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Revista Facultad Nacional de Agronomía

Biostimulation of *Moringa oleifera* Lam. seedlings with freshwater microalgae extract (Spirulina)

Bio-estimulación de plántulas de *Moringa oleifera* Lam. con extracto de microalga dulceacuícola (Spirulina)

https://doi.org/10.15446/rfnam.v79.119050

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Keywords:
Functional food
Mesoamerica
Moringaceae
Photosynthetic pigments

CITATION: Ledea-Rodríguez JL, Mayer-Félix EA, Ruiz-Espinoza FH and Guevara-Franco JA (2026) Biostimulation of *Moringa oleifera* Lam. seedlings with freshwater microalgae extract (Spirulina). Revista Facultad Nacional de Agronomía Medellín 79: e119050. doi: https://doi.org/10.15446/rfnam.v79.119050

ABSTRACT

The present study investigates the biostimulatory effect of extracts of the freshwater microalgae Spirulina on *Moringa oleifera* Lam. seedlings. There is a growing need to find sustainable alternatives in agriculture, especially for Moringa cultivation, which is known for its nutritional value and biomass production. This study aimed to evaluate the contribution of Spirulina extracts to the growth, development, and antioxidant components of *M. oleifera* seedlings grown in a greenhouse. The effect of the extract on plant and root morphology, leaf development indicators, and photosynthetic pigment content was evaluated using complete randomized blocks with four treatments and four replicates. The results showed that biostimulated seedlings with 2, 4, and 6 g L^{-1} Spirulina grew 13.57, 7, and 9% more compared to the absolute control (AC) ($P \leq 0.01$), respectively. The Gompertz, Logistics, and Bertalanffy models suggest that Moringa seedlings invest energy reserves from their cotyledons in the development of aerial organs, and this is compensated by active growth. Likewise, the experimental treatments increased the concentration of α, β, and total chlorophyll by 7, 16.6, and 11.4% compared to AC ($P \leq 0.05$). The application of Spirulina represents an effective strategy to optimize the growth of *Moringa oleifera* and enhance the functional and nutraceutical properties by increasing the content of photosynthetic pigments. This could have positive implications for sustainable agriculture and human and animal nutrition.

Palabras clave:
Alimento funcional
Mesoamérica
Moringaceae
Pigmentos fotosintéticos

RESUMEN

El presente estudio investigó el efecto bioestimulante de los extractos de la microalga dulceacuícola *Spirulina* en plántulas de *Moringa oleifera* Lam. Existe una creciente necesidad de encontrar alternativas sostenibles en la agricultura, especialmente en el cultivo de la Moringa, reconocida por su valor nutricional y la producción de biomasa. Se planteó como objetivo evaluar la contribución de los extractos de Spirulina al crecimiento, desarrollo y componente antioxidante de plántulas de *M. oleifera* cultivadas en condiciones de invernadero. Mediante un diseño de bloques completos al azar con cuatro tratamientos y cuatro réplicas se evaluó el efecto de los extractos en la morfología de la planta y de la raíz, en indicadores de desarrollo foliar y contenido de pigmentos fotosintéticos. Los resultados evidenciaron que las plántulas bio-estimuladas con 2, 4 y 6 g L^{-1} de Spirulina crecieron en 13,57, 7 y 9% más en comparación con el control (CA) ($P \leq 0,01$), los modelos de Gompertz, Logístico y Bertalanffy sugirieron que las plántulas de Moringa invierten las reservas energéticas del cotiledón en el desarrollo de órganos aéreos compensado con un crecimiento activo; igualmente los tratamientos experimentales incrementaron la concentración de clorofila α, β y total en 7, 16,6 y 11,4% respecto al CA ($P \leq 0,05$). La aplicación de Spirulina constituye una estrategia efectiva para optimizar el crecimiento de *Moringa oleifera*, y la modificación del carácter funcional o nutracéutico mediante el incremento del contenido de pigmentos fotosintéticos; lo que podría tener implicaciones positivas para la agricultura sostenible, nutrición del hombre y animales.

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Received: May 26, 2025; Accepted: September 22, 2025
Rev. Fac. Nac. Agron. Medellín 79: e119050. 2026. ISSN 0304-2847 / e-ISSN 2248-7026

Moringa oleifera Lam. is one of the most genus primarily due to its potential in terms of forage yield in dry matter per hectare, seed production, and quality of aerial biomass (Gandji et al. 2020). The production and quality of aerial biomass have been utilized in developing countries to mitigate nutritional deficiencies in the human population. This plant has also been used as a strategic supplement in animal feeding, contributing to the increase in the production of protein of high biological value. In developed countries, its consumption has become popular as part of a healthy lifestyle (Coles and du Toit 2020).

Due to the antioxidant properties of its leaves (Ma et al. 2020), and the high quality of the oil extracted from seeds, *M. oleifera* has received growing interest for its role in different industrial processes, especially cosmetics and pharmaceutics. It is a plant that promises important contributions to the various branches of knowledge and production systems (Gupta and Ahmed 2020).

Although *Moringa oleifera* is characterized by its rapid growth, a characteristic considered positive in various contexts in cropping systems intended for forage production, this trait can be considered a disadvantage. The highest aerial biomass production, without affecting the leaf/stem ratio, occurs between 45 and 60 days of growth (Ledea-Rodríguez et al. 2020). Its rapid growth means it requires nutrients such as water and minerals from the soil's aqueous fraction (Valdés Rodríguez et al. 2018).

For this reason, chemical and organic fertilization have been considered to increase biomass production and quality. However, these fertilizers modify the chemical composition and physical properties of the soil in the long term (FAO 2019), so alternatives within classical biotechnology, like the use of biostimulants, emerge as a sustainable strategy. This allows, among many uses and applications, the alternative of using microorganisms to promote plant growth and modify plant morphology (Zayed 2012).

Likewise, freshwater microalgae are considered bio-stimulants (Gemin et al. 2019), with positive effects documented in promoting plant growth (Petropoulos et al. 2019). However, their application in fast-growing woody plants remains poorly studied. Due to the diverse composition of elements such as nitrogen, macro and micro minerals, polysaccharides (glucans and rhamnose), and disaccharides (glucose, fructose, and sucrose) (Marjanović et al. 2024), microalgae can activate the induced systemic response (ISR) and stimulate the synthesis of secondary compounds in plant tissue (Ledea-Rodríguez 2022).This gives them new characteristics as food, resolving themselves as functional or nutraceutical.

Despite their bio-stimulant properties, microalgae have received little attention in scientific literature (De Saeger et al. 2020) and their application remains limited or non-existent in certain cultivation systems, such as *Moringa oleifera*. In this context, the present study aims to evaluate the contribution of Spirulina extracts to the growth, development, and antioxidant components of *M. oleifera* Lam. seedlings grown in greenhouses.

MATERIALS AND METHODS

Plant material

Moringa seeds were collected in La Paz, Baja California Sur, México (24° 07´45.47" N and 110° 20´14.72" W). Harvesting was carried out four months before sowing, and the seeds were stored at 5 °C in a domestic refrigerator until use.

Experimental conditions

This research was developed in the Agricultural Field of the Academic Department of Agronomy of the Universidad Autónoma de Baja California Sur under semi-protected greenhouse conditions, covered with 90% residential type shade netting, located at coordinates 24°59´00" N 110°19´04" W. Temperature and humidity variations during the experiment were measured every 6 hours for each variable starting at 6:00 a.m. using a HOBO 8K Pendant, part UA-001-08, and the relative humidity using a VEE GEE digital hydrometer.

The 55-gallon plastic containers were cut in half and used for seed sowing. Eight polyethylene bags (18×26 cm) were then placed inside, containing SOGEMIX-PGM® germination substrate, which consists of fine granulated Sphagnum peat that had been moistened for 24 hours prior to filling the bags. Once filled, the bags

were placed on oven-baked red clay bricks (6×12×24 cm), which were completely saturated with water. A 20 cm water film was maintained to make sure there was enough water for the plants.

Experimental design and treatments

The experiment used a completely randomized design with four replications. Treatments included a control (Tnt 1) with no extracts and distilled water irrigation, and three levels of lyophilized Spirulina application: 2 g L^{-1} (Tnt 2), 4 g L^{-1} (Tnt 3), and 6 g L^{-1} (Tnt 4), based on Ak (2012).

Freeze-dried Spirulina powder

Freeze-dried Spirulina powder was obtained through the online store "Estrella del Sur Spice Store" (Estrella del Sur Puebla - Your Online Spice Store - Dried Chilies and Seasonings). The product was imported as "Dried Spirulina Algae Powder" by Organic Herb Trading Co®, the nutritional composition is shown in Table 1.

Table 1. Microalgae powder composition.

Nutritional value for each 100 g	
Indicators	**Value**
Energy	290 kcal (1,213kJ)
Carbohydrates	23.9 g
Fats	5.38 g
• Saturates	2.65 g
• monounsaturated	0.675 g
• polyunsaturated	2.08 g
Proteins	57.47 g
Water	4.68 g
Retinol (Vit. A)	29 µg (3%)
• β-carotene	342 µg (3%)
Thiamine (Vit. B1)	2.38 g (183%)
Riboflavin (Vit. B2)	3.67 mg (245%)
Niacin (Vit. B3)	12.82 mg (85%)
Pantothenic acid (Vt. B5)	3.48 mg (70%)
Vitamin B6	0.364 (28%)
Vitamin E	5 mg (33%)
Calcium	120 mg (228%)
Iron	120 mg (228%)
Magnesium	195 mg (53%)
Manganese	1.900 mg (95%)
Phosporus	118 mg (17%)
Potasium	1,363 mg (29%)
Sodium	1,048 mg (70%)
Zinc	2 mg (20%)

Preparation and application of extracts

Spirulina solutions were prepared by weighing 2, 4, and 6 g of lyophilized powder using an AWS Gemini-20 analytical balance. Each amount was then dissolved in one liter of distilled water, resulting in concentrations of 0.2, 0.4, and 0.6%, respectively.

The extract application began 2 days after 100% germination of the seeds and was repeated every 5 days, to complete a total of six applications. Each time, 25 mL of extract was added to the base of the stem of each plant.

Plant measurements

Morphological variables

For each of the replicates in each of the treatments, 10 plants were randomly selected. Five were used for morphological measurements, and five for height measurements. Plant growth was monitored by height, and then plants were chosen randomly before applying the extract. In total, 20 plants were measured per treatment.

The height (cm) was considered from the base of the stem to the apical bud, taking the first measurement before the first application of the extract, and then every 5 days for 45 days. After 45 days, leaves were counted, and stem thickness was measured with a digital Vernier at 5 cm above the root collar. The length of the root was measured, from the collar to the cap, and the stem and root length values were used to calculate the stem/root ratio.

The robustness index was calculated by combining plant height and root collar diameter using Equation 1:

$$RI = \frac{PH_{cm}}{RCD_{mm}} \quad (1)$$

Where PH is the plant height and RCD is the root collar diameter.

Leaves, stems, and roots were collected and dried to constant weight. Then, the dry weight of each fraction was used to calculate quality indices as presented in Equations 2, 3, 4, and 5. The Dickson index (DI) and lignification index (LI) were calculated as described by Equations 6 and 7, respectively.

$$BP = \frac{\text{Aerial biomass}_g}{\text{Root biomass}_g} \quad (2)$$

$$IPP = \frac{\text{Leaf weight}_{(g)}}{\text{Plant weight}_{(g)}} \quad (3)$$

$$SPP = \frac{\text{Stem weight}_{(g)}}{\text{Plant weight}_{(g)}} \quad (4)$$

$$RPP = \frac{\text{Root weight}_{(g)}}{\text{Plant weight}_{(g)}} \quad (5)$$

$$DI = \frac{\text{Total dry mass}_{(g)}}{\frac{\text{Height}_{cm}}{\text{Diameter}_{mm}} + \frac{\text{Aerial mass}_g}{\text{Root weight}_g}} \quad (6)$$

$$LI = \frac{\text{Total dry mass}_g}{\text{Total wet mass}_g} \quad (7)$$

Productive variables

Dry Matter (DM) yield of leaves and whole plant

Dry weight values of leaves and stems were used to determine the percentage of DM according to the following Equation 8:

$$DM(\%) = \left[\frac{(FW - DW)}{FW}\right] \quad (8)$$

Where the FW is the fresh weight of the sample and the DW is the dry weight of the sample.

Morphophysiological variables

After 45 days, the length (cm) and width (average of the base, middle, and apical portions) of the first imparipinnate leaf were measured using a digital Vernier with a precision of 0.01 mm. In each treatment, the mean and longitudinal values of the leaves were determined, and the Leaf Area (LA) was calculated using multiple regression Equations (Table 2).

Table 2. Multiple regression expressions for calculating Leaf Area in each treatment.

Treatment (g L^{-1})	Regression expression	R	R^2	R^2_{ajust}	±SE
0	0.8(±0.03)+0.2(±0.03) L * W	0.99	0.98	0.98	3.6
2	0.5(±0.07)+0.5(±0.07) L* W	0.97	0.95	0.95	7.6
4	0.6(±0.04)+0.5(±0.04) L* A	0.99	0.98	0.998	7.2
6	0.7(±0.02)+0.5(±0.02) L * W	0.99	0.99	0.99	2.8

L: Length of leaves; W: Width of leaves; R: Coefficient of determination; R^2: Coefficient of determination squared; R^2_{ajust}: Adjusted coefficient of determination squared; ±SE: Standard error.

From the LA value, the following were calculated: Leaf Area Duration (LAD) and Leaf Area Index (LAI) according to Equations 9 and 10.

$$LAD = (LA_2 + LA_1) - \left(\frac{T_2 - T_1}{2}\right) \quad (9)$$

$$LAI = \frac{LA_2 + LA_1}{2 \times \left(\frac{1}{s}\right)} \quad (10)$$

Where LA is the leaf area; T1 is the start of plant growth, T2 is at the end of the experiment (45 days) and S is the area of soil covered.

Plant height measurements taken every 5 days after application of the first inoculum were used to estimate the Absolute Growth Rate (AGR) and Relative Growth Rate (RGR), described in Equations 11 and 12.

$$AGR\,(\text{cm per day}) = \left(\frac{L_2 - L_1}{T_2 - T_1}\right) \quad (11)$$

$$RGR\,(\text{cm cm}^{-1}\text{ per day}) = \left(\frac{L_2 - L_1}{T_2 - T_1}\right) x \left(\frac{1}{L_2}\right) \quad (12)$$

When relating FA, fresh weight of leaves, stems, roots, and the entire plant as the sum of the weight of the organs mentioned, the following variables are presented in Equations 13, 14, and 15.

$$ELA = \frac{LA_{(cm_2)}}{\text{Weight leaves fresh}_{(g)}} \quad (13)$$

$$NAR = \frac{LA_{(cm_2)}}{\text{Fresh weight of the plant}_{(g)}} \quad (14)$$

$$LAR = \left[\left(LA_{(leaves)} * \text{Leaves weight}_{(g)}\right)\right] x \left[\left(g_{(leaves)} * g_{(plant)}\right)\right] \quad (15)$$

Statistical analysis

A one-way ANOVA was performed by Statistic v 12.0, normality of the data was checked using the Kolmogorov-Smirnov test, homogeneity of variance was checked using the Bartlett criterion, and the Duncan test with a 95% confidence interval was used for the comparison of means.

An Analysis of Covariance (ANCOVA) was performed for plant height, with the height before treatment application (Spirulina extracts) as the covariate and final height as the dependent variable. Linear regression and correlation were used to verify the dependence and multicollinearity between the dependent variable and the covariate. The normality and homoscedasticity of the data were tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests, respectively. The analysis was conducted using the following mathematical model (Equation 16):

$$Y = \mu + T_i - bX_j + e_{ij} \quad (16)$$

Where μ is the model average, Ti is i-th treatment; b is the model constant, Xj is j-th height, and e is the estimation error.

Foliar area estimation was developed using multiple regression equations based on the following mathematical model (Equation 17):

$$Yi - \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} \ldots \beta_k X_{ki} + \varepsilon_i \quad (17)$$

Where Y_i is the represents FA as dependent variable, β_0 is Y- intercept or origin coordinate, β_1, β_2, $\beta_3 \ldots \beta_k$ are slopes of the equation, X_{1i} is the average effect of the average of the variable leaf length, X_{2i} is the average effect of the average of the leaf width variable, X_{3i} is the average effect of the average of the leaf area variable, $\beta_k X_{ki}$ is the regression line of Y on X_1, X_2, X_3..... X_k and ε_i is the error for each independent variable.

The analysis was performed using non-linear regressions (Equations 18, 19, and 20) between plant age and height to determine the curve that best fits shoot growth. Non-linear models were used, which are listed below:

Logistics

$$= \frac{a}{(1 + b * \exp^{(-c*t)})} \quad (18)$$

Where Y is the growth of the organism over time t, a is the maximum asymptotic growth, when t tends to infinity, b is the curvature parameter that expresses how fast it reaches maximum growth or lag time, c is the growth rate, and T is the time in days.

Gompertz

$$Y = a * \exp(\exp^{(b-c*t)}) \quad (19)$$

Where Y is the time response variable (t), a is the maximum growth and determines an asymptotic point, b is the positive number that shifts the model left or right, and c is the sets the intrinsic rate of growth.

Von Bertalanffy

$$Y = a * \exp^{(1-b*\exp^{(-c*t)^3})} \quad (20)$$

Where Y is the length of the individual at time (t), a is the maximum length of the individual (maximum asymptote), c is the curvature parameter that expresses how quickly the length reaches its maximum value and T is the time.

The model selection was based on a high R^2 value, strong statistical significance, low standard error of the parameters, estimates, the smallest mean square error, and a significant contribution of the equation parameters.

RESULTS AND DISCUSSION

Application of Spirulina extracts at 2, 4, and 6 g L^{-1} increased *M. oleifera* seedling height by 13.57, 7, and 9% compared to the control ($P \leq 0.01$). The effect decreased as the extract concentration was reduced ($P \leq 0.01$), suggesting a dose-response relationship (Figure 1).

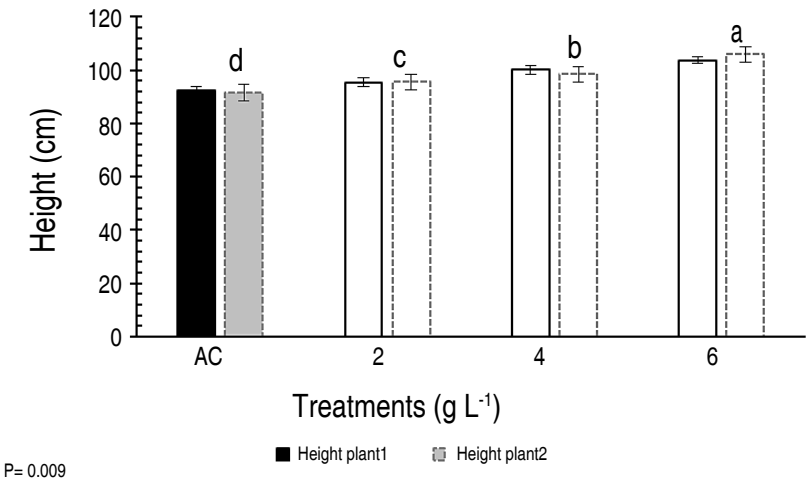

Figure 1. Covariance analysis for the effect of Spirulina extracts on promoting height-dependent growth in 45-day-old *M. oleifera* seedlings. Plant height 1 corresponds to actual means; plant height 2 corresponds to means with the fit value of 12.33, according to the ANCOVA regression equation. *P*=0.009 suggests the significance of the fit of the ANCOVA regression model. Vertical lines represent the standard error for actual and adjusted means.

Studies developed by Al Dayel and El Sherif (2021) considered biofertilization alternatives based on suspensions of *Chlorella vulgaris*, *Nanochloropsis salina*, and a beneficial microorganism to evaluate salinity tolerance in *M. oleifera*. Based on its height and structure, they observed that microalgae extract favors plant performance, as obtained in the present study. On the other hand, the favorable response of Moringa as a function of height is related to nitrogen availability in the microalga. In the case of Spirulina, the absence of cellulose cell walls facilitates greater bioavailability of nutrients. Also, it provides an important source of micro and macronutrients essential for plant development (Iqra-Saddique 2025). Likewise, the cofactors characteristic of biofertilizers (Shahrajabian et al. 2021) could have intervened in the absorption and metabolism of nitrogen so that *M. oleifera* showed a favorable response. To better understand this phenomenon, it is essential to analyze growth dynamics over time rather than just the seedlings' final height.

In this study, growth curves were analyzed using non-linear regression models, representing the first report of this approach in this Moringa variety. The evaluation revealed distinct growth responses to different concentrations of freshwater microalgae extract (Figure 2). Plants treated with 6 g L^{-1} of Spirulina extract followed the Gompertz model ($R^2_{adjusted}$=97%) (Figure 2A), which describes an initial slow growth phase, followed by accelerated growth, and then a gradual slowdown.

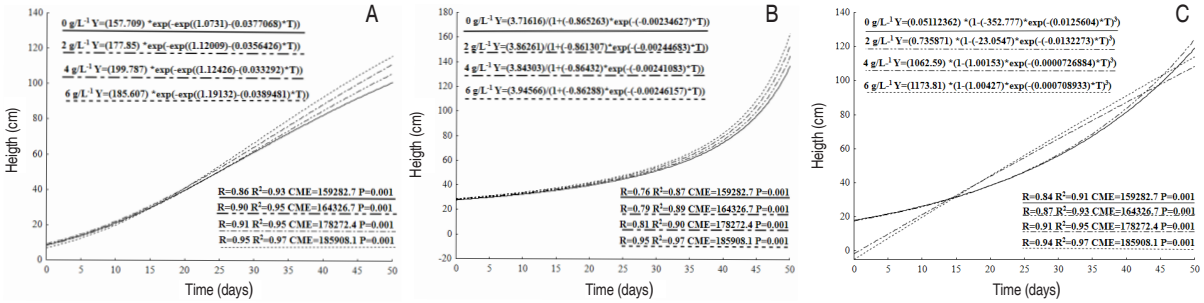

Figure 2. Growth dynamics of *M. oleifera* plants biofertilized with Spirulina extracts. **A** (Gompertz), **B** (Logistic), and **C** (Von Bertalanffy). R=Coefficient of determination, R^2: Coefficient of determination adjusted by the degrees of freedom, MSE: Mean square error, *P*: p-value.

Goodness-of-fit criteria and growth models analysis

Among the goodness-of-fit criteria, an increase in mean square error (MSE) was observed across levels, suggesting that Spirulina extracts influenced growth rates. This effect suggests a direct relationship between applied doses and the availability of nutrients, especially nitrogen. Similarly, studies have shown that microalgae extracts affect plant physiological activity, hormone regulation (Issa et al. 2019), and exposure to polysaccharides and oligosaccharides.

The contribution of the model parameters was highly significant (*P*=0.001) for the experimental treatments and the control. The latter obtained the lowest explanatory value of the model ($R^2_{adjusted}$=93%; Figure 2A), while in the experimental treatments, growth promotion was reflected in explanatory fits of the model between 95-97% as described by the Gompertz model (Tjørve and Tjørve 2017).

This behaviour reflects the prioritization of the seed's energy reserves for germination, emergence, and early growth. Moringa seeds are rich in essential oils, which serve as the primary carbohydrate reserve for radicle emergence and initial plant development, like other oil-bearing species (Ledea-Rodríguez et al. 2022). In this context, experimental treatments may have enhanced this process by increasing the availability of enzymatic precursors from trace minerals (Alfadhly et al. 2022), leading to a higher enzymatic activity that supports germination and early growth (Marjanović et al. 2024).

The logistic model (Figure 2B) displayed a convex curve with an exponential trend, improving in fit as the concentration of Spirulina extracts increased. The best fit was observed at 6 g L^{-1} (adjusted R^2=97%), followed by 4 g L^{-1} (90%), while the control and 2 g L^{-1} doses showed similar fits (87 and 89%, respectively). These results suggest a direct link to the bioavailability of nutrients, cofactors, and enzymatic precursors in the rhizosphere. According to Shahrajabian et al. (2021), these compounds—amino acids and soluble peptides—promote the synthesis of growth-related metabolites, acting similarly to plant hormones, which supports the validity of the logistic model in this study.

The Von Bertalanffy model showed an $R^2_{adjusted}$ between 91 and 97% fit, ranging from the experimental control to the 6 g L^{-1} Spirulina extract dose. Since this model had the highest fit and the best-adjusted descriptors among the three models, it can be inferred that Moringa plants allocate cotyledon reserves to the development of aerial organs while simultaneously maintaining active growth. This behaviour aligns with the premise of the Von Bertalanffy model, which describes growth as a balance between metabolism and catabolism (Bertalanffy 1957). In this context, Spirulina extracts appear to enhance this process by improving enzymatic, metabolic, and hormonal efficiency, while also providing essential trace elements such as Fe, Cu, Zn, and Mn (AlFadhly et al. 2022).

Morphological development of the plant

Morphological analysis of the plant (Figure 3) showed that the average leaf width was the only variable affected by the experimental treatments. Plants that were biostimulated with 4 g L^{-1} increased the average leaf width by 13.44 and 16.30% significantly ($P \leq 0.05$), compared to the

absolute control (AC) and those biostimulated with 2 g L^{-1}, respectively (Figure 3C).

This result is particularly relevant, as Moringa leaves contain a wide range of biologically significant compounds that are essential for human and animal nutrition. In addition to synthesizing bioactive compounds for plant growth and development through photosynthesis, leaf width expansion is closely linked to growth conditions, especially light intensity and irradiance (Azcón-Bieto and Talón 2013). In this study, the 4 g L^{-1} concentration appeared to promote this change, likely due to a hormonal effect. This may be attributed to the extract's diverse composition, which could regulate auxin-sensitive genes, either enhancing or inhibiting their expression.

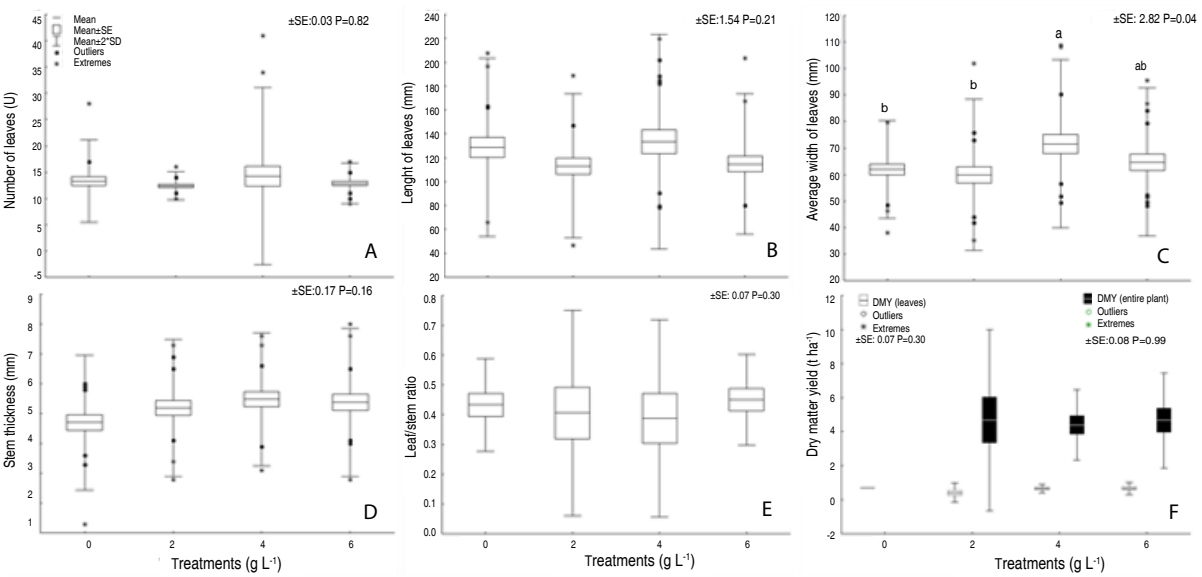

Figure 3. Leaf and stem morphology of *M. oleifera* plants at 45 days of age biostimulated with Spirulina extract. [a, b] Values with different superscript letters in each column are significantly different at $P \leq 0.05$ according to Duncan's test; significance for average leaf width was reached using $\sqrt{(x+2.75)}$.

The remaining morphological variables were not significantly affected by the treatments ($P \geq 0.05$). The number of leaves ranged from 12.4 to 14.2 (Figure 3A), while leaf length varied between 113.2 and 128.7 mm (Figure 3B). Stem thickness ranged from 4.7 to 5.48 mm (Figure 3D), and the leaf-to-stem ratio remained between 0.39 and 0.45 (Figure 3E), a favorable outcome as it maintains the proportional distribution of these organs, which defines the plant's nutritional value. Dry biomass yield (DBY) (Figure 3F) ranged from 0.41 to 0.69 t DBY ha^{-1} (leaves) and 4.4 to 4.79 t DBY ha^{-1} (whole plant).

Studies on Moringa crops under field conditions report dry matter (DM) yields of up to 6.61 t DM ha^{-1} when fertilized with cattle manure; however, this fertilization method is not ideal for Moringa forage production, as nutrient release is slow (Yalta Vela et al. 2024). Moringa grows rapidly and undergoes early structural modifications, including changes in the leaf-to-stem ratio, making timely nutrient availability crucial for optimal development.

Ledea-Rodríguez et al. 2022 considered the 1:1 ratio ideal for 45-day-old plants, though the leaf-to-stem ratio in this study remained balanced but was below 1. This limitation could potentially be overcome by increasing the concentration of antioxidants or nutrient compounds in the leaves. However, several factors must be considered, including differences in experimental conditions, spatial arrangement of trials, source of raw materials or experimental products (biostimulants, biofertilizers, or bio-inputs), soil and climate characteristics (Shahrajabian et al. 2021). Additionally, the genetic origin of the Moringa species plays a crucial role in determining the observed effects and outcomes compared to other studies (Peter et al. 2023).

Some leaf and growth indicators were affected by the Spirulina extracts (Table 3). Plants biostimulated with 2 g L^{-1} of extract showed a 34.06 and 70.17% increase in fresh weight (FW) compared to the absolute control (AC) and those treated with 6 g L^{-1} ($P \leq 0.001$).

The control plants (AC) showed a 12.34% increase in leaf area (LA) compared to those biostimulated with 6 g L^{-1} of microalgae extract ($P \leq 0.001$) (Table 1). This effect was also observed in other variables such as LAF, DFA, and ELA, which were promoted by the 2 g L^{-1} treatment

Table 3. Physiological leaf and growth indicators in 45-day-old *M. oleifera* plants biostimulated with Spirulina extracts.

	Spirulina extract (g L^{-1})					
Morpho-physiological variables	AC	2	4	6	**P**	**±SE**
LA (cm^2)	42.84±28.3^{b}	64.97±27.90^{a}	56.95±38.5^{ab}	19.38±74^{c}	0.001	0.31
LAI	43.49±29^{b}	67.04±28.6^{a}	59.82±39^{ab}	56.45±38.5^{ab}	0.001	0.32
DLA (mm^2 per day)	41.34±28.23^{b}	64.47±27.9^{a}	56.45±38.5^{ab}	19.88±74.1^{c}	0.001	0.31
ELA (mm^2 g^{-1})	63.26±4.18^{ab}	53.9±2926^{a}	75.66±22.4^{ab}	26.62±6.38^{b}	0.06	0.47
NAR (g mm^2 per day)	14.29±0.67	23.94±7.91	18.14±6.07	6.89±2.02	0.001	3.58
LAR (mm^2 g^{-1})	5.05±1.2	6.79±3.1	1.32±1.05	4.56±0.56	0.57	0.63
ROL ($g_{(leaves)}$ $g^{-1}{}_{(plan)}$)	0.25±0.02^{ab}	0.05±0.10^{b}	0.25±0.06^{ab}	0.30±0.01^{a}	0.018	0.04
SR ($g_{(stem)}$ $g^{-1}{}_{(plant)}$)	0.50±0.04	0.56±0.04	0.56±0.06	0.54±0.04	0.30	0.01
RR ($g_{(root)}$ $g^{-1}{}_{(plant)}$)	0.40±0.07	0.42±0.17	0.36±0.10	0.28±0.02	0.13	0.04
AGR (cm per day)	0.56±0.39^{b}	0.62±0.33^{ab}	0.72±0.34^{ab}	0.83±0.23^{a}	0.04	0.06
RGR (cm per day)*10^{-3}	0.160±0.96	0.154±0.03	0.154±0.007	0.159±0.005	0.92	0.001

$^{a, b, c}$ Values with different superscript letters in the same row are significantly different at $P \leq 0.05$ according to Duncan's test. AC: Absolute control; *P*: p-value; ±SE: Standard error; DLA was transformed using log (x+1); LA: Leaf area; LAI: Leaf area index; DLA: Duration of leaf area; ELA: Effective leaf area; NAR: Net assimilation rate; LAR: Leaf area ratio; POL: Ratio of leaves; SR: Stem ratio; RR: Root ratio AGR: Absolute growth rate; RGR: Relative growth rate.

compared to the AC. For LAI and DFA, the remaining treatments did not show significant differences ($P \geq 0.05$) from the AC. However, the plants biostimulated with 2 g L^{-1} increased the EFA by 64.8% ($P \leq 0.05$) compared to those treated with 6 g L^{-1}. Meanwhile, NAR and LAR did not show any significant differences ($P \geq 0.05$) in this study. Together, these variables are used to evaluate various agronomic traits that help us understand gas exchange and energy transformation into biomass.

Plants biostimulated with 6 g L^{-1} produced 64% more leaves than those treated with 2 g L^{-1} ($P \leq 0.05$). However, no significant differences were found compared to the control plants (AC) ($P \geq 0.05$). These positive effects are linked to the properties of microalgae cells, as shown in various studies (Parmar et al. 2023), which help improve the physiological and metabolic processes in *M. oleifera* plants. In this study, doses of 2 and 6 g L^{-1} increased the number of leaves while also causing a reduction in leaf size. This could benefit the plant's efficiency by improving gas exchange (Drake et al. 2013), photosynthesis, and its ability to tolerate stress. The morphology and physiology of the leaves have direct implications for leaf structure, as indicated by variables such as leaf area (LA), leaf area index (LAI), duration of leaf area (DLA), and effective leaf area (ELA).

Stems and roots proportion was not significantly affected by the treatments ($P \geq 0.05$), showing a morphological development that could favor forage production based on the number of leaves and fewer stems, a desired structure for plants used for animal and human consumption. Also, modifications were observed in root morphology and plant quality indicators due to the effects of Spirulina extracts. Plants bio-stimulated with 2 and 4 g L^{-1} showed an 18% increase in root collar diameter ($P \leq 0.01$) (Figure 4A) compared to the control and plants treated with 6 g L^{-1}. The increased root collar diameter suggests greater resistance in the plant, enhancing its survival under field and extensive cultivation conditions.

Root thickness was significantly reduced by 33.6% ($P \leq 0.001$) in plants bio-stimulated with 6 g L^{-1} compared to the other experimental treatments and the control plants (AC), with no significant differences observed among the latter ($P \geq 0.05$) (Figure 4B). No significant differences were found for root length, fresh and dry weight, and the stem/root ratio ($P \geq 0.05$). Their average values ranged from 75.8 to 90.42 cm (Figure 4C), 32.5 to 74.6 g, and 2.7 to 8.2 g (Figure 4E), and 10.8 to 18.8 mm (Figure 4D), respectively.

Regarding the variables that describe seedlings, the robustness index increased by 13-23% with the 6 g L^{-1} application compared to the control (AC) and the

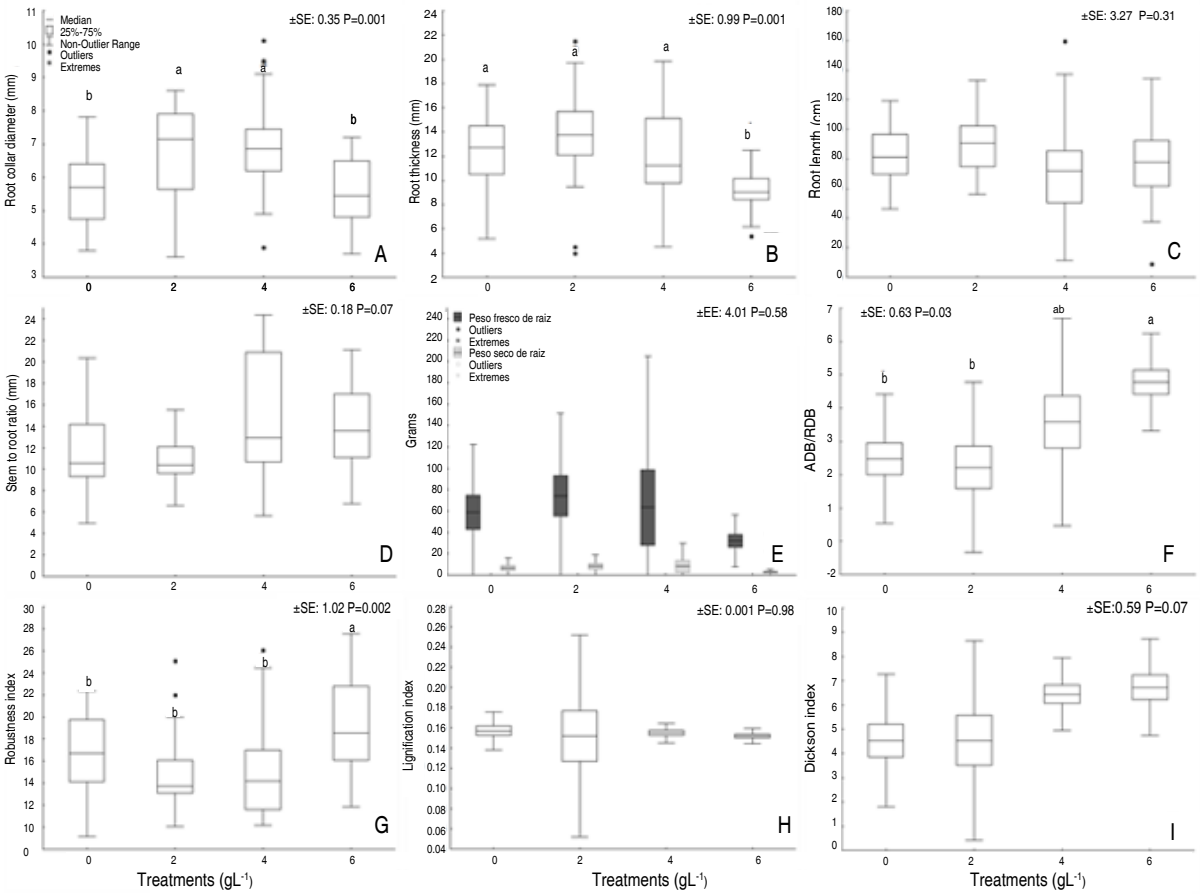

Figure 4. Root morphology and quality indices in 45-day-old *M. oleifera* plants biostimulated with Spirulina extracts. [a, b, c] Superscript letters with different values in the same row indicate significant differences at $P \leq 0.05$ according to Duncan's test. *1 Transformed using $\sqrt{(x+5.5)}$; *2 Transformed using $\sqrt{(x)}$. ADB/RDB: Aerial dry biomass/Root dry biomass; ±SE: Standard error.

2 g L^{-1} treatment ($P \leq 0.002$). In its interpretation, values below 6 are considered robust, while those above 6 are weaker. For this study, these plants likely have low lignin content in their stems, which improves their nutritional value. The lignification and Dickson indexes were not significantly affected by the treatments ($P \geq 0.05$), with values ranging from 0.15 to 0.16 (Figure 4H) and 4.5 to 6.7 (Figure 4I), respectively.

The results for photosynthetic pigment content as part of the antioxidant profile were encouraging (Figure 5). Chlorophyll α, β, and total chlorophyll levels increased by 7, 16.6, and 11.4%, respectively, for all experimental treatments compared to the control (AC) ($P \leq 0.05$). The AC showed a higher ratio of chlorophyll α to β, by 14-15%, compared to the 2 and 6 g L^{-1} treatments, respectively ($P \leq 0.01$).

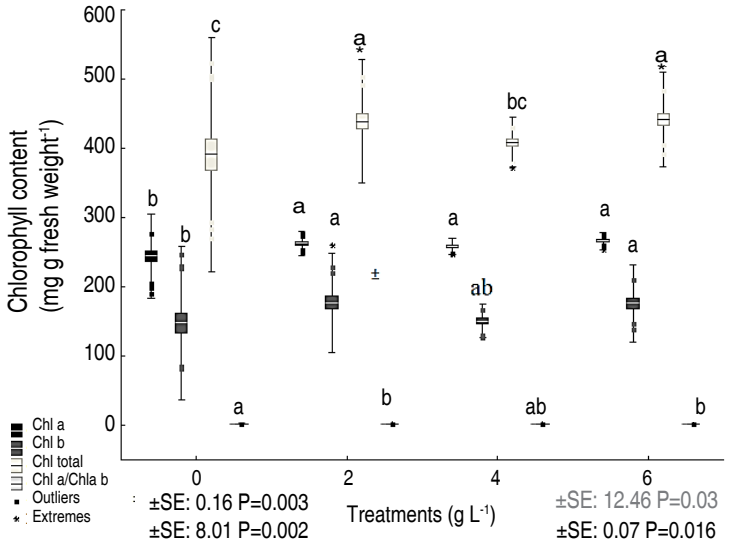

Figure 5. Chlorophyll content in the leaves of 45-day-old *M. oleifera* plants biostimulated with Spirulina extracts.

Similar results have been reported in *Vicia faba* L. crops (el-Sheekh and el-Saied A el-D 2000), *V. faba* and *Helianthus* (Mohammed et al. 2023), bio-stimulated with marine algae extracts. The effects on photosynthetic pigment content from microalgae extracts are linked to the presence of betaines (Baroud et al. 2019), and their ability to capture ions and increase leaf pigmentation (Mohammed et al. 2023). Other authors associate these effects with the availability of sugars, which serve as structural and storage compounds, respiratory substrates, intermediate metabolites in various metabolic activities, signaling molecules, and transporters (Ciereszko 2018). These compounds contribute to the plant tissue, enhancing new characteristics such as functional food or nutraceutical properties.

CONCLUSION

The experimental treatments using Spirulina extracts in *M. oleifera* plants stimulate growth, particularly in height. Low doses (2 and 4 g L^{-1}) promote the development of plants with physiologically active leaves, while higher doses (6 g L^{-1}) result in plants with greater leaf production and an improved leaf-to-stem ratio. The results suggest that the Spirulina extracts can be used as a sustainable strategy for the biostimulation of Moringa plants under controlled conditions. Future validation studies are required in open field agroecological spaces.

ACKNOWLEDGMENTS

Thanks are due to the Autonomous University of Baja California Sur for the grant of research project INV-IN/069 “Evaluation of a cation exchanger and biofertilizer of freshwater microalgae on germination, growth, development, stomatal morphology and tissue histology, and biomass production of Moringa oleifera Lam with nutraceutical value at two growth ages under salinity conditions”.

CONFLICTS OF INTEREST

No conflicts of interest.

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