



Effect of microalgae and a cyanobacteria mixture on germinative activity, growth, and cannabinoid production in *Cannabis sativa* plants

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Abstract

The enhancement in agricultural productivity has been achieved through the application of pesticides and nutrients, mainly of synthetic origin. This practice has led to a dependence on the use of agrochemicals in order to obtain better results. However, the overuse application has created environmental and toxicological problems, so other alternatives are needed. For this reason, this study focused on evaluating the biostimulation of cannabis (*Cannabis sativa*) plants used in the production of cosmetics, pharmaceuticals, and phytotherapeutics through the application of three microalgae: *Chlorella vulgaris*, *Ankistrodesmus falcatus*, and *Scenedesmus* sp. and one cyanobacterium: *Arthrospira platensis*. This strategy aims to increase the cannabinoids production through a practical and environmentally friendly approach. In this way, crop quality can be improved while reducing fertilizers use, thus promoting more sustainable and efficient agricultural practices. Treatments combining nutrients and microalgae at different concentrations, a control in which only water was applied and a treatment containing only nutrients were used to assess seed germination, plant growth and the production of ten cannabinoids and other compounds present in the plants. The microalgae treatment alone stimulated germination processes in cannabis by 93%. The mixture of microalgae and cyanobacteria and the nutrients positively affected the growth of cannabis plants, increasing their length by up to 40% more than the control. Cannabinoid production was increased when the 50% nutrient mixture was used with the microalgae and cyanobacteria, resulting in increased production of five cannabinoids (CBD, Δ^9 -THC, CBDA, THCA, and Δ^8 -THC). These results demonstrate the potential of microalgae and cyanobacteria as sustainable biostimulants for cannabis cultivation.

Keywords Microorganisms · Cannabis · Biostimulation · Secondary metabolites

Introduction

Cannabis plants have a high therapeutic and recreational potential, making them an attractive crop for the pharmaceutical industry (Pattnaik et al. 2022). These plants produce secondary metabolites called cannabinoids in the glandular

trichomes. Cannabinoids are products of plant metabolism that are mainly formed as carboxylic acids and then converted to decarboxylated analogs under light, heat, or prolonged storage (Bijelić et al. 2023). There are about 100 known phytocannabinoids, but Δ^9 -THC, cannabidiol (CBD), and cannabinol (CBN) are the most studied for the treatment of many diseases, including neurodegenerative diseases, cancer, neuropathic pain, inflammation, and obesity (Vijayakumar et al. 2016).

For the commercialization of cannabis products, the development time and quality of the products which significantly impact the chemical composition, are significant. Therefore, it is proposed to apply new methodological alternatives to manipulate the development and production of cannabis plants, including microalgae and cyanobacteria, which could lead to long-term increases in yields, harvest times, reduced production costs, and

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environmental impact (Chabili et al. 2024). Microalgae are photosynthetic, unicellular microorganisms with eukaryotic cells, unlike cyanobacteria, which are prokaryotic; both organisms are fast-growing and have few nutritional and environmental requirements for their development (Nunes et al. 2024).

Cyanobacteria and microalgae are currently being investigated for use as biofertilizers and biostimulants in agricultural practices (Álvarez-González et al. 2022), however, very few studies have been conducted using microalgae as biostimulants in cannabis plants. These applications induce plant protection mechanisms and increase crop yield and quality (Silva et al. 2022), enhancing nutrient uptake (Afkairin et al. 2021). The biostimulant activity of microalgal extracts is mainly related to the composition of their primary metabolites, such as carbohydrates, proteins, and lipids, as well as the presence of essential amino acids (such as arginine and tryptophan), vitamins, osmolytes (proline and betaine glycine) and polysaccharides such as β -glucan (Colla and Rouphael 2020). In addition, several microalgae strains have been characterized to produce phytohormone-like compounds, including auxins, cytokinins, gibberellins, abscisic acid, and brassinosteroids (Ferreira et al. 2024). The identification and selection of microalgae extracts containing natural phytohormones, especially auxins and cytokinins, could be a growing opportunity for the valorization of microalgae, as these compounds are key factors in improving plant growth, yield, and defense response, especially against abiotic stresses (Alling et al. 2023). Therefore, their cultivation through biorefineries could contribute to more sustainable agriculture and help meet the growing global demand for protein-rich products and plant growth promoters (Nunes et al. 2024), as well as the production of medicinal plants and metabolites.

This study explores alternative ways to meet the nutritional demands of *Cannabis sativa* plants in order to maintain optimal production and quality. Although biostimulation has been extensively studied in other crops, its application in cannabis remains limited. This work evaluates the effect of three microalgae (*Chlorella vulgaris*, *Ankistrodesmus falcatus*, and *Scenedesmus* sp.) and one cyanobacterium (*Arthrospira platensis*) on seed germination, plant growth, and the quality of flowers and fruits, with a particular focus on the production of primary cannabinoid-type secondary metabolites. The aim is to position biostimulation as a sustainable agricultural strategy that improves crop quality, reduces the use of chemical fertilizers, and contributes to a circular economy and the preservation of environmental resources.

Materials and methods

Chemicals, reagents and instruments

Phytocannabinoid standards (analytical grade > 99%) were provided by Innovateck (Colombia), 1 mg mL⁻¹ in methanol by RESTEK: Δ^8 -THC (Δ^8 -tetrahydrocannabinol) CAS 5957–75-5, Δ^9 -THC (Δ^9 -tetrahydrocannabinol) CAS 1972–08-3, THCV (tetrahydrocannabivarin) CAS 31262–37-0, THCA (tetrahydrocannabinol) CAS 23978–85-0, CBGA (cannabigerolic acid) CAS 25555–57-1, CBDA (cannabidiolic acid) CAS 1244–58-2, CBN (cannabinol) CAS 521–35-7, CBD (cannabidiol) CAS 13956–29-1, CBC (cannabichromene) CAS 20675–51-8, and CBG (cannabigerol) CAS 25654–31-3. The nutrients added to the culture media were purchased from Protochemistry (Colombia). The solvents used were Mili-Q water (18.2 M Ω cm⁻¹), acetonitrile CAS 75–05-8 Lichrosolv for HPLC from Supelco, Merck, and methanol CAS 67–56-1 for HPLC from Pan-Reac AppliChem, formic acid 98%–100% for LC–Ms CAS-84–18-6 LiChropur Merk.

Plant material and microalgae

To initiate germination experiments, seeds of the *Cannabis sativa* variety “Mango Biche” were obtained from Colombian growers in September 2023. The seeds were separated according to their morphological characteristics, such as size and color, and washed with a hypochlorite-water mixture for disinfection to start germination. The three species of microalgae (*Chlorella* sp., *Ankistrodesmus falcatus*, and *Scenedesmus* sp.) and the cyanobacterium *Arthrospira platensis* were taken from the culture collection of the Laboratory of Organic Chemistry of Natural Products of the University of Antioquia. *Arthrospira platensis* was purchased from the National University of Colombia. *Ankistrodesmus falcatus* was obtained from the Laboratory of Environmental Health Assessment and promotion, Oswaldo Cruz Institute (Fiocruz), Brazil. *Chlorella vulgaris* and *Scenedesmus* sp. were isolated from soil in El Carmen de Viboral (Colombia). Taxonomic identification was performed using literature for each microalga (Bellinger and Sigeo 2015).

Culture of microalgae

The selected microalgae were cultivated by taking a 20 mL sample from the stock cultures and inoculating them into 3 L sterilized glass flasks containing 2.5 L of sterile distilled water and the specific culture medium (Zarrouck's medium for *A. platensis*, ASM-1 for *A. falcatus*, and BBM

for *Chlorella* sp. and *Scenedesmus* sp.). These cultures were maintained under a photoperiod of 12 h light and 12 h dark, a light intensity of $17.2 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and constant aeration. After approximately 15 days of growth, the aeration was removed from the cultures to allow the microalgal biomass to settle. Finally, the water was removed using an electric suction pump to concentrate the sample. Microscopic monitoring was performed by mounting an object holder plate, adding 20 μL of the culture and observing under a microscope. Cell counts were performed using a Neubauer chamber, with samples taken every 48 h. Absorbance was measured at 630 nm and calibration curves were constructed to establish the linear relationship with cell density (Herrera et al. 2025).

Preparation of mixtures used as treatments in cannabis plants

The mixtures of microalgae and nutrients were established as shown in Table 1.

Germination tests

The five treatments described in Table 1 were used for germination tests. Twelve seeds were placed in 2 mL Eppendorf tubes containing 1.5 mL of each treatment in duplicate. The seeds treated with the solutions were left in the dark for 20 h at room temperature. Then, 10 seeds were placed in 15 cm Petri dishes with filter paper with 4 mL of each treatment solution added. They were kept at room temperature for 48 h (Rupawalla et al. 2022). The germination percentage was calculated by dividing the number of germinated seeds by the total number of seeds sown and multiplying the result by 100. The germinated seeds were used for growth assessment.

Plant growth assays

The experiment was conducted in an open field, exposed to natural sunlight. Shade netting was used to partially reduce direct solar radiation and prevent damage caused by extreme temperatures or water stress. The plant crop was in an area where temperatures varied between 22 °C and 29 °C during the day, and the average relative humidity was 60–75%. The experiment was carried out using a randomized block design with six treatments, each with six experimental units (plants). The space allocated to each treatment was 2 m by 2 m (4 m²) to ensure proper plant development and efficient management of the crop. Each treatment consisted of a set of six cannabis plants (*Cannabis sativa*), which were grown in individual pots.

The germinated seeds were transferred to 200 mL plastic containers which include 50 g of substrate with perlite and coconut fiber. The distribution of plants within each

block was done by maintaining a minimum distance of 40 cm between each plant to avoid excessive competition for resources such as light, water, and nutrients. 6 germinated seeds were used for each experiment, and 20 mL of each treatment solution added during the first 8 weeks and 50 mL from the following weeks until the 16th week. The measurements evaluated in the plants were plant length, number of leaves, leaf length, and flower dry weight. Measurements were taken with a caliper gauge twice a week during the 4 months of growth. The plants were watered every other day with the same amount of water or according to climatic factors. At the end of the trial, 3 flower (approximate size 7 cm) were cut from each treatment and weighed to determine the weight of the flowers.

Cannabis flower extracts

For the extraction, 3 flowers per treatment were selected and harvested in the 4th month of growth, ensuring that all flowers came from uniformly developed plants. Afterward, some leaves were removed to facilitate handling and sample preparation. The cannabis flowers were dried at 45 °C for 48 h. An equal number of fresh flowers (1 g), from each treatment, was weighed and transferred to 500 mL glass vials containing 99% ethanol for 24 h for metabolite extraction. The samples were placed in a ultrasound bath (Fisherbrand) for 20 min at room temperature. Finally, the samples were filtered (0.22 μm pore size) and the solvent was removed using a rotary evaporator at 40 °C to obtain a dry cannabis flower extract. These flower extracts were subjected to Thin-Layer Chromatography (TLC) analysis to identify the compounds of interest, and HPLC was used to detect and quantify the cannabinoids.

Evaluation of cannabinoids TLC

TLC was used to identify and analyze some chemical compounds present in the cannabis flower samples. Evaluation by TLC was performed using 300 μL methanol to dilute the 5 samples. TLC plates 5 cm $\times$ 5 cm wide used and 1 μL per sample was applied with a glass capillary. The plates were run in a mobile phase containing a mixture of dichloromethane and methanol in a ratio of 4:0.2. The plates were dried at room temperature, analyzed in an ultraviolet light chamber, and developed with the universal developer (Mano-Sousa et al. 2020).

High-performance chromatography-diode array detector (HPLC–PDA) analysis

Sample preparation. The samples for the analysis were prepared dissolving the extract in 2 mL methanol and homogenized using ultrasound bath. Then, the solution was filtered

Table 1 Content of the treatments used for testing with Cannabis plants

Treatment	Treatment Code	Nutrient content	Microalgae and cyanobacteria content	Quantity per Liter
Nutrients 100%	Nut 100%	100% Hoagland's stock	Yes	Hoagland's stock with 100% solution of the three microalgae and the cyanobacterium, corresponding to 25% of each species used
Nutrients 50%	Nut 50%	50% Hoagland's stock	Yes	Serial dilution of 50% Hoagland's stock and 50% with microalgae and cyanobacterium (each species at 12.5%)
Nutrients 25%	Nut 25%	25% Hoagland's stock	Yes	Serial dilution of 25% Hoagland's stock and 25% with microalgae and cyanobacterium (each species at 6.25%)
Nutrients	Nut	100% Hoagland's stock	No	1000 mL Hoagland's solution
Algae	Algae	No Hoagland's Solution	Yes	1000 mL algae solution (All at 25%) <i>A. platensis</i> : 1×10^5 cells mL ⁻¹ <i>Ankistrodesmus</i> : 8×10^7 cells mL ⁻¹ <i>Scenedesmus</i> : 3.3×10^8 cells mL ⁻¹ <i>Chlorella</i> : 8.3×10^8 cells mL ⁻¹
Control	Control	No Hoagland's stock	No	1000 mL water

using 0.22 µm filters into a 1.5 mL amber HPLC vial for analysis. The samples were stored at −4 °C until the analysis.

HPLC conditions: HPLC Shimadzu LC-2050C 3D equipped with a reverse phase C18 column, Phenomenex Luna 5 µm C18 100 Å 150×4.6 mm and UV detector (PDA). The method used a gradient of milli-Q water (0.1% HCOOH) and acetonitrile (ACN) (0.1% HCOOH). The elution gradient was: 0–4 min 70% ACN, 4–9 min ramp to 85% ACN, 9–11 min 85% ACN, 11–13 min ramp to 90% ACN, 13–13.8 min 90%, 13.8–14.5 min ramp to 70% ACN and 14.5–15 min 70% ACN. The column oven temperature was 35 °C, the detection wavelength was 220 nm for all cannabinoids, the injection volume was 5 µL and the flow rate was 1.5 mL min⁻¹. Three replicates were analyzed. The retention times for each cannabinoid standard are shown in Table 2 (Mandrioli et al. 2019).

Linearity: An initial solution was prepared from the cannabinoid standards (1000 µg mL⁻¹ methanol), and successive dilutions were obtained at five different levels of the curve. The five concentration levels of the cannabinoid standards were 250, 125, 62.5, 31.25, and 15.625 µg mL⁻¹.

To evaluate the linearity of the method, each point was analyzed in triplicate and linear regression was performed using the average of the areas and concentrations of the standards. The calibration curve was accepted on the condition that the r^2 value was not below 0.997. Standards were stored in the absence of light at −20 °C.

Data analysis

The difference between treatments was evaluated by ANOVA followed by Tukey's test. Normality (Shapiro–Wilk test) and homoscedasticity (Levene's test) were applied to the data. All tests were performed at a significant level of

0.05 and 95% confidence. Statistical calculations were performed using the R Core Team (2018) package.

ANOVA was performed to evaluate the effect of different treatments on the concentrations of different cannabinoids. Normality and homoscedasticity assumptions were tested using the Kolmogorov–Smirnov and Levene tests, respectively, and the independence of residuals was confirmed using the Runs test. The Tukey multiple comparison test (5%) was then used to identify specific treatments that differed significantly in the concentration of each cannabinoid.

Results

All the treatments were compared to the treatment that contained only the nutrients that are necessary for the fertilization of the Cannabis plants, called Nut.

Germination

The microalgae mixture significantly promoted germination compared to the control. The 100% nutrients (Nut) and microalgae in 50% nutrients (Nut 50%) increased germination although this was not significantly different from the control (Fig. 1). The other treatments had no effect in promoting germination.

Evaluation of *C. sativa* seedling growth during the first 3 weeks

During the first week, the treatment with the microalgae mixture (Algae) increased the number of leaves, which is very similar to the positive effect of the same treatment during germination (Fig. 2). None of the treatments

Table 2 Retention time (RT) of cannabinoid standards in the HPLC system

Cannabinoid	CBDA	CBGA	CBG	CBD	THCV	CBN	Δ^9 -THC	Δ^8 -THC	CBC	THCA-A
RT (min)	5.970	6.405	6.718	6.984	7.242	9.012	10.320	10.952	11.317	11.578

led to statistically significant differences in plant growth parameters, including leaf number, leaf length, or stem length. No consistent trends were observed across the different treatments, indicating that the application of nutrients, microalgae, or their combinations did not have a measurable impact on the vegetative development of the plants during the evaluation period.

Evaluation of stem length in *C. sativa* seedlings over 3 months

During the first 4 weeks, a 25% increase in stem length occurred with the treatment (Nut 100%) compared to nutrient-only treatment (Fig. 3-A). The Nut 100% treatment was statistically different from the other treatments. In the second month, the same trend was detected in the results (Fig. 3-B), an increase of 42% occurred with Nut 100% treatment and 12% with Nut 50%. In the third month, an increase in plant length of 39% was elicited in treatment that included 100% nutrients and contains the microalgae mixture (Nut 100%) and del 9% with Nut 50% (Fig. 3-C). Statistically significant differences were observed in the Nut 100% treatment compared to the other treatments.

Effect of treatments on the weight of dried cannabis flowers

The 25% nutrient samples (Nut 25%) were not evaluated further as the plants did not show optimal growth, and the data were not significant.

The flower weight increased slightly by 14% with the treatment containing 50% nutrients (Nut 50%). However, the weight was higher with the 100% nutrient treatment, with an induction percentage of 48% (Fig. 4). These results correlated with the increase in plant length. The 100% Nut treatment is statistically different from the other treatments.

Analysis of extract composition by TLC

Some of the treatments induced the formation more substances compared to the Nut treatment. The treatment with microalgae and cyanobacteria (Algae) induced the formation of a higher amount of substance at R_f 0.65. When the plate was observed in long-wave UV, the differences were more apparent with several yellow compounds detected at R_f 0.45 with the algae treatment that were absent from the Nut treatment (Fig. 5).

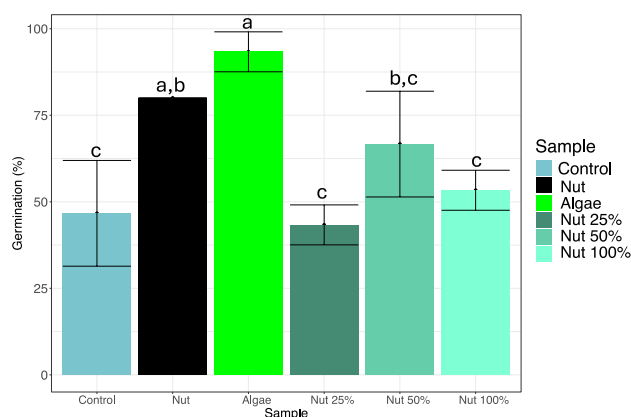


Fig. 1 Effects of mixtures of microalgae and nutrients in different proportions on the germination of *Cannabis sativa*. Results are presented as mean $\pm$ SE ($P < 0.05$). $n = 12$

Analysis of cannabinoid composition by HPLC–DAD

All ten cannabinoids were detected and quantified in the flower extracts of cannabis plants subjected to the different treatments. In plants treated with the 50% nutrient mixture (Nut 50%), CBD (cannabidiol) was detected at a concentration of $110.07 \mu\text{g mL}^{-1}$, and Δ^9 -THC (Δ^9 -tetrahydrocannabinol) was detected at $55.52 \mu\text{g mL}^{-1}$ (Fig. 6). Compared to the nutrient-only treatment (Nut), these values represent an increase of up to 200% for CBD and 85% for Δ^9 -THC. This treatment is considered statistically significant in the production of these two cannabinoids compared to the other treatments. The Nut 50% treatment also stimulated the synthesis of several other cannabinoids, including CBDA, THCA, and Δ^8 -THC. In the case of CBDA, significant differences were observed between the Nut 50% treatment and the other treatments.

The treatment with microalgae and cyanobacteria induced the production of CBG and THCV, achieving concentrations of $5.9 \mu\text{g mL}^{-1}$ and $15 \mu\text{g mL}^{-1}$, respectively. These values contrast with concentrations found in the Nut treatment, which were $0.95 \mu\text{g mL}^{-1}$ for CBG and $2.1 \mu\text{g mL}^{-1}$ for THCV. These findings are consistent with the thin-layer chromatography results (Fig. 5), which also showed an increase in compound production in the extracts from cannabis flowers treated with the microalgae mixture.

The highest concentrations of CBGA and CBN occurred in the Nut (nutrients-only) treatment. Some cannabinoids, such as CBGA, CBC, and Δ^8 -THC, showed a reduced response across nearly all treatments.

Discussion

Crop productivity can be increased with pesticides, fertilizers, and developmental factors such as hormones. However, this is not possible for all crops, such as medicinal plants, due to the risk of trace residues. An ecological and sustainable alternative is the use of microalgae and cyanobacteria as inert materials or their extracts, as they can provide nutrients and phytohormones on a sustained manner. This study is the first to evaluate the effect of mixtures of microalgae and a cyanobacterium on the biostimulation of *C. sativa* plants and the production of different cannabinoids. It used intact cells of most of the microalgae, and due to the greenish color of the medium, it was possible to observe their growth in the substrate. This was in contrast to other studies where the cells are sonicated to ensure the release of bioactive molecules and a faster action (Rupawalla et al. 2022).

In germination trials, the effect of microalgae on seed germination was significant (93%) (Fig. 1), suggesting a positive effect of microalgae and the cyanobacterium *A. platensis* on this process. Similarly, microalgae extracts of *Chlorella* and *Scenedesmus* increased spinach

germination by 1.7 times. These extracts contained plant growth regulators such as cytokinins, gibberellins, auxins, and abscisic acid (ABA), which were likely to be involved in the germination process (Rupawalla et al. 2022). However, we have not analyzed these compounds (phytohormones) in our microalgae and cyanobacterium. In addition, some studies have shown that microalgae can stimulate germination when plants are exposed to natural environmental conditions (Chanda et al. 2019). Microalgae are a rich source of nutrients such as vitamins, minerals, amino acids, and phytohormones, which could promote germination (Ouzakar et al. 2025). They have demonstrated promising effects on plant growth, nutrient use efficiency, and the mitigation of environmental impacts (Fragalà et al. 2023).

Regarding the growth of *C. sativa* seedlings exposed to the different treatments, the algal treatment containing only the microalgae and the cyanobacteria did not significantly affect plant growth (Fig. 2). When *Scenedesmus* was mixed with a fertilizer and applied to basil plants, the microalgae alone had no significant effect on plant growth, such as the number of leaves, shoot fresh and dry weight, leaf fresh

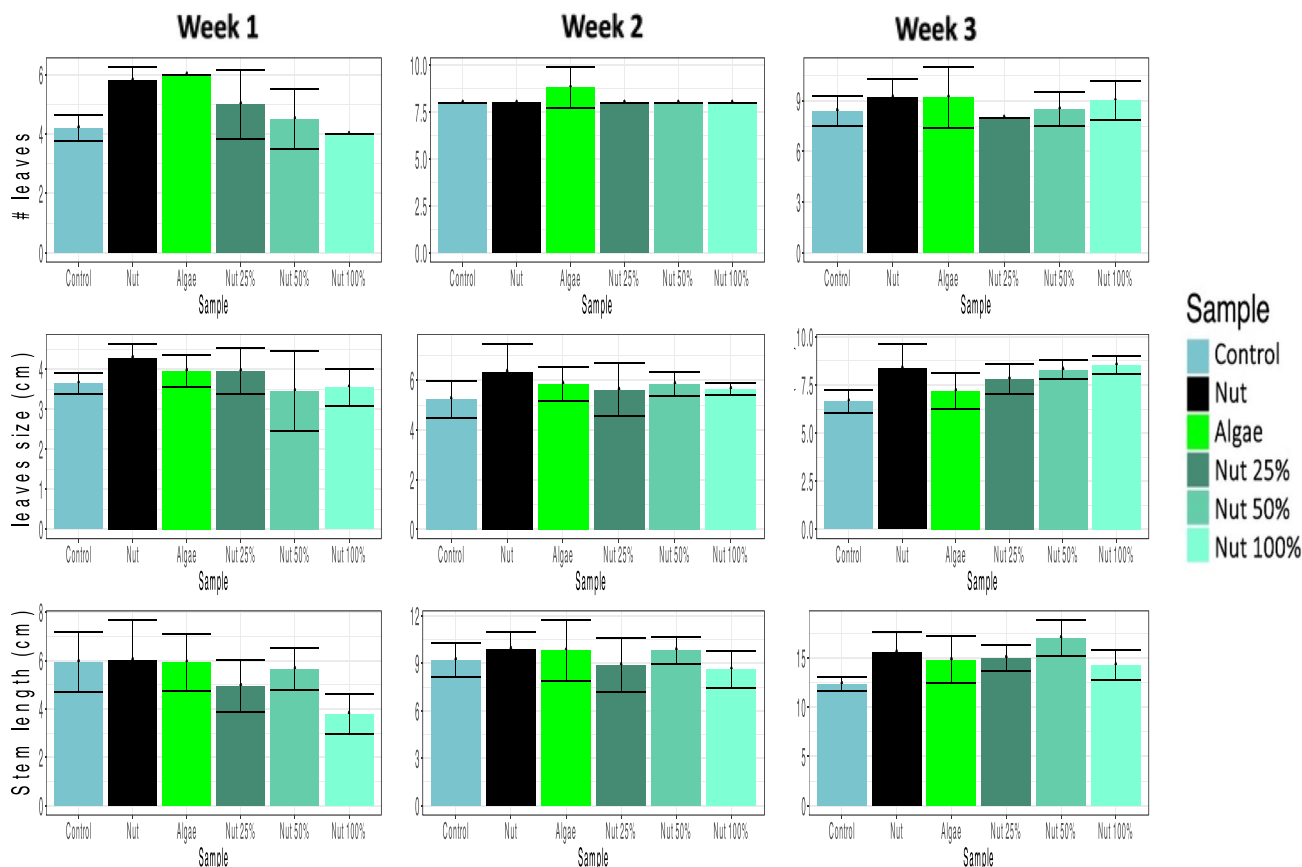
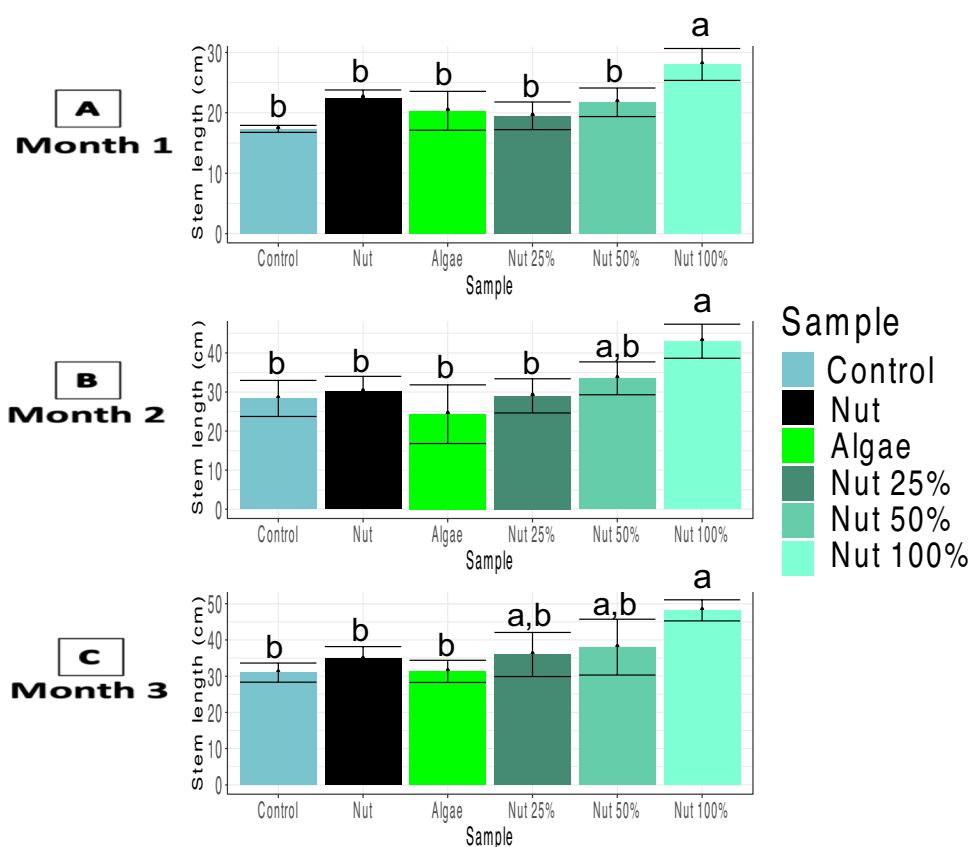


Fig. 2 Effect of treatments on cannabis plant growth during the first three weeks. Results are presented as mean ± SE (P < 0.05). n = 6

Fig. 3 Effect of treatments on stem growth of cannabis plants for three months. Different letters indicate significant differences. Results are presented as mean $\pm$ SE ($P < 0.05$). $n = 6$



weight, and chlorophyll content. The optimum treatment was a combination of microalgae and inorganic fertilizer (Álvarez-González et al. 2022). The present study results are equivalent because, after the first weeks of plant growth, there was a noticeable trend towards an increase in plant length when the microalgae mixture was combined with 100% nutrients. However, the plants exposed to the microalgae mixture alone (Algae) showed similar behavior to the control, indicating that the best alternative to biofertilizer and bio stimulation of the plants is to use the microalgae mixture together with the nutrients (Figs. 2 and 3). A similar study on lettuce demonstrated that combining microalgae with mineral fertilizers, significantly enhanced plant growth and led to improved crop quality (La Bella et al. 2024).

The stimulating effect of microalgae mixtures with nutrients could be favored by the components added to the plants (nitrogen and phosphorus) and by the constitution of the microalgae. Some have growth-promoting activity, while others act as elicitors of phytoalexins and modifiers of the synthetic processes of secondary metabolites (Chanda et al. 2019).

To date, no studies have evaluated the effect of microalgae on cannabinoid production in *C. sativa* plants. The medical cannabis industry requires plants with high cannabinoid production due to their high pharmaceutical

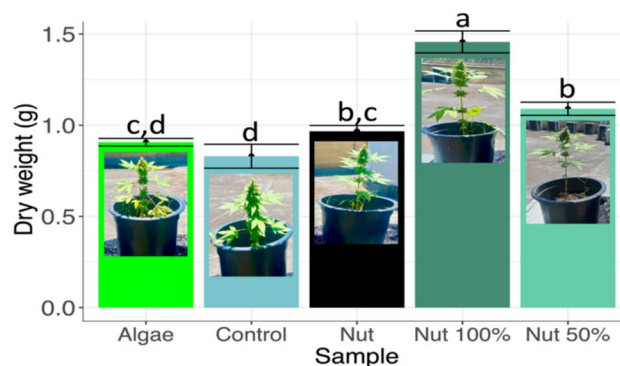


Fig. 4 Results of dry weight of cannabis flowers with the different treatments. Results are presented as mean $\pm$ SE ($P < 0.05$). $n = 3$

potential. In the present study, the 50% nutrient treatment (Nut 50%) with the microalgae mixture was the best option for application to plants, as it increased the production of secondary metabolites, specifically cannabinoids, in *C. sativa* plants (Fig. 6). This treatment mainly increased the synthesis of CBD and THC, as well as other cannabinoids such as CBDA, THCA, and Δ^8 -THC. By favoring the production of the main cannabinoids THC and CBD, these treatments can be used as alternatives to obtain better quality plants with higher concentrations of

these bioactive compounds. Both THC and CBD interact with receptors in the human body. However, due to the psychoactive effects of THC, the cultivation and use of *C. sativa* for recreational purposes is illegal and prohibited in many countries (Humeniuk et al. 2024). THC has been proposed for medical applications such as pain relief, appetite stimulation in cancer patients and reduction of chemotreatment-induced nausea (Manca et al. 2024), making biostimulation with mixtures of microalgae and cyanobacteria together with added nutrients an emerging option for growers of such plants.

However, there is very little information on the other cannabinoids as they are generally produced at lower levels (Hussain et al. 2021). In this work, low concentrations of Δ^8 -tetrahydrocannabinol (Δ^8 -THC), cannabichromene (CBC), cannabigerol (CBG), tetrahydrocannabinol (THCA), and tetrahydrocannabivarin (THCV) were detected at concentrations from $0.8 \mu\text{g mL}^{-1}$. Their production is significant in microalgae treatments. These results suggest that microalgae may induce cannabinoid production in plants (Wang et al. 2022). Due to the low concentrations of phytocannabinoids in plants, biotechnology offers promising alternatives for biosynthesis through in vitro culture or synthetic biology. However, several obstacles remain, such as the complexity of the cannabinoid biosynthetic pathway and the optimization of bioprocesses (Hesami et al. 2023). Increasing cannabinoid production in plants is one of the major challenges for the pharmaceutical industry (Shujat et al. 2024). Some studies have used genetic engineering techniques to introduce genes into microalgae to maximize cannabinoid expression (Fantino et al. 2024). No study has investigated the effect of media compositions and their

interactions on in vitro cannabinoid production. Experiments with medicinal plants show that optimization of media composition is an essential step for in vitro production of secondary metabolites (Niazian 2019). Although this research focused on the use of environmentally exposed cannabis cultures, it provides significant information for the treatment and maintenance of cannabis field cultures and the induction of cannabinoid production.

The use of microalgae and cyanobacteria offers an innovative solution to the main problems caused by synthetic agrochemicals, such as lack of sustainability, environmental persistence, and health risks. These microorganisms promote plant germination, growth, and development and enable the production of bioactive metabolites such as cannabinoids and high-value nutraceuticals. Although the use of microalgae in agriculture is still in its infancy, especially in biostimulation, there is a need to increase knowledge of these microorganisms as most existing studies focus on macroalgae as biostimulants and biofertilizers (Chanda et al. 2019). This approach opens new doors for a more sustainable agriculture rich in beneficial compounds.

Conclusions

In conclusion, microalgae and cyanobacteria favored the growth and development of *Cannabis sativa* plants. Microalgae were effective for germination processes. For vegetative, pre-flowering, and post-harvest development, it is recommended to use a mixture of nutrients at 50%

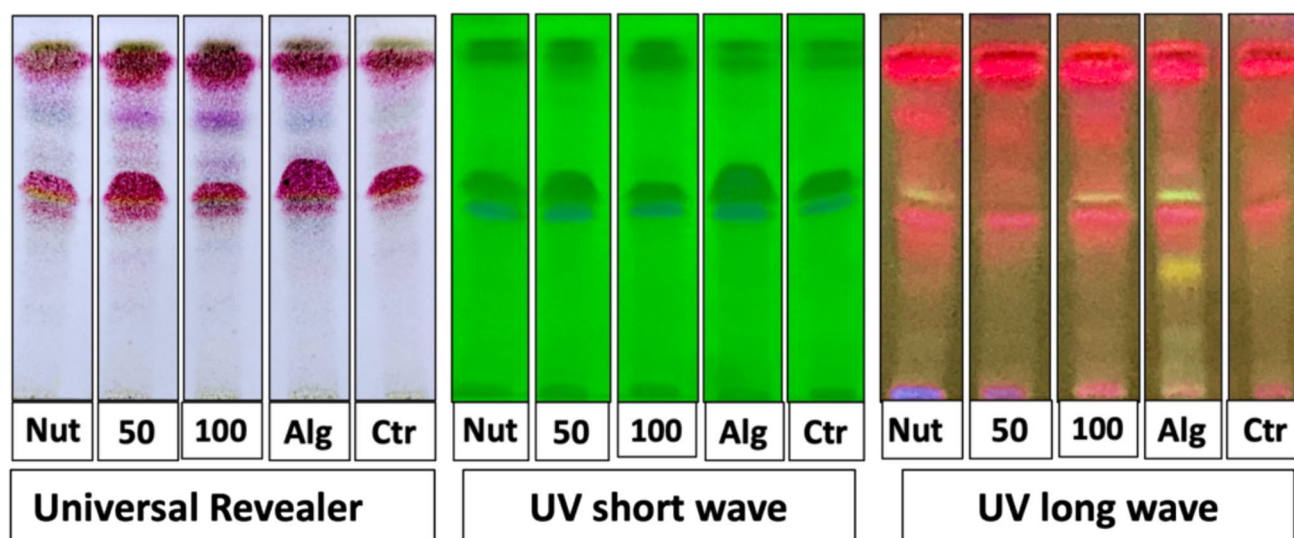


Fig. 5 Thin layer chromatography of each of the extracts obtained from the cannabis flower. Nut: nutrients only, 50: 50% nutrients (Nut 50%), 100: 100% nutrients (Nut 100%), Alg: a mixture of microalgae and cyanobacteria (Algae), and Ctr: control (Control)

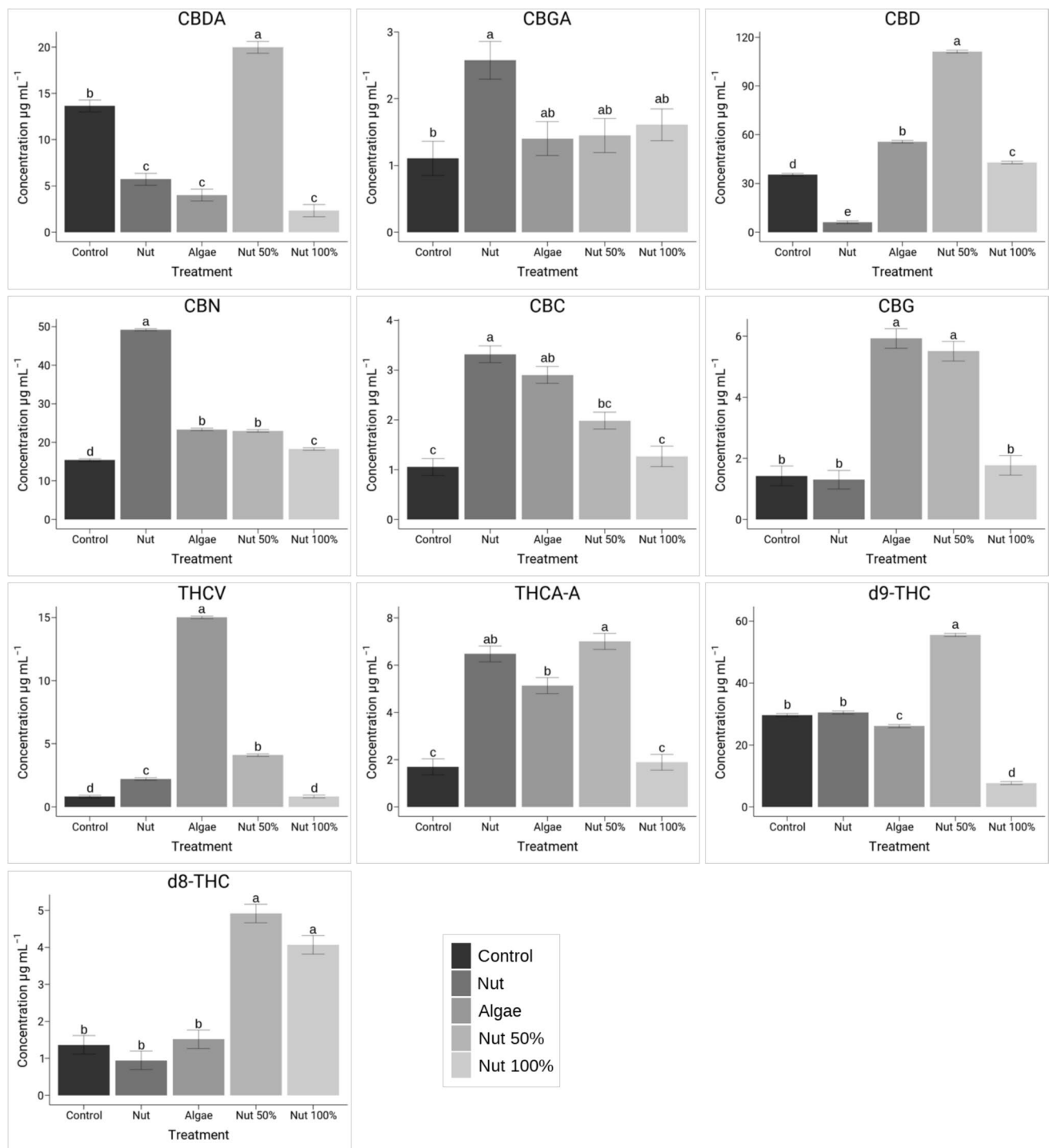


Fig. 6 Concentration (µg mL⁻¹) of phytocannabinoids in the extracts of the different treatments. Nut: nutrients only, 50%: 50% nutrients (Nut 50%), 100%: 100% nutrients (Nut 100%), Algae: a mixture of

microalgae and cyanobacteria, and control. The error bars represent the percentage error with an adjustment factor of 0.5. Results are presented as mean ± SE (P < 0.05). n = 3

together with microalgae. This treatment significantly induced the production of the main cannabinoids (CBD and THC). This type of work increases the value of the

circular economy, as it uses environmentally friendly microorganisms and improves the reduction of highly toxic products in agriculture.

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Authors contributions Natalia Herrera: Methodology, Investigation, Formal analysis, Conceptualization. Tatiana C. Puentes-Escobar: Methodology. Julian A. Álvarez-Pulido: Methodology Ronald Sanchez: Methodology Jelen Restrepo: Methodology Fernando Echeverri: Investigation, Conceptualization.

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Data Availability The data sets that were generated and analyzed in the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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