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Scientific Article

Biostimulant based on *Ascophyllum nodosum* in the formation of mint seedlings under salt stress

Bioestimulante à base de *Ascophyllum nodosum* na formação de mudas de hortelã sob estresse salino

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ABSTRACT - Mint stands out for its flavoring and therapeutic properties. However, its cultivation in the semi-arid region is limited due to the salinity in the irrigation water. As an alternative, to alleviate the harmful effects of salt stress, seaweed-based biostimulants have been used. Thus, the objective was to evaluate the effect of a biostimulant based on *Ascophyllum nodosum* on the levels of photosynthetic pigments, photochemical efficiency and growth of mint under irrigation with saline water. The experiment was conducted in a greenhouse from, using a randomized block design, in an incomplete factorial scheme, with five levels of electrical conductivity of irrigation water – ECw (0.50, 1.23, 3.00, 4.77 and 5.50 dS m^{-1}) and five concentrations of seaweed-based biostimulant (0.00, 1.45, 5.00, 8.55 and 10.0 mL L^{-1}). The biostimulant at concentrations of 6.30 and 7.31 mL L^{-1} alleviated salt stress effects on chlorophyll *b* content and Dickson quality index up to ECw of 3.41 and 2.71 dS m^{-1}. Foliar application of 10 mL L^{-1} stimulated the formation of leaf and shoot biomass under ECw of 0.5 dS m^{-1}. The increase in salinity did not compromise chlorophyll a and total chlorophyll contents, but reduced growth and biomass production. The highest quantum efficiency of photosystem II was obtained in plants subjected to ECw of 4.5 dS m^{-1}. Initial, variable and maximum fluorescences were reduced with the increase in ECw from 0.5 dS m^{-1}. Foliar application of up to 10 mL L^{-1} was efficient in reducing damage caused by salt stress in mint plants.

Keywords: *Mentha x piperita* L. Salinity. Abiotic stress. Growth regulators.

RESUMO - A hortelã se destaca pelas propriedades aromatizantes e terapêuticas. Contudo, o seu cultivo na região semiárida é limitado pela salinidade na água de irrigação. Assim, objetivou-se avaliar o efeito da aplicação de bioestimulante à base de *Ascophyllum nodosum* s teores de pigmentos fotossintéticos, a eficiência fotoquímica e o crescimento de hortelã sob irrigação com águas salinas. O experimento foi conduzido em casa de vegetação utilizando o delineamento de blocos casualizados, em esquema fatorial incompleto, com cinco níveis de condutividade elétrica da água de irrigação – CEa (0,50; 1,23; 3,00; 4,77 e 5,50 dS m^{-1}) e cinco concentrações de bioestimulante à base de alga marinha (0,00; 1,45; 5,00; 8,55 e 10,0 mL L^{-1}). O bioestimulante na concentração de 6,30 e 7,31 mL L^{-1} amenizou o estresse salino sobre o teor de clorofila *b* e no IQD até CEa de 3,41 e 2,71 dS m^{-1}. A aplicação foliar de 10 mL L^{-1} estimulou a formação de fitomassa das folhas e da parte aérea sob CEa de 0,5 dS m^{-1}. O aumento da salinidade não comprometeu os teores de clorofila *a* e total, porém, reduziu o crescimento e a produção de fitomassa. A maior eficiência quântica do fotossistema II foi obtido nas plantas submetidas a CEa de 4,5 dS m^{-1}. A fluorescência inicial, variável e máxima foram reduzidas com o aumento da CEa a partir de 0,5 dS m^{-1}. A aplicação foliar de até 10 mL L^{-1} foi eficiente em reduzir os danos promovidos pelo estresse salino nas plantas de hortelã.

Palavras-chave: *Mentha x piperita* L. Salinidade. Estresse abiótico. Reguladores de crescimento.

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INTRODUCTION

Mint (*Mentha x piperita* L.) is a species belonging to the Lamiaceae family and stands out for its aromatic and therapeutic properties, being widely used in the production of drugs and cosmetics, in traditional medicine and in cooking. It is a plant rich in secondary metabolites such as terpenoids and polyphenols and has strong biological effects (MAHENDRAN; RAHMAN, 2020), including the prevention of vomiting and nausea, as well as respiratory and digestive problems (OUNOKI et al., 2021).

Mint cultivation in semi-arid regions is limited by water scarcity, resulting from irregular rainfall, associated with high temperatures and low relative humidity, which leads to the use of groundwater, which often contains high levels of salts from the soil parent material and can compromise the development of crops (NÓBREGA et al., 2023; SILVA et al., 2024).

High levels of salts in the water stand out as one of the main abiotic stresses, causing a series of disturbances due to osmotic and ionic effects, mainly of Na^{+} and Cl^{-}, leading to changes in physiological functions, in the absorption and transport of water and nutrients, and consequently in plant growth (FÁTIMA et al., 2024). Accumulation of toxic ions causes damage to the photosynthetic apparatus, nutritional imbalance, and the intensification of reactive oxygen species, resulting in oxidative stress (ZAFAR et al., 2020; GUEDES et al., 2023).

In this context, an alternative to minimize the deleterious effects of salt stress on plants is the use of biostimulants due to the content of proteins, essential amino acids, vitamins, including the supply of macro and micronutrients, organic matter decomposers, hormone secretion, and neutralization of negative impacts of chemical fertilizers (MAHANTY et al., 2017).

Among the compositions of biostimulants, the use of microorganisms such as seaweed stands out, due to the favorable responses in nitrogen fixation, phosphorus and potassium solubilization, and protection against biotic and abiotic stresses (GOMES et al., 2024). Among the biostimulants, those obtained from microalgae, such as *Ascophyllum nodosum*, are capable of promoting improvements in the physiological processes of plants under stress conditions, such as salt stress, through compounds present in their constitution that act in the osmoregulation of processes, such as stomatal conductance, transpiration and photosynthesis, improving plant growth (DEEPIKO; ALI, 2020; FÁTIMA et al., 2024).

This beneficial effect of foliar application of *A. nodosum*-based biostimulant has been reported for other species, such as *Physalis peruviana* L., in which concentrations of up to 4.1 mL L^{-1} attenuated salt stress effects on the photochemical efficiency (NÓBREGA et al., 2021). Fátima et al. (2024) observed that foliar application of 6.67 mL L^{-1} attenuated the damage caused by salinity on gas exchange, growth and biomass accumulation of *Solanum aethiopicum* L. Rodrigues et al. (2025) found that the concentration of 5.0 mL L^{-1} attenuated the effects of salt stress on the growth and photochemical efficiency of *Moringa oleifera* Lam.

Considering the benefits of using seaweed-based biostimulants, it is essential to understand their role in attenuating the deleterious effects of salt stress on mint plants. Thus, the hypothesis of this study is that foliar application with the biostimulant can attenuate the damage caused by salt stress, due to the presence of compounds in the constitution of *A. nodosum* that stimulate the production of osmolytes that help in ionic homeostasis and osmoregulation, and due to the presence of growth regulators involved in the processes of signaling and gene expression of the plant's defense system.

Therefore, the objective of this study was to evaluate the effect of foliar application of biostimulant based on *Ascophyllum nodosum* on the contents of photosynthetic pigments, photochemical efficiency and growth of mint under saline irrigation.

MATERIAL AND METHODS

The experiment was carried out from February to April 2020 in a greenhouse belonging to the Department of Plant Science and Environmental Sciences of the Center for Agrarian Sciences, Federal University of Paraíba, Areia, PB, Brazil. The municipality is located at the geographic coordinates 6° 58' 00" S latitude and 35° 41' 00" W longitude, at an altitude of 575 m. The climate of the region, according to Köppen's classification, is As' type, which means dry and hot summer and rainfall in winter. The climatic conditions during the experiment are presented in Figure 1.

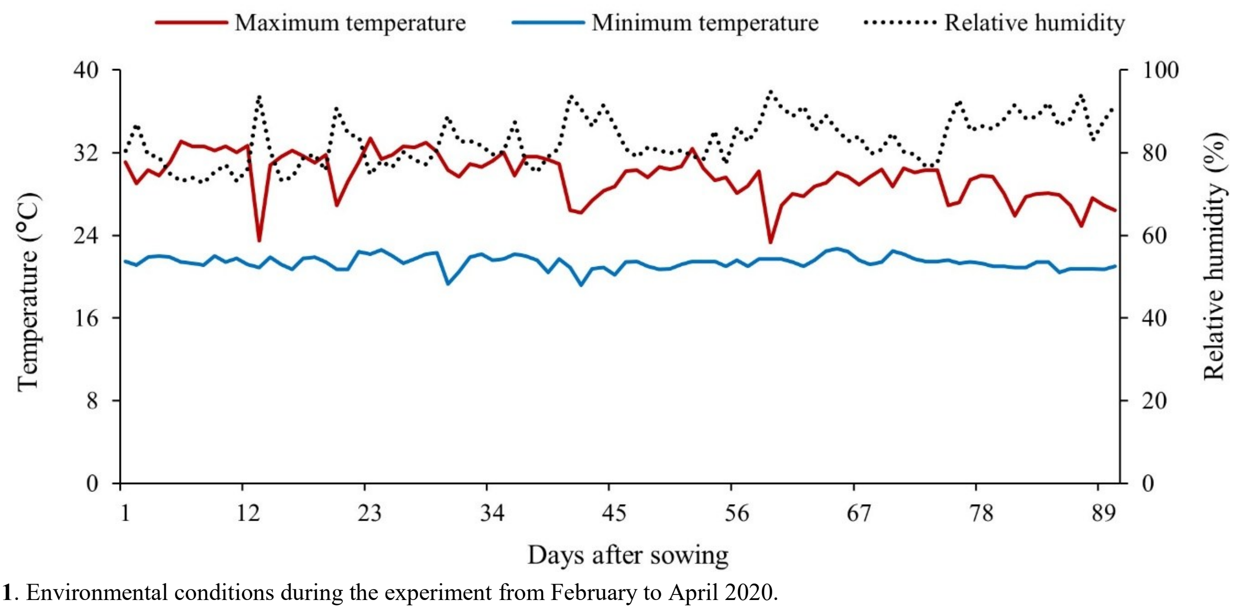


Figure 1. Environmental conditions during the experiment from February to April 2020.

The treatments were arranged in a randomized block design, in an incomplete factorial scheme, with five levels of electrical conductivity of irrigation water – ECw (0.50, 1.23, 3.00, 4.77 and 5.50 dS m^{-1}) and five doses of seaweed-based biostimulant (0.00, 1.45, 5.00, 8.55 and 10.0 mL L^{-1}), totaling nine combinations generated through the Central Box Compound matrix with four replicates and two plants per experimental plot, as shown in Table 1.

Table 1. Representative scheme of combinations and factors (ECw – electrical conductivity of water; Bio – concentrations of the biostimulant) used in the experiment.

Treatments	Levels		Levels	
	ECw	Bio	ECw (dS m^{-1})	BIO (mL L^{-1})
1	-1	-1	1.25	1.46
2	-1	1	1.23	8.54
3	1	-1	4.77	1.46
4	1	1	4.77	8.54
5	-1.41(α)	0	0.50	5.0
6	1.41(α)	0	5.50	5.0
7	0	-1.41(α)	3.00	10.0
8	0	1.41(α)	3.00	0.00
9	0	0	3.00	5.0

The plants were propagated from herbaceous cuttings of approximately 10 cm long of peppermint. The plant material was taken from healthy and pest-free plants grown in a greenhouse at CCE/UFPB. The cuttings were placed in 1.2-dm^{-3} polyethylene bags, filled with substrate formulated with soil classified as *Neossolo Regolítico* (Psamment), cattle manure and washed sand in the proportion of 3:1:1 (v:v), whose physical and chemical attributes were characterized according to the methodologies of Teixeira et al. (2017), as described in Table 2.

Table 2. Physical and chemical characterization of the substrate used in the experiment.

Physical	Value	Fertility	Value	Salinity	Value
Sand (g kg^{-1})	874	pH in water (1: 2.5)	8.1	pH	7.40
Silt (g kg^{-1})	91	P (mg dm^{-3})	65.16	ECse (dS m^{-1})	2.00
Clay (g kg^{-1})	35	K^+ (mg dm^{-3})	423.97	$SO4^{-2}$ (mmol$_c$ L^{-1})	2.17
Textural class	Sand	Na^+ (cmol$_c$ dm^{-3})	0.24	Ca^{+2} (mmol$_c$ L^{-1})	6.50
		Al^{+3} (cmol$_c$ dm^{-3})	0.00	Mg^{+2} (mmol$_c$ L^{-1})	17.50
		H^++Al^{+3} (cmol$_c$ dm^{-3})	0.99	Na^+ (mmol$_c$ L^{-1})	3.67
		Ca^{+2} (cmol$_c$ dm^{-3})	2.88	K^+ (mmol$_c$ L^{-1})	7.67
		Mg^{+2} (cmol$_c$ dm^{-3})	0.96	CO_3^{-2} (mmol$_c$ L^{-1})	0.00
		SB (cmol$_c$ dm^{-3})	5.17	HCO_3^{-2} (mmol$_c$ L^{-1})	17.50
		CEC (cmol$_c$ dm^{-3})	6.16	Cl^- (mmol$_c$ L^{-1})	10.00
		OM (dag kg^{-1})	15.00	SAR (mmol$_c$ L^{-1})	1.06
				ESP (%)	0.30
				Classification	Normal

OM = organic matter; SB = sum of bases; CEC = cation exchange capacity; ECse = Electrical conductivity of the saturation extract; SAR = Sodium adsorption ratio; ESP = Exchangeable sodium percentage.

The waters with different electrical conductivities were prepared by adding sodium chloride (NaCl) to water from the UFPB supply system (ECw = 0.5 dS m^{-1}), according to the pre-established treatments, considering the relationship between ECw and salt concentration described by Richards (1954), according to Equation 1:

$$C = 640 \times ECw \quad (1)$$

Where:
C = concentration of salts to be applied (mg L^{-1}); and,
ECw = electrical conductivity of water (dS m^{-1}).

Irrigation was carried out daily, with the application of saline water beginning at 15 days after setting of the cuttings, when the plants had the first two pairs of true leaves fully expanded, and the applied volume was established by the water balance, based on the difference between the amount applied and the amount drained.

The biostimulant based on *A. nodosum* (Acadian®) has in its constitution: N - 8.12; P - 6.82; K - 12.00; Ca - 1.60; Mg - 2.03; S - 8.16 g kg^{-1}; B - 5.74; Cu - 13.60; Fe - 11.5; Mn - 0.04; Zn - 24.40 and Na - 20000 mg kg^{-1}; potassium hydroxide, with 61.48 g L^{-1} of water-soluble K_2O; 69.60 g L^{-1} of total organic carbon; and a density of 1.16 g dm^{-3}. The biostimulant was applied 15 days after setting of the cuttings, being diluted in distilled water at the desired concentrations for the study, which were 0.00, 1.45, 5.00, 8.55 and 10.00 mL L^{-1}, with the doses based on Fátima et al. (2024). Four applications were carried out, every 15 days, at the end of the afternoon with a manual sprayer, using a plastic structure covering the plants, preventing the drift from reaching the other plants, applying a total of 100 mL of the biostimulant per plant.

The evaluations were carried out at 70 days after setting of the cuttings (DAS), with the determination of chlorophyll *a*, chlorophyll *b* and total chlorophyll indices by the non-destructive method, using a portable chlorophyll meter (ClorofiLOG®, model CFL 1030, Porto Alegre, RS), with the values expressed in Falker chlorophyll index (FCI).

Chlorophyll *a* fluorescence indices were determined with the aid of a pulse-modulated fluorometer (Sciences Inc.-Model OS-30p, Hudson, USA). For this, leaf clips were placed for 30 minutes to adapt the leaves to the dark, and the initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), F_v/F_0 ratio and quantum yield of photosystem II (F_v/F_m) were measured.

Growth evaluations were performed at 70 DAS, with the determination of plant height (PH), with a millimeter-graded ruler from the base of the plant to the apical meristem; and stem diameter (SD), with a digital caliper 2 cm above the collar. In the period from 15 to 70 DAS, the absolute and relative growth rates of plant height (AGR_{PH} and RGR_{PH}) and stem diameter (AGR_{SD} and RGR_{SD}) were calculated using the methodology proposed by Benincasa (2003).

Also at 70 DAS, the mint plants were collected, washed, fractionated in paper bags, placed in an air circulation oven at 65 ºC and kept for 72 hours. Subsequently, the samples were weighed on a 0.01 g precision scale to determine the leaf dry mass (LDM), stem dry mass (STDM), root dry mass (RDM) and shoot dry mass (SHDM), obtained by the sum of LDM + STDM. In the same period, the root/shoot ratio (R/S) was calculated based on the data of root dry mass and shoot dry mass. Seedling quality was determined through the Dickson quality index (DICKSON; LEAF; HOSNER, 1960), according to Equation 2:

$$DQI = \frac{(TDM)}{(PH/SD) + (SHDM/RDM)} \quad (2)$$

Where:
DQI = Dickson quality index;
PH = plant height (cm);
SD = stem diameter (mm);
TDM = total dry mass (g);
SHDM = shoot dry mass (g); and
RDM = root dry mass (g).

The data were subjected to the normality test (Shapiro-Wilk) and then to analysis of variance by the F test ($p \leq 0.05\%$); in cases of significance, polynomial regression analysis was applied. R statistical software version 4.0.1 was used to carry out the analysis. In cases where the interaction had a significant effect, the data were presented using response surfaces, constructed using the Sigma Plot® program, version 12.5.

RESULTS AND DISCUSSION

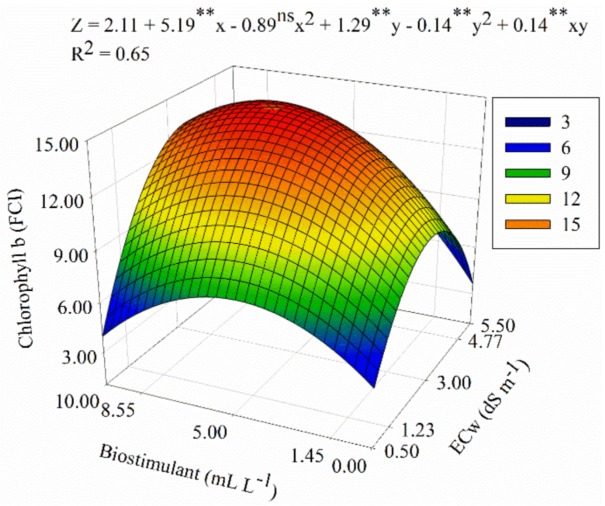
There was a significant effect of the interaction between factors (ECw × Bio) on the chlorophyll *b* contents and Dickson quality index of mint plants (Table 3). The water salinity levels and the doses of biostimulant based on *A. nodosum* significantly affected the chlorophyll *a* and total chlorophyll contents, Chl *a/b* ratio, initial, variable and maximum fluorescence indices, quantum yield of photosystem II and F_v/F_0 ratio, at 70 days after the application of the treatments.

Table 3. Summary of analysis of variance for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *Total*), Chl *a*/Chl *b* ratio (Chl *a/b*), initial fluorescence (F_0), variable fluorescence (F_v), maximum fluorescence (F_m), quantum yield of photosystem II (F_v/F_m), F_v/F_0 ratio (F_v/F_0) and Dickson quality index (DQI) of mint plants grown under saline water irrigation and application of *Ascophyllum nodosum*-based biostimulant.

Source of variation	DF	Mean Square									
		Chl *a*	Chl *b*	Chl *Total*	Chl.*a/b*	F_0	F_v	F_m	F_v/F_m	F_v/F_0	DQI
Blocks	3	1.54^{ns}	13.01^{ns}	0.61^{ns}	0.29^{ns}	128.6^{ns}	5141^{ns}	2709^{ns}	0.0008^{ns}	0.78^{ns}	0.0007^{ns}
Treatment	8	45.95^{**}	192.98^{**}	119.99^{**}	10.66^{**}	956.3^{**}	10784^{**}	12656^{**}	0.0059^{**}	1.47^{**}	0.0268^{**}
ECw (Linear)	1	6.48^{**}	3.09^{**}	9.13^{**}	0.18^{ns}	11.32^{ns}	44.97^{*}	62.47^{*}	0.050^{**}	0.74^{**}	0.131^{**}
ECw (Quadratic)	1	1.51^{ns}	0.002^{ns}	0.90^{ns}	0.20^{ns}	15.13^{*}	28.87^{ns}	30.17^{ns}	0.033^{*}	0.22^{ns}	0.009^{ns}
Bio (Linear)	1	1.18^{ns}	2.75^{**}	2.65^{ns}	0.89^{**}	27.73^{**}	11.96^{ns}	42.45^{ns}	0.008^{ns}	0.03^{ns}	0.113^{**}
Bio (Quadratic)	1	2.15^{*}	3.68^{**}	6.74^{**}	0.91^{**}	15.65^{*}	45.53^{*}	20.63^{ns}	0.031^{*}	0.15^{ns}	0.080^{**}
ECw x Bio	1	0.01^{ns}	0.45^{*}	0.28^{ns}	0.09^{ns}	4.71^{ns}	5.13^{ns}	7.20^{ns}	0.012^{ns}	0.14^{ns}	0.016^{*}
CV (%)		4.5	12.1	4.9	8.5	6.3	4.9	4.0	3.5	11.3	18.7

ECw – electrical conductivity of water; Bio – Biostimulant; DF - Degree of freedom; CV (%) - Coefficient of variation; (*) significant at $p \leq 0.05$ probability level; (**) significant at $p \leq 0.01$ probability level; (^{ns}) not significant.

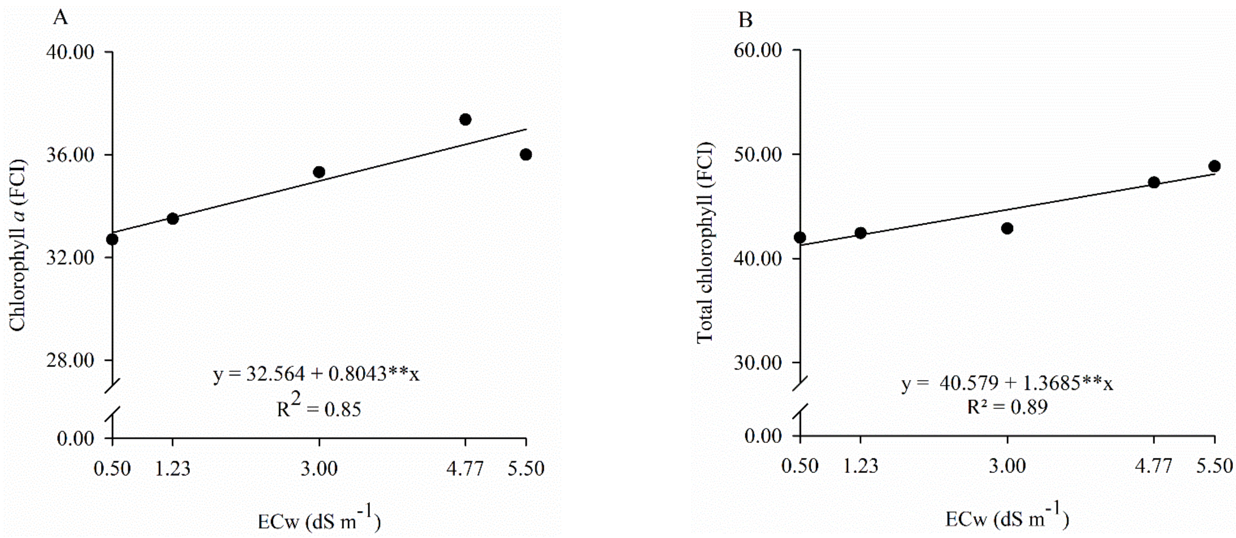
Chl *b* contents (Figure 2) were positively influenced by the foliar application of the biostimulant at a concentration of 6.30 mL L^{-1} associated with irrigation with water of 3.41 dS m^{-1} (15.03 FCI), exceeding by 55.27% the maximum value observed in plants without application of the treatment. On the other hand, the lowest value (3.72) was found under irrigation with water of 5.5 dS m^{-1} in plants without biostimulant application, indicating that the biostimulant reduces the harmful effect of salt stress on chlorophyll contents, which may be associated with the presence of amino acids and compounds that perform osmoprotective action in the composition of the seaweed *A. nodosum* (NÓBREGA et al., 2021), favoring the ability to transfer photochemical energy to the reaction centers in photosynthesis, so plants adapt even when subjected to stress conditions (OLIVEIRA et al., 2018).



X and Y – represent ECw = Electrical conductivity of irrigation water and biostimulant concentration, respectively. ** and * significant at $p \leq 0.01$ and $p \leq 0.05$; ns – Not significant.

Figure 2. Chlorophyll *b* contents of mint seedlings as a function of the interaction between irrigation water salinity levels and concentrations of biostimulants based on *Ascophyllum nodosum*.

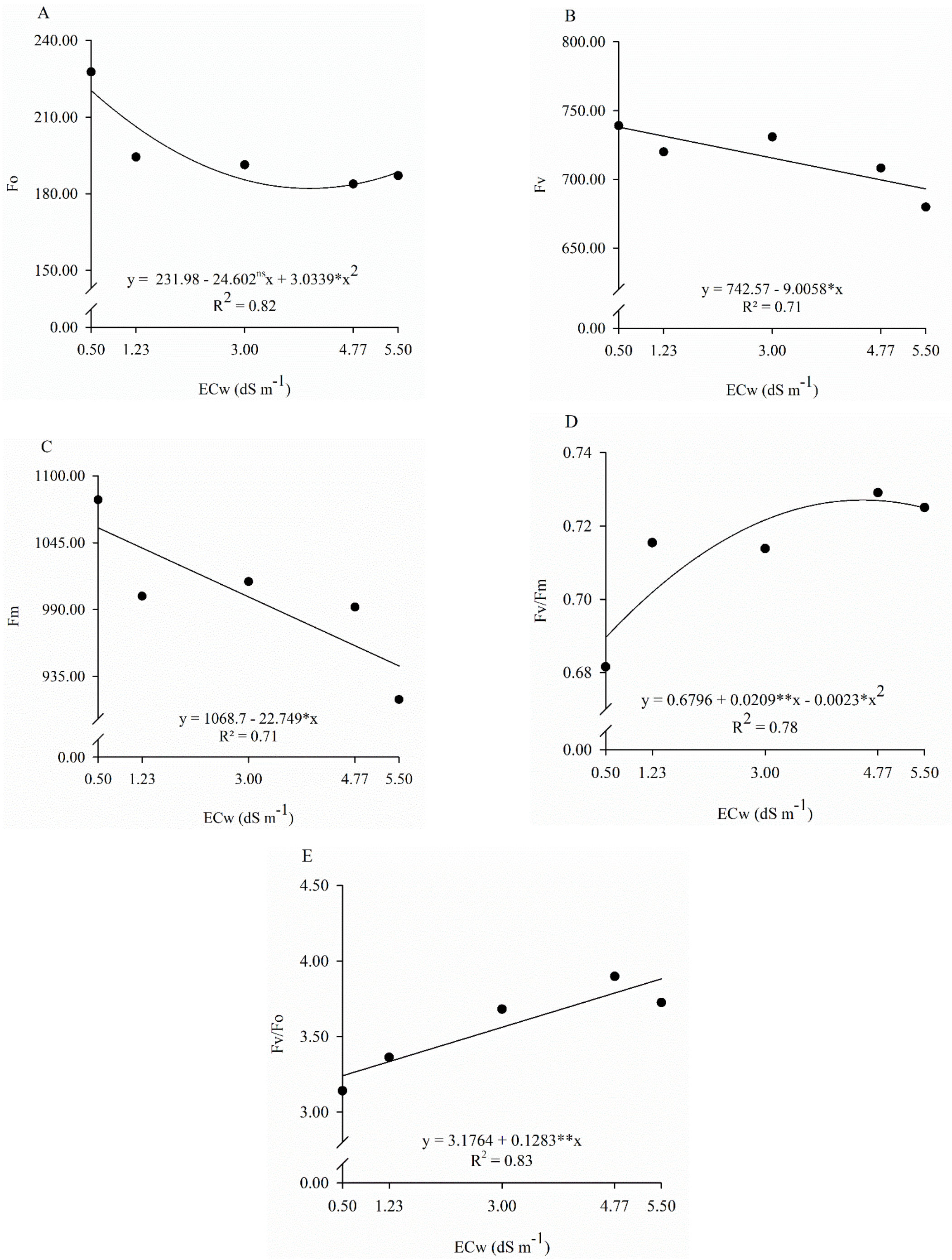
Irrigation water salinity contributed to increasing the chlorophyll *a* (Figure 3A) and total chlorophyll (Figure 3B) contents of mint plants at 70 DAS, with increments of 1.22 and 1.66% per unit increase in ECw. Plants subjected to ECw of 5.50 dS m^{-1} had increments in chlorophyll *a* and total chlorophyll contents of 12.20 and 16.58%, respectively, compared to those cultivated under water salinity of 0.5 dS m^{-1}. The increase in the contents of photosynthetic pigments may be associated with the adaptive mechanisms of plants, which tend to reduce their leaf area when subjected to stress, but that implies an increase in chlorophyll contents in the leaves (SOSNOWSKI et al., 2019).



**significant at $p \leq 0.01$

Figure 3. Contents of chlorophyll *a* - Chl *a* (A) and total chlorophyll - Chl *total* (B) of mint seedlings as a function of water salinity levels, at 70 DAS.

Water salinity reduced the initial fluorescence (F_0), with the maximum estimated value (220.43) being observed in plants under irrigation with ECw of 0.5 dS m^{-1}, followed by decreases with the increase in salinity levels, reaching the lowest value (182.11) under ECw of 4.0 dS m^{-1} (Figure 4A). The occurrence of this effect in F_0 is an indication that salt stress did not cause damage capable of affecting the photosynthetic apparatus of mint plants. Thus, possibly the period of exposure and the intensity of salt stress was not enough to damage the reaction centers of the photosystems (PSI and PSII) because, although salt stress causes damage to the photochemical efficiency of plants, some factors have a direct influence, such as the species and the intensity and duration of the stress conditions to which they are exposed (STEFANOVE et al., 2023).



** and * significant at $p \leq 0.01$ and $p \leq 0.05$ by the F test.

Figure 4. Initial fluorescence – F_0 (A), variable fluorescence – F_v (B), maximum fluorescence – F_m (C), quantum yield of photosystem II – F_v/F_m (D) and F_v/F_0 ratio (E) of mint seedlings as a function of water salinity levels, at 70 DAS.

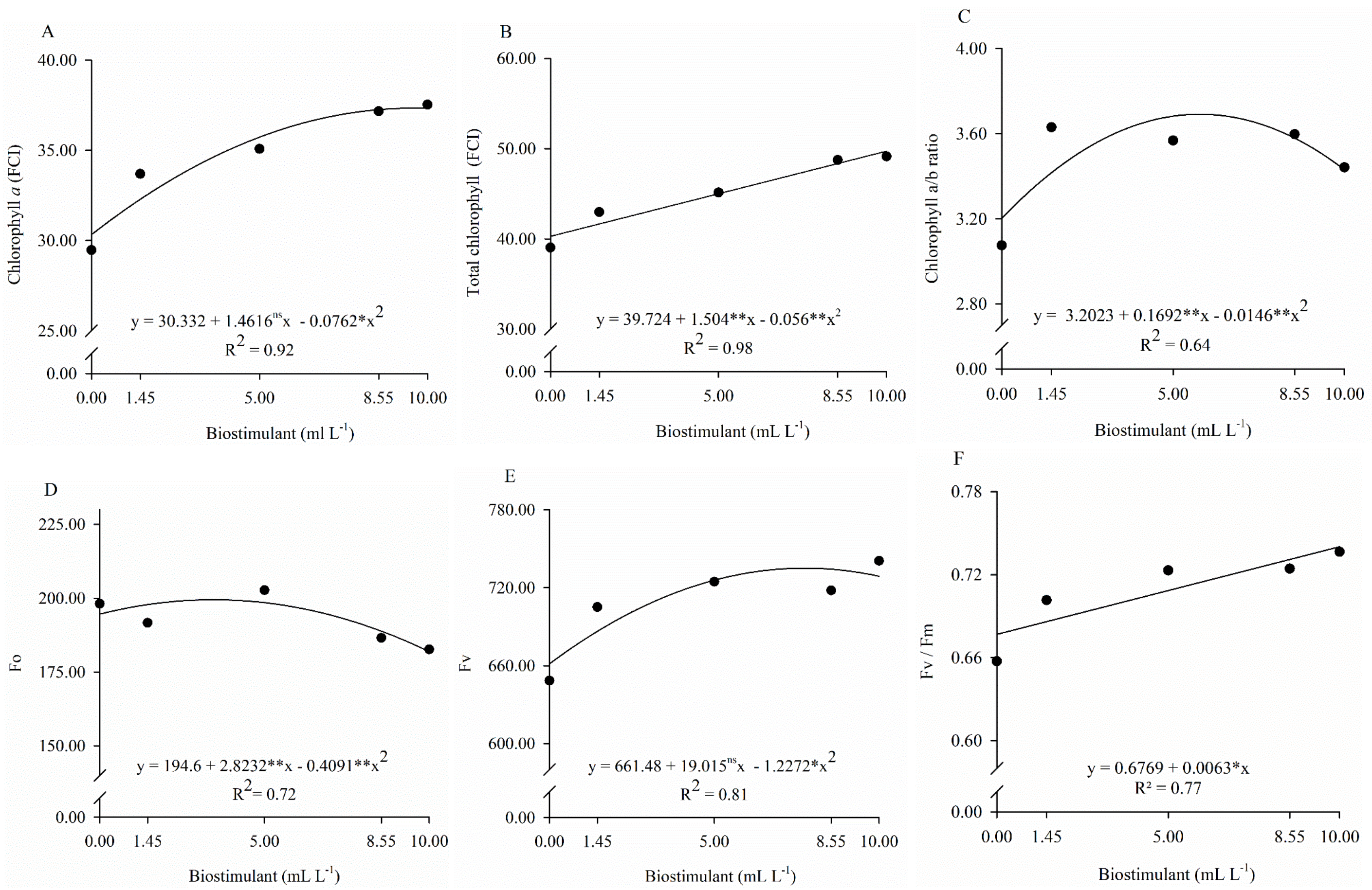
For variable fluorescence (F_v), a linear decrease was observed as the ECw levels increased, with a reduction of 0.61% per unit increase in water salinity (Figure 4B). The occurrence of this effect indicates that the increase in salinity, despite not having compromised the photosynthetic apparatus, limited the activation of the electron transport chain, resulting in the reduction of the photosynthetic capacity of plants, being associated with low efficiency in the dissipation of energy in the form of ATP and NADPH (LOTFI; GHASSEMI-GOLEZANI; PESSARAKLI 2020).

Salinity negatively affected maximum fluorescence (F_m), with a decrease of 1.07% per unit increase in water salinity, causing a reduction of 10.75% when comparing the values of plants subjected to the lowest and highest salinity (Figure 4C). This reduction is due to the excess accumulation of salts, which may have led to a deficiency in the photoreduction activity of quinone, which limits the influx of energy between the photosystems (LIMA et al., 2019).

The quantum yield of photosystem II (F_v/F_m) showed that mint plants were able to acclimatize to the stress conditions, with the highest value (0.7270) obtained at ECw of 4.5 dS m^{-1}, while the lowest value (0.6894) was obtained at the lowest ECw of 0.5 dS m^{-1} (Figure 4D). This effect is possibly associated with the plant's ability to acclimatize to the adverse conditions caused by salt stress, in order to be able to perform pigment synthesis and increase photochemical efficiency. This result was also observed by Nóbrega et al. (2023), who found that mint plants had the highest quantum efficiency of photosystem II when subjected to irrigation with water of 4.0 dS m^{-1}, values that are similar to those observed in the present study.

F_v/F_0 (Figure 4E), on the other hand, increased as the ECw increased, with an increment of 1.98 per unit increase in ECw. Regarding F_v/F_0, similar responses were found by Ounoki et al. (2021) in mint plants subjected to salinity of 50 mM NaCl, and these reductions are associated with changes in chlorophyll-protein complexes, especially in the fluorescence of the PSI complex.

The application of biostimulant increased the contents of chlorophyll *a* (Figure 5A), total chlorophyll (Figure 5B) and the chlorophyll *a*/*b* ratio (Figure 5C), with the highest values obtained (37.34, 49.16 and 3.69) when plants were subjected to concentrations of 9.6, 10 and 5.8 mL L^{-1}, promoting gains of 23.1, 23.8 and 15.31%, respectively, when compared to those without foliar application. The occurrence of this effect points to the beneficial effect of the application of the biostimulant on the synthesis of photosynthesizing pigments, which is directly associated with the seaweed extract and intrinsically related to the production of humic substances that can favor the increase in the contents of chlorophyll and photosynthetic pigments (LIMA NETO et al., 2018).



** and * significant at p ≤0.01 and p ≤0.05 by the F test.

Figure 5. Contents of chlorophyll *a* (A), total chlorophyll (B) and chlorophyll *a*/*b* ratio (C), initial fluorescence – F_0 (D), variable fluorescence (E) and quantum yield of photosystem II – F_v/F_m (F) of mint seedlings subjected to foliar application of biostimulant based on *Ascophyllum nodosum*, at 70 DAS.

Application of the biostimulant increased the F_0, with the maximum value (199.47) obtained in plants subjected to a dose of 3.45 mL L^{-1}, causing an increase of 2.5% compared to plants that did not receive foliar application of the biostimulant (Figure 5D). Despite having increased F_0, this effect is not harmful to mint plants; in addition, when the concentration of the biostimulant increased, F_0 was reduced, a result similar to that observed by Nóbrega et al. (2021) in *Physalis peruviana* L.

For F_v (Figure 5E), the maximum value (735.14) was obtained at the concentration of 7.75 mL L^{-1}, promoting a gain of 11.14% compared to that observed in the control (0.0 mL L^{-1}). A similar result was observed in the quantum yield of photosystem II (Figure 5F), with a linear increase as a function of the increase in the doses of the biostimulant, resulting in a maximum value of 0.74 at the dose of 10 mL L^{-1}, promoting a gain of 9.31% compared to that observed in plants without application of the biostimulant.

The occurrence of this behavior shows the beneficial action promoted by the biostimulant, which is directly associated with improvements in the synthesis of photosynthetic pigments, due to the presence of several compounds in its constitution, such as growth regulators, preserving the photosynthetic apparatus and stimulating the transfer of energy between the reaction centers, increasing the photochemical efficiency of the plant (NÓBREGA et al., 2021).

There was a significant effect of the interaction between irrigation water salinity levels and biostimulant concentrations on the variables relative growth rate in plant height, leaf dry mass, shoot dry mass, and Dickson quality index (Table 4). As a single factor, salinity had a significant effect on plant height, absolute and relative growth rates in stem diameter, root dry mass and stem dry mass. The biostimulant had an effect on all the variables evaluated.

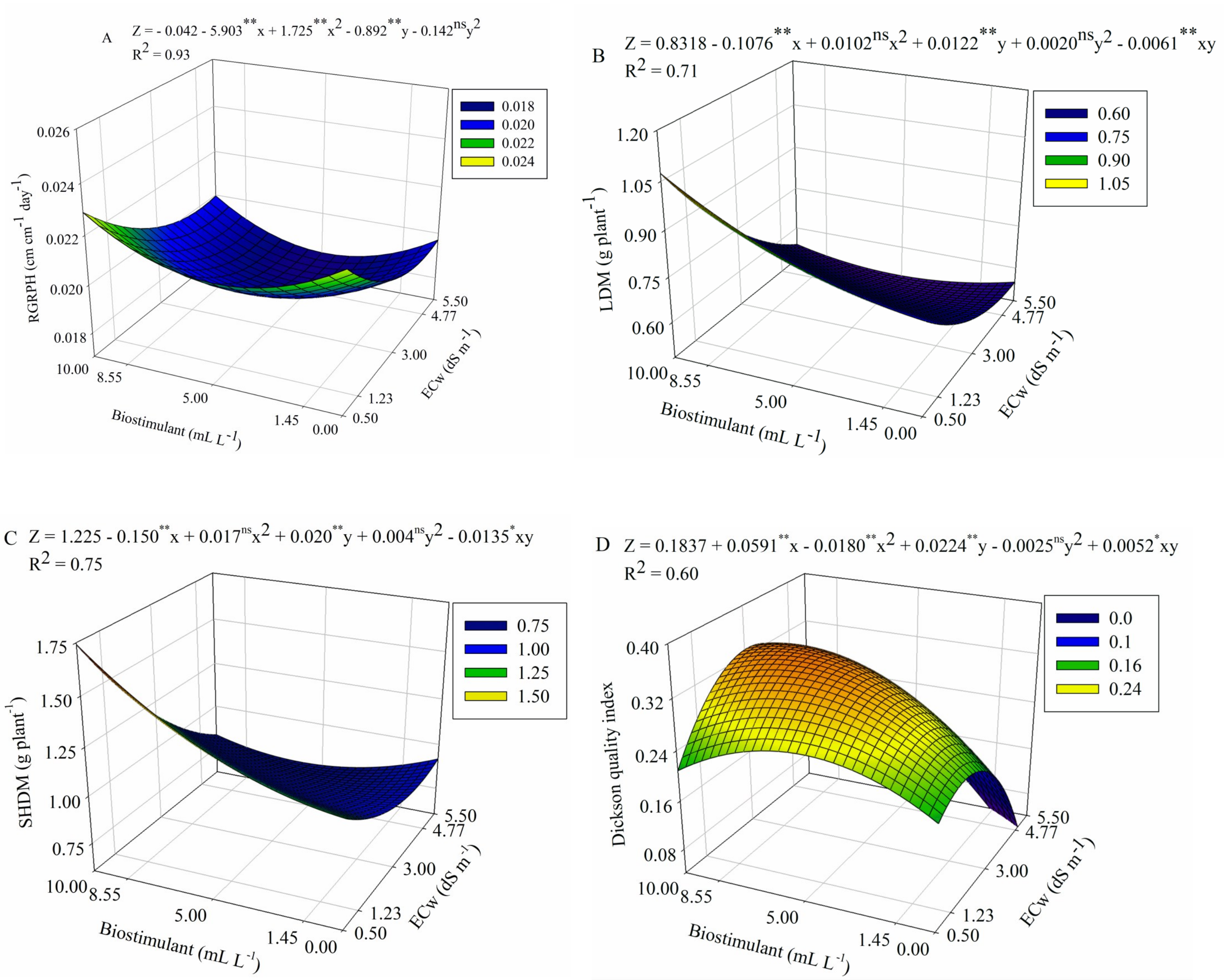
Table 4. Summary of the analysis of variance for plant height (PH), stem diameter (SD), absolute and relative growth rates in plant height (AGR_{PH} and RGR_{PH}) and stem diameter (AGR_{SD} and RGR_{SD}), leaf dry mass (LDM), root dry mass (RDM), stem dry mass (STDM), shoot dry mass (SHDM), root/shoot ratio (R/S) and Dickson quality index (DQI) of mint plants grown under saline water irrigation and application of biostimulant based on *Ascophyllum nodosum*, at 70 DAS.

Source of variation	DF	Mean Square										
		PH	SD	AGR_{PH}	RGR_{PH}	AGR_{SD}	RGR_{SD}	LDM	RDM	STDM	SHDM	DQI
Blocks	3	2.03^{ns}	0.04^{ns}	0.0003^{ns}	6.3e^{-7ns}	9.9e^{-7ns}	8.0e^{-7ns}	0.009^{ns}	0.14^{ns}	9.3e^{-4ns}	0.01^{ns}	0.0007^{ns}
Treatment	8	60.20**	0.52**	0.0096**	9.1e^{-6**}	1.6e^{-4ns}	3.9e^{-5**}	0.104**	2.88**	0.024**	0.24**	0.0268**
ECw (L)	1	2.40**	0.06^{ns}	0.005^{ns}	0.0002^{ns}	0.003**	0.003**	0.14**	1.24**	0.067**	0.25**	0.113**
ECw (Q)	1	0.81^{ns}	0.022^{ns}	0.013^{ns}	0.001^{ns}	0.006**	0.003**	0.01^{ns}	0.10^{ns}	0.017 ^{ns}	0.05^{ns}	0.080**
Bio (L)	1	9.81**	077**	0.126**	0.002**	0.014**	0.006**	0.38**	1.97**	0.191**	0.57**	0.131**
Bio (Q)	1	2.31**	0.02^{ns}	0.024**	4.8e^{-7ns}	4.3e^{-4ns}	2.8e^{-5ns}	0.03^{ns}	0.20^{ns}	0.024^{ns}	0.04^{ns}	0.009^{ns}
ECw x Bio	1	0.36^{ns}	0.02^{ns}	0.008^{ns}	0.0005*	2.3e^{-4ns}	1.3e^{-5ns}	0.02*	0.06^{ns}	0.002^{ns}	0.04**	0.016*
CV		4.3	9.5	4.8	6.6	13.9	16.3	9.0	17.4	11.0	9.8	18.7

ECw – electrical conductivity of water; Bio – biostimulant; DF - Degree of freedom; CV (%) - Coefficient of variation; (*) significant at $p \leq 0.05$ probability level; (**) significant at $p \leq 0.01$ probability level; (^{ns}) not significant.

For the relative growth rates (RGR_{PH}) in plant height (Figure 6A), it was observed that the foliar application of the biostimulant based on *A. nodosum* promoted improvements, with the maximum value (0.24 cm cm^{-1} day^{-1}) occurring in plants subjected to a dose of 0.35 mL L^{-1} and under salinity of 0.5 dS m^{-1}, whereas the lowest value (0.17 cm cm^{-1} day^{-1}) occurred in plants subjected to ECw of 5.5 dS m^{-1} and that did not receive foliar application of the biostimulant, which resulted in decreases of 38.2%. The beneficial effect of foliar application of the biostimulant is due to the presence of substances in the composition of *A. nodosum* seaweed that have an osmoprotective action, such as amino acids and growth regulators (NÓBREGA et al., 2021).

For leaf dry mass (Figure 6B) and shoot dry mass (Figure 6C), foliar application of 10 mL L^{-1} stimulated the production of biomass (1.07 and 1.74 g) under conditions of low salinity (0.5 dS m^{-1}), while the increase in ECw reduced these variables, with the lowest values (0.49 and 0.75 g) observed in plants subjected to ECw of 5.5 dS m^{-1} and at concentrations of 5.17 and 5.86 mL L^{-1}, causing reductions of 54.2 and 56.8%, respectively, when comparing the maximum and minimum values obtained. Application of exogenous substances such as the biostimulant based on *A. nodosum* may contribute to the processes of cell division and expansion, since the biostimulant contains plant growth regulators, which may have stimulated the production of biomass in mint plants under low salinity conditions. This has also been reported by Figueiredo et al. (2021), who found that foliar application promoted increases in gas exchange and growth of *P. peruviana* L. under salt stress conditions. In *Solanum aethiopicum* L., Fátima et al. (2024) observed that the application of 6.67 mL L^{-1} attenuated the effects of salinity on gas exchange and biomass production.

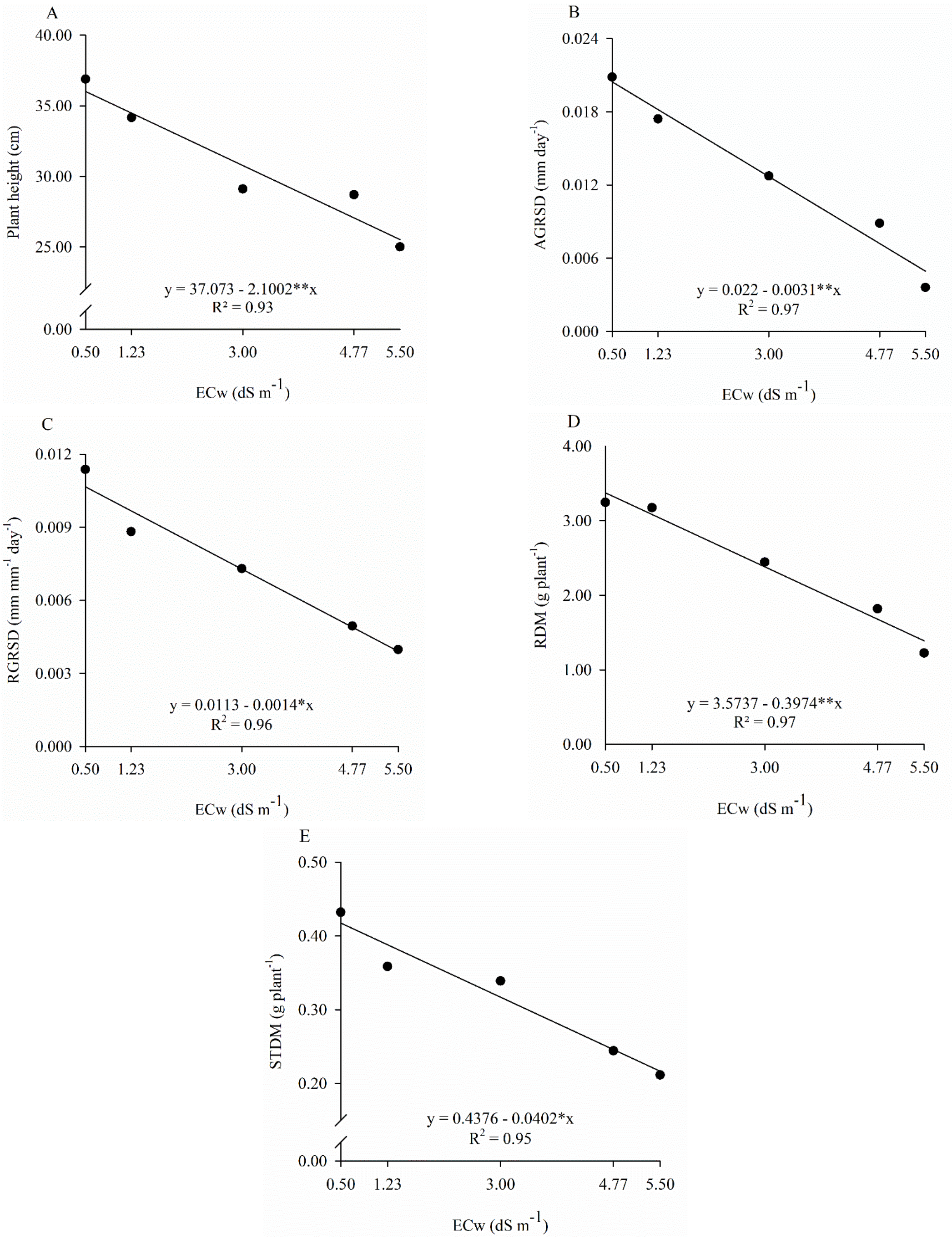


X and Y – represent ECw = Electrical conductivity of irrigation water and biostimulant concentration, respectively. ** and * significant at $p \leq 0.01$ and $p \leq 0.05$; ns – Not significant.

Figure 6. Relative growth rate in plant height - RGR_{PH} (A), leaf dry mass (B), shoot dry mass (C) and Dickson Quality Index - (D) of mint seedlings as a function of the interaction between salinity levels of irrigation water and concentrations of biostimulants based on *Ascophyllum nodosum*, at 70 DAS.

In general, it can be seen that the application of the biostimulant resulted in an increase in DQI under all conditions of irrigation with saline water, with the highest value (0.35) observed at the biostimulant concentration of 7.31 mL L^{-1} and in plants irrigated with water of 2.71 dS m^{-1} (Figure 6D), promoting an increase of 42.85% compared to plants subjected to the lowest salinity (0.5 dS m^{-1}). These values are within the limits established (>0.2) by Dickson, Leaf and Hosner (1960) as indicators of quality seedlings. This positive effect on the quality of mint seedlings comes from the improvement in the physiological state, directly contributing to biomass production and consequently to plants with greater vigor (PARMAR et al., 2023), which is possibly associated with the presence of substances in the composition of the biostimulant, such as macro and micronutrients and phytohormones that regulate physiological processes, resulting in improvements in plant growth (NASIRI et al., 2025).

Salt stress compromised the growth of mint plants, and linear reductions were observed as ECw increased, causing a decrease in plant height of 2.92% per unit increase in ECw and a decrease of 29.2% when comparing the values obtained at the highest and lowest salinity levels (Figure 7A). This reduction in growth rates is due to the water restriction imposed by the reduction in the osmotic potential of the soil, resulting in disturbances in physiological processes, including the processes of cell division and expansion (NÓBREGA et al., 2022). In addition, the toxicity of some ions such as Na^{+} and Cl^{-}, contribute to reducing cellular processes, compromising photosynthetic activity and, consequently, plant growth (NOOR et al., 2024).

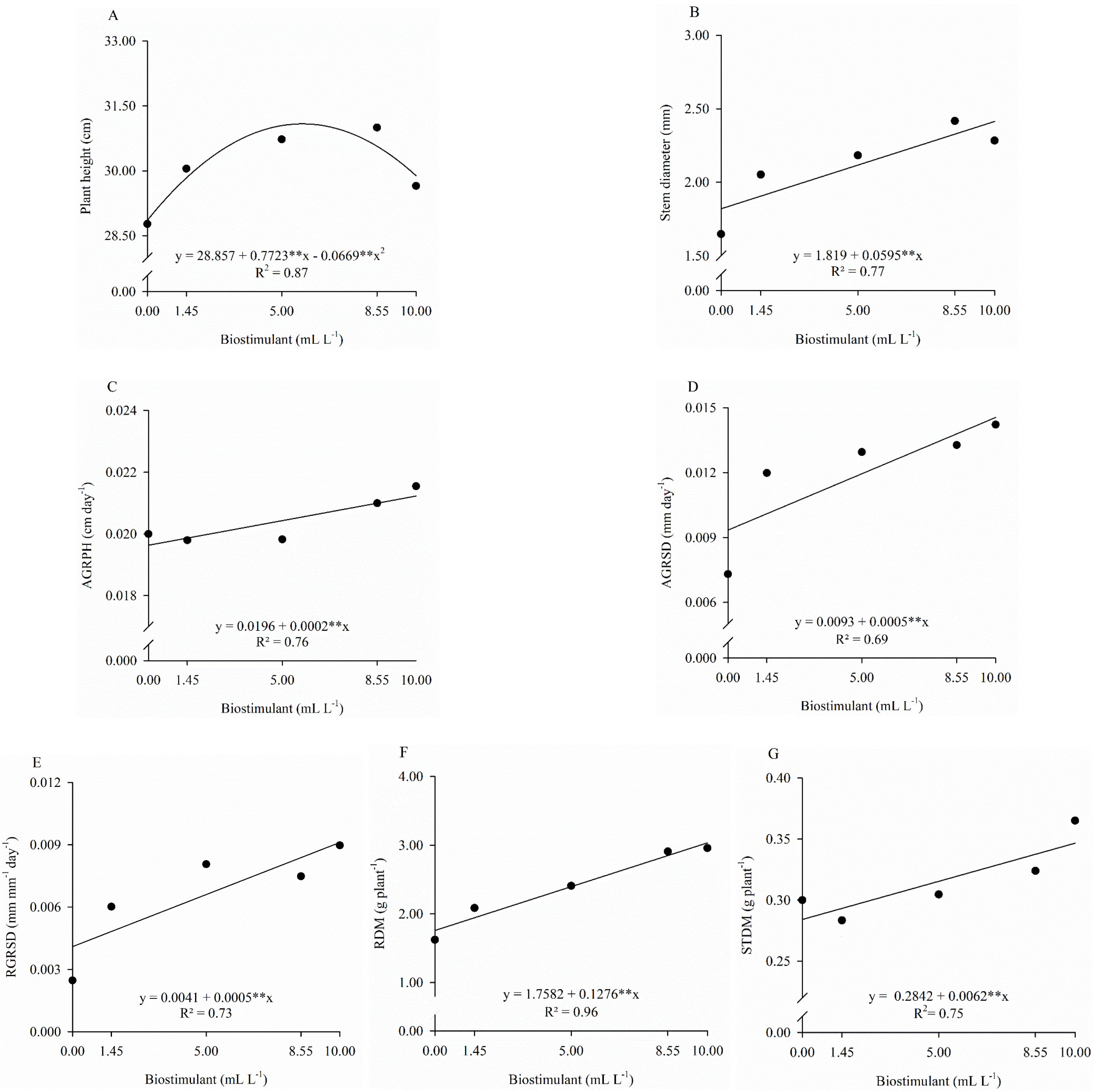


** and * significant at p ≤0.01 and p ≤0.05 by the F test.

Figure 7. Plant height (A), absolute (B) and relative (C) growth rates in stem diameter, root dry mass (D) and stem dry mass (E) of mint seedlings subjected to different levels of salinity of irrigation water at 70 DAS.

The absolute (AGR_{SD}) and relative (RGR_{SD}) growth rates were reduced with the increase in salinity levels, with decreases of 7.57 and 6.60% per unit increase in ECw, resulting in decreases of 75.8 and 66% when comparing the values obtained at the highest and lowest salinity (5.5 and 0.5 dS m^{-1}), respectively (Figures 7B and 7C). The same occurred for root dry mass and stem dry mass (Figures 7D and 7E), as the increase in ECw caused reductions of 58.9 and 48.1%, respectively, when comparing the values obtained at the highest and lowest salinity. The this severe inhibition in growth rates and biomass production is a consequence of the osmotic, ionic and oxidative effects triggered by the excess of salts in the irrigation water, which compromise the production of photoassimilates by the plant, due to a higher energy expenditure to maintain membrane integrity, as well as promoting the production of organic substances that act in cell osmoregulation, compromising both the growth and the dry mass accumulation in plants (QUEIROGA et al., 2023).

The isolated effect of the foliar application of the biostimulant was beneficial for plant height (Figure 8A), and a quadratic effect was observed as the concentration increased, with the maximum value (31.09 cm) in plants that received 5.8 mL L^{-1} of the biostimulant, promoting an increase of 7.20% compared to the lowest concentration, which led to the lowest value (28.85 cm). For stem diameter, in turn, there was a linear increase as a function of the foliar application of the biostimulant, with increments of 3.27% per unit increase in concentration, resulting in gains of 32.7% when comparing the values obtained at the highest (10 mL L^{-1}) and lowest (0 mL L^{-1}) concentration (Figure 8B).



** and * significant at p ≤0.01 and p ≤0.05 by the F test.

Figure 8. Plant height (A), stem diameter (B), absolute growth rate in plant height (C), absolute (D) and relative (E) growth rates in stem diameter (F), root dry mass (F) and stem dry mass (G) of mint seedlings subjected to foliar application of biostimulant based on *Ascophyllum nodosum*, at 70 days DAS.

For the absolute growth rate in plant height (AGR_{PH}) and absolute (AGR_{SD}) and relative (RGR_{SD}) growth rates in stem diameter, foliar application of the biostimulant based on *A. nodosum* promoted linear increments with the increase in concentration, leading to gains of 10.2, 53.76 and 121.95%, respectively, when comparing the values obtained at the highest (10 mL L^{-1}) and lowest (0.0 mL L^{-1}) concentration (Figures 8C, 8D and 8E). The increase in growth is a consequence of the presence of regulators in the composition of *A. nodosum*, which act by regulating physiological processes, including cell division and expansion (GOMES et al., 2024), consequently promoting higher growth rates in mint plants.

Similar results were observed for root dry mass (Figure 8F) and stem dry mass (Figure 8G), with linear increments as a function of the increase in the concentration of the biostimulant, promoting gains of 72.57 and 21.81% when comparing the values obtained at the highest and lowest concentration. Seaweed-based biofertilizers have the ability to promote direct improvements in plant growth, through the presence of organic acids in their constitution, increasing P absorption capacity and supplying N to the plant (DEEPIKO; ALI, 2020).

Beneficial effect of the *A. nodosum*-based biostimulant on plant growth has been reported by other authors, such as Fátima et al. (2024) in scarlet eggplant (*S. aethiopicum* L.), with the use of up to 6.67 mL L^{-1} being able to improve gas exchange and growth under salt stress conditions. Vila et al. (2023) found that the application of 2.0 mL of the biostimulant promoted an increase in growth and yield in tomato (*Solanum lycopersicum* L.). Shakya et al. (2023) in strawberry (*Fragaria x anamassa*) observed that the application of 3.0 mL L^{-1} promoted an increase in plant biomass and in the number of flowers and fruits when plants were subjected to 50% water deficit, and Nóbrega et al. (2021) found in Cape gooseberry (*P. peruviana* L.) that the application of up to 9.9 mL L^{-1} promoted an increase in biomass production and seedling quality.

CONCLUSIONS

Irrigation water salinity above 0.5 dS m^{-1} compromises the photochemical efficiency, growth and biomass production of mint seedlings.

Foliar application of the biostimulant based on *A. nodosum* up to 3.14 mL L^{-1} is efficient in attenuating the deleterious effects caused by salt stress on the contents of photosynthetic pigments, growth and quality of mint plants.

REFERENCES

BENINCASA, M. M. P. **Análise de crescimento de plantas, noções básicas**. 2. ed. Jaboticabal, SP: FUNEP, 2003. 41 p.

DEEPIKO, P.; ALI, D. M. Production and assessment of microalgal liquid fertilizer for the enhanced growth of four crop plants. **Biocatalysis and Agricultural Biotechnology**, 28: 101701, 2020.

DICKSON, A.; LEAF, A. L.; HOSNER, J. F. Quality appraisal of white spruce and white pine seedling stock in nurseries. **Forestry Chronicle**, 36: 10-13, 1960.

FÁTIMA, R. T. et al. Seaweed extract biofertilizer modulates scarlet eggplant tolerance to salt stress. **Arid Land Research and Management**, 38: 46-61, 2024.

FIGUEIREDO, F. R. A. et al. Impact of biostimulant and saline water on cape gooseberry (*Physalis peruviana* L.) in Brazil. **Physiology and Molecular Biology of Plants**, 27: 2141-2150, 2021.

GOMES, F. A. L. et al. Morphophysiology of *Punica granatum L.* under microalgae biomass stimulation. **Comunicata Scientiae**, 15: e4142, 2024.

GUEDES, M. A. et al. Hydroponic cultivation of laranja cherry tomatoes under salt stress and foliar application of hydrogen peroxide. **Agriculture**, 13: 1688, 2023.

LIMA NETO, A. J. D. et al. Biometric variables and photosynthetic pigments in tamarind seedlings irrigated with saline water and biofertilizers. **Semina: Ciências Agrárias**, 39: 1909-1920, 2018.

LIMA, G. S. et al. Eficiência fotoquímica, partição de fotoassimilados e produção do algodoeiro sob estresse salino e adubação nitrogenada. **Revista de Ciências Agrárias**, 42: 214-225, 2019.

LOTFI, R.; GHASSEMI-GOLEZANI, K.; PESSARAKLI, M. Salicylic acid regulates photosynthetic electron transfer and stomatal conductance of mung bean (*Vigna radiata* L.) under salinity stress. **Biocatalysis and Agricultural Biotechnology**, 26: 101635, 2020.

MAHANTY, T. et al. Biofertilizers: a potential approach for sustainable agriculture development. **Environmental Science and Pollution Research**, 24: 3315-3335, 2017.

MAHENDRAN, G.; RAHMAN, L. U. Ethnomedicinal, phytochemical and pharmacological updates on Peppermint (*Mentha × piperita* L.) - A review. **Phytotherapy Research**, 34: 2088-2139, 2020.

NASIRI, Y. et al. Effect of Ascophyllum nodosum extract and chemical fertilizers on the growth, yield and composition of *Satureja hortensis* L. essential oil. **Pesquisa Agropecuária Tropical**, 55: e81275, 2025.

NÓBREGA, J. S. et al. Photochemical efficiency, biomass and chlorophyll of phyális under salinity and biostimulant. **Revista Brasileira de Ciências Agrárias**, 16: 1-7, 2021.

NÓBREGA, J. S. et al. Ecophysiology of *Mesosphaerum suaveolens* (L.) Kuntze (Lamiaceae) under saline stress and salicylic acid. **Ciência Rural**, 52: e20210389, 2022.

NÓBREGA, J. S. et al. Ecophysiology of *Mentha piperita* under saline stress and biostimulant in the Brazilian semiarid region. **Revista Brasileira de Ciências Agrárias**, 18: e2203, 2023.

NOOR, J. et al. Enhancing saline stress tolerance in soybean seedlings through optimal NH_4^+/NO_3^- ratios: a coordinated regulation of ions, hormones, and antioxidant potential. **BMC Plant Biology**, 24: 572, 2024.

OLIVEIRA, F. Í. F. et al. Biomass and chloroplast pigments in jackfruit seedlings under saline stress and nitrogen fertilization. **Revista Caatinga**, 31: 622-631, 2018.

OUNOKI, R. et al. Salt stress affects plastid ultrastructure and photosynthetic activity but not the essential oil composition in spearmint (*Mentha spicata* L. var. crispa "Moroccan"). **Frontiers in Plant Science**, 12: 2253, 2021.

PARMAR, P. et al. Microalgae as next generation plant growth additives: Functions, applications, challenges and circular bioeconomy based solutions. **Frontiers in Plant Science**, 14: 1073546, 2023.

QUEIROGA, C. M. et al. Formation of guava seedlings under irrigation with water of different cationic natures and salicylic acid. **Revista Caatinga**, 36: 650-662, 2023.

RICHARDS, L. A. **Diagnosis and improvement of saline and alkali soils**. 1. ed. Washington: U.S. Department of Agriculture, 160 p. (Agriculture Handbook, 60), 1954.

RODRIGUES, M. H. B. S. et al. Photochemical efficiency and growth of moringa irrigated with brackish water and foliar application of biostimulant. **Water Resources and Irrigation Management**, 14: 1-13, 2025.

SHAKYA, R. et al. Effect of two biostimulants, based on *Ascophyllum nodosum* extracts, on strawberry performance under mild drought stress. **Agriculture**, 13: 2108, 2023.

SILVA, J. M. et al. Avaliação e espacialização de parâmetros físico-químicos em águas subterrâneas na bacia hidrográfica do rio Piauí Nordeste, Brasil. **Scientia Plena**, 20: 097201, 2024.

SOSNOWSKI, J. et al. Effect of *Ecklonia maxima* extract on photosynthesis activity and chlorophyll content of *Medicago × varia* Martyn leaves. **Chilean Journal of Agricultural Research**, 79: 257-265, 2019.

STEFANOV, M. A. et al. Protective effects of sodium nitroprusside on photosynthetic performance of *Sorghum bicolor* L. under salt stress. **Plants**, 12: 832, 2023.

TEIXEIRA, P. C. et al. **Manual de métodos de análise de solo**. 3.ed. Brasília, DF: Embrapa, 2017. 573 p.

VILA, V. V. et al. Seaweed extract of *Ascophyllum nodosum* applied in tomato crop as a biostimulant for improving growth, yield and soil fertility in subtropical condition. **Journal of Applied Phycology**, 35: 2531-2541, 2023.

ZAFAR, Z. et al. Acclimatization of *Terminalia Arjuna* saplings to salt stress: characterization of growth, biomass and photosynthetic parameters. **Journal of Sustainable Forestry**, 39: 76-91, 2020.