



Advances in biology through agronomy, aquaculture, coastal and environmental sciences

— Volume II —

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Editors

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**Advances in biology through
agronomy, aquaculture, coastal and
environmental sciences**
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Advances in Biology through Agronomy, Aquaculture, Coastal and Environmental Sciences, Volume II, is an e-book that continues the work of the first version (<https://editorapantanal.com.br/ebooks/2023/advances-in-biology-through-agronomy-aquaculture-coastal-and-environmental-sciences/ebook.pdf>), published in 2023 by Leandris Argente-Martínez and Ofelda Peñuelas-Rubio.

Our e-book compiles 25 manuscripts that present scientific results and reviews based on biological aspects. All of them contribute to explaining the responses of living organisms to agricultural, aquaculture, and coastal ecosystems, as well as those facing fragility or threat of climate change. This e-book was edited by Leandris Argente-Martínez, Ofelda Peñuelas-Rubio, and Joe Luis Arias-Moscote, members of the Consolidated Academic Group ITVAYA-CA-3.

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Hydrolysate of the alga *Spirulina* influences the proliferation, growth, and development of *Spathiphyllum uxpanapense* *in vitro*

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 10.46420/9786585756792cap18Verónica Gómez Entzin¹ Antonia Gutiérrez Mora¹ Nutan Prasad Rout^{1*} 

ABSTRAC

Spathiphyllum uxpanapense is an ornamental species of commercial interest, but its propagation is limited by slow growth, low shoot production, and a lack of suitable seed-based propagation material. In this study, the effects of *Spirulina* hydrolysate on the *in vitro* proliferation and growth of axillary shoots of *S. uxpanapense* cv. were evaluated. *Spirulina* hydrolysate was obtained through acid and alkaline hydrolysis, and the growth variables included total plant length, number of roots, root thickness, number of shoots, number of leaves, survival rate, and photosynthetic pigments. Although acid and alkaline hydrolysis did not differ significantly, alkaline hydrolysis at a concentration of 1 M was found to be the most favorable mineral profile for subsequent biological evaluation. In the establishment trials, low hydrolysate concentrations, especially 5% and 10%, improved root development and thickness, plant vigor, and overall plant quality; conversely, high concentrations reduced survival and growth. The contents of chlorophyll a, chlorophyll b, and carotenoids did not differ significantly among the treatments but were higher in most cases. Overall, the *spirulina* hydrolysate acted more clearly as an additive that promoted growth and development and promoted plant rooting and as a direct stimulator of shoot proliferation. These results support the use of low concentrations of *spirulina*-derived hydrolysate as a complementary component in the *in vitro* culture of *S. uxpanapense*. This can be used as a biostimulant in agricultural practices after field trials.

Keywords: *Spathiphyllum uxpanapense*; *Spirulina* sp.; axillary buds; biostimulant; *in vitro* culture.

INTRODUCTION

The genus *Spathiphyllum* comprises 47 species, 94% of which are found in the Neotropics, from Mexico to northern Bolivia and Brazil (Cardona, 2016). It is the second best-selling houseplant in the world and of commercial interest because of its attractive foliage and white spathe-shaped inflorescences (Cardona, 2016). The use of synthetic growth promoters and mineral fertilizers has been practiced for many decades. Nevertheless, their continued use has led to negative effects, including soil chemical alteration and reduced plant growth (Srijaya et al., 2010). Seaweed extracts have gained prominence and

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are used as nutritional supplements, biostimulants, or biofertilizers in agriculture and horticulture (Hernández et al., 2013). Consequently, new natural materials are being developed and can be used as fertilizers or plant growth biostimulants. Plant biostimulants, regardless of their nutrient content, contain substance(s), compound(s), and/or microorganism(s) whose functional use, when applied to plants or the rhizosphere, improves crop development, vigor, yield, and/or quality by stimulating natural processes that promote growth and responses to biotic stress (Palazon, 2013). The presence of at least six types of cytokinins and their precursors has been scientifically demonstrated in cyanobacteria, microalgae, and three types of marine macroalgae, such as *cis*- and *trans*-zeatin riboside, *trans*-zeatin, dihydro-Zetina N6 (isopentenyl) adenine and its 9- β -ribosides, as well as in microalgae and cyanobacteria (Blunden; Wildgoose, 1977; Meeting et al., 1988; Mooney; Van Staden, 1987; Zhang et al., 1991; Crouch; Van Staden, 1993).

Various studies have demonstrated the potential of these bioactive substances as alternatives to synthetic hormones used in plant micropropagation. Among them, the use of Pectimorf®, obtained from a mixture of oligosaccharides of peptidic origin (Cabrera, 1999), has been employed to promote various processes in plants; hormonal effects have been reported both *in vitro* and *ex vitro*, in somatic embryogenesis in coffee, sugarcane, and potato, and as a rooting agent for rootstocks in *Ficus sp.* and guava cuttings, among others (Ramírez et al., 2003). The addition of seaweed extract (SWE) with and without growth hormones significantly increased total biomass production, total plant length, number of shoots, root length, and number of roots in *Picrorhiza kurooa* plants; furthermore, this is the first report demonstrating the potential of SWE as an inducer to increase the growth and yield of *P. kurooa* (Sharma et al., 2014). Currently, various extraction processes are employed to obtain stimulants from seaweed.

Methods for producing seaweed extracts can generally be divided into three groups: biological methods (enzymatic degradation), physical extraction methods (high-pressure and cold processes and supercritical fluid extraction), and chemical hydrolysis methods (organic and inorganic solvents) (Kim; Chojnacka, 2015). Enzymatic extraction has emerged as a promising alternative for isolating bioactive compounds from algae. This method uses enzymes; however, its large-scale application is limited by the high cost of these enzymes (Barzana et al., 2002). The disadvantages include the high volumes of solvent required and the prolonged extraction time (Ramnluckan et al., 2014). Acid hydrolysis: The purified material is mixed with deionized water at a specific acid concentration. Sulfuric acid is generally used, as it produces a negative surface charge on the particles and stabilizes the suspension (Habibi et al., 2010).

Alkaline hydrolysis: In this process, the algal biomass is first dried and then mixed with alkaline solutions. The most commonly used compounds are sodium hydroxide or potassium hydroxide, either alone or in combination (Harun; Danquah, 2011). Marine algae products can be classified as extracts or suspensions depending on the production process. The term “extract” is used to refer to products obtained by using chemical agents to hydrolyze the algae, while the term “suspension” refers to those obtained without chemical agents (Chojnacka et al., 2012). Toxicity tests are used to study the

relationships between dose or concentration and effect, and between dose or concentration and the dose–response effect: a biological change that can be evaluated on a scale of intensity or severity; response: the proportion of the exposed population that exhibits a defined effect (Cárdenas, 2009).

Spirulina platensis, also known as *Arthrospira*, is a filamentous, microscopic cyanobacterium (blue–green algae) with a long history of use as a food source and as a biofertilizer. An evaluation of *Spirulina platensis* as a biostimulant for organic farming systems revealed that it contains 6.7% N, 2.47% P, and 2.14% K, up to 60% protein, and adequate amounts of the trace elements necessary for plant nutrition. This biostimulant was inoculated under *ex vitro* conditions, resulting in higher yields (Aly; Esawy, 2008; Spínola et al., 2024).

Spathiphyllum sp. is an important tropical ornamental plant. Other species are distinguished within their genus; one of them is *S. uxpanapense*. It is of commercial interest, but its production requires large quantities of plants. In vitro culture enables the production of these compounds in relatively short periods, underscoring the need to standardize a micropropagation protocol for this species. On the other hand, *Spirulina platensis* is a potential biostimulant microalga. The use of extracts in crops has positive effects; therefore, it is essential to verify whether these extracts can increase the proliferation of *S. uxpanapense* in vitro. Consequently, new biostimulant alternatives based on the development of natural products that mitigate the harmful effects of chemical substances are needed.

MATERIALS AND METHODS

Collection of plant material and induction of in vitro culture

Explants (axillary buds) from *Spathiphyllum uxpanapense*, cultivar Callisto, were used to induce shoots. The explants were subsequently sown in MS media (Murashige; Skoog, 1962) supplemented with 30 g·L⁻¹ sucrose and 7.5 g·L⁻¹ agar, and the pH was adjusted to 5.8 with concentrations of 2 mg·L⁻¹ benzyl aminopurine (BA) and 2 g·L⁻¹ naphthaleneacetic acid (NAA). The cultures were incubated under a 16-hour light/8-hour dark cycle; shoot numbers were evaluated after 30 days, and treatment means were compared using analysis of variance and Tukey's HSD test.

Preparation of a hydrolyzed extract of Spirulina sp.

The hydrolyzed extract was obtained by varying the acid and alkaline hydrolysis conditions from *Spirulina* sp. biomass. The samples underwent heat treatment, centrifugation, and pH neutralization, as described by Setyaningsih (2012). Subsequently, with minor modifications, the optimal hydrolysis was adjusted. The yields from acid and alkaline hydrolysis were analyzed to determine the most effective hydrolysis process, and the CIATEJ Analytical and Metrological Services Unit determined the essential mineral contents.

Establishment of Cultures with Hydrolyzed (Extracts of *Spirulina* sp.)

The effect of the hydrolyzed extract of *Spirulina* sp. (5%, 10%, 20%, 30%, 40%, and up to 100%) was evaluated both alone and in combination with MS media with and without BA. Treatments without the hydrolyzed extract were used as controls (Table 1). Each treatment consisted of 5 replicates; the media were adjusted to a pH of 5.8; 25 ml was poured into each test tube and sterilized for 15 min at 121 °C. The cultures were maintained at 25 ± 2 °C under a 16 h light/8 h dark photoperiod. The effects of hydrolyzed spirulina extract (HE) on the plants were evaluated every 30 days. The variables evaluated were the number of shoots per explant, survival rate, height, root thickness, number of shoots, number of leaves, chlorophyll content, and concentration of the hydrolyzed extract.

Table 1. Composition of media containing different percentages of hydrolyzed extract (HE): Medium 1 (M1), (water + HE); Medium 2 (M2), (MS + HE); and Medium 3 (M3), (MS + BA + HE).

Treatments	Medium 1	Medium 2	Medium 3
Control	WATER + EH (0)	MS + EH (0)	MS + BA + EH (0)
T1	WATER + EH (5)	MS + EH (5)	MS + BA + EH (5)
T2	WATER + EH (10)	MS + EH (10)	MS+BA + EH (10)
T3	WATER + EH (20)	MS + EH (20)	MS + BA + EH (20)
T4	WATER + EH (30)	MS + EH (30)	MS + BA + EH (30)
T5	WATER + EH (40)	MS + EH (40)	MS + BA + EH (40)

Measurement of chlorophyll a, b, and carotene

A modified method by Wellburn (1994) was used to measure chlorophyll a, b, and carotene levels. Three replicates of each treatment were used. A leaf sample of approximately 20 mg was taken from the explant, cut into small pieces, and placed in test tubes. Two milliliters of absolute methanol were added, and the samples were incubated in the dark at 4 °C for 24 hours until all the tissue became white. The optical density at 666, 653, and 470 nm was subsequently measured using a spectrophotometer. The concentrations of chlorophyll a and b and the carotenoid content were calculated for each sample using the formula of Wellburn (1994).

Statistical analysis

The evaluation results were analyzed using ANOVA, and the treatment means were compared using Tukey's HSD test at the 5% significance level in the STATGRAPHICS program.

RESULTS

Analysis and Selection of the Best Hydrolyzed Extract

An analysis of the yields from acid and alkaline hydrolysis revealed that compared with the other hydrolysis methods, 1 M alkaline hydrolysis yielded the greatest amount of minerals of interest (Table 2). Compared with the other alkaline and acid hydrolysis methods, the 1 M and 0.4 M alkaline hydrolysis

methods result in a large amount of phosphorus (P); for calcium (Ca), potassium (K), and iron (Fe), 1 M alkaline hydrolysis is superior to the other hydrolysis methods, except for the contents of two minerals: magnesium (Mg) and sulfur (S). The 0.4 M acid hydrolysis resulted in a higher magnesium content, and the 1 M acid hydrolysis resulted in a high amount of sulfur.

Table 2. Performance analysis of hydrolysis methods for the determination of major and minor elements.

Minerals mg/kg	Acid Hydrolysis 0.4 M	Acid Hydrolysis 0.6 M	Acid Hydrolysis 1 M	Alkaline Hydrolysis 0.4 M	Alkaline Hydrolysis 0.6 M	Alkaline Hydrolysis 1 M
Phosphorus	971.1	336	874.3	1851	576.9	1798
Calcium	53.39	37.71	42.47	38.59	48.92	59.68
Magnesium	133.2	108.9	107	40.96	34.69	45.02
Sulfur	13410	14370	23160	244.5	258	309.2
Potassium	1721	979.8	1610	12570	9989	38520
Iron	7.4	9.82	8.38	10.05	7.3	10.96

Hydrolyzed Extract Toxicity Test

A toxicity bioassay was performed 15 days after sowing, and chlorosis was observed only in the leaves of *S. uxpanapense* at extract concentrations greater than 40%. After 30 days, the previously chlorotic plants exhibited necrosis and complete plant death (Figure 1B). A suitable concentration without any toxicity was observed at concentrations of nearly 10% or less than 20% (Figure 1C).

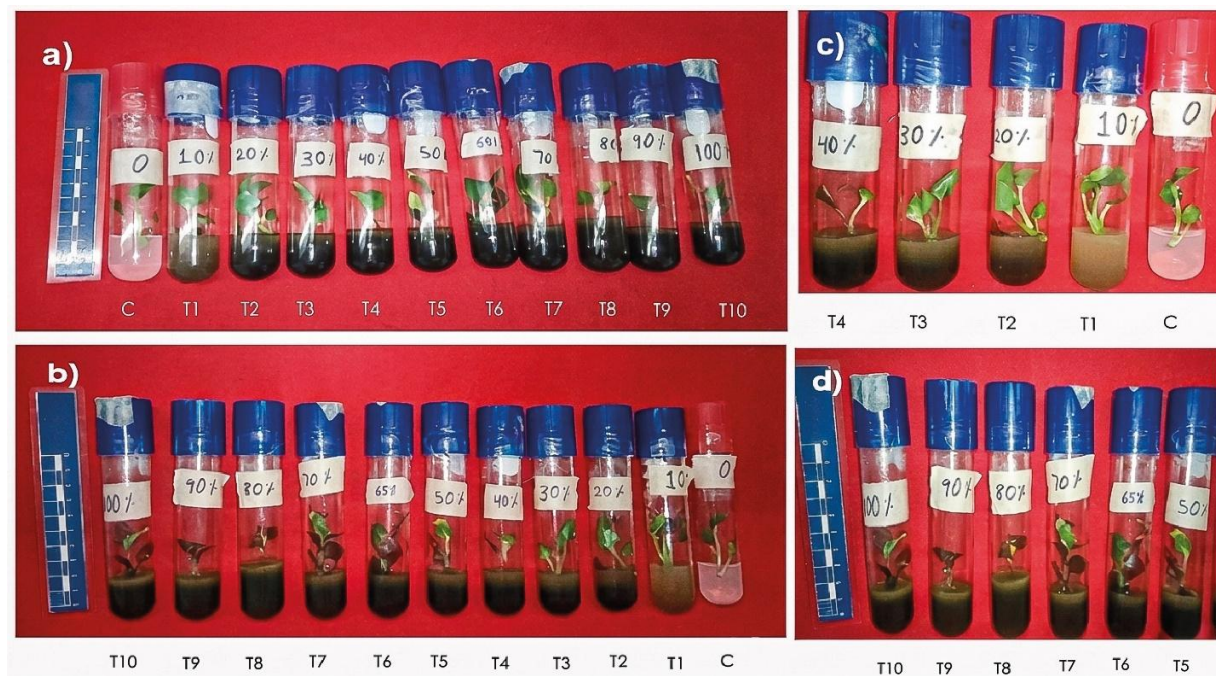


Figure 1. Effects of different concentrations of hydrolyzed extract on the axillary buds of *S. uxpanapense*. (a) Day 1 after sowing: control (C 0%) and treatments with 10, 20, 40, 50, 60, 70, 80, 90, and 100% hydrolyzed extract (T1–T10); (b) evaluation 15 days after sowing under the same concentrations; (c) evaluation at 30 days for treatments of 0–40% (C–T4); (d) evaluation at 30 days for treatments of 50–100% (T5–T10).

Effect of the hydrolyzed extract on the establishment of *in vitro* cultures

The effects of the extract on the *in vitro* culture of *S. uxbanapense* revealed that the type of medium and the treatment percentage significantly affected ($p \leq 0.05$) plant length. Moreover, their interaction was not significant ($p \geq 0.05$), indicating that both factors independently influence plant length. With respect to root number and thickness, the type of medium did not significantly differ ($p \geq 0.05$). In contrast, the treatment percentage and its interaction did ($p \leq 0.05$), indicating that these variables depend primarily on the treatment level and its interaction with the medium (Fig 2). In contrast, the number of shoots, the number of leaves, and the survival rate were significantly affected by the type of medium, the treatment percentage, and their interaction ($p \leq 0.05$), indicating that the plant's response depends on the combination of both factors (Figure 2).

In continuation, the analysis of means using Tukey's test ($\alpha=0.05$) showed that the plant length was highest in media M3 (3.81 cm) and M1 (3.69 cm), with no statistically significant difference between them, and both were greater than M2 (3.21 cm). No significant differences were observed among the three media evaluated for root number and root thickness. For the number of shoots, medium M3 produced a significant number of shoots (3.0), while M1 and M2 produced no shoots. Regarding the number of leaves, M3 also showed the highest value (6.17), statistically higher than that of the other treatments. Finally, in terms of survival rate, medium M3 had the highest value (71.36%), followed by M1 (64.70%) and M2 (58.41%), with significant differences among them (Figure 2).

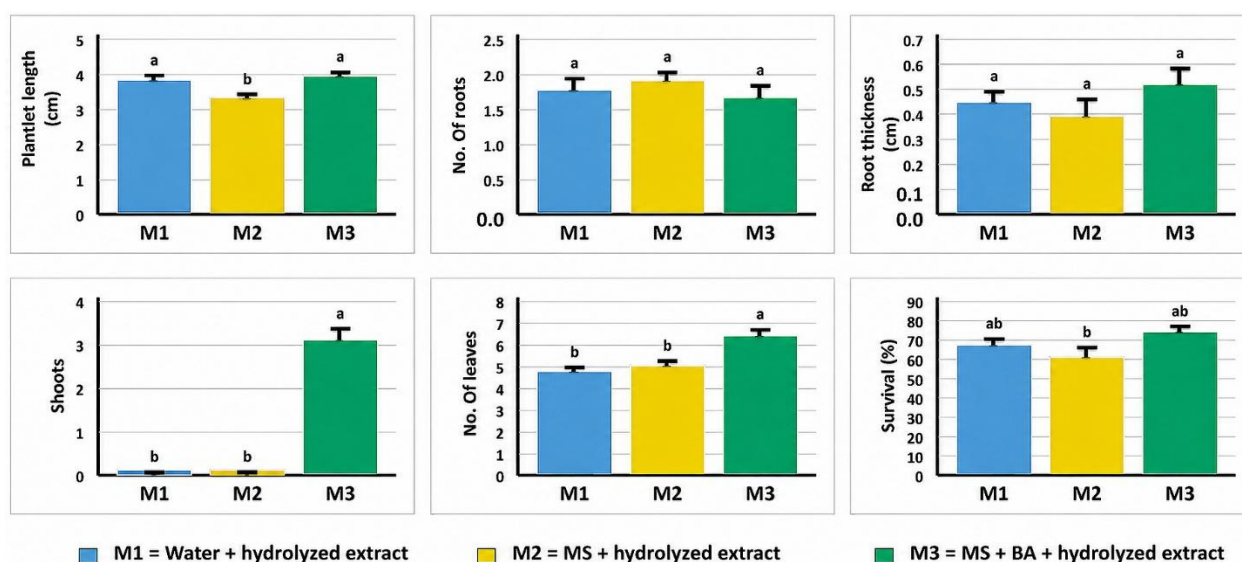


Figure 2. Multiple comparison of means for medium type and extract concentrations regarding total plant length, number of roots, root thickness, shoots, number of leaves, and (%) survival of axillary buds of *S. uxbanapense*. Medium 1 (M1) Water + Hydrolyzed extract, Medium 2 (M2) MS + Hydrolyzed extract, Medium 3 (M3) MS + BA + Hydrolyzed extract. Using the Tukey HSD test. Groups with the same letter do not differ significantly from one another. $p=0.05$.

The effect of different extracts of hydrolyzed extract on in vitro cultures

On the other hand, the extract concentration had varying effects depending on the variable evaluated, as seen in Table 3. For plant length, the 20% concentration (3.97 cm) yielded the highest value, though it was not statistically different from most treatments, whereas the 40% concentration (3.16 cm) yielded the lowest value. For root numbers, the 0%, 5%, and 10% concentrations showed the highest values, with no significant differences, whereas a significant decrease was observed at 20%.

Table 3. The effect of different hydrolyses of *Spirulina* extract (from 5 to 40%, 0 is without extract) on total plant length, number of roots, root thickness, shoots, number of leaves, and (%) survival of axillary buds of *S. uxpapapense*. Using the Tukey HSD test. Groups with the same letter do not differ significantly from one another. $p=0.05$.

% of Extract	Length of Plantlets	No. of Roots	Thickness of Roots	Shoots	No of Leaves	Survival %
0	3.44 ab	3.87 a	0.69 b	4.8 a	6.9 a	92.21 a
5	3.66 ab	3.4 a	0.7 ab	1 b	5.83 ab	77.96 a
10	3.66 ab	2.67 a	0.8 a	0.2 b	5.2 bc	80.94 a
20	3.97 a	0.13 b	0.0 c	0 b	5.13 bc	76.47 a
30	3.55 ab	0.2 b	0.03 c	0 b	4.17 cd	43.93 b
40	3.16 b	0.0 b	0.0 c	0 b	3.8 d	17.44 c

Regarding root thickness, 10% concentration had the highest value (0.8), whereas higher concentrations significantly reduced it. As for shoots, the greatest formation was observed at the 0% concentration; it also had the highest value (6.9), with a decreasing trend as the extract concentration increased. Finally, the survival rate was highest at the 0%, 5%, and 10% concentrations, while the 30% and 40% concentrations significantly reduced survival, with 40% showing the lowest rate (17.44%) (Table 3).

The effect of the combination of Medium with hydrolyzed extracts

The results of the effect of different concentrations of hydrolyzed extract on the growth of axillary buds of *S. uxpapapense* in combination with three different culture media show very interesting results (Figure 3). The treatment of extract without nutrients (M1: Water + hydrolyzed extract), like MS salts, showed better growth compared to nutrients (M2: MS medium + hydrolyzed extract). The medium containing a growth regulator offers the best of the three (M3: MS medium + BA + hydrolyzed extract) (Figure 3).



Figure 3. Effect of different concentrations of hydrolyzed extract on the growth of axillary buds of *S. uxpanapense* in three culture media. (a) Medium 1 (M1): Water + hydrolyzed extract, (b) Medium 2 (M2): MS medium + hydrolyzed extract, (c) Medium 3 (M3): MS medium + BA + hydrolyzed extract. Six treatments were evaluated in each medium: control without extract (c) and concentrations of 5, 10, 20, 30, and 40% hydrolyzed extract (T1–T5), respectively.

Effect of Hydrolysate of Spirulina on Chlorophyll a, b, and Carotene

The results of pigmentation on the influence of different concentrations of the hydrolysate of spirulina are presented in Figure 4. The Chlorophyll concentration in the three media was not statistically significant (Figure 4); however, in medium 1 (water + hydrolyzed extract), the 20% treatment showed a higher level of chlorophyll content than the other media and their treatments. For chlorophyll b, higher amounts were found in media 1 and 2 with a 40% treatment, and in medium 3 with a 10% treatment. Regarding carotene content, higher levels were observed in medium 1 with treatment percentages of 20% and 40%; in medium 2, carotene content did not differ significantly; and in medium 3, with treatment percentages of 30% and 40%, higher carotene levels were observed.

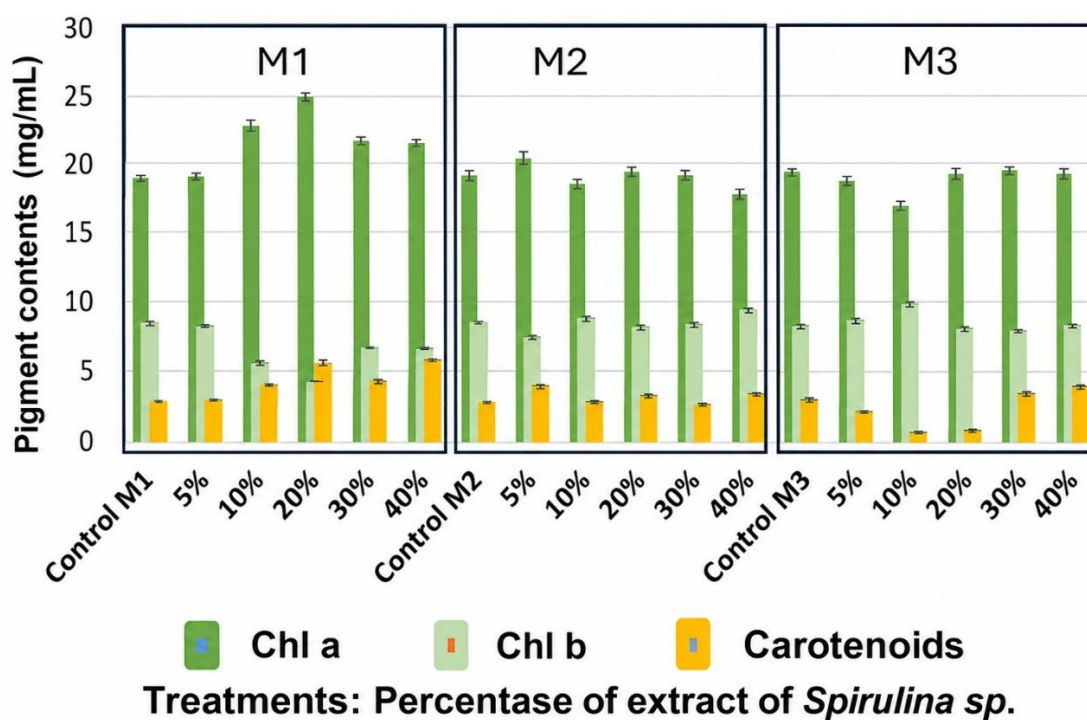


Figure 4. Chlorophyll (Chl) a, b, and carotenoid content of *S. uxpanapense* cultured in different media (M): M1, water control (water + EH), M2, MS control (MS + EH), M3, MS + BA (MS + BA + EH). Where hydrolyzed extract is represented as EH, Murashige Skoog as MS, and Benzylaminopurine as BA.

DISCUSSION

Estimation of hydrolyzed extract yield

The results of the statistical analysis indicated that the type of hydrolysis and concentration did not have a significant effect; however, mineral content differed, with higher concentrations of Ca, K, Fe, and Mg in the alkaline hydrolysis. These results are consistent with those reported by Kadam et al. (2013), who noted that alkaline conditions and high temperatures promote cell wall disruption and the release of bioactive compounds compared to other extraction methods.

In this study, alkaline hydrolysis at 80 °C and pH 12 (1 M) yielded an extract with a higher mineral content, supporting its use as a supplement in *in vitro* culture media. However, other authors have noted that extreme pH conditions can degrade certain sensitive compounds (Michalak and Chojnacka, 2015), suggesting the need to optimize extraction conditions.

Toxicity levels of the hydrolyzed extract

The toxicity assessment showed that concentrations of the hydrolyzed *Spirulina sp.* extract exceeding 40% were harmful, causing chlorosis in the explants. This behavior is consistent with the findings of Khan et al. (2009), who reported that algal-derived biostimulants exhibit dose-dependent effects, with high concentrations capable of causing phytotoxicity. Likewise, Calvo et al. (2014) note that excessive accumulation of bioactive compounds can alter the physiological balance of plants, which explains the negative effects observed at high concentrations. Therefore, the range of 10% to 40% was considered appropriate for evaluation.

*Effect of the hydrolyzed extract of *Spirulina sp.* on the *in vitro* cultures of *S. uxpanapense**

The results showed that the hydrolyzed *Spirulina* extract had positive effects on *in vitro* cultures of *S. uxpanapense*, as demonstrated by increased *S. uxpanapense* growth and higher values for plant height, leaf number, and root development, especially at low concentrations of hormones. These results are consistent with those reported by Zhang and Ervin (2008), who observed that algal extracts promote plant growth by stimulating physiological and metabolic processes.

The very promising effect on the root system is consistent with Craigie's (2011) findings, which noted that algal extracts contain auxins and cytokinins that promote root formation and elongation. However, the extract in this study did not show a significant effect on shoot formation, unlike the findings of Kavipriya et al. (2011), who observed increased shoot formation in other species. This difference may be due to the species evaluated, the concentration used, or interaction with the growth medium. The superior performance at low concentrations and negative effects at high concentrations reinforce Du Jardin's (2015) findings that biostimulants act within specific concentration ranges, where inappropriate doses can inhibit growth. Taken together, these results confirm the potential of the hydrolyzed extract of

Spirulina sp. as a biostimulant in vitro culture, constituting a viable alternative to synthetic growth regulators, as reported in the literature.

CONCLUSIONS

Among the hydrolysis methods of acid and alkaline processes, the alkaline hydrolysate extracts at 0.6 and 1 M were found to have higher mineral content than those from acid hydrolysis. The hydrolysate of *Spirulina sp.* was tested in an *in vitro* culture of *S. uxpanapense*, with 5% and 10% hydrolyzed microalgae extracts, alone or combined with MS or MS plus BA, which influenced root proliferation, producing greater root mass and improving plant development by stimulating vigor, bud growth, and root thickness. Importantly, the hydrolysate of *Spirulina sp.* without nutrients has been effective for establishing and in vitro culturing of *S. uxpanapense*.

Modifying this hydrolysis process can improve the yield of the *Spirulina* extraction process. Further investigation is needed to evaluate the hydrolyzed extract across different plant species, both in vitro and in the field, to determine its potential as a bioestimulant.

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