REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.12.e1797
ISSN 2007-3380

Original article/Artículo original

Effect of seaweed extracts on the germination and growth of tomato seedling

Efecto de extractos de algas marinas en la germinación y el crecimiento de plántulas de tomate

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ABSTRACT

In the quest for innovative and sustainable solutions to tackle the challenges of modern agriculture, seaweed emerges as a promising alternative to traditional chemical inputs. This study aimed to evaluate the effects of aqueous extracts from the seaweeds *Gracilaria huavensis* and *Chaetomorpha antennina* on tomato germination and seedling growth (*Solanum lycopersicum*) under laboratory conditions. The effects of different concentrations of seaweed extracts (0.25 %, 0.5 %, and 1.0 %) were assessed in comparison to a control group (distilled water). The application of all seaweed extracts enhanced tomato seed germination and early seedling growth. Specifically, seed treatments with 0.25 % *G. huavensis* extract and 0.5 % *C. antennina* extract increased the germination percentage, germination rate, germination energy, and seedling vigor index compared to the control. Additionally, seedling length and biomass accumulation improved with both seaweed treatments. Obtained data highlight the potential of aqueous extracts from *G. huavensis* and *C. antennina* as effective biostimulants in the early stages of tomato plant development.

KEY WORDS: . *Chlorophyta*, *Rhodophyta*, germination percentage, vigor index, bioactive extracts.

Please cite this article as/Como citar este artículo: Espinosa-Antón, A.A., Acosta-Calderón, J.A., Hernández-Herrera, R.M. (2025). Effect of seaweed extracts on the germination and growth of tomato seedling. *Revista Bio Ciencias*, 12, e1797. https://doi.org/10.15741/revbio.12.e1797

Article Info/Información del artículo
Received/Recibido: October 21th 2025.
Accepted/Aceptado: May 21th 2024.
Available on line/Publicado: June 06th 2025.

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Revista Bio ciencias
ISSN 2007-3380

RESUMEN

En la búsqueda de soluciones innovadoras y sostenibles para afrontar los retos de la agricultura actual, las algas marinas emergen como una fuente prometedora de productos alternativos a los insumos químicos tradicionales. El objetivo de este estudio fue evaluar los efectos de los extractos acuosos de las algas marinas *Gracilaria huavensis* y *Chaetomorpha antennina* sobre la germinación y el crecimiento de las plántulas de tomate (*Solanum lycopersicum*) en condiciones de laboratorio. Se evaluaron los efectos de diferentes concentraciones de extractos de algas marinas (0.25, 0.5 y 1.0 %) en comparación con un grupo de control (agua destilada). La aplicación de todos los extractos de algas marinas mejoró la germinación de las semillas de tomate y el crecimiento temprano de las plántulas. Específicamente, los tratamientos de semillas con 0.25 % de extracto de *G. huavensis* y 0.5 % de extracto de *C. antennina* aumentaron el porcentaje de germinación, la tasa de germinación, la energía de germinación y el índice de vigor de las plántulas en comparación con el control. Además, la longitud de las plántulas y la acumulación de biomasa mejoraron con ambos tratamientos de algas marinas. Los resultados de este estudio indican el potencial de los extractos acuosos de *G. huavensis* y *C. antennina* como bioestimulantes efectivos en las primeras etapas del desarrollo de las plantas de tomate.

PALABRAS CLAVE: *Chlorophyta*, *Rhodophyta*, porcentaje de germinación, índice de vigor, extractos bioactivos.

Introduction

Modern agriculture faces the constant challenge of increasing crop productivity and sustainability in a global context marked by the growing demand for food and the effects of climate change (Hernández-Herrera *et al*., 2018; Chanthini *et al*., 2022). In this scenario, the search for biologically active products derived from local raw materials has become a priority to minimize the use of synthetic agrochemicals (Espinosa-Antón *et al.,* 2020). Among the various emerging strategies, the seaweed extracts application has achieved recognition as plant biostimulants due to its key effects, such as enhanced nutrient assimilation, accelerated seed germination, increased seedling vigor, improved root development, higher crop yields, greater stress resistance, and extended post-harvest shelf life of fruits and vegetables (Khan *et al.,* 2009; Hernández-Herrera *et al.,* 2014; Di Filippo-Herrera *et al.,* 2019; Chanthini *et al.,* 2019; 2022).

These beneficial effects are attributed to its high content of bioactive compounds, including minerals, various types of carbohydrates, amino acids, phytohormones, and secondary metabolites (Hernández-Herrera *et al.,* 2023). In recent years, interest in seaweed extract-based biostimulants as a sustainable crop management strategy has increased in Mexican agriculture and horticulture (Sariñana-Aldaco *et al*., 2022; Hernández-Herrera *et al.,* 2023). Although seaweeds are a diverse and abundant local resource along Mexico's coastlines, most commercially available agricultural extracts are derived from brown algae (*Phaeophyceae*) (Hernández-Herrera *et al.,* 2018). Furthermore, while Mexico lacks specific legislation on biostimulants, according to the Official Mexican Standard for Plant Nutrient Labeling (NOM-182-SSA1-2010), seaweed extracts are marketed as type 1 growth regulators.

On the other hand, available information on the biostimulant potential of extracts derived from red (Rhodophyta) and green (Chlorophyta) algae in Mexico remains limited, only two studies have reported that alkaline extracts from the red algae *Acanthophora spicifera*, *Gelidium robustum*, and *Gracilaria parvispora* promote seed germination and seedling vigor in mung bean (*Vigna radiata*) (Di Filippo-Herrera *et al.,* 2019; Hernández-Herrera *et al.,* 2022). Similarly, extracts from *Ulva lactuca* and *Caulerpa sertularioides* have been found to promote seed germination and early seedling growth in tomato (*Solanum lycopersicum*) (Hernández-Herrera *et al.,* 2014; 2016) and mung bean (*V. radiata*) (Castellanos-Barriga *et al.,* 2017).

Building on the existing knowledge of the bioactivity of seaweed extracts, it is of great interest to assess other species of red (Rhodophyta) and green (Chlorophyta) algae from Mexico to determine the potential of seaweeds in agriculture. In this context, the red macroalga *G. huavensis* Abbott and the green macroalga *C. antennina* are perennial species distributed along the Mexican Pacific coast. In the coastal environments of Oaxaca, these species are essential components of the region's phycological diversity (Rosas-Alquicira *et al.,* 2019). Thus, this study aimed to evaluate the effects of aqueous extracts from *G. huavensis* and *C. antennina* on seed germination and their potential as growth promoters for tomato seedlings.

Material and Methods

For the extract preparation, dried seaweed biomass was supplied by the Biological Oceanography Laboratory at Universidad del Mar, sourced from the coast of Oaxaca in the tropical Mexican Pacific. The samples were dried in a convection oven at 60 °C for 25 hours and ground in an electric mill to obtain a 0.50 mm powder. Aqueous extracts were prepared following the methodology described by Hernández-Herrera *et al.* (2014). Briefly, 10 g of seaweed powder from each species was individually added to 1 L of distilled water under constant agitation for 15 minutes, followed by autoclaving at 121 °C for 1 hour at 1.5 kg cm^{-2}. The hot extracts were filtered through Whatman No. 40 filter paper and stored at 4 °C until use.

For the germination bioassay, experiments were conducted using certified tomato seeds (*S. lycopersicum* cv. Rio Fuego; Kristen Seed®, San Diego, CA, USA). The germination bioassays followed the guidelines of the Association of Official Seed Analysts (AOSA, 1996). Seeds were surface-disinfected by immersion in a 4 % sodium hypochlorite (NaClO) solution for 10 minutes and rinsed three times in sterile distilled water for 1 minute. Seeds were then placed on Whatman

No. 5 filter paper inside 90 mm diameter Petri dishes. Subsequently, 5 mL of seaweed extract at concentrations of 0.25 %, 0.5 %, and 1.0 % were applied, with distilled water serving as the control. Petri dishes were incubated in a growth chamber at 25 ± 2 °C, with 85 % relative humidity and a 16:8 h (light:dark) photoperiod.

Germination was daily observed for eight days following the methods of the Association of Official Seed Analysts (AOSA, 1996). Seeds were considered germinated when the emerging radicle exceeded 2 mm in length. Germination parameters, including germination percentage, mean germination time, germination rate, germination energy, and seedling vigor index, were calculated according to Castellanos-Barriga *et al.* (2017). After 12 days of growth, radicle length, hypocotyl length, and total seedling length were measured using a digital Vernier caliper (Electrónica Steren S.A. de C.V., Mexico City, Mexico). Fresh and dry weights were determined using an analytical balance (HR-200, A & D Company, Ltd., Michigan, USA).

Experiments were conducted independently for each seaweed extract in a completely randomized factorial design. Germination was evaluated using a total of 10 replicates (Petri dishes), each containing 10 seeds, for a total of 100 seeds per treatment and concentration. For seedling growth measurements, 50 seedlings per treatment and concentration were randomly selected. Data were analyzed using one-way analysis of variance (ANOVA, $p < 0.05$) followed by Tukey's multiple comparison test ($p < 0.05$) in Statgraphics® Centurion XVI software.

Results and Discussion

Germination assays constitute a rapid and straightforward tool for evaluating seaweed extracts as plant growth biostimulants and predicting their potential effects on plants (Di Filippo-Herrera *et al.,* 2019). In this study, a differential response to the two types of extracts was observed in seed germination and seedling growth. Tomato seeds treated with low concentrations at 0.25 % of *Chaetomorpha antennina* showed improved performance in terms of germination percentage and germination rate (16 % and 19 %), which were associated with a shorter mean germination time (4.73 days), higher germination energy (24 %), and, consequently, greater seedling vigor (37 %) compared to the control group. Conversely, the application of *Gracilaria huavensis* aqueous extract at a 0.50 % concentration also significantly increased the germination percentage (GP) (17 %), germination rate (GR) (19 %), germination energy (GE) (23 %), and seedling vigor index (SVI) (52 %) compared to the control group (Table 1). In contrast, the 1.0 % concentration of both extracts did not show significant differences in germination when compared to the control.

Revista Bio ciencias ISSN 2007-3380

Table 1. Effects of aqueous extracts on seed germination of tomato

Variables	Germination percentage (%)	Mean germination time (days)	Germination rate	Germination energy (%)	Seedling vigor index
Control	81±1.87 [b]	4.78±0.10 [a]	14.38±0.46 [b]	71±3.67 [b]	587.72±39.92 [b]
GH 0.25 %	88±3.39 [ab]	4.78±0.05 [a]	15.72±0.40 [ab]	75±3.16 [ab]	735.66±52.41 [ab]
GH 0.50 %	**95±1.58 [a]**	4.71±0.02 [a]	**17.14±0.61 [a]**	**87±2.55 [a]**	**895.25±56.69 [a]**
GH 1.00 %	89±3.67 [ab]	4.70±0.03 [a]	16.75±0.76 [ab]	86±3.67 [ab]	794.84±54.37 [ab]
CA 0.25 %	**94±1.87 [a]**	4.73±0.06 [a]	**17.05±0.80 [a]**	**88±3.74 [a]**	**802.92±32.59 [a]**
CA 0.50 %	86±3.31 [ab]	4.73±0.01 [a]	16.10±0.62 [ab]	84±4.30 [ab]	651.52±51.21 [ab]
CA 1.00 %	86±3.31 [ab]	4.76±0.05 [a]	15.83±0.34 [ab]	83±2.00 [ab]	610.30±43.42 [b]

Gracilaria huavensis (GH) and *Chaetomorpha antennina* (CA). Values are mean ± standard error (n=10). Different letters indicate significant differences between the means of the same column, according to Tukey's test ($p < 0.05$).

The observed seed germination behavior is consistent with previous studies using green seaweed extracts from *Caulerpa sertularioides* and *Ulva lactuca* at 0.20 % (Hernández-Herrera *et al.,* 2014), as well as Di Filippo-Herrera *et al.* (2019), who reported that treatment with red algae *G. parvispora* increased mung bean seed germination by 3 %. Conversely, in a study by Chanthini *et al.* (2019), the application of *C. antennina* extracts at a 10 % concentration accelerated seed germination by reducing the mean germination time and increasing germination energy, germination rate, and seedling vigor index in tomato plants. Similarly, liquid extracts from *Gracilaria edulis* at a 20 % concentration resulted in a 100 % germination rate in tomatoes (Vinoth *et al.,* 2012). These differences in effective concentrations may be attributed to variations in the quantitative composition of the bioactive compounds in seaweed extracts, which might fluctuate depending on the biomass harvest season, geographic location, and extraction methods and conditions (Castro-González *et al.,* 1996; Khan *et al.,* 2009; Santos *et al*., 2023). Additionally, the effect of seaweed extracts is variable within the same crop depending on the assessed variety (Espinosa-Antón *et al.,* 2020).

Furthermore, seaweed extracts have been shown to improve the root-to-shoot ratio and biomass accumulation in tomato seedlings by stimulating growth (Crouch & Van Staden, 1992). Regarding tomato seedling growth, *G. huavensis* extract did not significantly affect hypocotyl and radicle length (Figure 1a, b). However, treatment with *C. antennina* extract at 0.50 % significantly enhanced seedling growth, increasing radicle length (3.55 cm), hypocotyl length (6.63 cm), and total seedling length (10.9 cm) compared to control seedlings, which measured 2.34 cm, 4.89 cm, and 7.24 cm, respectively. Similarly, seedlings treated with the 1.0 % extract concentration showed an increase in hypocotyl length (Figure 1c, d).

Revista Bio ciencias
ISSN 2007-3380

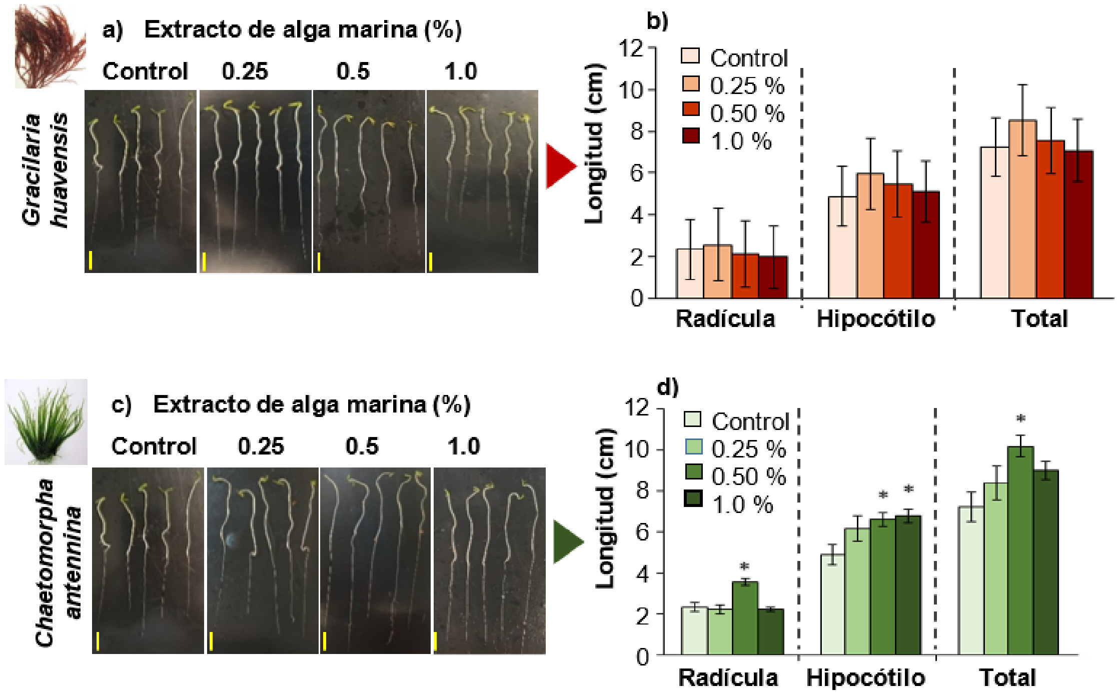

Figure 1. Effect of extracts of (a,b) *Gracilaria huavensis* and (c,d) *Chaetomorpha antennina* at concentrations of 0.25, 0.50, and 1.0 % on growth of radicle, hypocotyl, and total length. (Bar = 1 cm).

However, although different concentrations of the aqueous *G. huavensis* extract did not significantly affect tomato seedling growth, they did have a notable effect on seedling weight. The 0.25 % and 0.50 % concentrations significantly increased fresh weight (0.77 g) and dry weight (0.03 g) compared to control seedlings (0.23 g and 0.01 g, respectively) (Figure 2a). Similarly, alkaline extracts of *C. antennina* at 0.50 % increased both fresh and dry weight in tomato seedlings (Figure 2b).

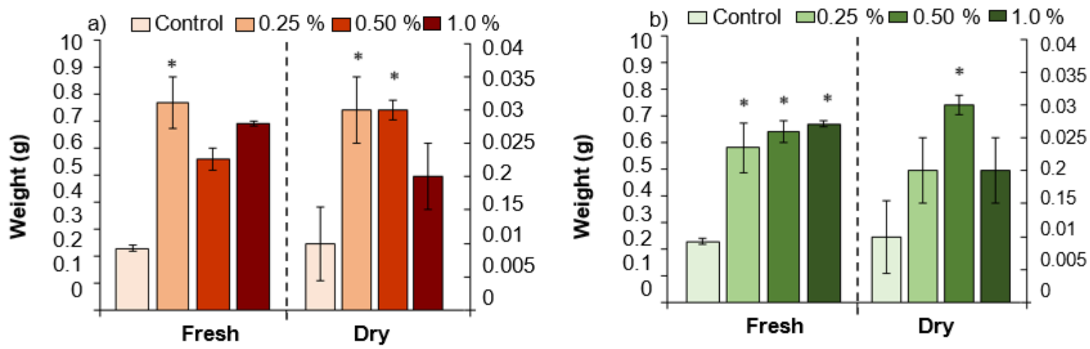

Figure 2. Effect of aqueous extracts of (a) *Gracilaria huavensis* and b) *Chaetomorpha antennina* at concentrations of 0.25, 0.5, and 1.0 % on fresh and dry weight of tomato seedling.

The favorable effects of *G. huavensis* and *C. antennina* extracts on seed germination and seedling growth parameters observed in this study may be related to their chemical composition, which includes small amounts of phytohormones (gibberellins, auxins, and cytokinins), carbohydrates, and proteins. These compounds regulate key physiological processes such as reserve mobilization, embryo axis elongation and differentiation, and the development of essential seedling structures (Chanthini *et al.,* 2019; Di Filippo-Herrera *et al.,* 2019; Espinosa-Antón *et al.,* 2020; Hernández-Herrera *et al.,* 2014; 2023). Additionally, osmotically active solutes, such as mineral salts (NO_3^-, NH_4^+, Na^+, K^+, Cl^-, Mg^{2+}, Ca^{2+}), may promote water imbibition in seeds at low concentrations, thereby triggering germination (Bewley & Black, 1994). Conversely, seaweed extracts at higher concentrations (>1 %) significantly inhibit tomato seed germination by reducing the ability of seeds to absorb water (Castellanos-Barriga *et al.,* 2017).

Similarly, the 1.0 % seaweed treatments evaluated in this study could have affected the osmotic potential, thereby preventing the development of turgor pressure in the seed, one of the key factors initiating root growth during germination (Maia & Rainer, 2001). The obtained results suggest that both aqueous extracts demonstrated bio-stimulant activity during the early developmental stages of tomato seedlings. However, further research on green and red algae is necessary to explore their potential as an underutilized resource. Increasing knowledge of their characteristics and properties could offer valuable benefits for Mexican agriculture, particularly as sustainable alternatives to conventional agrochemicals.

Conclusions

The aqueous extracts of *G. huavensis* at 0.25 % and *C. antennina* at 0.5 % demonstrated positive effects during the early developmental stages of tomato cultivation. These findings highlight the biotechnological potential of algae, not only as sources of renewable energy but also as bio-stimulants or high-value-added products. The aqueous extracts of *G. huavensis* and *C. antennina* could serve as alternative bio-stimulant products, promoting more sustainable agricultural production in coastal communities with access to these resources. However, more research is required to identify the biochemical composition of these extracts to enhance our understanding of the mechanisms driving their bio-stimulant properties. Future studies should also assess their effects on tomato plant growth and development in agricultural settings, considering different doses and application methods.

Author Contributions

Work conceptualization, Espinosa-Antón, A.A., Hernández-Herrera, R.M.; development of the methodology, Espinosa-Antón, A.A., Acosta-Calderón, J.A.; software management, Espinosa-Antón, A.A., Hernández-Herrera, R.M.; experimental validation, Hernández-Herrera, R.M., and Acosta-Calderón, J.A.; analysis of results, Espinosa-Antón, A.A., Hernández-Herrera, R.M.; data management, Espinosa-Antón, A.A.; writing and preparation of the manuscript, Espinosa-Antón, A.A., Hernández-Herrera, R.M.; writing, review, and editing, Acosta-Calderón, J.A., Hernández-Herrera, R.M.; project manager, Hernández-Herrera, R.M.; funding acquisition, Acosta-Calderón, J.A., Hernández-Herrera, R.M.

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

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest.

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