











## Algae biostimulant improves salt tolerance and visual quality of tropical ornamental herbaceous species<sup>1</sup>

### Bioestimulante de algas melhora a tolerância à salinidade e a qualidade visual de espécies herbáceas ornamentais tropicais

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#### HIGHLIGHTS:

Salinity negatively affects the morphophysiology of *Catharanthus roseus*.

Salinity up to 2.5 dS m<sup>-1</sup> favors the visual quality of *Celosia argentea*.

Extract of seaweed *Ascophyllum nodosum* partially mitigates the harmful effects of salt stress.

**ABSTRACT:** The use of biostimulants may mitigate the deleterious effects of salt stress on plant growth and physiological variables. Thus, the present study aimed to evaluate the growth, physiology, organic solutes, and visual characteristics of two tropical ornamental species (*Celosia argentea* and *Catharanthus roseus*) cultivated with brackish waters and treated with *Ascophyllum nodosum* extract. The experiment was performed in greenhouse conditions. The experimental design was a complete randomized block arranged in a split-plot scheme. The plots comprised three levels of electrical conductivity of the irrigation water (EC<sub>w</sub> - 0.5, 2.5, and 4.5 dS m<sup>-1</sup>), the subplots four concentrations of algae extract (0, 400, 800, and 1200 mg L<sup>-1</sup>), and the sub-subplots two tropical ornamental herbaceous species [*C. argentea* and *C. roseus*]. The salinity of the irrigation water negatively affects the morphological and physiological characteristics of the two species studied, mainly *C. roseus*. Irrigation with water up to 2.5 dS m<sup>-1</sup> favored visual quality and increased the purchasing preference for *C. argentea*. The use of intermediate concentrations (400 mg L<sup>-1</sup>) of algae extract (*A. nodosum*) promoted partial mitigation of the effects of salt stress on biomass production and leaf gas exchange, especially for *C. argentea*, but these positive responses decreased or disappeared with the intensification of salt stress and the increase in algae extract concentration.

**Key words:** *Ascophyllum nodosum*, irrigation, ornamental plants, photosynthesis, salt stress

**RESUMO:** O uso de bioestimulantes pode mitigar os efeitos deletérios do estresse salino no crescimento e variáveis fisiológicas. Assim, objetivou-se com o presente estudo avaliar o crescimento, fisiologia, solutos orgânicos e características visuais de duas espécies ornamentais tropicais (*Celosia argentea* e *Catharanthus roseus*) cultivadas utilizando-se águas salobras e tratadas com extrato de *Ascophyllum nodosum*. O experimento foi conduzido em casa de vegetação, em delineamento experimental em blocos ao acaso, dispostos em parcelas subdivididas, sendo as parcelas constituídas por três níveis de condutividade elétrica da água de irrigação (CE<sub>a</sub> - 0,5, 2,5 e 4,5 dS m<sup>-1</sup>), as subparcelas quatro concentrações de extrato de algas (0, 400, 800 e 1200 mg L<sup>-1</sup>) e as subsubparcelas de duas espécies herbáceas ornamentais tropicais: *C. argentea* e *C. roseus*. A salinidade da água de irrigação afeta negativamente as características morfológicas e fisiológicas das duas espécies estudadas, principalmente *C. roseus*. A irrigação com água de até 2,5 dS m<sup>-1</sup> favoreceu a qualidade visual e aumentou a preferência de compra por *C. argentea*. O uso de concentrações intermediárias (400 mg L<sup>-1</sup>) de extrato de alga (*A. nodosum*) promoveu mitigação parcial dos efeitos do estresse salino na produção de biomassa e nas trocas gasosas foliares, especialmente para *C. argentea*, mas essas respostas positivas diminuíram ou desapareceram com a intensificação do estresse salino e com o aumento da concentração do extrato de algas.

**Palavras-chave:** *Ascophyllum nodosum*, irrigação, plantas ornamentais, fotossíntese, estresse salino

## INTRODUCTION

Irrigated agriculture accounts for 70% of freshwater withdrawals worldwide (FAO, 2018). This represents a major challenge for the agricultural sector, given the effects of climate change and the lack of more effective recycling policies, which can make water resources increasingly limited qualitatively and quantitatively (Rosenzweig et al., 2020; Leal Filho et al., 2022).

Among the productive sectors that depend on irrigation, ornamental gardens and landscaped areas could be irrigated mainly with brackish or municipal-treated wastewater, especially during the dry season (García-Caparrós & Lao, 2018; Neves et al., 2018; Oliveira et al., 2018; Markovic et al., 2022).

The *Celosia argentea* L. species belongs to the Amaranthaceae family and is an important tropical ornamental species; it is appreciated for its beauty and is often used in ornamental gardens and floral arrangements (Alam et al., 2022). *Cataranthus roseus*, belonging to the Apocynaceae family, is highly valued in the horticultural industry, often planted in gardens, flowerbeds, or pots as summer flowering plants (Markovic et al., 2022).

The use of brackish water for irrigation can help conserve freshwater sources, which are essential for human supply and aquatic ecosystems. This is particularly relevant in regions where freshwater is limited, as in the Brazilian semi-arid region (Cavalcante et al., 2022). In addition, the integrated use of management practices can mitigate the effects of salinity (García-Caparrós & Lao, 2018).

These cultivation practices include the use of nutrients in organic or mineral form and the use of biostimulants (Lacerda et al., 2021). Biostimulants based on algae extracts have been used in agriculture in several regions of the world with beneficial effects on crop yield, notably under conditions of biotic and abiotic stresses (Barone et al., 2018; Kopta et al., 2018; Fernandes et al., 2019). However, responses to the use of biostimulants are quite variable depending on the application method, dose, environmental conditions, and the type of plant evaluated (Carvalho & Castro, 2014).

The little information in the literature indicates the need to expand studies with biostimulants in ornamental species, especially in systems irrigated with brackish water. Thus, the objective of the present study was to evaluate the growth, physiology, organic solutes, and visual characteristics of two tropical ornamental species (*C. argentea* and *C. roseus*) cultivated with brackish waters and treated with *Ascophyllum nodosum* extract.

## MATERIAL AND METHODS

The experiment was conducted from June to August 2021 in a greenhouse in Fortaleza (3° 44' 44" S and 38° 34' 50" W, 20 m a.s.l.), Ceará, Brazil. The temperature and relative air humidity data were monitored using a Data Logger (model HOBO<sup>®</sup> U12-012 Temp/RH/Light/Ext). The mean air temperature ranged from 27.4 to 31.1 °C, while the mean relative air humidity of air ranged from 56.7 to 67.5%. The average values of daily light ranged from 13,739.5 to 22,474.8 Lux. Due to Fortaleza's

proximity to the Equator, the photoperiod was close to 12 hours of light per day throughout the experimental period.

The experimental design was randomized blocks arranged in a split-plot scheme with four replications. The plots comprised three levels of electrical conductivity of the irrigation water (EC<sub>w</sub> - 0.5, 2.5, and 4.5 dS m<sup>-1</sup>), the subplots comprised four concentrations of algae extract (0, 400, 800, and 1200 mg L<sup>-1</sup>), and the sub-subplots two species of tropical ornamental plants (*C. argentea* var. Plumosa and *C. roseus*). These species are moderately salt tolerant and moderately salt sensitive, respectively (Bezerra et al., 2020). Each experimental unit consisted of three plants, with one plant per pot.

The preparation of saline treatments was carried out using salts of NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O, and MgCl<sub>2</sub>·6H<sub>2</sub>O, in an equivalent proportion of 7:2:1, among the cations Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>, following the relationship between EC<sub>w</sub> and its concentration (EC<sub>w</sub> = mmol<sub>c</sub> L<sup>-1</sup>/10). Sowing was carried out in polyethylene pots with a capacity of 700 mL, filled with a substrate composed of fine sand and vermicompost in the proportion of 2:1 (v/v), respectively.

The substrate had the following chemical attributes: organic matter - 14.07 g kg<sup>-1</sup>; N - 0.89 g kg<sup>-1</sup>; Ca<sup>2+</sup> - 4.3 cmol<sub>c</sub> kg<sup>-1</sup>; K<sup>+</sup> - 0.83 cmol<sub>c</sub> kg<sup>-1</sup>; Mg<sup>+</sup> - 1.9 cmol<sub>c</sub> kg<sup>-1</sup>; Na<sup>+</sup> - 0.36 cmol<sub>c</sub> kg<sup>-1</sup>; sum of exchangeable bases - 7.39 cmol<sub>c</sub> kg<sup>-1</sup>; cation exchange capacity - 8.88 cmol<sub>c</sub> kg<sup>-1</sup>; base saturation - 83%; P - 342 mg kg<sup>-1</sup>; pH - 6.3; and electrical conductivity of the soil saturation extract - 1.76 dS m<sup>-1</sup>.

Twelve days after sowing (DAS), thinning was performed, leaving one plant per pot. Fertilization was carried out with nitrogen, phosphorus, and potassium using the 10-10-10 NPK formulation (1.0 g per pot), using urea (45% N), single superphosphate (18% P<sub>2</sub>O<sub>5</sub>, 25% CaO, and 12% S), and potassium chloride (62% of K<sub>2</sub>O), according to Bezerra et al. (2020). The seedlings were irrigated with low-salinity water (0.5 dS m<sup>-1</sup>) up to 14 days after sowing.

The foliar spray with algae extract began at 14 DAS and was performed weekly with a total of five applications. 10 mL of the extract was applied in each application. The four concentrations of the algae extract (0, 400, 800, and 1200 mg L<sup>-1</sup>) were defined based on the recommendation proposed by the manufacturer. The solutions presented the electrical conductivity of 0, 0.2, 0.4, and 0.6 dS m<sup>-1</sup>, respectively.

A commercial algae *Ascophyllum nodosum* (Alga95<sup>®</sup>) extract was used as a water-soluble powder. This extract had a pH between 9.0 and 10.5 and contained 20% soluble potassium and 1% soluble nitrogen as macronutrients, chelated micronutrients, carbohydrates (alginates and mannitol), amino acids (alanine and proline), antioxidant substances, plant growth stimulants (auxin and cytokinin), and soluble sodium (3-5%).

From 15 to 50 DAS, all plants were irrigated with their respective treatments of water salinity. This period was defined based on the time required to produce plants for commercialization. Irrigations were performed manually every other day, and the drainage lysimeter method determined the amount of water, in which the water volume to be applied in irrigation was estimated through the difference between the volume of water applied and the volume drained in the last

irrigation event. In each irrigation, a leaching fraction of 0.15 was adopted to avoid the excessive accumulation of salts in the root zone.

At 51 DAS, the following physiological variables were measured: stomatal conductance (gs, mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), photosynthetic rate (A, μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), and leaf transpiration (E, mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>). Measurements were performed on fully expanded leaves using a portable infrared gas analyzer (model LI-6400XT, Licor, USA) with a light intensity of 1400 μmol m<sup>-2</sup> s<sup>-1</sup>. The readings were taken between 08:00 a.m. and 10:00 a.m., under ambient temperature, CO<sub>2</sub> concentration (400 μmol mol<sup>-1</sup>), and relative air humidity.

Stem diameter (SD) measurements were performed approximately 2 cm above the soil surface. The plants were collected and separated into flowers, leaves, stems, and roots. The leaves were measured in a leaf-area integrator (Area meter, LI-3100, Licor, USA) to obtain leaf areas (LA, cm<sup>2</sup> plant<sup>-1</sup>). The plant material was placed in a forced-air oven adjusted at 65 °C until it reached constant weight to obtain the dry mass production. The determinations of free proline and soluble carbohydrates were performed in aqueous extracts using the methods described by Bates et al. (1973) and Dubois et al. (1956), respectively.

For the sensory analysis (visual quality), 24 plants were randomly selected, one for each treatment. Sensory analysis was performed following the methodology described by Ureña et al. (1999), adapted for qualitative assessment of salt damages (Neves et al., 2018). A hedonic scale with nine numerical points was used: 1. I disliked it extremely; 2. I disliked it a lot; 3. I disliked it moderately; 4. I disliked it slightly; 5. indifferent; 6. I liked it slightly; 7. I liked it moderately; 8. I liked it a lot, and 9. I liked it extremely. A total of 156 judges recorded their opinions according to this scale, and the scores assigned to the plants were converted into weighted averages.

The data were subjected to analysis of variance (p≤0.05), and the means were compared by the Tukey test (p≤0.05). The effects of algae extract concentrations were tested by regression analysis, adopting the mathematical model based on the value of the determination coefficient. Statistical analyses were performed using the software ASSISTAT (Silva & Azevedo, 2016).

## RESULTS AND DISCUSSION

Based on the results obtained with the analysis of variance, it can be seen that there was a significant interaction between the factors evaluated for all the variables analyzed. The algae extract concentrations that resulted in the best leaf gas exchange also varied with the plant species. For the stomatal conductance (gs) of *C. argentea*, the quadratic polynomial model best fitted the data, in which the ECw of 0.5, 2.5, and 4.5 dS m<sup>-1</sup> corresponded to the maximum gs of 0.18, 0.097, and 0.048 (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) for the algae extract concentrations of 556, 875, and 625 mg L<sup>-1</sup> (Figure 1A), respectively. However, for *C. roseus* (Figure 1B), the increase of the algae extract concentration, when combined with the ECw of 2.5 and 4.5 dS m<sup>-1</sup>, resulted in linear decreases of gs of up to 34.1 and 86.1% when comparing the lowest (0 mg L<sup>-1</sup>) with the highest concentration of algae extract (1200 mg L<sup>-1</sup>).

The ECw of 0.5 dS m<sup>-1</sup> promoted a maximum gs of 0.41 (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) when combined with the application of 625 mg L<sup>-1</sup> of algae extract.

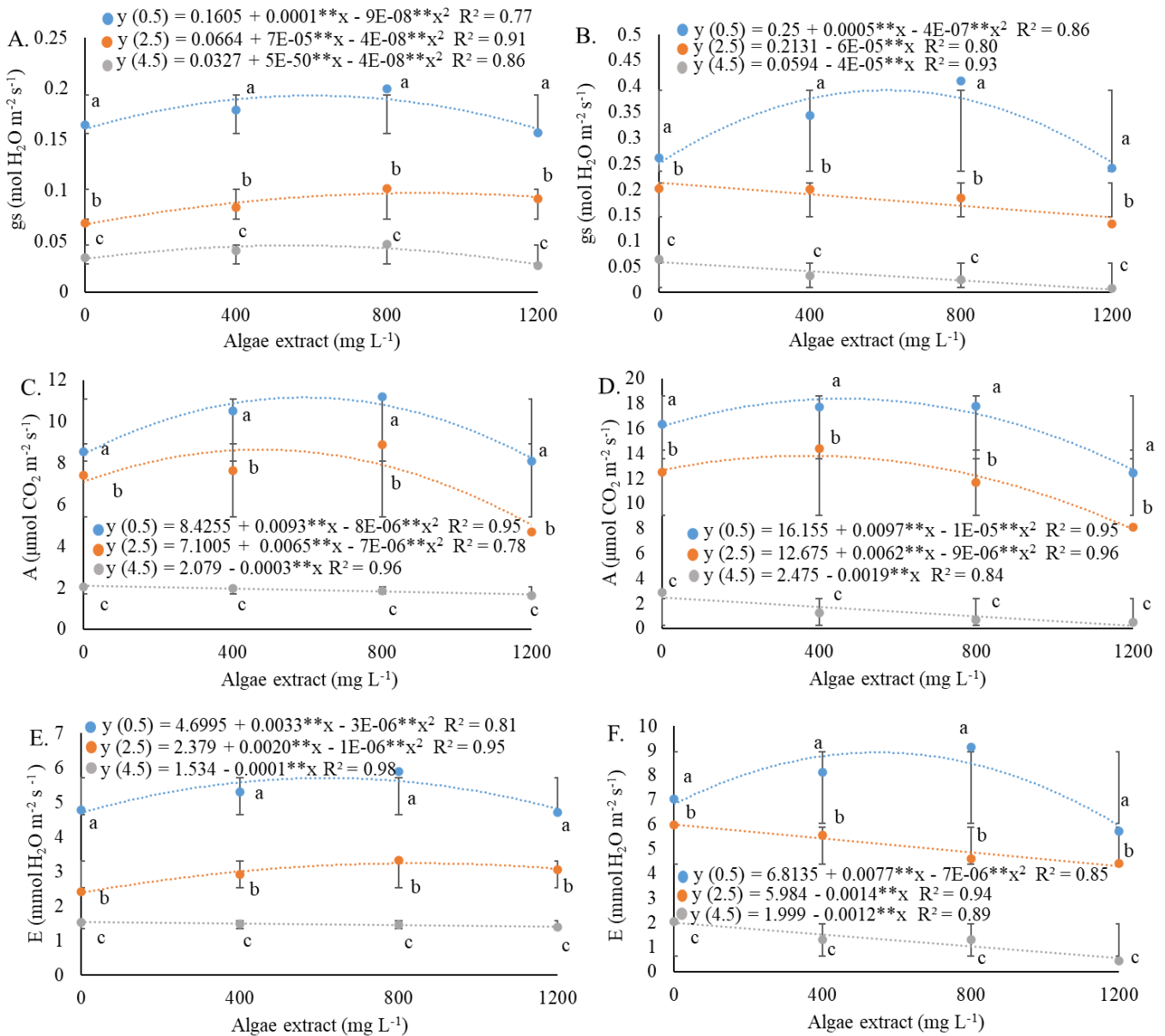
The maximum values for the photosynthetic rate of *C. argentea* (Figure 1C) were 11.1 and 8.6 μmol of CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> for the treatments of 0.5 and 2.5 dS m<sup>-1</sup>, and for the calculated algae extract concentrations of 581.25 and 464.28 mg L<sup>-1</sup>, respectively. However, plants irrigated with ECw 4.5 dS m<sup>-1</sup> had linear decreases in photosynthetic rates with increasing extract concentrations, with a decrease of 20% when comparing the lowest with the highest concentration. A similar trend was observed in the *C. roseus* (Figure 1D), in which the maximum rates of photosynthesis (18.5 and 13.7 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) were obtained when plants were irrigated with ECw of 0.5 and 2.5 dS m<sup>-1</sup> and provided algae extract at concentrations of 485.0 and 344.44 mg L<sup>-1</sup>. For the highest level of salinity (ECw of 4.5 dS m<sup>-1</sup>), increasing concentrations of algae extract caused a linear reduction in photosynthesis, with a decrease of 81.8% between 0 and 1200 mg L<sup>-1</sup> of algae extract.

Figure 1E illustrates that the increase in algae extracts concentrations at 550 and 1000 mg L<sup>-1</sup> promoted maximum transpirations of 5.61 and 3.37 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> for the electrical conductivities of water of 0.5 and 2.5 dS m<sup>-1</sup>, respectively. However, the ECw of 4.5 dS m<sup>-1</sup> caused a unit reduction of 0.0001 mmol H<sub>2</sub>O m<sup>-1</sup> s<sup>-1</sup> in the transpiration of *C. argentea*. Figure 1F shows that an ECw of 0.5 dS m<sup>-1</sup> promoted a maximum transpiration rate of 8.93 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> for *C. roseus* for the concentration of algae extract of 550 mg L<sup>-1</sup>. However, when the ECw increased from 0.5 to 2.5 and 4.5 dS m<sup>-1</sup>, there were linear decreases in transpiration of 26.2 and 78.4%, respectively, due to the increase in the concentration of algae extract of 0 and 1200 mg L<sup>-1</sup>.

Based on the decomposition of the qualitative factors within the quantitative, it is possible to state that, in general, the use of ECw of 2.5 and 4.5 dS m<sup>-1</sup> promoted reductions in gas exchange (gs, A and E) for both species, with a more severe effect on *C. roseus*, it is also highlighted that these effects were enhanced with the substantial increase in the concentration of the algae extract.

The application of algae extracts resulted in improved leaf gas exchange, especially under low and moderate salinity levels (Figure 1). These results are supported by other studies, which show that algae-based biostimulants are resources that, when applied in small concentrations, may improve physiological processes, resulting in better plant nutrition, mainly due to ionic homeostasis provided by K and N, favoring greater tolerance to stress (Barone et al., 2018; Kopta et al., 2018). However, with the substantial increase in extract concentrations, there was a decline in A, gs, and E, regardless of the water salinity level.

Photosynthesis and transpiration rates had similar trends as gs, where algae extract mitigated salinity effects only up to moderate levels. These positive results can be explained by the high potassium concentration (20% K<sub>2</sub>O) of the algae extract. The beneficial effects of algae extracts are justified based on the premise that, according to Melo et al. (2014), the accumulation of K (a nutrient in greater quantity in the extract) in plants favors an osmotic gradient that facilitates the



Vertical bars represent the standard deviation ( $n=4$ ); \*\* and \* - Significant at  $p \leq 0.01$  and  $p \leq 0.05$ , respectively, by the F-test. Lowercase letters differentiate the salinity levels using the Tukey's test ( $p \leq 0.05$ )

**Figure 1.** Stomatal conductance -  $g_s$  (A and B), net photosynthesis - A (C and D), and transpiration rate - E (E and F) of *C. argentea* (A, C, and E) and *C. roseus* (B, D, and F) irrigated with brackish waters and treated with algae extract (*Ascophyllum nodosum*)

movement of water in plant tissues, according to their study in banana cultivation.

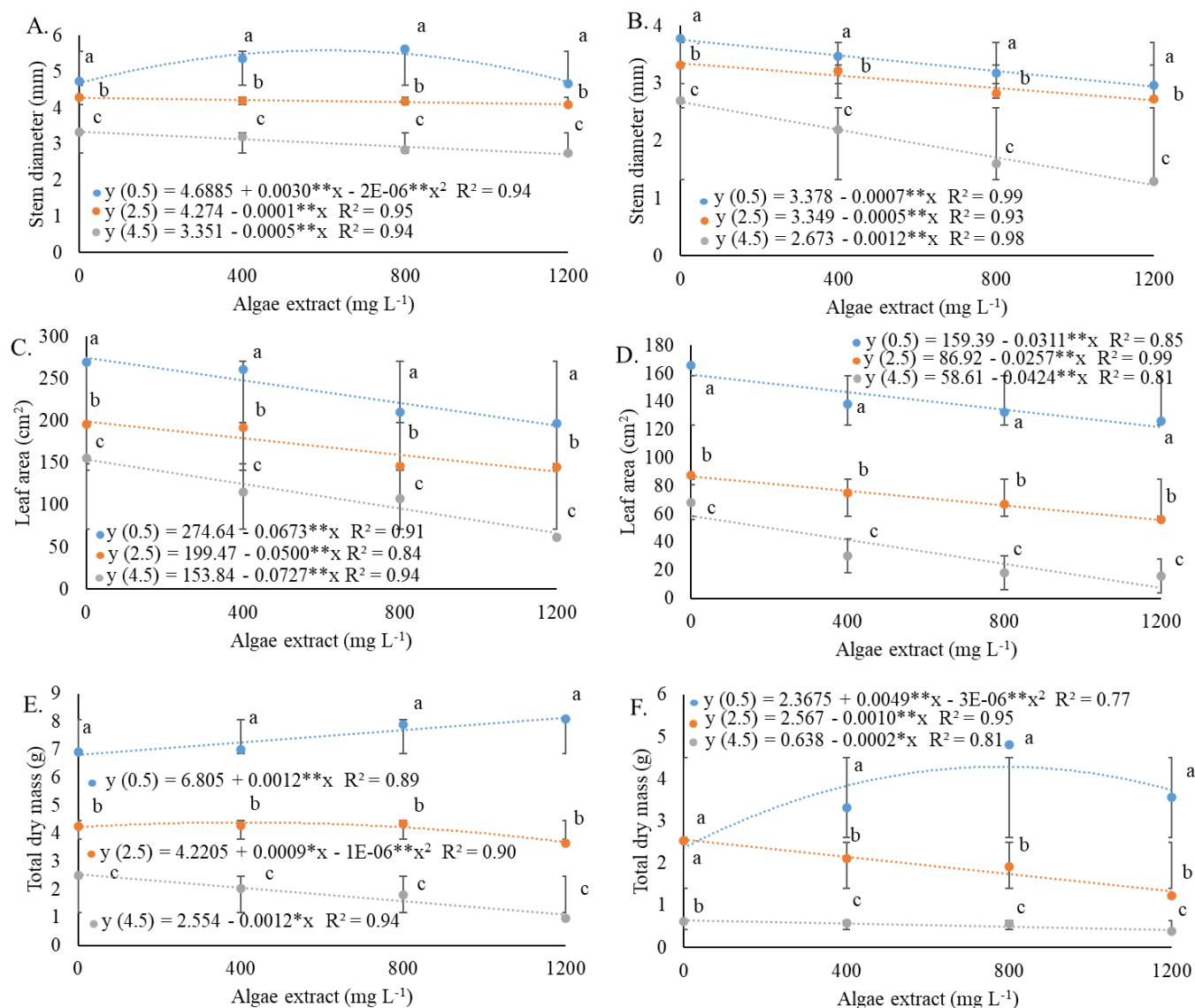
This water circulation helps to regulate stomatal opening and plays a vital role in cellular turgor, enabling greater net assimilation of  $CO_2$  (Taiz et al., 2021). However, the negative influence of high concentrations of algae extracts on the leaf gas exchange of *C. argentea* and *C. roseus* under saline conditions is due, at least in part, to its high concentration of  $Na^+$  (3-5%). High sodium accumulation in the plant tissues can inhibit physiological processes, such as the  $CO_2$  assimilation rate (Munns & Tester, 2008). However, the accumulation of enough K in leaf tissues can prevent the absorption and accumulation of Na in certain species, as reported for two salt-tolerant spinach cultivars that favored the absorption of K over Na when K was provided at sufficient levels (Ferreira et al., 2020).

The reductions in transpiration imposed by salinity are due to the osmotic effect of salts, reducing stomatal conductance as

a way of regulating the amount of water absorbed by the roots as a response mechanism meant to reduce water losses (Álvarez & Sánchez-Blanco, 2015; Taiz et al., 2021). However, limitations in stomatal opening can also affect photosynthetic capacity, which restricts the availability of  $CO_2$  for carboxylation reactions. Reduction in photosynthesis is also related to non-stomatal effects, such as inhibition of enzymatic activity or photochemical reactions in chloroplasts, such as chlorophyll degradation (Gururani et al., 2015; Lacerda et al., 2020).

Stem diameter (SD) and leaf area (LA) decreased linearly with increasing algae extract concentration for both species, except for *C. argentea* under salinity of 0.5  $dS\ m^{-1}$ , which presented a maximum SD of 5.81 mm for algae extract concentration of 750  $mg\ L^{-1}$  (Figure 2A). Plants of this species, irrigated with water of ECw 2.5 and 4.5  $dS\ m^{-1}$ , had decreased stem diameters (4.2 and 17.3%, respectively) when comparing the smallest (0  $mg\ L^{-1}$ ) and the largest (1200  $mg\ L^{-1}$ ) extract concentrations. For *C. roseus* (Figure 2B), these total reductions





Vertical bars represent the standard deviation (n=4); \*\* and \* - Significant at  $p \leq 0.01$  and  $p \leq 0.05$ , respectively, by the F-test. Lowercase letters differentiate the salinity levels using the Tukey test ( $p \leq 0.05$ )

**Figure 2.** Stem diameter (A and B), leaf area (C and D), and total dry mass (E and F) of *C. argentea* (A, C, and E) and *C. roseus* (B, D, and F) irrigated with brackish waters and treated with algae extract (*Ascophyllum nodosum*)

in stem diameter reached 21.4% (0.5 dS m<sup>-1</sup>), 17.7% (2.5 dS m<sup>-1</sup>), and 52.2% (4.5 dS m<sup>-1</sup>).

For the leaf area, the reductions were more expressive when the concentration of algae extract was increased, especially at the highest level of salinity. Plants of *C. argentea* irrigated with EC<sub>w</sub> of 0.5, 2.5, and 4.5 dS m<sup>-1</sup> had leaf area reductions of 26.9, 26.2, and 60.5% when comparing the lowest (0 mg L<sup>-1</sup>) and highest (1200 mg L<sup>-1</sup>) algae extract concentrations, respectively (Figure 2C). For *C. roseus* (Figure 2D), these reductions were 23.8, 36.1, and 76.8%, respectively.

The responses regarding total biomass showed divergent results compared to stem diameter and leaf area measurements. *Celosia argentea* irrigated with low salinity water showed a unit increase of 0.0012 g for each increase in algae extract concentration (Figure 2E). For an EC<sub>w</sub> of 2.5 dS m<sup>-1</sup>, the maximum value of total dry mass was observed for the extract concentration of 450 mg L<sup>-1</sup>. However, at the highest salinity level (4.5 dS m<sup>-1</sup>), the increase in algae extract (from 0 to 1200 mg L<sup>-1</sup>) provided a decrease of 61.6% in total biomass production.

On the other hand, for *C. roseus* (Figure 2F) irrigated with low-salinity water, the model that best fitted the data was the polynomial, in which the maximum total dry mass value was 4.36 g when the concentration of algae extract was 816.7 mg L<sup>-1</sup>. However, when the EC<sub>w</sub> was increased to 2.5 and 4.5 dS m<sup>-1</sup>, there were reductions of 51.5 and 37.7%, respectively, when comparing the limits of extract concentration (0 and 1200 mg L<sup>-1</sup>).

From the breakdown of qualitative factors, it is possible to conclude that plant growth and biomass accumulation are harmed by increased electrical conductivities (0.5 to 4.5 dS m<sup>-1</sup>). These decreases affect the two species evaluated but are more noticeable in the *C. roseus*. It is also verified that in moderate and high salinity, the increase in concentrations of algae extracts also promotes reductions in the analyzed variables.

There was a strong negative interaction between irrigation-water salinity and the concentration of algae extracts, especially for biomass production (Figure 2). The beneficial effect of the biostimulant application alone was restricted to plants irrigated with fresh water.

The beneficial effect of the algae extract on biomass production was observed only for the species that were moderately tolerant to salinity (*C. argentea*). Previously, this positive effect of algae extracts was observed in plants subjected or not to abiotic stresses (Neumann et al., 2017; Fernandes et al., 2019), and it was postulated to be associated with antioxidant protection and the presence of plant growth-regulating hormones such as auxin and cytokinin (Saeger et al., 2020).

The reduction in stem diameter, leaf area, and total dry mass caused by salinity has been widely reported in several studies because of osmotic and ionic effects that impact the processes of cell division and expansion (Munns & Tester, 2008). Such effects were potentiated with the increased concentration of algae extract, especially in *C. roseus*, which is more sensitive to salinity. The high alkalinity of the algae extracts, associated with the high concentrations of salts, may have intensified the nutritional imbalance and the inhibition of vegetative growth. It is worth mentioning that algae of the species *A. nodosum* contain 3 to 5% of Na<sup>+</sup> in their composition, and the distribution of this cation via phloem may have contributed negatively to cell expansion and division of growing tissues (Saeger et al., 2020).

The foliar proline concentrations were lower in control plants than in those irrigated with saline water (Figure 3A), and proline values increased with the application of algae extract, regardless of the level of salinity of the irrigation water. For plants irrigated with control salinity (0.5 dS m<sup>-1</sup>), there was a linear increment of 32.9% between the lowest and the highest concentration of the algae extract. For ECw of 2.5 and 4.5 dS

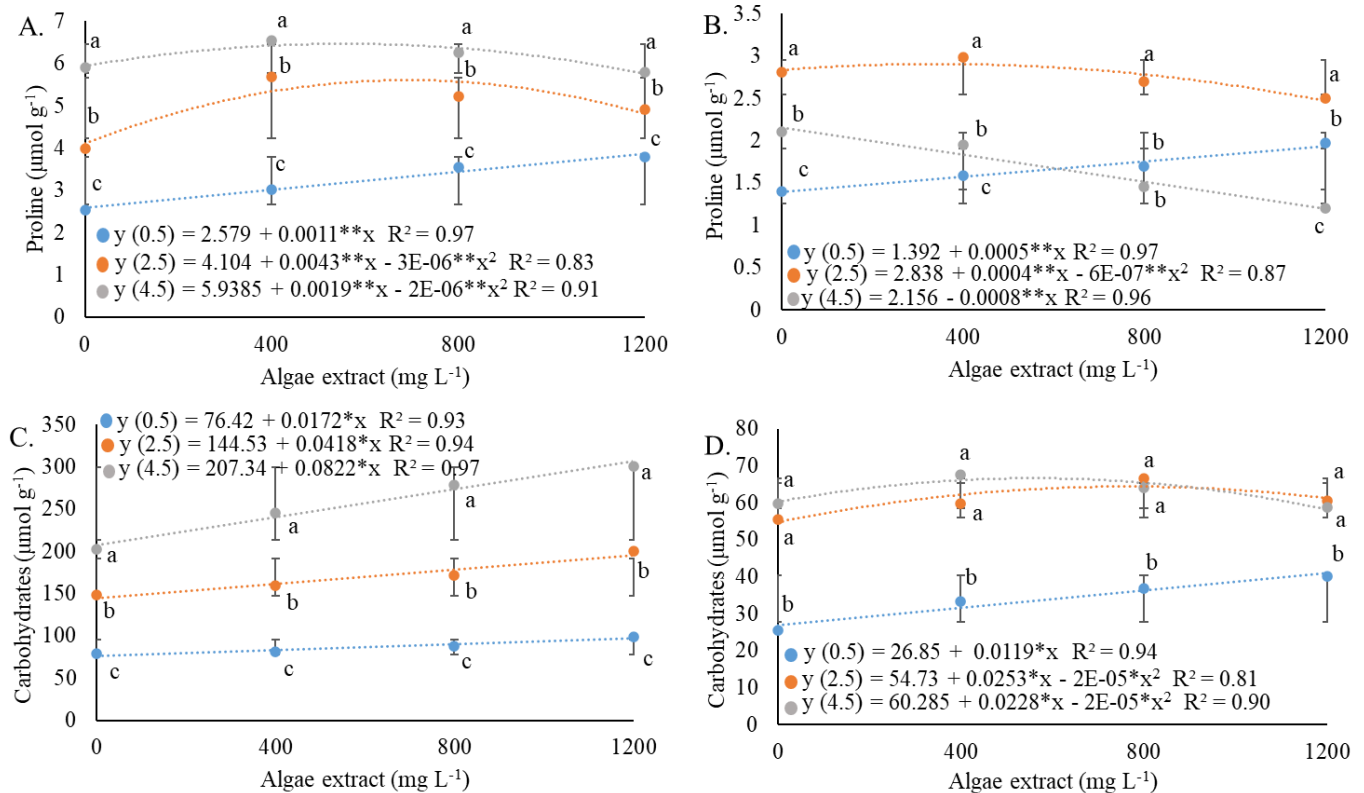
m<sup>-1</sup>, maximum proline levels of 5.64 and 6.38 μmol g<sup>-1</sup> were obtained using concentrations of 717 and 475 mg L<sup>-1</sup> of algae extracts, respectively.

In Figure 3B, contrasting proline values were observed for *C. roseus*, in which, for an ECw of 0.5 dS m<sup>-1</sup>, there was a linear increase of 28.9%, while for an ECw of 4.5 dS m<sup>-1</sup>, there was a linear decrease of 42.8% between the treatments with zero and the maximum concentration of the algae extract (1200 mg L<sup>-1</sup>). Irrigation water of 2.5 dS m<sup>-1</sup> promoted a maximum proline concentration of 2.89 μmol g<sup>-1</sup> of fresh matter for algae extract concentration of 286 mg L<sup>-1</sup>, with mean values higher than those observed for plants irrigated with fresh water (0.5 dS m<sup>-1</sup>) and high salinity (4.5 dS m<sup>-1</sup>).

For the soluble carbohydrates, the increase was more expressive when the concentration of algae extract was increased, especially at the highest level of salinity. Plants of *C. argentea* irrigated with ECw of 0.5, 2.5, and 4.5 dS m<sup>-1</sup> showed soluble carbohydrate increases of 20.9, 25.9, and 32.8% between no algae (0 mg L<sup>-1</sup>) and the highest (1200 mg L<sup>-1</sup>) algae extract concentrations, respectively (Figure 3C).

For *C. roseus* (Figure 3D), the ECw of 0.5 dS m<sup>-1</sup> promoted a linear increase in carbohydrate concentration of 36.5% between no algae (0 mg L<sup>-1</sup>) and the highest concentration of algae extract (1200 mg L<sup>-1</sup>). The increase of the algae extract concentration, when combined with the ECw of 2.5 and 4.5 dS m<sup>-1</sup>, promoted a maximum carbohydrate content of 62.73 and 66.78 μmol g<sup>-1</sup> of fresh matter when submitted to the application of 632 and 570 mg L<sup>-1</sup> of algae extract.

Contrary to previous results, the unfolding of qualitative factors within the quantitative factor allowed us to observe



Vertical bars represent the standard deviation (n=4); \*\* and \* - Significant at  $p \leq 0.01$  and  $p \leq 0.05$ , respectively, by the F-test. Lowercase letters differentiate the salinity levels using the Tukey test ( $p \leq 0.05$ )

**Figure 3.** Proline (A and B) and soluble carbohydrate (C and D) of *C. argentea* (A and C) and *C. roseus* (B and D) irrigated with waters of electrical conductivities of 0.5, 2.5, and 4.5 dS m<sup>-1</sup> and treated with algae extract (*Ascophyllum nodosum*)

that the content of organic solutes increases due to the increase in the salinity of the irrigation water (0.5 to 4.5 dS m<sup>-1</sup>), with more expressive results for *C. argentea*.

The accumulation of organic solutes in response to salinity has been pointed out as an important mechanism for the osmotic adjustment of the cell and for the protection of cellular structures and functions (Munns & Tester, 2008; Li et al., 2018). Accumulation of proline may be due to synthesis, decreased degradation, lower utilization, or hydrolysis of proteins, and contributes to the stabilization of subcellular structures, scavenging of free radicals, and cellular redox potential buffering (Kaur & Asthir, 2015). Decreased levels of carbohydrates in leaves are often associated with disturbances in their biosynthesis or translocation to other plant organs (Taiz et al., 2021).

The increase in proline and soluble carbohydrates concentrations (Figure 3) due to the increase in the concentrations of the algae extract under conditions of moderate salinity (2.5 dS m<sup>-1</sup>) can be explained by the fact that the algae extract contains these substances in its composition. However, with the increase of EC<sub>w</sub> to 4.5 dS m<sup>-1</sup>, there was a significant decrease in leaf proline, mainly for *C. roseus*.

This was possibly due to the combined effect of salts from the irrigation water with high sodium present in the algae extract, leading to a reduction of leaf proline, indicating that *C. roseus* is more sensitive to salt stress than *C. argentea* and that, under excessive saline conditions; the algae extract does not improve salt tolerance. Some authors demonstrated a positive correlation between proline accumulation and salt tolerance in plants (García-Caparrós & Lao, 2018; Lacerda et al., 2020), which is in accordance with our results since the species that showed the best physiological results under saline stress showed the highest leaf proline concentration.

Table 1 presents the scores assigned to the general appearance (visual quality) of *C. argentea* and *C. roseus* plants. The scores varied from species to species and decreased with increases in the electrical conductivity of the irrigation water and the concentration of algae extract. The treatment of 2.5 dS m<sup>-1</sup> combined with the concentrations of 0 and 400 mg L<sup>-1</sup> of algae extract promoted higher scores for the general appearance of *C. argentea* (8.48 and 8.02), which are numerically superior to the obtained by plants under low salinity (7.93 and 7.77).

The lowest average scores for general appearance (5.58 and 5.25) of *C. argentea* were assigned to plants cultivated under the highest salinity (4.5 dS m<sup>-1</sup>) and provided with the highest concentrations of algae extract (800 and 1200 mg L<sup>-1</sup>),

respectively. The highest scores attributed to *C. roseus* plants were observed for the control treatment (0.5 dS m<sup>-1</sup>) and the low concentrations of algae extract (0 and 400 mg L<sup>-1</sup>), with averages of 9.05 and 8.23, respectively.

The increase in salinity associated with a high concentration of algae extract severely reduced the general commercial appearance of *C. roseus*. These results demonstrate the deleterious effect of the interaction between high salinity and high concentrations of algae extract on the visual quality of tropical ornamental species. Low scores were due to the poor aesthetic quality of the plants, which had a lower number of leaves and flowers and unattractive coloring, all considered serious defects according to the quality standards of commercial ornamental plants (IBRAFLO, 2021).

The beneficial action of the algae extracts on the visual quality of *C. argentea* was recorded up to 2.5 dS m<sup>-1</sup>, possibly due to the antioxidant protection, better nutritional balance, and presence of specific algae polysaccharides, betaines, polyamines, and phenolic compounds (Saeger et al., 2020). On the other hand, no salinity mitigating effect of the algae extract was observed for *C. roseus*, possibly due to the greater sensitivity of this species to salt stress. The greater accumulation of sodium in the leaves of *C. roseus* is a characteristic related to the sensitivity of this species to salt stress (Lacerda et al., 2020), which can cause toxicity and necrosis of leaf tissue (Munns & Tester, 2008; Taiz et al., 2021).

## CONCLUSIONS

1. Salinity of the irrigation water negatively affects the morphological and physiological characteristics of the two species studied, mainly *C. roseus*. Irrigation with EC<sub>w</sub> up to 2.5 dS m<sup>-1</sup> favored visual quality and increased the purchasing preference for *C. argentea*.

2. The use of intermediate concentrations (400 mg L<sup>-1</sup>) of algae extract (*Ascophyllum nodosum*) promoted partial mitigation of the effects of salt stress on biomass production and leaf gas exchange, especially for *C. argentea*, but these positive responses decreased or disappeared with the intensification of salt stress and the increase in algae extract concentration.

3. The algae extract can be a valuable amendment in producing ornamental plants of good quality if used in low concentrations (≤ 400 mg L<sup>-1</sup>) when the irrigation water available has low to moderate salinity (2.5 dS m<sup>-1</sup>) and the ornamental species has some degree of salt tolerance.

**Table 1.** Average score assigned by judges for the general appearances of *C. argentea* and *C. roseus* plants irrigated with brackish water and increasing concentrations of algae extract

EC <sub>w</sub> (dS m <sup>-1</sup> )	Concentration of algae extract (mg L <sup>-1</sup> )			
	0	400	800	1200
<i>Celosia argentea</i>				
0.5	7.93 Ba ± 0.21	7.77 Ba ± 0.20	7.46 Bb ± 0.19	7.42 Ab ± 0.18
2.5	8.48 Aa ± 0.41	8.02 Aa ± 0.39	7.81 Ab ± 0.38	7.29 Bb ± 0.36
4.5	6.25 Ca ± 0.59	6.01 Ca ± 0.58	5.58 Cb ± 0.54	5.25 Cb ± 0.57
<i>Catharanthus roseus</i>				
0.5	9.05 Aa ± 0.23	8.23 Ab ± 0.22	7.56 Ac ± 0.21	7.19 Ac ± 0.20
2.5	6.58 Ba ± 0.39	6.39 Ba ± 0.39	6.23 Ba ± 0.38	5.17 Bb ± 0.36
4.5	4.54 Ca ± 0.58	3.92 Cb ± 0.59	2.66 Cc ± 0.61	2.64 Cc ± 0.62

Means followed by the same lower-case letter in the row and upper-case in the column do not differ statistically from each other according to the Tukey's test ( $p \leq 0.05$ ),  $n = 4$

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