











ORIGINAL PAPER

**Photochemical efficiency and growth of moringa irrigated with brackish water and foliar application of biostimulant**

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**Abstract:** Salinity affects the photosynthetic capacity of plants, reducing their efficiency and productivity. Therefore, it is necessary to seek alternatives to mitigate these negative effects, such as the application of biostimulants based on *Ascophyllum nodosum*, which can help restore photosynthetic function. This study aimed to evaluate the effects of foliar application of the biostimulant on mitigating salt stress on the photochemical efficiency of *Moringa oleifera*. The experiment was conducted at the Federal University of Paraíba, Areia, Paraíba, Brazil. The experimental design was a randomized block design, with four replicates and two plants per plot, with five levels of irrigation water electrical conductivity (EC<sub>w</sub> – 0.50, 1.88, 5.25, 8.62, and 10.00 dS m<sup>-1</sup>) and five concentrations of foliar-applied biostimulant (0.00, 1.45, 5.00, 8.55, and 10.00 ml L<sup>-1</sup>). The brackish water caused reductions in photochemical efficiency and stem diameter of moringa seedlings, with significant damage starting at an EC<sub>w</sub> of 5.25 dS m<sup>-1</sup>. The application of the biostimulant at concentrations of 5 mL L<sup>-1</sup> and above improved photochemical activity and growth of moringa seedlings under salt stress of up to 10.0 dS m<sup>-1</sup> at 60 days after sowing.

**Keywords:** *Ascophyllum nodosum*, *Moringa oleifera*, photosynthesis, salt stress.

### Introduction

Soil salinity is one of the main challenges for global agriculture, particularly in semi-arid regions such as northeastern Brazil. In these areas, irregular water availability often leads to increased use of lower-quality water for agriculture

(Castro and Santos, 2020; Pessoa et al., 2022; Ahmed et al., 2024).

The use of water with high salt concentrations has caused significant negative impacts on plants, compromising their photosynthetic efficiency and leading to the overproduction of reactive oxygen species (ROS), which affect photosynthetic

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activity, metabolism, and, ultimately, plant growth (Hasanuzzaman et al., 2021a).

In this context, *Moringa oleifera*, belonging to the Moringaceae family, emerges as a promising crop for areas affected by salinity due to its relative tolerance to adverse conditions (Mahaveerchand and Salam, 2024). Additionally, moringa is a highly nutritious food source, with leaves that provide significant amounts of  $\beta$ -carotene, vitamin C, calcium, protein, potassium, and iron, surpassing the levels found in carrots, oranges, milk, peas, bananas, and spinach (Alam et al., 2020).

Moreover, the growing demand for solutions to mitigate the harmful effects of salt stress has drawn attention to biostimulants (Hasanuzzaman et al., 2021b; Zuzunaga-Rosas et al., 2023; Abdelkhalik et al., 2024). These compounds contain bioactive substances, such as phytohormones, amino acids, seaweed extracts, humic acids, chitosan, beneficial microorganisms, vitamins, and antioxidants, which have demonstrated the ability to modulate plant metabolism and enhance their resistance to salt stress (Sanchez et al., 2019; Malik et al., 2021).

A notable example of a biostimulant is the seaweed *Ascophyllum nodosum*, which is rich in carbohydrates and essential nutrients. It stimulates the synthesis of phytohormones such as auxins and gibberellins, triggering genetic responses for defense and hormonal regulation. This enhances the plants' ability to tolerate salinity (Silva et al., 2016; Hadia et al., 2020). Its effectiveness in mitigating salt-induced damage, promoting higher photosynthetic rates, and consequently increasing plant growth, has been demonstrated in crops such as tomato (Dell'aversana et al., 2021), rice (Shahzad et al., 2023), jack bean (Sales et al., 2024), and soybean (Silva et al., 2024). However, its effect on moringa remains unknown.

Considering the potential of *M. oleifera* in the restoration of degraded areas and the significant relevance of using biostimulants

to maximize plant production, this study aimed to evaluate the effects of foliar application of the biostimulant on mitigating salt stress on the photochemical efficiency of *M. oleifera*.

## Materials and Methods

### Experimental place

The experiment was conducted from May to August 2019 in a protected environment at the Center for Agricultural Sciences, Federal University of Paraíba, Areia, Paraíba, Brazil (6° 58' 00" S and 35° 41' 00" W, with an altitude of 575 m). According to the Köppen classification, the region's climate is As', characterized by a dry, hot summer and rainfall in the winter (Alvares et al., 2013). The average temperature observed during the experiment was 27.5°C, with maximum and minimum temperatures ranging between 36.2 and 18.8°C, respectively.

### Experimental design and treatments

The experimental design was a randomized block design, with four replications and two plants per plot, arranged according to the Central Composite Design (Mateus et al., 2001). The treatments included five levels of electrical conductivity of irrigation water (ECw) and five concentrations of foliar-applied biostimulant (Table 1).

### Plant material and treatments

The seedlings were produced from seeds obtained from healthy plants free from pest attacks, located in the municipality of Areia-PB. The seeds were sown in black polyethylene bags with a capacity of 1.2 dm<sup>3</sup>, filled with a substrate consisting of 85% Latosol, 10% washed fine sand, and 5% cattle manure (v:v). The substrate was analyzed for physical and chemical characteristics, fertility, and salinity using methodologies proposed by Richards (1954) and Teixeira et al. (2017), as indicated in Table 2.

Table 1: Schematic representation of the combinations and factors (ECw – electrical conductivity of irrigation water; Bio – biostimulant concentrations) used in the experiment

Treatments	Levels		Doses	
	ECw	Bio	ECw (dS m <sup>-1</sup> )	Bio (mL L <sup>-1</sup> )
1	-1	-1	1.88	1.45
2	-1	1	1.88	8.55
3	1	-1	8.62	1.45
4	1	1	8.62	8.55
5	-1.41(α)	0	0.50	5.00
6	1.41(α)	0	10.00	5.00
7	0	-1.41(α)	5.25	10.00
8	0	1.41(α)	5.25	0.00
9	0	0	8.25	5.00

Table 2: Physical and chemical composition of the substrate used in the experiment

Physical	Value	Fertility	Value	Salinity	Value
Sand (g kg <sup>-1</sup> )	639	pH in water (1:2.5)	7.00	pH	7.30
Silt (g kg <sup>-1</sup> )	227	P (mg dm <sup>-3</sup> )	146.32	ECse (dS m <sup>-1</sup> )	0.73
Clay (g kg <sup>-1</sup> )	134	K <sup>+</sup> (mg dm <sup>-3</sup> )	633.29	SO <sub>4</sub> <sup>-2</sup> (mmol <sub>c</sub> L <sup>-1</sup> )	1.02
Textural class	Sandy	Na <sup>+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.27	Ca <sup>2+</sup> (mmol <sub>c</sub> L <sup>-1</sup> )	16.00
	loam	Al <sup>3+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.00	Mg <sup>2+</sup> (mmol <sub>c</sub> L <sup>-1</sup> )	16.75
		H <sup>+</sup> + Al <sup>3+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	2.84	K <sup>+</sup> (mmol <sub>c</sub> L <sup>-1</sup> )	6.90
		Ca <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	5.53	CO <sub>3</sub> <sup>-2</sup> (mmol <sub>c</sub> L <sup>-1</sup> )	0.00
		Mg <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	1.70	HCO <sub>3</sub> <sup>-2</sup> (mmol <sub>c</sub> L <sup>-1</sup> )	40.00
		SB (cmol <sub>c</sub> dm <sup>-3</sup> )	9.12	Cl <sup>-</sup> (mmol <sub>c</sub> L <sup>-1</sup> )	30.00
		CEC (cmol <sub>c</sub> dm <sup>-3</sup> )	11.96	Na <sup>+</sup> (mmol <sub>c</sub> L <sup>-1</sup> )	0.89
		OM (cmol <sub>c</sub> dm <sup>-3</sup> )	26.69	SAR (mmol <sub>c</sub> L <sup>-1</sup> )	0.94
				ESP (%)	2.25
				Classification	No-saline

SB – sum of bases (Na<sup>+</sup> + K<sup>+</sup> + Ca<sup>2+</sup> + Mg<sup>2+</sup>); CEC – cation exchange capacity [SB + (H<sup>+</sup> + Al<sup>3+</sup>)]; OM – organic matter; ECse – electrical conductivity of the saturation extract; SAR – sodium adsorption ratio: Na<sup>+</sup> × [(Ca<sup>2+</sup> + Mg<sup>2+</sup>)/2] – 1/2; ESP – exchangeable sodium percentage (100 × Na<sup>+</sup>/CEC).

The electrical conductivity of each irrigation water (ECw) above 0.5 dS m<sup>-1</sup> was achieved by diluting sodium chloride (NaCl) with water from the supply system (ECw of 0.5 dS m<sup>-1</sup>) to the predetermined value using a portable microprocessor-based conductivity meter model CD-860 (Instrutherm® Instrumentos de Medição Ltda., São Paulo, SP, Brazil). The values of irrigation water electrical conductivity were selected based on Tavares Filho et al. (2020), who observed inhibitory effects of salinity on moringa seedlings irrigated with ECw ranging from 0.24 to 10.00 dS m<sup>-1</sup>.

Irrigation was carried out daily, with brackish water application starting at 10 days after emergence (DAE). The volume applied was determined using the drainage

lysimetry method, based on the difference between the applied and drained amounts, to maintain substrate moisture at field capacity levels. The basic formula (Equation 1) used was according Bernardo et al. (2019).

$$P + I = ET + D + \Delta S \quad (1)$$

Where: P – precipitation, in mm; I – irrigation (water applied manually), in mL; ET – evapotranspiration, in mm; D – drainage; ΔS – variation in water storage in the pot's substrate, in mL.

The biostimulant doses were applied starting from 10 days after emergence (DAE), using a seaweed extract from the species *Ascophyllum nodosum* of the

Acadian<sup>®</sup> commercial product (Agritech, Dryden, ON, Canada), composed of: N – 8.12, P – 6.82, K – 12.00, Ca – 1.60, Mg – 2.03, and S – 8.16 g kg<sup>-1</sup>; B – 5.74, Cu – 13.60, Fe – 11.50, Mn – 0.04, Zn – 24.40, and Na – 20000 mg kg<sup>-1</sup>; potassium hydroxide, with 61.48 g L<sup>-1</sup> of soluble K<sub>2</sub>O; 69.60 g L<sup>-1</sup> of total organic carbon; and a density of 1.16 g dm<sup>-3</sup> (Silva et al., 2016). Fertilizations were divided into six foliar applications to avoid leaching or toxicity to the plants, performed weekly in the late afternoon using sprayers, applying approximately 100 mL of solution of the respective doses per plant.

### Variables analyzed

At 60 DAE, assessments of photosynthetic pigments were conducted by determining chlorophyll indices *a*, *b*, total, and the chlorophyll *a/b* ratio using non-destructive methods. This involved using a ClorofiLOG<sup>®</sup> CFL1030 portable meter (Falker, Porto Alegre, RS, Brazil), with values expressed in Falker chlorophyll index (FCI). Additionally, chlorophyll fluorescence was measured using a modulated fluorometer model OS-30p (Sciences Inc., Hudson, NH, USA). Prior to measurements, leaf clips were applied for 30 min to adapt leaves to darkness, and measurements included initial fluorescence (*F*<sub>0</sub>), maximum fluorescence (*F*<sub>m</sub>), variable fluorescence (*F*<sub>v</sub> = *F*<sub>m</sub> – *F*<sub>0</sub>), *F*<sub>v</sub>/*F*<sub>0</sub> ratio, and the quantum yield of photosystem II (*F*<sub>v</sub>/*F*<sub>m</sub>).

The relative growth rates of plant height (RGR<sub>ph</sub>) and stem diameter (RGR<sub>sd</sub>) were obtained between 10 and 60 DAE using the methodology proposed by Benincasa (2003), as presented in Equation 2.

$$\text{RGR} = \frac{\ln A_2 - \ln A_1}{t_2 - t_1} \quad (2)$$

Where: RGR – relative growth rate, in cm cm<sup>-1</sup> day<sup>-1</sup> for plant height and mm mm<sup>-1</sup> day<sup>-1</sup> for stem diameter; A<sub>2</sub> – plant height (cm) or stem diameter (mm) at 60 DAE; A<sub>1</sub> – plant height (cm) or stem diameter (mm)

at 10 DAE; t<sub>2</sub> – t<sub>1</sub> – time difference (days) between evaluations; ln – natural logarithm.

### Statistical analysis

The data were subjected to a normality test using the Shapiro-Wilk test and a homogeneity test using Bartlett's test. The data were subjected to analysis of variance and regression using the statistical software R (R Core Team, 2023).

### Results and Discussion

Based on the variance analysis, there was a significant interaction between the factors electrical conductivity of irrigation water (EC<sub>w</sub>) and biostimulant (Bio) for chlorophyll *b* (Chl. *b*), total chlorophyll (Chl. To), chlorophyll *a/b* ratio (Chl. *a/b*), initial fluorescence (*F*<sub>0</sub>), maximum fluorescence (*F*<sub>m</sub>), variable fluorescence (*F*<sub>v</sub>), and the *F*<sub>v</sub>/*F*<sub>m</sub> ratio, as well as for the relative growth rates of plant height (RGR<sub>ph</sub>) and stem diameter (RGR<sub>sd</sub>) in moringa irrigated from 10 to 60 days after emergence (DAE). The isolated effect of the factors was observed in chlorophyll *a* (Chl. *a*) index at 60 DAE.

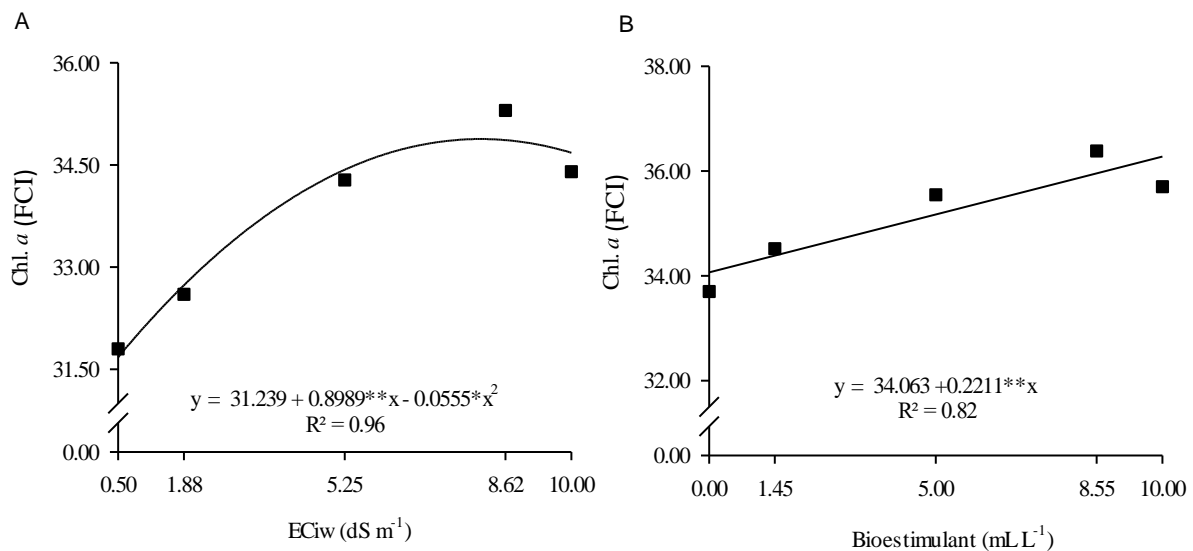
The chlorophyll *a* index in moringa plants also increased with the rise in EC<sub>w</sub>, following a quadratic pattern, and reached an estimated maximum of 34.87 FCI when irrigated with 7.89 dS m<sup>-1</sup>, before subsequently decreasing (Figure 1A). This initial increase in chlorophyll *a* is due to the activation of defense mechanisms that enhance the plant's acclimation to stress conditions, such as the synthesis of osmolytes and hormonal regulation, which can stimulate chlorophyll *a* production to maximize photosynthetic efficiency.

However, at higher EC<sub>w</sub> levels, the salt stress becomes excessive, causing damage to chloroplasts, metabolic dysregulation, and increased production of reactive oxygen species (ROS), which degrade chlorophyll (Ameen et al., 2024; Bezerra et al., 2024).

The biostimulant increased the chlorophyll *a* by 6.49% when applied at a concentration of 10 mL L<sup>-1</sup> (Figure 1B).

This effect is attributed to the biostimulant's ability to protect chloroplasts via cytokinins (Wally et al., 2013; Kałużewicz et al., 2017). Cytokinins are plant hormones that protect chloroplasts by delaying senescence and maintaining their functionality, which explains their effect when applied via biostimulants. They reduce oxidative stress by regulating antioxidant enzyme activity, minimizing the accumulation of reactive oxygen species that damage chloroplasts.

Additionally, they regulate the expression of genes involved in chloroplast structure and function. Study developed by Saeger et al. (2020) and Ali et al. (2022), the *A. nodosum*-based biostimulant has been shown to contribute to chlorophyll synthesis and reduce ROS activity by providing essential nutrients and precursors necessary for chlorophyll biosynthesis.



\*\* , \* – significant at  $p < 0.01$  and  $p < 0.05$ , respectively.

Figure 1: Chlorophyll *a* (Chl. *a*) in moringa as a function of the electrical conductivity of irrigation water – ECiw (A) and biostimulant (B) at 60 days after emergence.

For chlorophyll *b*, an increase was observed in response to ECw up to 3.0 dS m<sup>-1</sup>, reaching a maximum value of 8.91 FCI. This point represented the highest gain, while the lowest gain occurred at an ECw of 10 dS m<sup>-1</sup>, resulting in a value of 7.3 FCI, reflecting an 18% reduction in Chl. *b* index. However, the application of the biostimulant showed positive responses regarding the increase in irrigation ECw, with the most significant enhancement noted at 10 dS m<sup>-1</sup> when using a concentration of 10 mL L<sup>-1</sup> (8.6 FCI), which led to a 17.81% increase compared to plants without biostimulant application at the same salinity (Figure 2A).

For total chlorophyll, a decrease of 16.9% was observed as the ECw increased, dropping from 42.14 to 35.02 FCI between ECw of 0.5 and 10 dS m<sup>-1</sup>. However, the

application of the biostimulant led to an increase in total chlorophyll content, with the highest value of 44.41 FCI recorded in plants irrigated with water at 0.5 dS m<sup>-1</sup> and treated with a concentration of 5.96 mL L<sup>-1</sup> of biostimulant. At the highest salinity, it was found that increasing the biostimulant concentration resulted in greater chlorophyll synthesis, showing a 16.56% increase compared to plants that did not receive biostimulant treatment (Figure 2B).

The observed increase in chlorophyll content in plants treated with the *A. nodosum*-based biostimulant can be explained by its composition, which includes essential nutrients and bioactive compounds such as amino acids, humic and fulvic acids, and plant growth regulators. These substances play a pivotal role in enhancing the activity of chlorophyll

synthase, the enzyme responsible for chlorophyll synthesis, while simultaneously reducing its degradation. As chlorophylls are key photosynthetic pigments and sensitive indicators of stress-induced changes in photosynthetic processes, this improvement in chlorophyll content suggests enhanced photosynthetic efficiency and better plant resilience under salinity stress (Lanfer-Márquez, 2003; Saeger et al., 2020; Smiderle et al., 2022).

The chlorophyll *a/b* ratio in moringa plants decreased as the EC<sub>w</sub> increased, reaching a value of 3.59 when irrigated with 4.4 dS m<sup>-1</sup>, which represents a reduction of 6.26%. Subsequently, this ratio increased until an EC<sub>w</sub> of 10 dS m<sup>-1</sup>, reaching 4.20. A similar trend was observed in plants treated with the biostimulant, particularly at a concentration of 10 mL L<sup>-1</sup> under salt stress conditions of 0.5 dS m<sup>-1</sup> (4.89), resulting in a 21.26% increase in the chlorophyll *a/b* ratio compared to plants without biostimulant application (Figure 2C). This increase in the chlorophyll *a/b* ratio is associated with a rise in chlorophyll *a* production relative to chlorophyll *b*, which can be linked to chlorophyll *a* being the primary reaction center of the photosystem, while chlorophyll *b* enhances the absorption of longer wavelengths of light (Huihui et al., 2020). Such behavior has been documented in studies on salinity effects in moringa (Farooq et al., 2022; Azeem et al., 2023), as well as in research involving *A. nodosum*-based biostimulants (Raja and Vidya, 2023; Melo et al., 2024).

The increase in biostimulant doses also resulted in a 22.37% reduction in initial fluorescence ( $F_0$ ) between the doses of 0 and 10 mL L<sup>-1</sup> at an EC<sub>w</sub> of 0.5 dS m<sup>-1</sup> (Figure 2D). This reduction in  $F_0$  occurs as a response to adjustments in the photosynthetic apparatus, involving an increase in the efficiency of photosystem II (PSII), reflecting a protective response or damage to chloroplasts under high salinity levels (Gulzar et al., 2020). The maintenance of energy utilization for activating the reaction centers of the

photosystem observed with the application of the biostimulant can be attributed to its ability to enhance the efficiency of light capture and energy transfer within PSII. By supporting the structural and functional stability of PSII under stress conditions, the biostimulant mitigates potential damage to the photosynthetic apparatus. This results in improved light-use efficiency, ensuring that the energy absorbed is effectively utilized for photochemical processes, thereby protecting the photosystem and sustaining photosynthetic performance, even under adverse conditions (Santaniello et al., 2017).

Regarding the maximum fluorescence ( $F_m$ ) of moringa plants (Figure 2E), it was observed that as the EC<sub>w</sub> increased, there was a significant reduction in  $F_m$ , decreasing from 828.27 at an EC<sub>w</sub> of 0.5 dS m<sup>-1</sup> to 334.57 at 10 dS m<sup>-1</sup>, which represents a 59.6% decline. A similar trend was observed with the application of the biostimulant at an EC<sub>w</sub> of 0.5 dS m<sup>-1</sup>, showing a reduction of 23.75% with a concentration of 10 mL L<sup>-1</sup> (631.52) compared to plants without biostimulant application at the same salinity (828.27). However, at a salinity level of 10 dS m<sup>-1</sup>, applying the biostimulant at a concentration of 10 mL L<sup>-1</sup> (643.02) resulted in a 37.2% increase compared to plants that did not receive the treatment.

Thus, the decrease in maximum fluorescence due to salt stress is attributed to reduced energy capture at the reaction centers, caused by disturbances in the plant's metabolic activity, which lead to the generation of ROS. This, in turn, limits the energetic activity of photosynthetic pigments (Zhang et al., 2010). Consequently, during salt stress, plants require metabolic adjustments to protect themselves from oxidative damage, with plant hormones playing a crucial role in defense signaling by regulating ROS. In this context, studies such as those by Omidbakhshfard et al. (2020) have shown that bioactive compounds from the *A. nodosum* biostimulant activate hormonal

biosynthesis pathways, such as auxins and gibberellins, which reduce oxidative stress and provide protection to the plants.

The variable fluorescence ( $F_v$ ) also experienced the effects of  $EC_w$  (Figure 2F), decreasing from 634.51 to 213.91 between the lowest and highest  $EC_w$  levels, representing a reduction of 66.28%. When comparing plants without biostimulant application to those receiving a concentration of 10 mL L<sup>-1</sup>, the biostimulant resulted in a 29.49% reduction in  $F_m$  at an  $EC_w$  of 0.5 dS m<sup>-1</sup>. In contrast, under higher salinity (10 dS m<sup>-1</sup>), the biostimulant led to a 45.48% increase in  $F_v$  with the same concentration compared to plants that did not receive the treatment. Since  $F_v$  represents the active potential energy in the photosystem, any loss in its value indicates limitations in the activation of the electron transport chain, which is responsible for ATP and NADPH production, potentially leading to decreased efficiency in the photosystem (Silva et al., 2018; Lotfi et al., 2020). This behavior is common in plants experiencing salt stress (Bashir et al., 2021).

The biostimulant led to an increase in  $F_m$  when the plants were under salt stress, likely because the protein hydrolysates and humic acids present in brown algae extracts (*A. nodosum*) promote greater antioxidant activity in essential enzymes (Colla and Rouphael, 2015), such as catalase and superoxide dismutase, while also reducing the accumulation of H<sub>2</sub>O<sub>2</sub> in leaf tissues. This behavior was also observed by Amor et al. (2005) in *Crithmum maritimum*.

The increase in the salinity of irrigation water for plants without biostimulant application maintained the quantum efficiency of the photosystem up to an  $EC_w$  of 4.43 dS m<sup>-1</sup>, rising from 0.765 at 0.5 dS m<sup>-1</sup> to 0.766, followed by a subsequent reduction of 7.57% up to an  $EC_w$  of 10.00 dS m<sup>-1</sup> (Figure 2G). However, the application of the biostimulant led to improvements in  $F_v/F_m$ , contributing to the maximum value of this variable (0.791) at an  $EC_w$  of 4.43 dS m<sup>-1</sup> with a concentration

of 4.5 mL L<sup>-1</sup>. Additionally, at an  $EC_w$  of 10 dS m<sup>-1</sup>, a concentration of 6.6 mL L<sup>-1</sup> resulted in a 7.11% increase in  $F_v/F_m$ , approaching the value of 0.766 obtained in plants under lower salinity conditions.

Exposure of plants to salt stress leads to a decrease in the maximum quantum yield of photosystem II ( $F_v/F_m$ ) due to ionic toxicity, suggesting that the reaction centers of PSII may suffer partial damage or even inactivation as a result of stress (Shi-chu et al., 2019; Shahzad et al., 2021). Conversely, the application of the biostimulant has demonstrated the ability to increase  $F_v/F_m$  values even during periods of salt stress, likely reducing the damage caused by photoinhibition and thus preserving the photosynthetic capacity of the plants (Akhter et al., 2021).

The relative growth rate of plant height (RGRph) in moringa plants was significantly affected by irrigation with brackish water (Figure 3A), showing a reduction of 3.1% as it decreased from 0.02199 cm cm<sup>-1</sup> day<sup>-1</sup> with 0.5 dS m<sup>-1</sup> to 0.02129 cm cm<sup>-1</sup> day<sup>-1</sup> at 10 dS m<sup>-1</sup>. However, the application of the biostimulant had beneficial effects on RGRph, with the greatest contribution observed in plants irrigated with freshwater. The maximum growth rate recorded was 0.0284 cm cm<sup>-1</sup> day<sup>-1</sup> with a concentration of 9.17 mL L<sup>-1</sup>, which was 22.57% higher than that of plants without biostimulant application at an  $EC_w$  of 0.5 dS m<sup>-1</sup>.

The maintenance of plant height under salt stress may be related to signaling processes that detect high levels of Na<sup>+</sup> and hyperosmolarity, leading to alterations in phospholipid composition. This results in adaptive processes to alleviate stress, such as maintaining ionic and osmotic balance, inducing phytohormones, and regulating cytoskeletal dynamics and cell wall structure (Zhao et al., 2021). These processes help mitigate the growth effects on plants, as observed by Farooq et al. (2022) and Azeem et al. (2023) in *M. oleifera*.

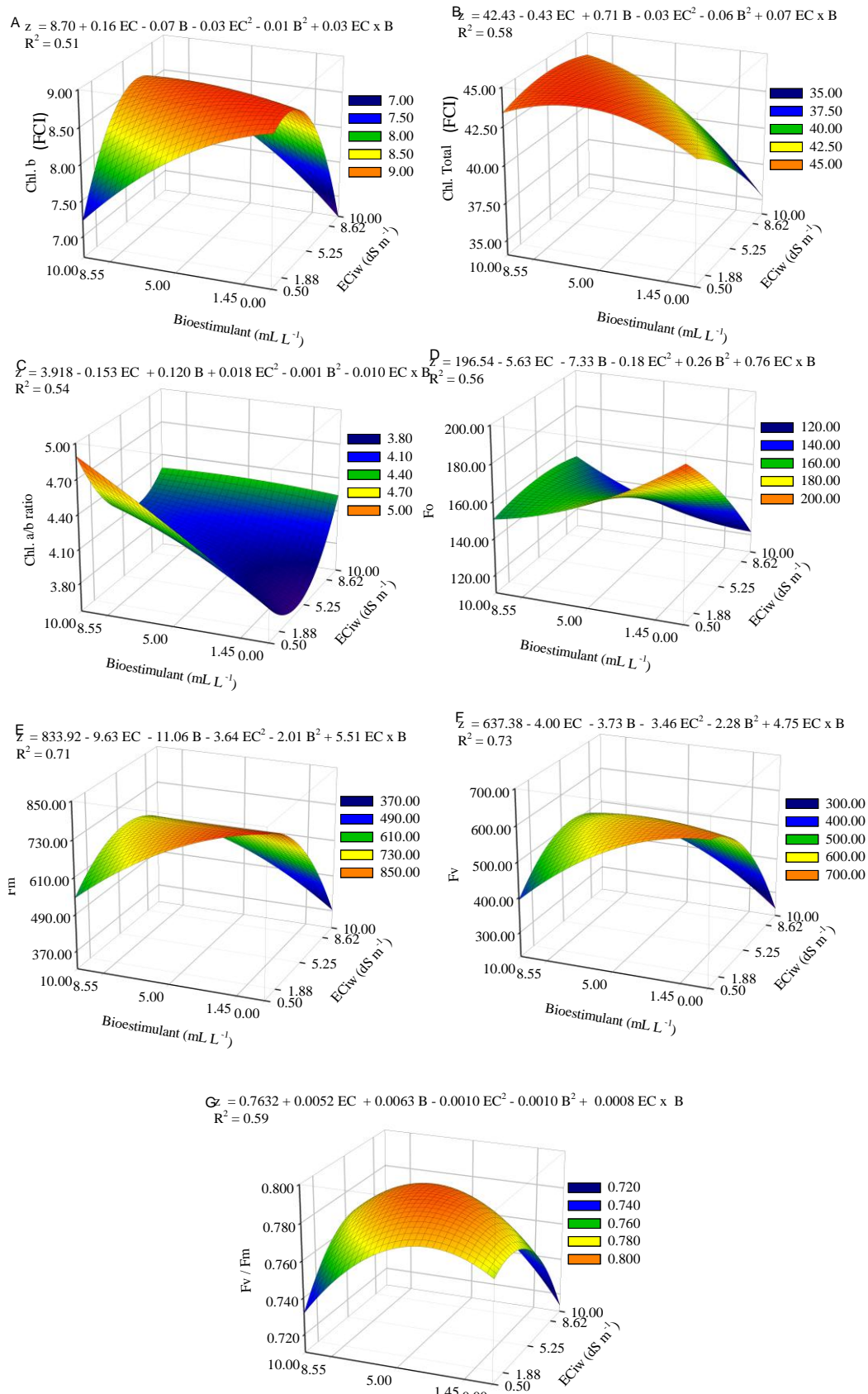


Figure 2: Chlorophyll *b* (A), total chlorophyll (B), chlorophyll *a/b* ratio (C), initial fluorescence (D), maximum fluorescence (E), variable fluorescence (F), and  $F_v/F_m$  ratio (G) in moringa as a function of the electrical conductivity of irrigation water –  $EC_{iw}$  and biostimulant application at 60 days after emergence.



The increase in salinity levels had more severe effects on the relative growth rate of stem diameter (Figure 3B), resulting in a 5.46% loss by the time the salinity reached 10 dS m<sup>-1</sup>. This may be related to cellular turgor, which is impacted by the restriction of water absorption due to the osmotic effects of salts in the soil, leading to reduced cell expansion (Lu and Fricke, 2023) and, consequently, affecting stem diameter.

The application of the biostimulant had a positive impact on the relative growth rate of stem diameter, with the greatest

contribution observed at an EC<sub>w</sub> of 3.73 dS m<sup>-1</sup>. Applying 4.36 mL L<sup>-1</sup> resulted in the maximum growth rate of 0.02286 mm mm<sup>-1</sup> day<sup>-1</sup>, which was 12.69% higher than that of plants without biostimulant under the same EC<sub>w</sub> (Figure 3B). This outcome can be attributed to the role of the *A. nodosum* biostimulant in maintaining the water potential of the plants, indicating an osmoprotective strategy against water loss due to water deficit (Martynenko et al., 2016).

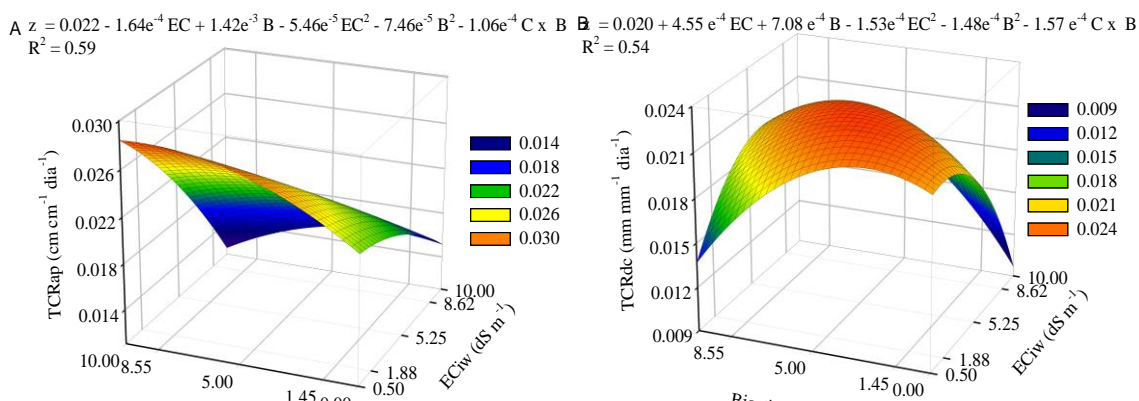


Figure 3: Relative growth rates of plant height (A) and stem diameter (B) of moringa as a function of the electrical conductivity of irrigation water – EC<sub>w</sub> and biostimulant application during the period from 10 to 60 days after emergence.

## Conclusions

Salinity in water leads to losses in photochemical efficiency and stem diameter in moringa seedlings, with significant damage starting at an electrical conductivity of irrigation water (EC<sub>w</sub>) of 5.25 dS m<sup>-1</sup>.

The application of a biostimulant at a concentration of 5 mL L<sup>-1</sup> enhances photochemical activity and growth of moringa seedlings under salt stress of up to 10 dS m<sup>-1</sup> by 60 days after sowing.

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