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Original Research Article

Evaluation of biostimulant capacity by phytohormonal extracts from native microalgae of the Barú and Alanje Regions, Chiriquí Province, Panamá

[Evaluación de la capacidad bioestimulante por extractos fitohormonales provenientes de microalgas nativas de las Regiones de Barú, Alanje, Provincia de Chiriquí, Panamá]

Andrea Polo[1234*], Byron Álvarez[1234], Miguel J. Vega-Quiel[1234], Ariadna Batista[1234]

[1] Universidad Autónoma de Chiriquí (UNACHI)
[2] Maestría en Ciencias Químicas con Énfasis en Inocuidad Alimentaria
[3] Facultad de Ciencias Naturales y Exactas
[4] Centro de Investigación de Productos Naturales y Biotecnología (CIPNABIOT), David, Panamá.

(*Autor de correspondencia: andrea.polo@unachi.ac.pa)

Abstract:

This study evaluated the effects of different concentrations of extracts from microalgal strains identified as *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus quadricauda* and *Scenedesmus almeriensis*, in germination bioassays using rice (*Oryza sativa*), tomato (*Solanum lycopersicum*) and watercress (*Nasturtium officinale*) seeds to assess the germination index (GI). The biostimulant effects were tested at different concentrations of the algal extracts coded as Al_2, Bar_1, Bar_4 and SA at 0.1 g/L and 0.5 g/L each. The extract of strain Bar_4 at 0.1 g/L exhibited the strongest biostimulant effect in most of the assays, with 19 out of 25 germinated seeds and a GI of 137±0.24% in rice seeds. The remaining extracts showed a germination-promoting effect ranging from 119% to 133%. In contrast, strain SA displayed a mild inhibitory effect, with GI of 84% and 85% at 0.1 and 0.5 g/L, respectively, both below the negative control. In the tomato seed assay, Bar_4 resulted in 18±1.89 germinated seeds and a GI of 220±0.41%. The other extracts also showed germination-promoting activity, with values ranging from 187% to 216%. Finally, in the watercress seed assay, Bar_4 at 0.1 g/L again demonstrated the highest biostimulant effect, reaching 10±3.21 germinated seeds and a GI of 319±38%. Considering the phytohormones present in microalgae as a key factor in the valorization of these microscopic organisms opens promising perspectives; it represents not only a growing opportunity but also an innovative approach that could significantly contribute to improving efficiency and sustainability in agriculture.

Keywords: *biostimulants, biotechnology, extracts, phytohormones, microalgae.*

Resumen:

Este estudio evaluó los efectos de distintas concentraciones de extractos de microalgas identificadas como *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus quadricauda* y *Scenedesmus almeriensis* en bioensayos de germinación de semillas de arroz (*Oryza sativa*), tomate (*Solanum lycopersicum*) y berro (*Nasturtium officinale*) evaluando el índice de germinación (IG). Los efectos bioestimulantes fueron evaluados a distintas concentraciones de los extractos microalgales con códigos Al_2, Bar_1, Bar_4 y SA respectivamente a 0.1g/L y 0.5 g/L cada una. El extracto de la cepa Bar_4 a 0.1 g/L exhibió el mayor efecto bioestimulante en la mayoría de los ensayos, con un número de semillas germinadas de 19 de 25 y un IG de 137±0.24% en las semillas de arroz. Los extractos restantes presentaron un efecto promotor de la germinación en un rango desde 119 a 133%. En contraste, la cepa SA mostró un efecto inhibitorio leve, con IG de 84% y 85% a 0.1 y 0.5 g/L, respectivamente, por debajo del control negativo. Bar_4 obtuvo un número de germinación de 18±1.89% y un IG de 220±0.41% en el ensayo con semillas de tomate. Los extractos restantes presentaron un efecto promotor de la germinación en un rango desde 187 a 216%. Finalmente, la cepa Bar_4 a 0.1 g/L nuevamente presentó el mayor efecto bioestimulante en el ensayo con semillas de berro, obteniendo un número de germinación de 10±3.21 y un IG de 319±38%. La consideración de las fitohormonas presentes en las microalgas como un factor clave en la valorización de estos organismos microscópicos abre perspectivas prometedoras; no solo se trata de una oportunidad creciente, sino también de un enfoque innovador que podría contribuir significativamente a mejorar la eficiencia y la sostenibilidad en la agricultura.

Palabras clave: *bioestimulantes, biotecnología, extractos, fitohormonas, microalgas.*

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1. Introduction

According to the European Biostimulants Industry Council (EBIC), biostimulants enhance nutrient use efficiency and improve plant tolerance to abiotic stress, resulting in improved crop quality and yield (EBIC, 2016). In this regard, Drobek *et al*. (2019) emphasize that biostimulants should not be considered biofertilizers, as they do not directly supply nutrients but instead facilitate their uptake through modifications of the rhizosphere and plant metabolism. This mechanism contributes to greater nutrient utilization efficiency, increased tolerance to abiotic stress, and improved quality of agricultural products.

Within this group, phytohormones have been widely recognized as biostimulant agents. These are low-molecular-weight organic compounds that regulate vital processes in plant cells and allow growth adaptation under adverse environmental conditions (Ohri *et al*., 2015). They act as chemical messengers involved in a wide spectrum of physiological and biochemical processes in higher plants, even at very low concentrations. To date, ten major groups of phytohormones have been identified: auxins, cytokinins (CK),

gibberellins (GA), abscisic acid (ABA), ethylene (ETH), brassinosteroids (BR), salicylates (SA), jasmonates (JA), strigolactones (SL), and peptide hormones. Of these, the first five (auxins, CK, GA, ABA, and ETH) are traditionally classified as "classical," while the remaining groups represent emerging families in phytohormonal regulation (Su *et al*., 2017).

In microalgae, the biological functions of auxins are analogous to those observed in higher plants (Stirk and van Staden, 1996). Indole-3-acetic acid (IAA) is the most relevant auxin in plant cells, along with indole-3-butyric acid and indole-3-acetamide, which have been identified in at least 46 microalgal species belonging to Cyanophyta and Chlorophyta (Wang *et al*., 2021). Cytokinins, in turn, stimulate cell division in microalgae, promote the accumulation of photosynthetic pigments, and optimize photosynthetic efficiency, thereby contributing to biomass increase (Mousavi *et al*., 2016). Their concentration exhibits circadian variations, with low levels during dark periods and higher levels during the light phase (Schmülling *et al*., 2003).

Gibberellins are involved in cell elongation and regulate processes associated with carbon metabolism, influencing both growth and metabolism in microalgae. In higher plants, these hormones control key processes such as seed germination, stem elongation, leaf expansion, and flower and seed development (Tarakhovskaya *et al*., 2007). Abscisic acid, a 15-carbon sesquiterpenoid, accumulates primarily in senescent organs and tissues, where it exerts an inhibitory effect on cell growth (Anantharaman and Aravind, 2001). In microalgae, ABA also plays a crucial role in inducing dormant states under adverse conditions, thereby promoting survival and increasing resistance to environmental stress (Yoshida *et al*., 2004). This study aimed to evaluate the biostimulant capacity of phytohormonal extracts from native microalgae from Barú and Alanje, applied in seed germination bioassays with rice, tomato, and watercress. Extracts were tested at different concentrations and compared with negative and positive controls to determine their potential as natural biostimulants and assess their implications for more sustainable agriculture.

2. Materials and Methods

2.1 Strain isolation

Sampling was carried out in two locations in the province of Chiriquí, Panama: the district of Barú (La Esperanza) and the district of Alanje (El Tejar). At each site, shallow bodies of water associated with rice farms were selected as sources of inoculum. The collected samples were then taken to the laboratory while keeping them refrigerated with ice, isolated and cultivated at our institute, and subsequently identified at the Center for Research in Marine Sciences and Limnology (CIMAR, UCR). *Chlorella vulgaris* (Bar_4), *Chlorella sorokiniana* (Al_2) and *Scenedesmus quadricauda* (Bar_1) were identified in samples (Table 1). Biomass was cultivated in 10 L reactors operated in batch mode, with continuous aeration and pH control maintained between 7 and 8. Conventional LED lamps were used to provide light, applying a photoperiod of 12 h light/12 h darkness. Growth was monitored in triplicate using UV-Vis spectrophotometry, recording the optical density at 680 nm. The cultures were scaled up to reach cell densities of 0.7908, 2.666 and 3.0203 g/L for strains Bar_4, Al_2, and Bar_1 (*S. quadricauda*–

Barú), respectively. The *Scenedesmus almeriensis* (SA) strain, from the BIO173 group (Almería), was included as a reference, reaching a cell density of 1.782 g/L. The biomass of each strain was recovered by centrifugation (6000 rpm, 5 min) in 50 mL conical tubes, freeze-dried, and stored at -20 °C until the extracts were prepared.

2.2 Preparation of the extract

From the lyophilized biomass of each strain (Al_2, Bar_1, Bar_4 and SA), stock solutions were prepared at a concentration of 2 g/L by dissolving 0.4 g of freeze dry biomass in 200 mL of sterile distilled water. Each suspension was then subjected to cold ultrasonic treatment using a Branson 5800 ultrasonic bath for 1 hour at 100% amplitude with constant agitation, in order to promote cell disruption and metabolite release. From these stock solutions, serial dilutions were prepared to obtain the final concentrations required for the bioassays (0.5 g/L and 0.1 g/L) (Table 1). The solutions were adjusted to the required volume for each assay and kept refrigerated until use.

Table 1. Strains, origin and concentration of microalgae used in bioassays.

Microalgal strain code	Species	Origin	Concentration [g/L]	
Bar_4	*Chlorella vulgaris*	Barú, Panamá	0.1	0.5
Al_2	*Chlorella sorokiniana*	Alanje, Panamá	0.1	0.5
Bar_1	*Scenedesmus quadricauda*	Barú, Panamá	0.1	0.5
SA	*Scenedesmus almeriensis*	Almería, España	0.1	0.5

2.3 Germination of tomato and rice seeds under the effect of gibberellic acid

Tomato (*Solanum lycopersicum*), rice (*Oryza sativa*), and watercress seeds were disinfected by immersion in a 30% (v/v) hydrogen peroxide (H_2O_2) solution for 5 minutes. Subsequently, 2 mL of the corresponding extract (0.1 and 0.5 g/L) were placed in 90-mm Petri dishes containing sterile filter paper. Twenty-five seeds were sown per plate, with four replicates per treatment and concentration. Sterile distilled water was used as a negative control, while gibberellic acid (GA_3) at 3 mg/L served as the positive control.

Plates were incubated at 25 °C for 3 days. Seeds were considered germinated when radicles of ≥ 2 mm in length developed. Seedling length was determined using ImageJ software, complemented by manual measurements with a millimeter ruler. Germination rate and shoot length were evaluated for each treatment, comparing them with controls and with the gibberellic acid calibration curve. The germination index (GI) was calculated according to Equation 1:

$$\text{GI}\ (\%) = \frac{\text{G} * \text{L}}{\text{Gw} * \text{Lw}} * 100$$

Equation 1. Calculation of GI (%)

GI: Germination Index
G: Number of germinated seeds (with extract)
L: Shoot length (with extract)
Gw: Number of germinated seeds (with distilled water)
Lw: Shoot length (with distilled water)

This index integrates two parameters: the proportion of seeds that germinate and the growth of the resulting seedlings. In this way, it enables a comparative evaluation of the biostimulant effect of the treatments, in contrast with the negative control. A GI value > 100% indicates a promotive effect on germination and growth, whereas GI values < 100% suggest an inhibitory effect.

2.4 Statistical analysis

Results were expressed as mean ± standard deviation (SD). Differences among treatments were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple mean comparisons. Differences were considered statistically significant at $p<0.05$.

3. Results and Discussion

3.1 Germination of rice seeds

Table 2 and Fig. 1 illustrate the response of rice seeds to the application of microalgal extracts at different concentrations (0.1 and 0.5 g/L). The germination index (GI), calculated relative to the negative control (H_2Od, GI=100%), allowed for the evaluation of both germination induction capacity and the biostimulant effect of the treatments.

Among the extracts tested, strain Bar_4 at 0.1 g/L stood out by promoting the highest number of germinated seeds (19±2.94 out of 25), longer shoots (2.6±0.29 cm), and an elevated GI (137±0.18).

Table 2. Evaluation of the number of germinated rice seeds in response to the tested samples.

Sample	Concentration (g/L)	Germination Index (%)
H_2Od	-	100
GA_3	0.003	195
Bar_4	0.1	137
	0.5	119
Al_2	0.1	122
	0.5	120
Bar_1	0.1	137
	0.5	118
SA	0.1	85
	0.5	84

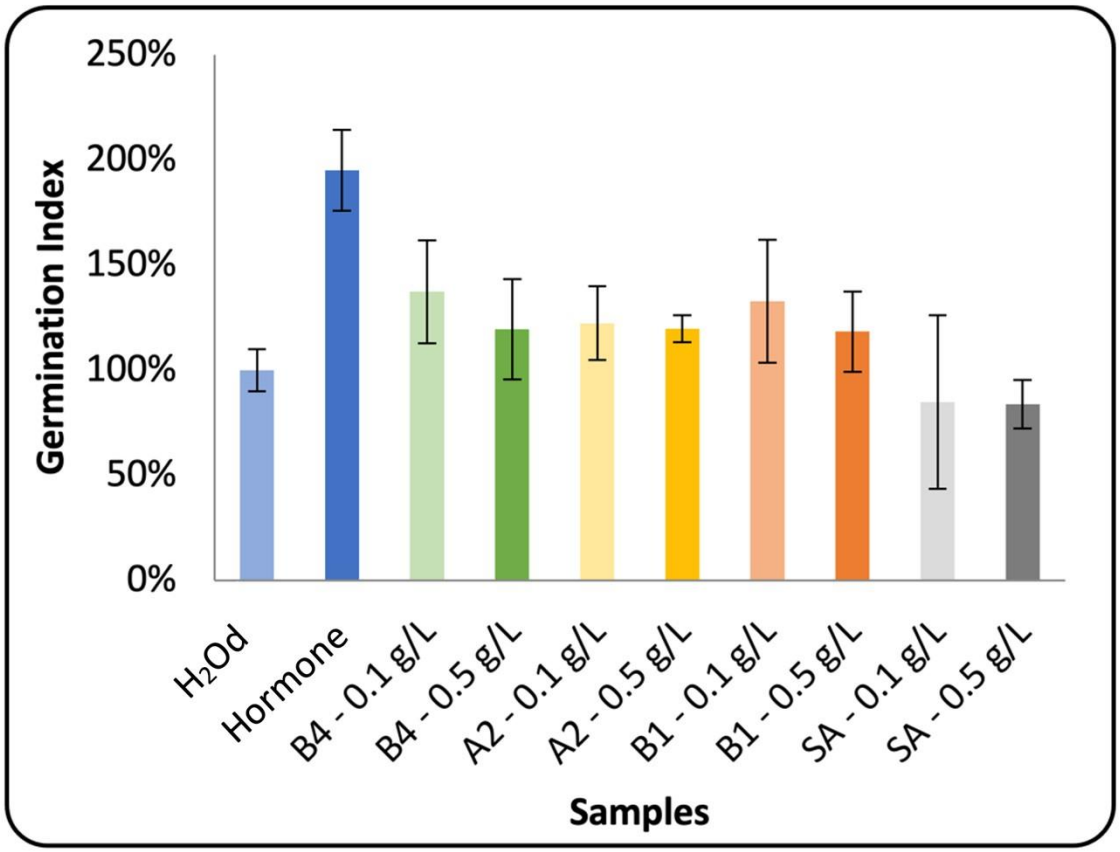

Figure 1. Percentage of rice seed germination under exposure to extracts.

This behavior, similar to that reported by Santos *et al*. (2019), who found that the application of biostimulants based on *Ascophyllum nodosum* extract in ornamental sunflower requires an optimal concentration to increase germination rates, suggests that low doses of the extract enhance efficiency in the germination process, possibly due to improved availability of bioactive compounds at the root level, without causing osmotic stress or an accumulation of secondary metabolites that could inhibit germination. In contrast, higher concentrations (0.5 g/L) did not show proportional increases in GI, suggesting a dose-dependent effect with an optimum near 0.1 g/L. This pattern is consistent with previous reports in which microalgal extracts enhanced early plant development at low doses, while higher concentrations tended to reduce the stimulatory response due to saturation or mild phytotoxicity (Mutum *et al*., 2023).

However, despite the observed trends, one-way ANOVA and Tukey's test indicated that differences in GI among treatments and the negative control were not statistically significant ($p>0.05$). This result indicates that, although mean germination values increased under certain treatments, such changes did not reach the statistical robustness required to confirm them as consistent effects at this experimental stage. Similar behavior has been reported in studies evaluating pre-germination treatments in *Acacia* species, where improvements in germination did not always translate into statistically significant differences (Alzandi *et al*., 2025). This highlights that early

germination assays—even when showing positive trends—may not reach statistical significance when the magnitude of the response is modest, as observed in our rice seed assays with microalgal extracts.

Overall, the results suggest that the Bar_4 extract at 0.1 g/L exhibits an initial biostimulant potential on rice seeds, as reflected by the positive trends observed in germination index and seedling growth. Although these effects did not reach statistical significance at this stage, the response pattern is consistent with findings reported in other microalgae–plant systems.

3.2 Germination of tomato seeds

Table 3 and Fig. 2 show the biostimulant effect of microalgal extracts on tomato seed germination. Results indicate that the GI was influenced both by the strain used and by the applied concentration, confirming a dependency on these two factors.

Overall, all strains produced positive increases compared to the negative control (H_2Od, GI=100%), suggesting the presence of bioactive compounds with activity analogous to that of the positive control (GA_3 at 0.003 g/L, GI=276%). Gibberellins, known for their essential role in seed germination and stem elongation, appear to have a functional counterpart in the metabolites present in the evaluated extracts, supporting their potential use as natural biostimulants.

The analysis revealed that strain Bar_4 at 0.1 g/L exhibited the highest GI (220%), even surpassing the GA_3 positive control, followed by strain SA 0.1 g/L (207%) and Bar_1 at 0.1 g/L (203%). Similarly, Alling *et al*. (2023) reported in their study on tomato seeds that all treatments with *Chlorella vulgaris* led to significantly faster germination compared with the water control, achieving at least a 0.5-day reduction in germination time. These findings support the notion that low doses of microalgal extracts can enhance early germination processes across different plant species.

Table 3. Evaluation of the number of germinated tomato seeds in response to the tested extracts.

Sample	Concentration (g/L)	Germination Index (%)
H_2Od	-	100
GA_3	0.003	276
Bar_4	0.1	220
	0.5	200
Al_2	0.1	100
	0.5	189
Bar_1	0.1	203
	0.5	216
SA	0.1	207
	0.5	167

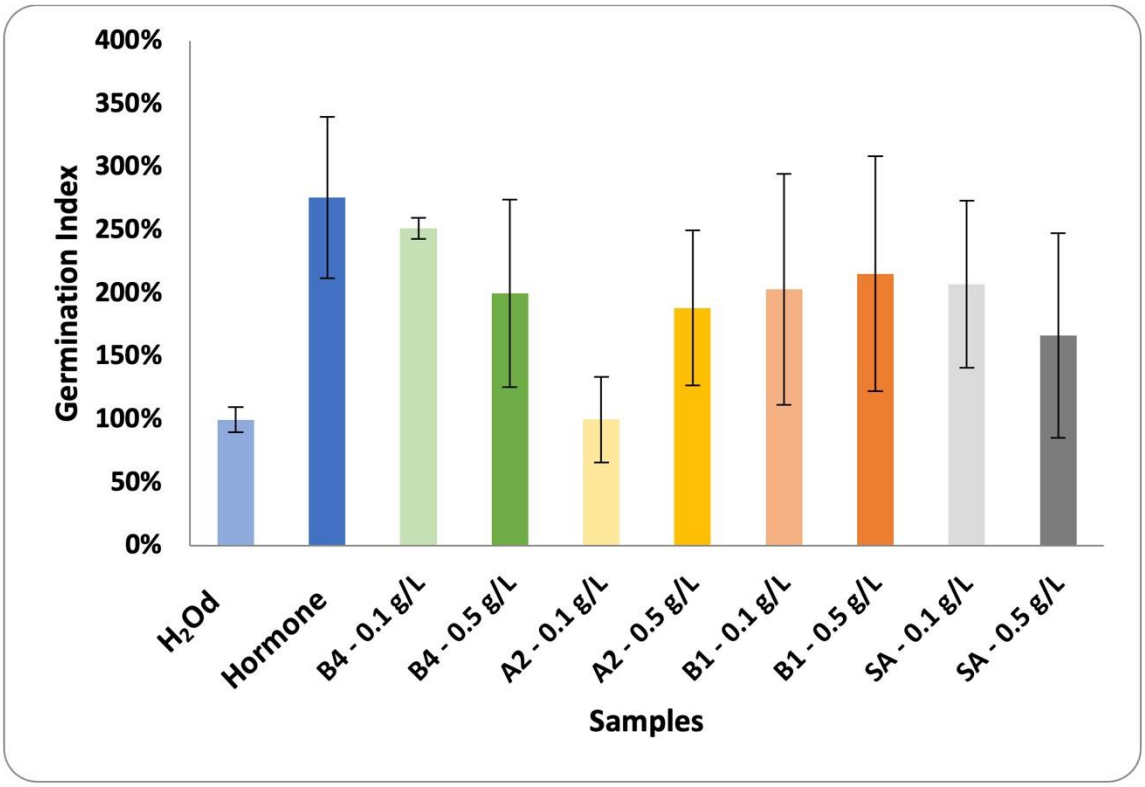

Figure 2. Percentage of tomato seed germination under exposure to extracts.

Nevertheless, further replication, cross-species validation, and assessment of additional physiological parameters—such as enzymatic activity, hormonal balance, or pigment accumulation—are necessary to confirm the biostimulant effect of Bar_4 and to better elucidate its underlying mechanisms. This pattern suggests that, at low concentrations, the extracts may create a favorable environment for germination and early development, possibly by stimulating the synthesis of hydrolytic enzymes and the mobilization of energy reserves.

In contrast, higher concentrations (0.5 g/L) did not always result in a proportional increase in GI, suggesting a possible saturation threshold or even inhibitory effects associated with excessive levels of bioactive compounds. This type of dose-dependent response has been widely documented in studies involving plant-based and microalgal extracts, where low concentrations tend to enhance germination, whereas higher doses can diminish or nullify the biostimulant effect. Consistent with this pattern, Puglisi *et al*. (2020) reported that intermediate extract concentrations achieved the highest germination and vigor indices, while both lower and higher doses failed to outperform the control. These findings reinforce the notion that the biostimulant response operates within an optimum range, beyond which physiological benefits are attenuated or reversed.

Regarding statistical analysis, one-way ANOVA and Tukey's test confirmed that only strain Bar_4 at 0.1 g/L showed significant differences compared to the negative control ($p=0.03<0.05$). The other treatments, although higher than the control, did not demonstrate statistically significant differences.

3.3 Germination of watercress seeds

Table 4 and Fig. 3 illustrate the biostimulant effect of microalgal extracts on watercress seed germination. Results indicate that treatments at 0.1 g/L yielded GI increases ranging from 165% to 319%, whereas at 0.5 g/L values ranged from 130% to 207%. These findings confirm that extracts, at both concentrations, promoted germination, although with varying magnitude depending on the strain tested.

A consistent pattern of greater biostimulant efficacy at lower concentrations (0.1 g/L) was observed across most treatments, indicating a clear dose-dependent response in which reduced doses enhanced germination more effectively than higher ones. This behaviour reflects a well-documented pattern in seed bioassays: moderate concentrations of microalgal extracts tend to stimulate germination and early growth, whereas elevated doses often lead to saturation or partial inhibitory effects. Similar trends have been reported in sugar beet, where optimal germination responses occurred at intermediate extract concentrations and declined as the dose increased (Puglisi *et al.*, 2020). Consistent evidence has also been documented for watercress, in which *Chlorella* extracts at 0.1 g/L maximised the GI, while higher concentrations progressively reduced the response (Morillas-España *et al.*, 2022). The only exception in the present study was strain SA, which displayed an opposite trend by maintaining or even reducing its efficacy at low concentrations. This deviation may reflect intrinsic differences in the profile of secondary metabolites or the presence of compounds with dual activity—promotive at certain thresholds and inhibitory when concentrations fall outside their optimal range. Such duality has been previously suggested for specific microalgal and plant-derived biostimulants, whose effects depend strongly on both concentration and biochemical composition.

Table 4. Evaluation of the number of germinated watercrees seeds in response to the tested samples.

Sample	Concentration (g/L)	Germination Index (%)
H_2Od	-	100
GA_3	0.003	295
Bar_4	0.1	319
	0.5	130
Al_2	0.1	165
	0.5	144
Bar_1	0.1	192
	0.5	207
SA	0.1	166
	0.5	145

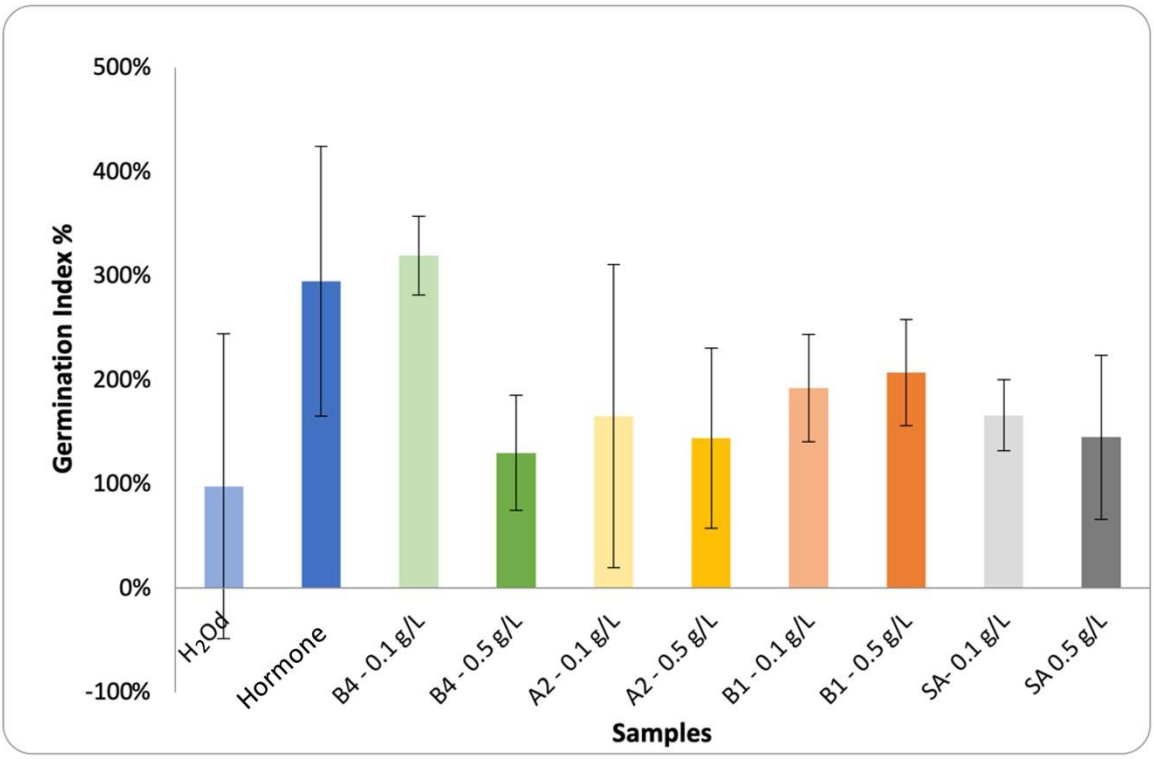

Figure 3. Percentage of watercress seed germination under exposure to extracts.

Despite the observed trends, statistical analyses using one-way ANOVA and Tukey's test revealed that differences in GI among treatments and the negative control were not statistically significant ($p>0.05$). This implies that, although mean values suggest a positive effect on germination, additional replicates and complementary analyses are required to confirm the robustness of these differences and to understand the biochemical mechanisms involved.

4. Conclusions

The species investigated *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus quadricauda* y *Scenedesmus almeriensis* appear to be a highly promising source of biostimulant compounds, exhibiting outstanding performance in the trials conducted compared with the control treatment. The findings indicate that microalgal extracts exert a beneficial and distinctive influence on seed germination and early seedling development, thereby confirming their potential as valuable inputs for sustainable agriculture.

Importantly, the concentration of microalgae in the tested extracts (0.1 g/L) had a clear and significant impact on the extent of the biostimulant response, underscoring the necessity of defining precise dosage protocols and optimal application conditions to maximize their effectiveness. This observation is in agreement with previous research suggesting that both the chemical profile of microalgal metabolites and the applied concentration are decisive factors in determining plant physiological responses.

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