively known as brassinosteroids, which are essentially plant growth hormones. Brassinosteroids play a vital role in plant metabolism, regulating processes such as photosynthesis, cell division, and cell expansion (Rachidi *et al.*, 2020). In a related study, La Bella *et al.* (2021) demonstrated that foliar application of a *Chlorella vulgaris*-derived biostimulant on lettuce leaves resulted in increased plant weight. This positive effect is believed to be associated with the higher pigment and protein content provided by the biostimulant.

Table 1. Characterization of pig wastewater used for microalgae culture.

Time (d)	CODs (mg L ⁻¹)	Removal (%)	Ammonium (mg L ⁻¹)	Removal (%)	Phosphorus (mg L ⁻¹)	Removal (%)	Nitrates (mg L ⁻¹)	Removal (%)
0	2372.22	0	302.55	0	274	0	30.654	0
1	552.22	49.73	207.11	12	104.33	62.04	0.823	97.3
2	1641	11.78	237.7	8	229.33	16.30	0.781	97.44
3	874.4	33.98	134.9	27	180.00	13.50	0.506	98.34
4	46.33	93.78	107.7	35	158.67	34.30	0.443	98.56
5	63.33	91.63	123.13	30	109.67	60.21	0.549	98.20

Source: Own elaboration based on the results of this study.

Table 2. Protein, lipids, and carbohydrate content ($\mu\text{g mL}^{-1}$) on the biostimulant at 0.1, 0.5, and 1.0 % of microalgae biomass content.

Proteins			Lípidos			Carbohidrates		
0.1%	0.5%	1.0%	0.1%	0.5%	1.0%	0.5%	1.0%	0.1%
204	1019	2038	269.69	1325	2700	71.62	350	710
± 4.9	± 25	± 246	± 9.06	± 51	± 105	± 3.8	± 25	± 55

Source: Own elaboration based on results of this study.

On the other hand, the effect of each factor, application method, and biostimulant concentration, as well as their interaction on tomato cultivation, was evaluated. Regarding the concentration factor, the treatments showed a similar trend across the three application methods: foliar, soil, and combined. For chlorophyll, foliar dry weight, and yield, only the 0.1 % treatment was significantly different ($p \leq 0.05$) from the 1.0 % and 0.5 % concentrations, with the latter producing the best results. For plant height, root dry weight, and fruit weight, all concentrations tested were significantly different, except between 1.0 % and 0.1 %. In other words, the concentration factor had a significant effect on most of the evaluated variables, with 0.5 % and 1.0 % producing the best results. Analysis of the application method factor revealed that, at 0.5 % concentration, significant differences ($p \leq 0.05$) were observed for all variables, except for foliar and root dry weight, in which no difference was detected between soil and combined application. The same trend was recorded at 0.1 % and 1.0 % concentrations for plant height, foliar dry weight, and root dry weight. Thus, for most variables, the application method had a significant effect, with foliar application producing significantly lower results compared to soil and combined application. When analyzing factor interactions, significant differences ($p \leq 0.05$) were found for all variables, except for chlorophyll. This indicates that the effects of the factors were maximized through their interaction, with foliar and combined application at 0.5 % and 1.0 % delivering the best results.

In addition, the effect of applying the microalgae-based biostimulant on tomato cultivation was evaluated. Regarding plant height (Figure 1), T1 (81.5 ± 2.1 cm) and T2 (81.6 ± 5.7 cm) achieved the greatest heights during the first three phenological stages (vegetative growth, flowering, and fruit set), being significantly different ($p \leq 0.05$) from the control (70.5 ± 3.97 cm). During the fruit ripening stage, the highest plant heights were recorded in T4, T7, and T8, with 111.1 ± 4.51 cm, 110.2 ± 4.49 cm, and 109.9 ± 2.73 cm, respectively, significantly higher ($p \leq 0.05$) than the control (94.6 ± 6.74 cm). Similarly, for chlorophyll content expressed in SPAD units (Figure 2), T4 showed significant differences ($p \leq 0.05$) compared to the control, with values of 42.32 ± 3.2 at the flowering stage. However, at weeks 15 and 24, all treatments showed higher values than the control, with significant differences ($p \leq 0.05$). The evaluation of chlorophyll content in SPAD units is a reliable indicator of photosynthetic activity, nitrogen, and leaf chlorophyll content. The increase in chlorophyll in plants results from efficient nitrogen and

phosphorus assimilation, indicating that plants received adequate nutrition (Weisser *et al.*, 2024). Favorable results have been reported in plants receiving nitrogen and phosphorus supplied by algae (Gitau *et al.*, 2022).

These results demonstrate that the application of the *C. sorokiniana*-based biostimulant exerts beneficial effects on the vegetative development of tomato crops. This agrees with previous studies reporting significant improvement in tomato growth with *C. vulgaris*-based biostimulants at 1 mg L⁻¹ (Barone *et al.*, 2019). Foliar application of microalgae-based biostimulants has been shown to enable faster nutrient absorption and more efficient correction of deficiencies compared to soil application (Garcia-Gonzalez & Sommerfeld, 2016). In addition, microalgae can influence metabolic processes such as cellular respiration, photosynthesis, and synthesis of growth-regulating hormones, leading to apical elongation and dominance. Soil application can also improve nutrient availability and uptake, enhance soil water retention, increase antioxidant content, improve cellular metabolism, and raise leaf chlorophyll levels (Ronga *et al.*, 2019); However, in this study, combined application, soil plus foliar, provided the best results, potentiating the benefits of both individual methods. Similar results were obtained by Mostafa *et al.* (2024) with *C. vulgaris* and *Spirulina platensis* biostimulants in tomato plants, increasing chlorophyll a content by more than 70 % compared to the control. Calderón-Arias *et al.* (2024) also reported that values above 31 SPAD units are adequate for tomato cultivation, which aligns with the chlorophyll levels in this study, which ranged between 38 to 44 SPAD units. Photosynthetic capacity depends on leaf chlorophyll content and overall plant health. Chlorophyll is the primary constituent enabling photosynthesis, imparting the characteristic green color and allowing light energy absorption for the process (Zulkarnaini *et al.*, 2019).

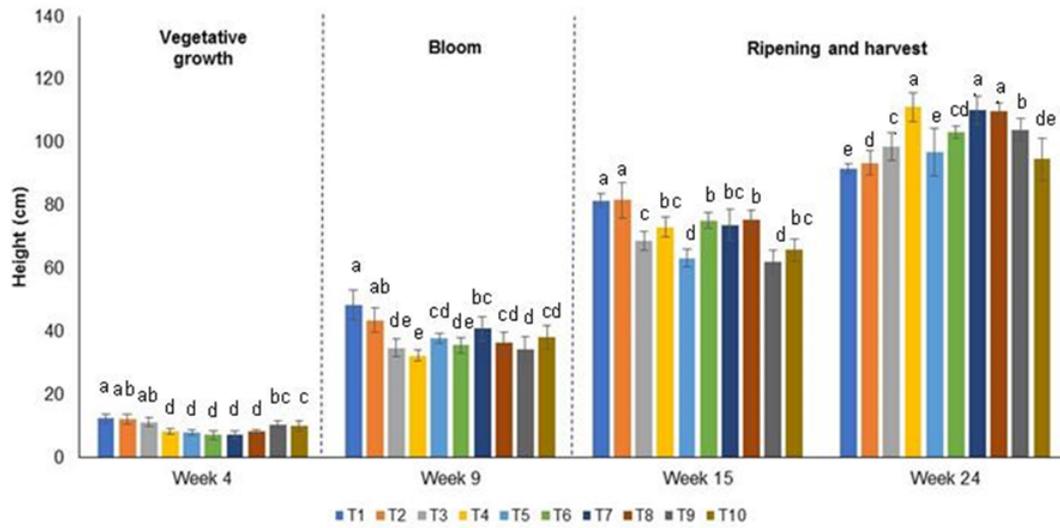


Figure 1. Plant height at different phenological stages of the tomato plant.

Values with different letters show significant differences ($p \leq 0.05$).

Source: Own elaboration based on results of this study.

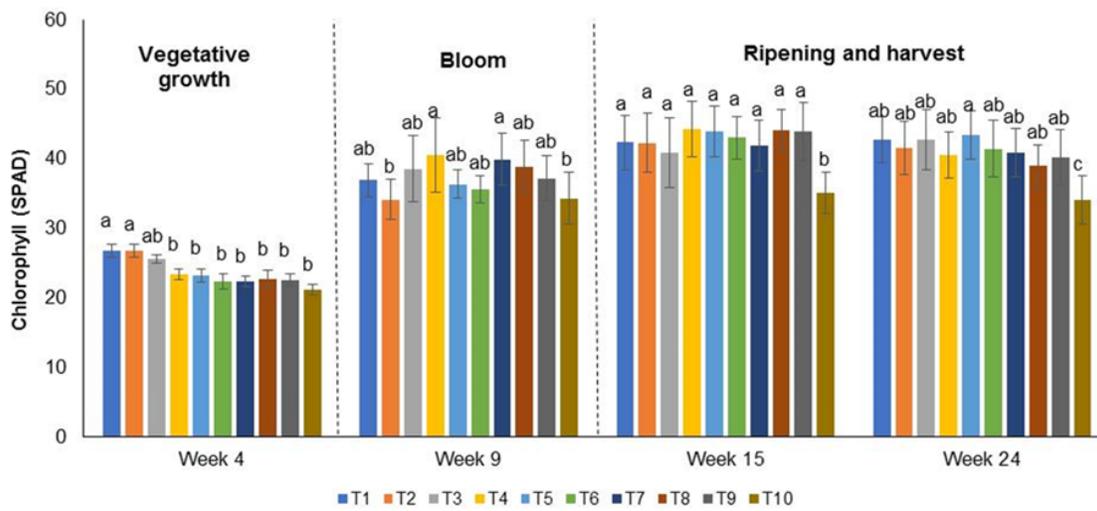


Figure 2. Chlorophyll index (SPAD units) at different phenological stages of tomato cultivation.

Values with different letters show significant difference ($p \leq 0.05$).

Source: Own elaboration.

Figure 3 shows fruit weight across all applied treatments. Foliar and soil applications yielded better effects than combined application, though not significantly different, with a tendency toward increased fruit weight as the concentration of microalgae in the biostimulant rose from 0.1 % to 0.5 %. However, at 1.0 %, the effect decreased. The best treatment was T5 (67 g \pm 0.08), which was significantly higher ($p \leq 0.05$) than the control (44.3 \pm 3.60 g), indicating improved assimilation and the effect of microalgal extracts applied to the soil. Likewise, Figure 4 shows yield results, with the highest values in T5 (13.01 \pm 2.17 t ha⁻¹), significantly higher ($p \leq 0.05$) than the control (5.47 \pm 0.63 t ha⁻¹) and soil-applied treatments (T1, T2, and T3). Yield analysis should consider plant components such as fruit number and fruit weight. These results indicate improved assimilation and the effect of soil-applied microalgal extracts at medium concentration, consistent with fruit weight outcomes. This can be explained by foliar uptake of stimulants through stomata, where they enter plant metabolism and promote increases in leaf area (Battacharyya *et al.*, 2015). Microalgae have also been shown to enhance uptake and accumulation of nutrients (N, P, K, Ca, etc.) in plants (Mostafa *et al.*, 2024), which can improve fruit weight and reduce the incidence of hollow or puffed tomato (Weisser *et al.*, 2024), characterized by a lack of gelatinous tissue surrounding seeds.

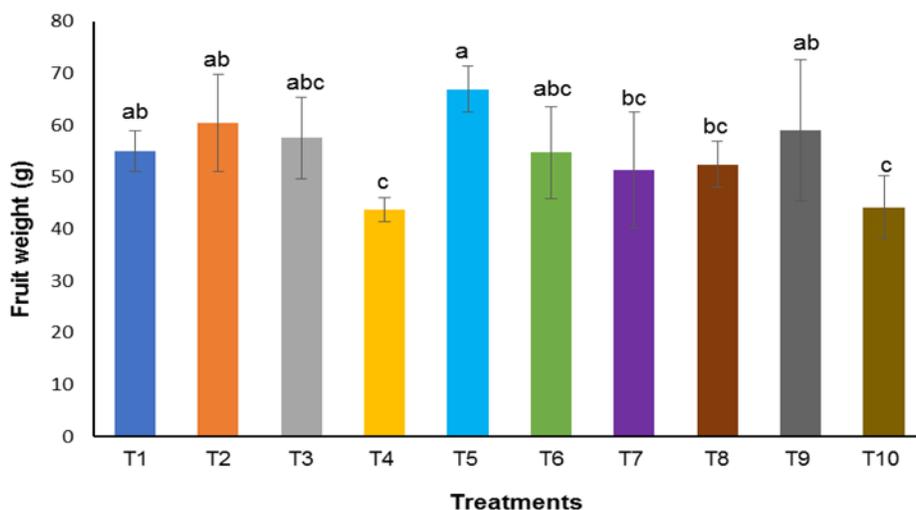


Figure 3. Effect of biostimulant on fruit weight tomato.

Values with different letters show significant difference at $p \leq 0.05$.

Source: Own elaboration.

Similar results were reported with *Nannochloropsis oculata* in tomatoes, yielding greater fruit weight compared to conventional inorganic fertilizers (Coppens *et al.*, 2016). These authors

suggest supplementing conventional fertilizers (macronutrient source) with microalgae to obtain high-quality fruits with satisfactory yields, since microalgae stimulate nitrogen uptake and improve market value. Microalgal polysaccharides can act as biostimulants by enhancing nutrient uptake, yield, growth, and plant physiology through the root system (Chanda *et al.*, 2019).

Figures 5 and 6 present foliar and root dry weight, respectively. The highest values were obtained in soil and combined applications, with dry weights increasing as the microalgal concentration increased, all of which were significantly higher ($p \leq 0.05$) than the control. A similar trend was observed in foliar applications, particularly at a concentration of 0.5 %. Plants under foliar application grew taller until week 15, after which soil and combined applications showed greater heights, consistent with recorded foliar and root dry weights. Similar observations were reported by Kumari *et al.* (2011), where higher algal extract concentrations promoted plant growth. In contrast, Hernández-Herrera *et al.* (2014) reported shorter plants and reduced shoot length under foliar sprays of algal extracts above 0.4 %.

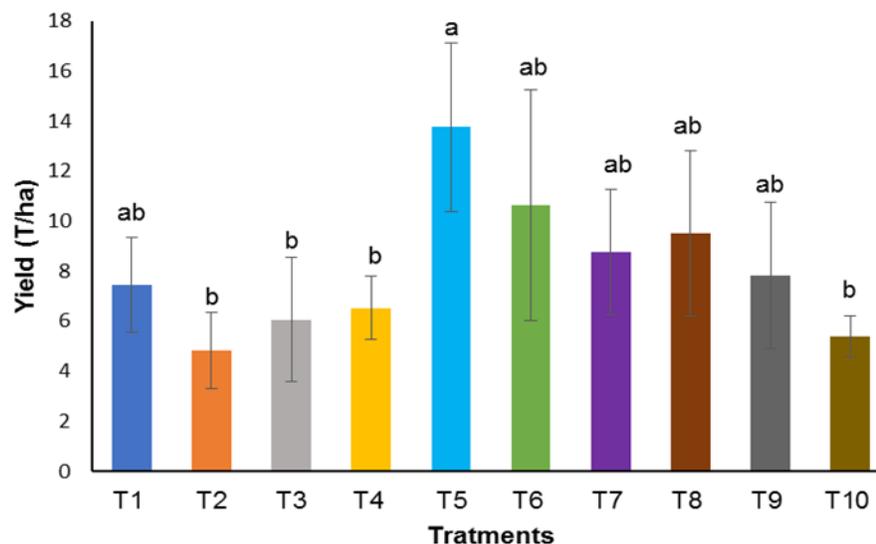


Figure 4. Effect of biostimulant on yield (ton ha⁻¹) of tomato culture.

Values with different letters show significant difference at $p \leq 0.05$.

Source: Own elaboration.

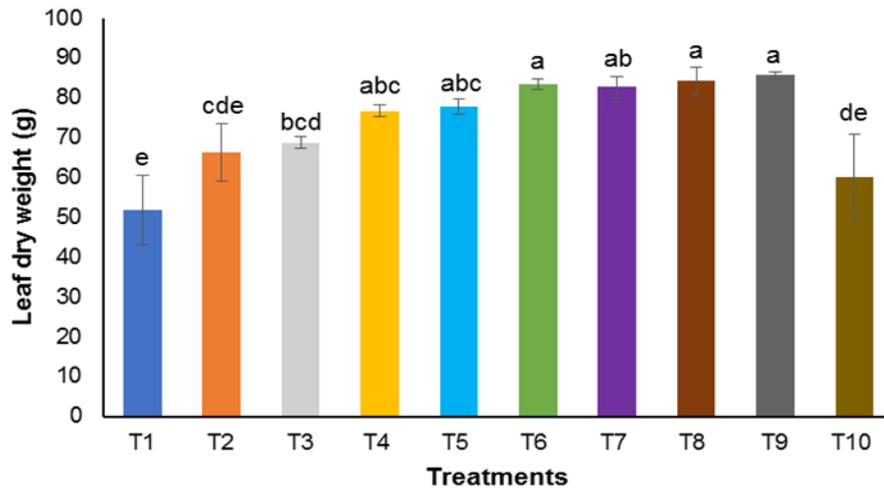


Figure 5. Effect of biostimulant on leaf dry weight of tomato culture.

Values with different letters show significant difference at $p \leq 0.05$.

Source: Own elaboration.

These results align with Garcia-Gonzalez & Sommerfeld (2016), who reported variation in lateral root number depending on the extract concentration of *Acutodesmus dimorphus*, showing a direct relationship between root number and extract concentration. This represents a benefit for plants, as higher root numbers improve nutrient and water absorption capacity.

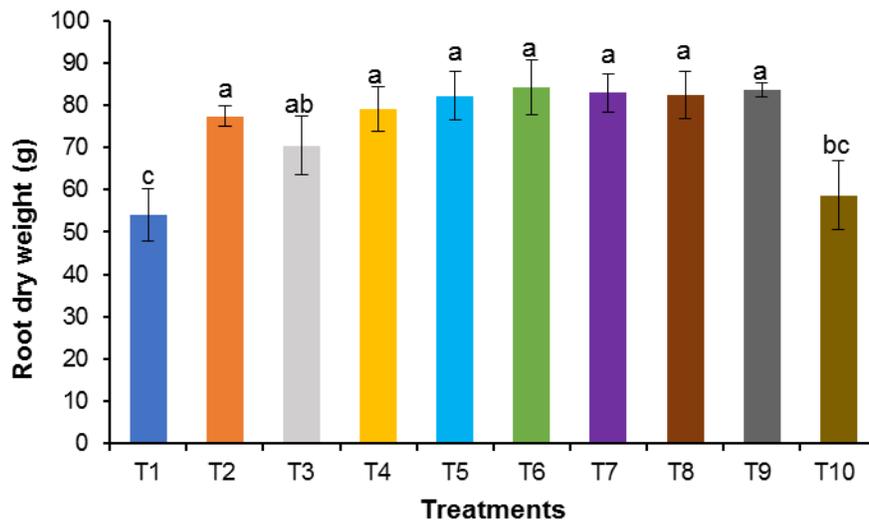


Figure 6. Effect of biostimulant on root dry weight of tomato culture.

Values with different letters show significant difference at $p \leq 0.05$.

Source: Own elaboration.

This study evaluated the effect of applied doses of biostimulant. Low concentrations, as low as 0.1 % (w/v), and preferably between 0.5 % and 1.0 %, of microalgal biomass were shown to have a greater impact on plant development. Minaoui *et al.* (2024) demonstrated dose-dependent effects in all treatments, with 25 % being the most effective biostimulant effect; conversely, the highest dose (50 %) negatively affected the germination process. Several authors obtained similar results in their studies of the effect of doses of algae extracts applied to crops such as wheat, radish, and tomato (Hernández-Herrera *et al.*, 2014; Kumar & Sahoo, 2011; Godlewska *et al.*, 2019). This may result from imbalances in phytohormones and alterations in mineral homeostasis. Excessive application may also induce negative responses or phytotoxicity (Gharib *et al.*, 2024; Ferreira *et al.*, 2021). High concentrations of microalgal extracts have also been associated with lower phenol and flavonoid content, antioxidant compounds that positively affect plant development (Chanda *et al.*, 2019). However, the exact mode of action of these compounds has not been fully explored (Mantzorou *et al.*, 2018) since the effects result not from a single compound but from complex interactions among compounds in the biomass, complicating interpretation. Hence, increasing microalgal biomass concentration in formulated biostimulants may cause antagonism among compounds, leading to inhibitory effects on plant development, as observed in some variables in this study.

Plant nutrition is a key factor for development. In this regard, Table 3 presents leaf contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) per treatment at the flowering stage. N and P values were within the normal ranges for tomato

cultivation established by Jones *et al.* (1991), who defined “sufficient” ranges as 2.50-2.99 % for N, 3.0-3.9 % for P, and 4.0-4.99 % for K.

For nitrogen, all treatments fell within the “sufficient” range. In contrast, phosphorus values were lowest in T7 (0.64 %) and the control (0.74 %). Regarding K values, all treatments were “high,” except for the control. This may be attributed to stimulation of micronutrient uptake, particularly K, Ca, and Cu, by microalgae in plant leaves. Ca and Mg levels were within “sufficient” ranges (1.5-2.4 %) and (0.32-0.8 %), respectively. Likewise, the foliar content of micronutrients was determined (Table 3). In some treatments, “high” values (>300 ppm) of iron were found, according to the ranges established above. This may be due to the microalgae’s high capacity to absorb Fe, which is reflected in the high values of this nutrient (Mantzorou *et al.*, 2018). The zinc values obtained in the samples were “low” (between 8 and 14 ppm). This behavior can be attributed to an antagonistic Fe/Zn effect, where Fe was provided in the foliar applications of micronutrients and in the applications of microalgae treatments. Therefore, increasing iron doses generates competition between Fe and Zn in their absorption and transport within plant cells. In this sense, iron applied foliar is more available to be mobilized by the plant to the fruit, which limits zinc absorption because these minerals use the same transporters and have low mobility in the plant (Félix *et al.*, 2024).

At the maturation and harvest stage, the analysis of macronutrients in leaf tissue showed that N and P contents remained constant from the beginning to the end of the plant cycle (Table 4), thus staying within the “sufficient” range. In contrast, K content decreased from the “high” to the “sufficient” range in the microalgae treatments, while the control showed an 8.33 % reduction. A similar trend was observed for Ca and Mg. This deficit may be attributed to plant stress caused by low temperatures (<15 °C) during the maturation stage. Micronutrients exhibited the same pattern previously observed at the flowering stage.

Table 3. Foliar content of macronutrients (N, P, K, Ca, Mg in percentage) and micronutrients (Fe, Cu, and Zn in ppm) in the indicator leaf in the early flowering stage.

Treatments	N	P	K	Ca	Mg	Fe	Cu	Zn
	(%)						(ppm)	
T1	3.94	1.19	5.60	1.67	0.35	180	5.80	10
T2	3.15	0.80	5.70	2.00	0.45	230	4.30	10
T3	3.15	1.10	5.30	1.83	0.47	150	3.20	10
T4	3.97	0.79	5.20	1.91	0.46	211	3.00	8
T5	3.60	0.82	5.70	1.62	0.88	145	4.20	14
T6	3.97	0.73	5.00	1.86	0.45	274	6.70	12
T7	3.67	0.64	5.90	1.51	0.65	346	8.90	10
T8	3.97	0.75	5.30	1.56	0.46	359	6.00	12
T9	3.96	1.05	5.60	1.66	0.56	346	8.90	10
T10	2.52	0.74	4.80	1.32	0.44	314	5.50	8

Source: Own elaboration based on the results of this study.

Table 4. Foliar content of macronutrients (N, P, K, Ca, Mg in percentage) and micronutrients (Fe, Cu, and Zn in ppm) measured in the indicator leaf at the maturation and harvest stages.

Treatment	N	P	K	Ca	Mg	Fe	Cu	Zn
	(%)						(ppm)	
T1	3.45	0.69	4.3	1.21	0.14	137	7.4	8
T2	3.1	0.89	4.2	1.13	0.6	218	7.4	7
T3	3.2	0.69	4.7	1.3	0.47	180	7.6	7
T4	2.99	0.55	5.3	1.47	0.28	255	11	7
T5	2.97	0.59	5.1	1.01	0.67	136	8.4	8
T6	3.45	0.57	5.4	1.69	0.66	206	5.8	10
T7	3.76	0.9	5.4	1.78	0.34	152	9.6	7
T8	3.66	0.94	5.5	1.02	0.55	122	9.6	10
T9	3.4	0.7	5.4	1.73	0.44	136	8.8	10
T10	2.99	0.53	4.4	1.11	0.59	126	6	8

Source: Own elaboration based on the results of this study.

Conclusion

The results demonstrated that growth variables were positively influenced by soil-applied microalgae treatments, which significantly enhanced fruit size and weight. This confirms that even low concentrations of microalgal extract applied to the crop stimulate both plant growth and yield. Furthermore, the content of micro- and macronutrients remained within sufficient ranges after microalgae applications, suggesting a stimulatory effect on nutrient assimilation by the plant.

Author contributions

Work conceptualization, Y.K.P.V., G.U.M., and M.A.G.C.; Methodology, Y.K.P.V. and C.A.D.Q.; Software, Y.K.P.V. and A.M.R.M.; Experimental validation, Y.K.P.V., G.U.M., and M.A.G.C.; Data analysis, Y.K.P.V. and A.M.R.M.; Data curation, Y.K.P.V. and C.A.D.Q.; Writing-original draft preparation, Y.K.P.V. and G.U.M.; Writing-review and editing, Y.K.P.V., G.U.M., P.G.M., and C.A.D.Q.; Project administration, G.U.M.; Funding acquisition, G.U.M.

All authors have read and agreed to the published version of the manuscript.

Funding

“CONAHCYT funded this research through a doctoral fellowship (No. 852858) awarded to Y.K. Padilla-Valle and by project CF-2023-G1278. Additional support was provided by the Research Strengthening Program through project PROFAPI-2023-0485.”

Conflict of interest

“The authors declare no conflict of interest.”

Referencias

- Alcántar González, G. & Sandoval Villa G. (1999). *Manual de análisis químico de tejido vegetal: Guía de muestreo, preparación, análisis e interpretación*. México, D. F. Sociedad Mexicana de la Ciencia del Suelo. <https://www.sidalc.net/search/Record/KOHA-OAI-TEST:23162/Description>
- American Public Health Association. (1926). *Standard methods for the examination of water and wastewater* (Vol. 6). American public health association.
- Arioli, T., Mattner, S. W., Islam, M. T., Tran, T. L. C., Weisser, M., Winberg, P., & Cahill, D. M. (2024). Applications of seaweed extracts in agriculture: An Australian perspective. *Journal of Applied Phycology*, 36(2), 713-726. <https://doi.org/10.1007/s10811-023-03120-x>

- Barone, V., Puglisi, I., Fragalà, F., Lo Piero, A. R., Giuffrida, F., & Baglieri, A. (2019). Novel bioprocess for the cultivation of microalgae in hydroponic growing system of tomato plants. *Journal of Applied Phycology*, 31(1), 465-470. <https://doi.org/10.1007/s10811-018-1518-y>
- Battacharyya, D., Babgohari, M. Z., Rathor, P., & Prithiviraj, B. (2015). Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae*, 196, 39-48. <https://doi.org/10.1016/j.scienta.2015.09.012>
- Calderón-Arias, C., Calle-Carmen, K., Carreño-Farfán, C., Estela-Campos, C., Carbajal-Gamarra, F. M., Barturén-Quispe, A. P., Chirinos-Cuadros, H. Y., & Sánchez-Purihuamán, M. N. (2024). Efecto de bacterias rizosféricas de *Prosopis limensis* Benth. En el desarrollo de *Solanum lycopersicum* L. bajo estrés salino. *Bioagro*, 36(1), 49-60. <https://doi.org/10.51372/bioagro361.5>
- Campobenedetto, C., Agliassa, C., Mannino, G., Vigliante, I., Contartese, V., Secchi, F., & Berteà, C. M. (2021). A Biostimulant Based on Seaweed (*Ascophyllum nodosum* and *Laminaria digitata*) and Yeast Extracts Mitigates Water Stress Effects on Tomato (*Solanum lycopersicum* L.). *Agriculture*, 11(6), 557. <https://doi.org/10.3390/agriculture11060557>
- Chanda, M., Merghoub, N., & EL Arroussi, H. (2019). Microalgae polysaccharides: The new sustainable bioactive products for the development of plant bio-stimulants? *World Journal of Microbiology and Biotechnology*, 35(11), 177. <https://doi.org/10.1007/s11274-019-2745-3>
- Coppens, J., Grunert, O., Van Den Hende, S., Vanhoutte, I., Boon, N., Haesaert, G., & De Gelder, L. (2016). The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. *Journal of Applied Phycology*, 28(4), 2367-2377. <https://doi.org/10.1007/s10811-015-0775-2>
- Farid, R., Mutale-joan, C., Redouane, B., Mernissi Najib, E., Abderahime, A., Laila, S., & Arroussi Hicham, E. (2019). Effect of Microalgae Polysaccharides on Biochemical and Metabolomics Pathways Related to Plant Defense in *Solanum lycopersicum*. *Applied Biochemistry and Biotechnology*, 188(1), 225-240. <https://doi.org/10.1007/s12010-018-2916-y>
- Federation, W. E., & Aph Association. (2025). *Standard methods for the examination of water and wastewater*. (Vol. 21). American Public Health Association (APHA). Washington DC, USA. https://books.google.com.mx/books/about/Standard_Methods_for_the_Examination_of.html?id=buTn1rmfSI4C&redir_esc=y
- Félix, J. W., Sánchez-Chávez, E., Tosquy-Valle, O., Preciado-Rangel, P., & Márquez-Quiroz, C. (2024). Biofortificación de Frijol (*Phaseolus vulgaris* L.) Variedad Verdín con Quelato y Sulfato de Hierro. *Revista Terra Latinoamericana*, 42. <https://doi.org/10.28940/terra.v42i0.1831>
- Ferreira, A., Melkonyan, L., Carapinha, S., Ribeiro, B., Figueiredo, D., Avetisova, G., & Gouveia, L. (2021). Biostimulant and biopesticide potential of microalgae growing in piggery wastewater. *Environmental Advances*, 4, 100062. <https://doi.org/10.1016/j.envadv.2021.100062>
- García-González, J., & Sommerfeld, M. (2016). Biofertilizer and biostimulant properties of the microalga *Acutodesmus dimorphus*. *Journal of Applied Phycology*, 28(2), 1051-1061. <https://doi.org/10.1007/s10811-015-0625-2>
- Gharib, F. A. E. L., Osama, K., Sattar, A. M. A. E., & Ahmed, E. Z. (2024). Impact of *Chlorella vulgaris*, *Nannochloropsis salina*, and *Arthrospira platensis* as bio-stimulants on common bean plant growth, yield and antioxidant capacity. *Scientific Reports*, 14(1), 1398. <https://doi.org/10.1038/s41598-023-50040-4>
- Gitau, M. M., Farkas, A., Ördög, V., & Maróti, G. (2022). Evaluation of the biostimulant effects

- of two Chlorophyta microalgae on tomato (*Solanum lycopersicum*). *Journal of Cleaner Production*, 364, 132689. <https://doi.org/10.1016/j.jclepro.2022.132689>
- Godlewska, K., Michalak, I., Pacyga, P., Baśladyńska, S., & Chojnacka, K. (2019). Potential applications of cyanobacteria: *Spirulina platensis* filtrates and homogenates in agriculture. *World Journal of Microbiology and Biotechnology*, 35(6), 80. <https://doi.org/10.1007/s11274-019-2653-6>
- Gracida-Valdepeña, M. L., Navarro-Aguirre, F., Herrera-Acosta, K., Ulloa-Mercado, G., Meza-Escalante, E., Plascencia-Jatomea, R., González-Meza, G., & Serrano-Palacios, D. (2020). Nutrient removal from swine wastewater using a thermotolerant strain of *Chlorella* sp. Grown under outdoor conditions. *Journal of Chemical Technology & Biotechnology*, 95(4), 1243-1249. <https://doi.org/10.1002/jctb.6313>
- Hernández-Herrera, R. M., Santacruz-Ruvalcaba, F., Ruiz-López, M. A., Norrie, J., & Hernández-Carmona, G. (2014). Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). *Journal of Applied Phycology*, 26(1), 619-628. <https://doi.org/10.1007/s10811-013-0078-4>
- Jones, J. J., Wolf, B., & Mills, H. A. (1991). *Plant analysis handbook. A practical sampling, preparation, analysis, and interpretation guide. Pp 213*. <https://www.cabidigitallibrary.org/doi/full/10.5555/19921969819>
- Krid, A., El Hallabi, M., Ennoury, A., Nhhala, N., Aberkani, K., Nhiri, M., & Zerrouk, M. H. (2023). The potential of seaweed extracts as a biostimulant for improving salt stress tolerance of *Solanum lycopersicum* L. *South African Journal of Botany*, 161, 305-316. <https://doi.org/10.1016/j.sajb.2023.08.020>
- Kumar, G., & Sahoo, D. (2011). Effect of seaweed liquid extract on growth and yield of *Triticum aestivum* var. Pusa Gold. *Journal of Applied Phycology*, 23(2), 251-255. <https://doi.org/10.1007/s10811-011-9660-9>
- Kumari, R., Kaur, I., & Bhatnagar, A. K. (2011). Effect of aqueous extract of *Sargassum johnstonii* Setchell & Gardner on growth, yield and quality of *Lycopersicon esculentum* Mill. *Journal of Applied Phycology*, 23(3), 623-633. <https://doi.org/10.1007/s10811-011-9651-x>
- La Bella, E., Baglieri, A., Rovetto, E. I., Stevanato, P., & Puglisi, I. (2021). Foliar Spray Application of *Chlorella vulgaris* Extract: Effect on the Growth of Lettuce Seedlings. *Agronomy*, 11(2), 308. <https://doi.org/10.3390/agronomy11020308>
- Mantzorou, A., Navakoudis, E., Paschalidis, K., & Ververidis, F. (2018). Microalgae: A potential tool for remediating aquatic environments from toxic metals. *International Journal of Environmental Science and Technology*, 15(8), 1815-1830. <https://doi.org/10.1007/s13762-018-1783-y>
- Minaoui, F., Hakkoum, Z., Chabili, A., Douma, M., Mouhri, K., & Loudiki, M. (2024). Biostimulant effect of green soil microalgae *Chlorella vulgaris* suspensions on germination and growth of wheat (*Triticum aestivum* var. Achtar) and soil fertility. *Algal Research*, 82, 103655. <https://doi.org/10.1016/j.algal.2024.103655>
- Mostafa, M. M., Hammad, D. M., Reda, M. M., & El-Sayed, A. E.-K. B. (2024). Water extracts of *Spirulina platensis* and *Chlorella vulgaris* enhance tomato (*Solanum lycopersicum* L.) tolerance against saline water irrigation. *Biomass Conversion and Biorefinery*, 14(17), 21181-21191. <https://doi.org/10.1007/s13399-023-04460-x>
- Mutale-joan, C., Rachidi, F., Mohamed, H. A., Mernissi, N. E., Aasfar, A., Barakate, M., Mohammed,

- D., Sbabou, L., & Arroussi, H. E. (2021). Microalgae-cyanobacteria-based biostimulant effect on salinity tolerance mechanisms, nutrient uptake, and tomato plant growth under salt stress. *Journal of Applied Phycology*, 33(6), 3779-3795. <https://doi.org/10.1007/s10811-021-02559-0>
- Mutale-joan, C., Redouane, B., Najib, E., Yassine, K., Lyamlouli, K., Laila, S., Zeroual, Y., & Hicham, E. A. (2020). Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L. *Scientific Reports*, 10(1), 2820. <https://doi.org/10.1038/s41598-020-59840-4>
- Navarro-López, E., Ruíz-Nieto, A., Ferreira, A., Ación, F. G., & Gouveia, L. (2020). Biostimulant Potential of *Scenedesmus obliquus* Grown in Brewery Wastewater. *Molecules*, 25(3), 664. <https://doi.org/10.3390/molecules25030664>
- Rachidi, F., Benhima, R., Sbabou, L., & El Arroussi, H. (2020). Microalgae polysaccharides bio-stimulating effect on tomato plants: Growth and metabolic distribution. *Biotechnology Reports*, 25, e00426. <https://doi.org/10.1016/j.btre.2020.e00426>
- Ranglová, K., Lakatos, G. E., Câmara Manoel, J. A., Grivalský, T., Suárez Estrella, F., Ación Fernández, F. G., Molnár, Z., Ördög, V., & Masojídek, J. (2021). Growth, biostimulant and biopesticide activity of the MACC-1 *Chlorella* strain cultivated outdoors in inorganic medium and wastewater. *Algal Research*, 53, 102136. <https://doi.org/10.1016/j.algal.2020.102136>
- Ronga, D., Biazzi, E., Parati, K., Carminati, D., Carminati, E., & Tava, A. (2019). Microalgal Biostimulants and Biofertilisers in Crop Productions. *Agronomy*, 9(4), 192. <https://doi.org/10.3390/agronomy9040192>
- Shukla, P. S., Mantin, E. G., Adil, M., Bajpai, S., Critchley, A. T., & Prithiviraj, B. (2019). Ascophyllum nodosum-Based Biostimulants: Sustainable Applications in Agriculture for the Stimulation of Plant Growth, Stress Tolerance, and Disease Management. *Frontiers in Plant Science*, 10. <https://doi.org/10.3389/fpls.2019.00655>
- Stirk, W. A., Ördög, V., Novák, O., Rolčík, J., Strnad, M., Bálint, P., & van Staden, J. (2013). Auxin and cytokinin relationships in 24 microalgal strains¹. *Journal of Phycology*, 49(3), 459-467. <https://doi.org/10.1111/jpy.12061>
- Supraja, K. V., Behera, B., & Balasubramanian, P. (2020). Efficacy of microalgal extracts as biostimulants through seed treatment and foliar spray for tomato cultivation. *Industrial Crops and Products*, 151, 112453. <https://doi.org/10.1016/j.indcrop.2020.112453>
- Weisser, M., Mattner, S. W., Southam-Rogers, L., Hepworth, G., & Arioli, T. (2024). Effect of a Fortified Biostimulant Extract on Tomato Plant Productivity, Physiology, and Growing Media Properties. *Plants*, 13(1), 4. <https://doi.org/10.3390/plants13010004>
- Zulkarnaini, Z. M., Sakimin, S. Z., Mohamed, M. T. M., & Jaafar, H. Z. E. (2019). Relationship between chlorophyll content and soil plant analytical development values in two cultivars of fig (*Ficus carica* L.) as brassinolide effect at an open field. *IOP Conference Series: Earth and Environmental Science*, 250(1), 012025. <https://doi.org/10.1088/1755-1315/250/1/012025>