



## Pyroligneous acid extract as an attenuator of salt stress in Surinam cherry<sup>1</sup>

### Extrato de ácido pirolenhoso como atenuador do estresse salino em pitangueira

Adriana dos S. Ferreira<sup>2</sup>, Vander Mendonça<sup>2</sup>, Antônio G. de L. Souto<sup>2</sup>,  
Francisco V. da S. Sá<sup>3</sup> & João E. da S. Ribeiro<sup>2</sup>

<sup>1</sup> Research developed at Universidade Federal Rural do Semi-Árido, Mossoró, RN, Brazil

<sup>2</sup> Universidade Federal Rural do Semi-Árido, Mossoró, RN, Brazil

<sup>3</sup> Universidade Estadual da Paraíba, Catolé do Rocha, PB, Brazil

#### HIGHLIGHTS:

*The 2% extract concentration promoted an increase in the number of leaves under low salinity conditions.*

*Salt stress affects the growth and quality of Surinam cherry seedlings.*

*Surinam cherry increases proline synthesis under salt stress.*

**ABSTRACT:** Pyroligneous acid extract can reduce the effects of excess toxic salts on Surinam cherry plants, especially in the Brazilian Northeast, where soil and water salinity is a limiting factor for agricultural production. Thus, this study aimed to evaluate the effect of pyroligneous acid extract as an attenuator on the growth and synthesis of osmoregulators of Surinam cherry irrigated with saline waters. The experiment was conducted in a greenhouse with the treatments distributed in a randomized block design under the factorial scheme  $4 \times 3$ , referring to four levels of salinity of the irrigation water (0.5, 2.5, 4.5, and  $6.5 \text{ dS m}^{-1}$ ) and application of three concentrations of pyroligneous acid extract (0, 1, and 2%). High salinity levels, of 4.5 and  $6.5 \text{ dS m}^{-1}$ , significantly reduced growth, chlorophyll, and soluble sugars. However, the application of 2% pyroligneous acid extract was practical in mitigating salt stress effects up to  $2.5 \text{ dS m}^{-1}$ , resulting in improvements in the number of leaves, proline contents, dry mass production and quality of the seedlings.

**Key words:** *Eugenia uniflora* L., irrigation, water salinity, seedling quality, organic solutes

**RESUMO:** O ácido pirolenhoso pode reduzir os efeitos do excesso de sais tóxicos no cultivo de pitangueiras, principalmente no Nordeste brasileiro, onde a salinidade do solo e/ou da água é um fator limitante para produção agrícola. Assim, objetivou-se avaliar o efeito do ácido pirolenhoso como atenuador sobre o crescimento e na síntese de osmorreguladores da pitangueira irrigadas com águas salinas. O experimento foi conduzido em ambiente protegido com os tratamentos distribuídos no delineamento em blocos casualizados, sob o esquema fatorial  $4 \times 3$ , referentes à quatro níveis de salinidade da água salobras (0,5; 2,5; 4,5 e  $6,5 \text{ dS m}^{-1}$ ) e aplicação de três concentrações do ácido pirolenhoso (0, 1 e 2%). Altos níveis de salinidade, de 4,5 e  $6,5 \text{ dS m}^{-1}$ , reduziram significativamente o crescimento, a clorofila e os açúcares solúveis. No entanto, a aplicação de extrato de ácido pirolenhoso a 2% foi prática na mitigação dos efeitos salinos até  $2,5 \text{ dS m}^{-1}$ , resultando em melhorias no número de folhas, teores de prolina, produção de massa seca e qualidade das mudas.

**Palavras-chave:** *Eugenia uniflora* L., irrigação, salinidade da água, qualidade de mudas, solutos orgânicos



## INTRODUCTION

Surinam cherry (*Eugenia uniflora* L.) is found in several regions of Brazil due to its adaptability and the growing interest in the sustainable cultivation of this fruit (Nofal et al., 2024). Its fruits are nutritious and rich in vitamins C and A, while the leaves have anti-inflammatory and antioxidant properties, used in traditional medicine to treat stomach problems and aid in wound healing (Fidelis et al., 2022). It is a fruit tree that is widely exploited in the Brazilian Northeast.

The production of Surinam cherry seedlings is challenged by soil salinity, which affects water relations. In addition, using water sources with high salinity in irrigation significantly limits growth and compromises the quality of seedlings (Arif et al., 2020). Salt stress affects the ability of plants to absorb water from the soil, causes toxicity by specific ions, such as sodium and chloride, in addition to the damage caused by photooxidative stress (Goharrizi et al., 2021). Thus, strategies must be implemented to mitigate the effects of salt stress on Surinam cherry plants.

In this context, pyroligneous acid extract, also known as wood vinegar or pyroligneous extract, emerges as a promising alternative to mitigate abiotic stress (Zhu et al., 2021; Abbaszadeh et al., 2022). This acid extract is produced through controlled thermal decomposition without oxygen, derived from the pyrolysis of plant biomass (Fačková et al., 2020) and can mitigate salt stress. The benefits of the acidic extract for plants are attributed to its bioactive compounds, especially phenolic compounds, due to their antioxidant capacity to neutralize reactive oxygen species (ROS) (Ofoe et al., 2022).

Recent studies highlight the potential of pyroligneous acid extract in promoting plant growth and yield, under both normal environmental conditions and stress (Ofoe et al., 2022). The effectiveness of this extract has been observed in inducing salt tolerance, mitigating oxidative damage, and protecting the photosystem II in canola cultivars (*Brassica napus* L.) (Ma et al., 2022). Additionally, pyroligneous acid has shown positive effects on various crops, such as peanut calendula (*Calendula officinalis* L.) (Abbaszadeh et al., 2022), and sunflower (*Helianthus annuus* L.) (Ferreira et al., 2024), with significant results in saline soils.

The hypothesis of this study is that the application of pyroligneous extract at least at one of the tested concentrations can mitigate the effects of salt stress on Surinam cherry seedlings, promoting improvements in growth and physiological processes of the plants. In view of the above, the study aimed to evaluate the effect of pyroligneous acid extract as an attenuator on the growth and synthesis of osmoregulators of Surinam cherry plants irrigated with saline water.

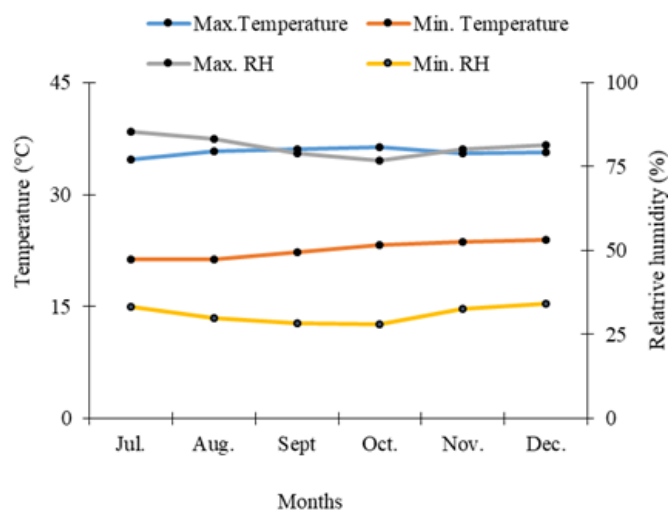
## MATERIAL AND METHODS

The research was carried out from July to December 2023 in a greenhouse belonging to the Federal Rural University of the Semi-Arid Region (UFERSA), municipality of Mossoró, in the state of Rio Grande do Norte, Brazil (5° 11' 31" S and 37° 20' 40" W), with an average altitude of 18 m. The region's climate is semi-arid, classified as BSwh, hot and dry (Alvares et al., 2013).

Meteorological data regarding air temperature (minimum and maximum) and relative humidity (minimum and maximum) during the experimental period were collected daily (Figure 1) through the Automatic Meteorological Station of UFERSA, located in Mossoró, Rio Grande do Norte, at the external part of the greenhouse.

The treatments were distributed in a randomized block design, in a 4 × 3 factorial scheme, with four replicates and one plant per plot, totaling 48 experimental units. The treatments were composed of four levels of salinity of the irrigation water (0.5, 2.5, 4.5, and 6.5 dS m<sup>-1</sup>) and the application of three concentrations of the pyroligneous acid extract (0, 1, and 2%). The saline treatments applied to Surinam cherry are based on the methodology proposed by Rodrigues-Filho et al. (2023) for guava (*Psidium guajava* L.).

Plastic pots, with a capacity of 4 dm<sup>3</sup>, were filled with a substrate composed of a 2:1 (by volume) ratio of soil and commercial substrate (pine bark, ash, vermiculite, peat, sawdust, and bio-stabilizers and additives). At the beginning of the experiment, a sample of this mixture was collected and analyzed to determine its chemical attributes regarding fertility and physical attributes (Teixeira et al., 2017), as shown in Table 1.



**Figure 1.** Mean values of air temperature and relative humidity of air (RH) (maximum and minimum) during the experiment (July 2 to December 29, 2023)

**Table 1.** Chemical attributes regarding fertility and physical characteristics of the substrate used in the experiment before the application of the treatments

Coarse sand	Fine sand	Sand		Silt	Clay	Textural classification		ECse	pH	
		(g kg <sup>-1</sup> )						(dS m <sup>-1</sup> )	(H <sub>2</sub> O)	
530	290	820		70	110	Sandy Soil		0.41	5.70	
P <sup>1</sup>	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H <sup>+</sup> + Al <sup>3+</sup>	SB	CEC	V	ESP
(mg dm <sup>-3</sup> )										(%)
185.4	0.55	0.68	6.31	1.75	0.04	3.14	9.30	12.43	75	4

pH - Hydrogen potential; P, K<sup>+</sup>, Na<sup>+</sup>: Mehlich 1 Extractant; SB - Sum of exchangeable bases (Ca<sup>2+</sup> + Mg<sup>2+</sup> + K<sup>+</sup> + Na<sup>+</sup>); CEC - Cation exchange capacity [SB + (H<sup>+</sup> + Al<sup>3+</sup>)]; V% = (SB/CEC) × 100; ESP - Exchangeable sodium percentage = [Na<sup>+</sup>]/CEC × 100

Surinam cherry seeds were obtained from fruits harvested at full maturity of mother plants located in the didactic orchard of UFERSA (5° 12' 20" S and 37° 19' 17" W, 15 m). After pulp removal, the seeds were manually extracted, selected, washed under running water and dried on paper towels, and then sown. In each plastic pot, three seeds were sown and watered once a day. Thinning was carried out 90 days after sowing (DAS), with the maintenance of the more vigorous plant. Simultaneously, 100 mg of P dm<sup>-3</sup> from single superphosphate was applied for nutritional management, as per Corrêa et al. (2003). At 180 DAS, with the plants already developed, the application of the treatments began.

Irrigation was carried out daily using the weighing lysimeter method, to determine the volume of water evaporated or transpired over 24 hours, maintaining field capacity at 60% (Girardi et al., 2016).

Water with electrical conductivity (ECw) of 0.5 dS m<sup>-1</sup> (control) came from the supply system of the UFERSA campus. The solutions with electrical conductivities of 2.5, 4.5, and 6.5 dS m<sup>-1</sup> were obtained by the addition of sodium chloride (NaCl) to the water of 0.5 dS m<sup>-1</sup>, following the relationship between ECw and the concentration of salts (mg L<sup>-1</sup> ≈ 640 × ECw) as described by Richards (1954). The electrical conductivity of the irrigation waters was monitored using a portable conductivity meter (Akso EC BASIC, TDS % ppm EC SALT) to ensure the accuracy of the adjustments. In addition, each water sample was subsequently sent to a laboratory for chemical characterization regarding the concentration of anions and cations, as shown in Table 2.

The pyroligneous acid extract (PAE) was subjected to a detailed chemical analysis. The product sample had the following characteristics: pH = 2.6; EC = 1.15 dS m<sup>-1</sup>; Organic matter = 8.07 g L<sup>-1</sup>; C = 4.68 g L<sup>-1</sup>; N = 1.45 g L<sup>-1</sup>; P = 0.06 g L<sup>-1</sup>; K = 2.5 g L<sup>-1</sup>; Mg = 0.20 g L<sup>-1</sup>; Ca = 1.20 g L<sup>-1</sup>; Na = 0.30 g L<sup>-1</sup>; C/N = 3.23; Fe = 52.0 mg L<sup>-1</sup>; Cu = 5.0 mg L<sup>-1</sup>; Mn = 4.0 mg L<sup>-1</sup>; B = 91.0 mg L<sup>-1</sup>; Zn = 2.0 mg L<sup>-1</sup>. The concentrations of pyroligneous acid extract used in the experiment were 0, 1, and 2%, which were selected based on the recommendations on the product label (SP Pesquisa e Tecnologia).

During the experiment, five applications of the pyroligneous acid extract were carried out, with intervals of 15 days between each application. PAE was diluted at concentrations of 0% (0 mL PAE: 1000 mL water), 1% (10 mL PAE: 990 mL water), and 2% (20 mL PAE: 980 mL water). The 1 and 2% PAE solutions showed mean electrical conductivities of 0.59 and 0.61 dS m<sup>-1</sup> and pH of 4.89 and 4.03, respectively.

The evaluation of plant growth was carried out on the day after the application of the pyroligneous acid extract. The parameters evaluated were plant height, measured with a millimeter tape from the substrate level to the apical meristem;

stem diameter, measured with a digital caliper (MTX, 316119) accurate to 0.01 mm; number of leaves; and leaf area, calculated using images of leaves arranged on a surface with a numerical scale, processed by the public domain software ImageJ.

The analyses to determine the chlorophyll index were conducted in the final phase of the experiment (80 days after the application of the treatments). The measurements of chlorophyll a, chlorophyll b and chlorophyll total, obtained by the sum of the values of a and b, were carried out from three readings in different positions (basal, median and apical), in leaves located in the middle third of the plant. Reading on the leaves' midrib was avoided during the process. The readings were taken using a portable ChlorofiLOG<sup>®</sup> 1030 meter from Falker, and the results were expressed as Falker Chlorophyll Index (FCI).

Proline (PRO) and total soluble sugars (TSS) were determined 30 days after the conclusion of the experiment. For the analysis, 15 fresh leaves per plant were selected and macerated to obtain the crude extract. The extract was vortex-stirred in a 0.1 M monobasic phosphate buffer solution and then centrifuged. The resulting supernatant was removed and stored in an ultra-freezer at -20 °C. Proline content was determined by the method of Bates et al. (1973), using a standard curve with 0 to 0.10 µg mL<sup>-1</sup> concentrations, with readings taken in a spectrophotometer at 520 nm. The results were expressed in micrograms of proline per gram of fresh material.

Total soluble sugars (TSS) content was quantified following the methodology of Yemm & Willis (1954), using the anthrone reagent in the quantitative analysis of sugars. A reference curve was prepared for the determination with concentrations from 0 to 60 µg mL<sup>-1</sup>. The results were expressed in milligrams of soluble sugars per gram of fresh material.

After 24 hours from the end of the experiment, the plant material was separated into shoots and roots, placed in a paper bag identified with the respective treatments and dried in an oven at 65 °C for 72 hours until a constant mass was reached to quantify shoot dry mass (SDM), root dry mass (RDM), and total dry mass (TDM). The material was weighed on a semi-analytical scale (0.001 g), and the results were expressed in grams per plant. The Dickson quality index (DQI) was determined according to the methodology proposed by Dickson et al. (1960), as shown in Eq. 1.

$$DQI = \frac{TDM}{\left(\frac{SDM}{RDM}\right) + \left(\frac{PH}{SD}\right)} \quad (1)$$

where:

TDM - total dry mass (g),

**Table 2.** Chemical characteristics of irrigation water used in irrigation in terms of anion and cation concentrations

Samples (dS m <sup>-1</sup> )	pH (H <sub>2</sub> O)	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Cl <sup>-</sup>	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	SAR (mmol L <sup>-1</sup> ) <sup>0.5</sup>	Cations	Anions	Hardnes (mg L <sup>-1</sup> )
		(mmol <sub>c</sub> L <sup>-1</sup> )										
0.5	8.50	0.26	4.59	0.74	1.46	2.40	0.10	3.50	4.4	7.0	6.0	110
2.5	8.10	0.25	27.11	4.50	4.70	23.0	0.40	3.20	12.6	36.6	26.6	460
4.5	8.30	0.25	48.13	2.00	3.70	39.0	0.40	3.00	28.5	54.1	42.4	285
6.5	8.30	0.25	67.30	2.00	4.00	55.0	0.40	3.00	38.9	73.5	58.4	300

EC - Electrical conductivity; pH - Hydrogen potential; SAR - Sodium adsorption ratio; CO<sub>3</sub><sup>2-</sup> - Carbonate; HCO<sub>3</sub><sup>-</sup> - Bicarbonate. Ions - Potassium, sodium, calcium, magnesium, chloride (K<sup>+</sup>; Na<sup>+</sup>; Ca<sup>2+</sup>; Mg<sup>2+</sup>; Cl<sup>-</sup>), respectively

SDM - shoot dry mass (g),  
RDM - root dry mass (g),  
PH - plant height (cm); and,  
SD - stem diameter (mm).

The results were evaluated by the Shapiro-Wilk and Cochran tests for normality and homogeneity of variances, followed by an analysis of variance ( $p \leq 0.05$ ). The means for the salinity of the irrigation water were subjected to polynomial regression ( $p \leq 0.05$ ), and those for the pyroligneous extract were compared by the Tukey test ( $p \leq 0.05$ ). The statistical program SISVAR-ESAL, version 5.6 (Ferreira, 2019), was used for data analysis.

## RESULTS AND DISCUSSION

The interaction between salinity and pyroligneous acid extract exerted a significant effect on the number of leaves, root dry mass, Dickson quality index and proline content ( $p \leq 0.05$ ), in addition to a significant effect on total dry mass ( $p \leq 0.01$ ). Irrigation water salinity significantly influenced all the variables analyzed ( $p \leq 0.01$ ). At the same time, pyroligneous acid extract had a significant effect on plant height, total chlorophyll index, and shoot dry mass ( $p \leq 0.05$ ) (Table 3).

Salt stress reduced the number of leaves in Surinam cherry plants, as indicated by the regression equation (Figure 2A). However, plants irrigated with saline water at  $2.5 \text{ dS m}^{-1}$  showed an increase in NL compared to those subjected to the maximum electrical conductivity (EC) of  $6.5 \text{ dS m}^{-1}$ . In the absence of PAE, the increase was 11.59% (88 leaves), while with the application of 1% PAE, the increment reached 24.48% (97.54 leaves).

The quadratic function analysis revealed that the number of leaves reached a maximum of 108.80 with the application of 2% PAE when plants were irrigated with water at an EC of  $1.41 \text{ dS m}^{-1}$ . These results demonstrate that, under low salinity conditions, pyroligneous extract mitigated the negative effects of salt stress on Surinam cherry plants. Similarly, Ma et al. (2022) reported that the application of pyroligneous acid alleviated growth inhibition in *B. napus* cultivars induced by

salinity, promoting physiological adjustments dependent on the dose and cultivar used.

Increasing salinity in irrigation water resulted in a linear reduction in plant height (PH), stem diameter (SD), and leaf area (LA) of Surinam cherry seedlings, as indicated by the regression equations (Figure 2B, D, and E, respectively). At a moderate salinity level ( $2.5 \text{ dS m}^{-1}$ ), PH and SD were 4.89 and 6.52% lower than the values found in the control, respectively. However, these values were 5.13 and 10.27% (PH) and 6.98 and 14.34% (SD) higher than those observed at the higher salinity levels of 4.5 and  $6.5 \text{ dS m}^{-1}$ , respectively. Similar to the growth variables, the leaf area at EC levels of 0.5, 2.5, 4.5, and  $6.5 \text{ dS m}^{-1}$  showed estimated values of 4.07, 3.50, 3.11, and  $2.63 \text{ cm}^2$ , respectively. These results indicate that Surinam cherry seedlings can be irrigated with moderately saline water ( $2.5 \text{ dS m}^{-1}$ ), showing better performance compared to higher salinity levels.

Nofal et al. (2024) evaluated the tolerance of Surinam cherry (*E. uniflora*) transplants to irrigation water salinity and observed that the salt tolerance index improved at salinity levels of 2.0 and  $4.0 \text{ g L}^{-1}$ . However, as the salt concentration increased to  $10.0 \text{ g L}^{-1}$ , a significant reduction in the index percentages was recorded, reaching 81.5 and 73.7%, respectively.

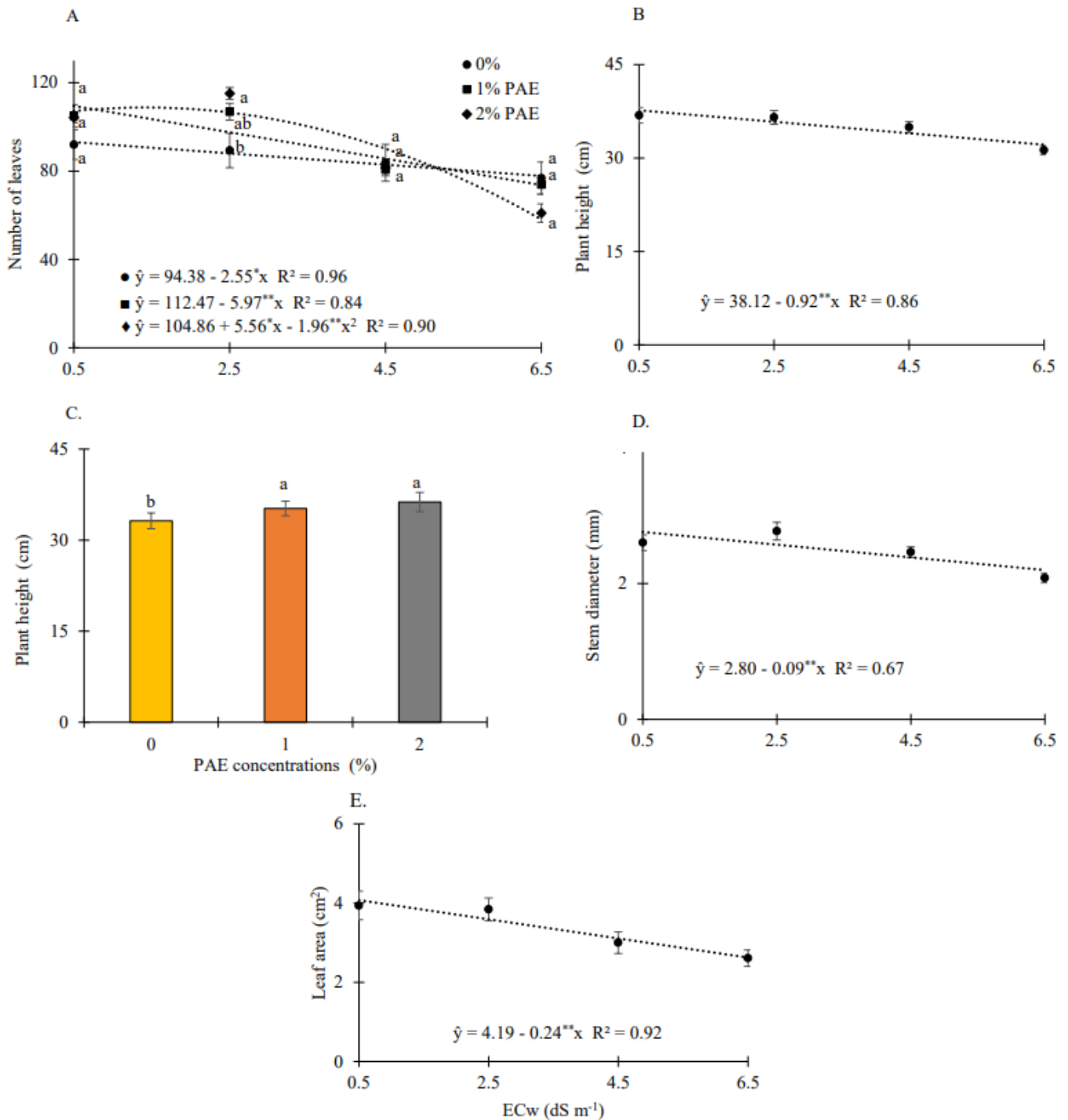
This behavior is common in fruit tree seedlings, such as guava (*P. guajava* L.), cultivar 'Crioula', as the high salinity of  $3.5 \text{ dS m}^{-1}$  severely impairs their stem diameter, leaf area, and plant height, reducing seedling development (Rodrigues-Filho et al., 2023). Similar behavior was observed in guava and bael plants (*Aegle marmelos* Correa), as water electrical conductivity of  $6.0 \text{ dS m}^{-1}$  affected plant growth due to excessive accumulation of toxic ions in soil and leaf tissues (Singh et al., 2018).

The application of pyroligneous acid extract at a concentration of 2% promoted increases of 8.54 and 2.92% in seedling height, respectively, compared to 0 and 1% concentrations (Figure 2C). Wu et al. (2022) reported that pyroligneous acid increased the height of *H. annuus* by 6.1 to 9.0% in saline soils in the Yellow River Delta, China. The observed increases in seedling growth can be attributed to the presence of compounds in the pyroligneous acid extract, such as phenols, esters, and acetic acids, which are associated

**Table 3.** Analysis of variance (F test) for growth, chlorophyll and osmolyte indices in Surinam cherry seedlings irrigated with saline waters and under application of pyroligneous acid extract concentrations at 80 days after the application of the treatments

Source of variation	Mean squares				Residual	CV (%)
	Salinity (S)	Concentrations (C)	S × C	Blocks		
DF	3	2	6	3	33	
NL	2954.19**	171.10 <sup>ns</sup>	344.06*	84.50 <sup>ns</sup>		11.20
Proline	0.09**	10 <sup>-4ns</sup>	0.003*	$2 \times 10^{-4ns}$		12.32
RDM	1.88**	0.01 <sup>ns</sup>	0.27*	0.06 <sup>ns</sup>		12.58
TDM	16.01**	1.26*	1.42**	0.21 <sup>ns</sup>		8.34
DQI	0.08**	0.003 <sup>ns</sup>	0.006*	0.0006 <sup>ns</sup>		10.51
TSS	$4.69 \times 10^{-8**}$	$3.95 \times 10^{-9ns}$	$3.12 \times 10^{-9ns}$	$2.98 \times 10^{-9ns}$		11.88
PH	79.31**	39.88**	3.82 <sup>ns</sup>	2.82 <sup>ns</sup>		4.19
LA	5.00**	0.09 <sup>ns</sup>	0.36 <sup>ns</sup>	0.56 <sup>ns</sup>		16.81
SD	1.01**	0.02 <sup>ns</sup>	0.03 <sup>ns</sup>	0.02 <sup>ns</sup>		8.64
Chl a	65.05**	7.56 <sup>ns</sup>	2.67 <sup>ns</sup>	3.88 <sup>ns</sup>		6.54
Chl b	5.70**	0.30 <sup>ns</sup>	0.25 <sup>ns</sup>	0.84 <sup>ns</sup>		8.69
Chl a+b	107.96**	10.86*	3.49 <sup>ns</sup>	5.71*		5.77
SDM	7.16**	1.31*	0.54 <sup>ns</sup>	0.15 <sup>ns</sup>		11.05

DF - Degrees of freedom; CV - Coefficient of variation; ns, \*, \*\* - Non-significant, significant at  $p \leq 0.05$ , and  $p \leq 0.01$  by the F-test, respectively. NL - Number of leaves, RDM - Root dry mass, TDM - Total dry mass, DQI - Dickson quality index, TSS - Total soluble sugar, PH - Plant height, LA - Leaf area, SD - Stem diameter, Chl a - Chlorophyll a index, Chl b - Chlorophyll b index, Chl (a+b) index - Total chlorophyll, and SDM - Shoot dry mass



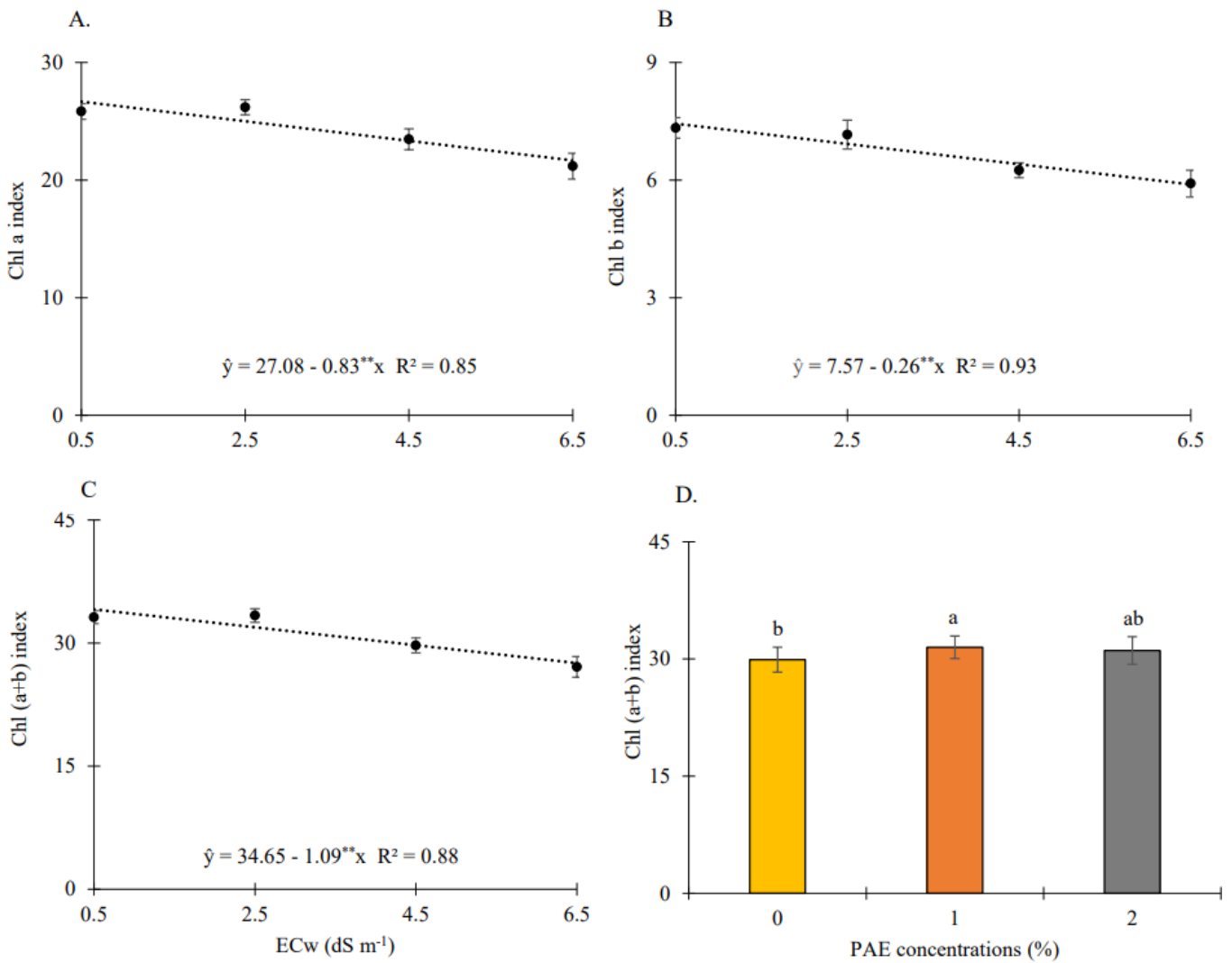
\*Same letters do not differ for different concentrations of pyroligneous acid extract (PAE) within each salinity level by Tukey's test ( $p \leq 0.05$ ). Vertical bars represent the standard error ( $n = 4$ ). Distinct lowercase letters indicate differences between the means of the PAE concentrations factor by Tukey's test at 5% probability level

**Figure 2.** Number of leaves of Surinam cherry irrigated with saline waters and under application of pyroligneous acid extract concentrations (A); plant height (B), stem diameter (D) and leaf area (E) of Surinam cherry seedlings irrigated with saline waters; height of Surinam cherry seedlings under application of pyroligneous acid extract (C), at 80 days after the application of the treatments

with plant growth-promoting properties (Grewal et al., 2018). Defining adequate concentrations of growth stimulants is of great importance, and these may or may not vary between plant species (Ghalati et al., 2020).

The regression equation for the chlorophyll indices (Chl a, Chl b, and total Chl) (Figures 3A, B, and C) revealed that the linear model was the most appropriate to describe the relationship with irrigation water salinity. The results indicated a positive effect on chlorophyll indices up to the salinity level

of 2.5 dS m<sup>-1</sup>, with values of Chl a (26.19 FCI), Chl b (7.16 FCI), and total Chl (33.36 FCI). At the highest salinity level (6.5 dS m<sup>-1</sup>), a reduction was observed in the indices Chl a (21.18 FCI), Chl b (5.91 FCI), and total Chl (27.10 FCI). These results may be attributed to the osmotic stress caused by the salt, which can destabilize the activity of enzymes essential for chlorophyll synthesis, such as protoporphyrin reductase, inhibiting critical steps in the formation of this pigment (Arif et al., 2020). Corroborating these positive results for Surinam



Vertical bars represent the standard error (n = 4). Distinct lowercase letters indicate differences between the means of the PAE concentrations factor by Tukey's test at 5% probability level

**Figure 3.** Chlorophyll a (A), chlorophyll b (B) and total chlorophyll (C) indices in Surinam cherry seedlings irrigated with saline water and total chlorophyll in Surinam cherry seedlings under pyroligneous acid extract (PAE) concentrations (D) at 80 days after the application of the treatments

cherry with EC of 2.5 dS m<sup>-1</sup>, Lacerda et al. (2022) reported that ECw of 3.2 dS m<sup>-1</sup> reduced the levels of chlorophyll a and b in *P. guajava*.

The reduction in enzyme activity is linked to the osmotic effect and toxic ions, such as sodium and chloride, which leads to dysfunctions in energy metabolism (ATP and NADPH) and oxidative stress by reactive oxygen species (Ma et al., 2022).

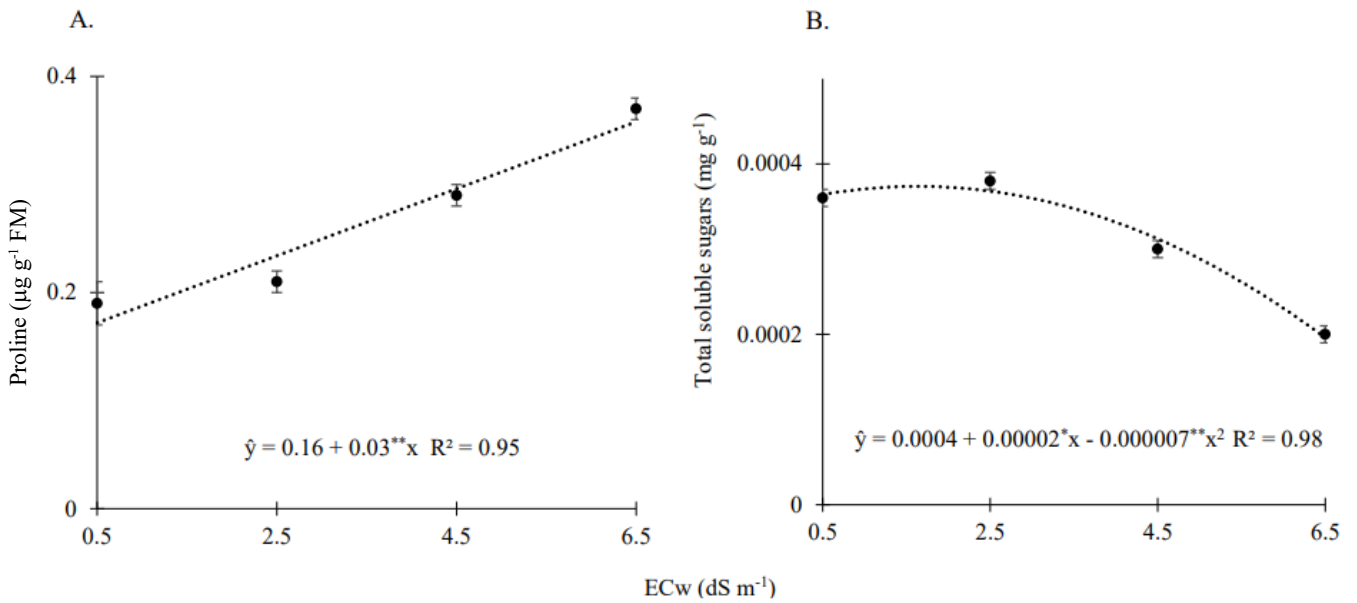
When analyzing the effect of the application of the pyroligneous acid extract, the total chlorophyll index in the Surinam cherry seedlings was higher with the application of the extract at 1% (31.08 FCI) but without showing statistical differences from the treatment with the application of the attenuator at a concentration of 2% (Figure 3D). Pyroligneous acid extract stimulates chlorophyll biosynthesis by providing nitrogen and magnesium, essential elements for the structure of chlorophyll molecules (Fačková et al., 2020). Ferreira et al. (2024) reported that the application of pyroligneous acid extract at the maximum concentration of 1% resulted in a 20.47% increase in the total chlorophyll index in sunflower plants.

Proline content in the leaves increased progressively as a function of the saline treatments, reaching a 48.65% increase

at an EC of 6.5 dS m<sup>-1</sup> compared to the control treatment (0.5 dS m<sup>-1</sup>), with 0.19 μg g<sup>-1</sup> of fresh material (Figure 4A). The increase in proline, associated with higher electrical conductivity, reduced the number of leaves in Surinam cherry, impairing the translocation of assimilates and diverting resources from development, which may result in lower yield (Hosseinifard et al., 2022).

According to the results of the regression analysis for the total soluble sugar content (Figure 4B), a quadratic decrease was observed with the increase in water salinity. At the maximum point of the equation, with an electrical conductivity (EC) of 1.43 dS m<sup>-1</sup>, the estimated value was 0.0003 mg g<sup>-1</sup>, representing a 33.33% increase compared to the EC of 6.5 dS m<sup>-1</sup>. Under low salt stress conditions, Surinam cherry plants showed an increase in the synthesis of primary metabolites related to growth, such as sugars, which play a crucial role in osmotic balance and maintaining cellular functionality (Arif et al., 2020).

The results corroborate those obtained by Singh et al. (2018) in seedlings of *P. guajava* and *A. marmelos* under salt stress, as irrigation with water of 6.0 dS m<sup>-1</sup> resulted in a 30% reduction in total soluble sugars in both species. However,



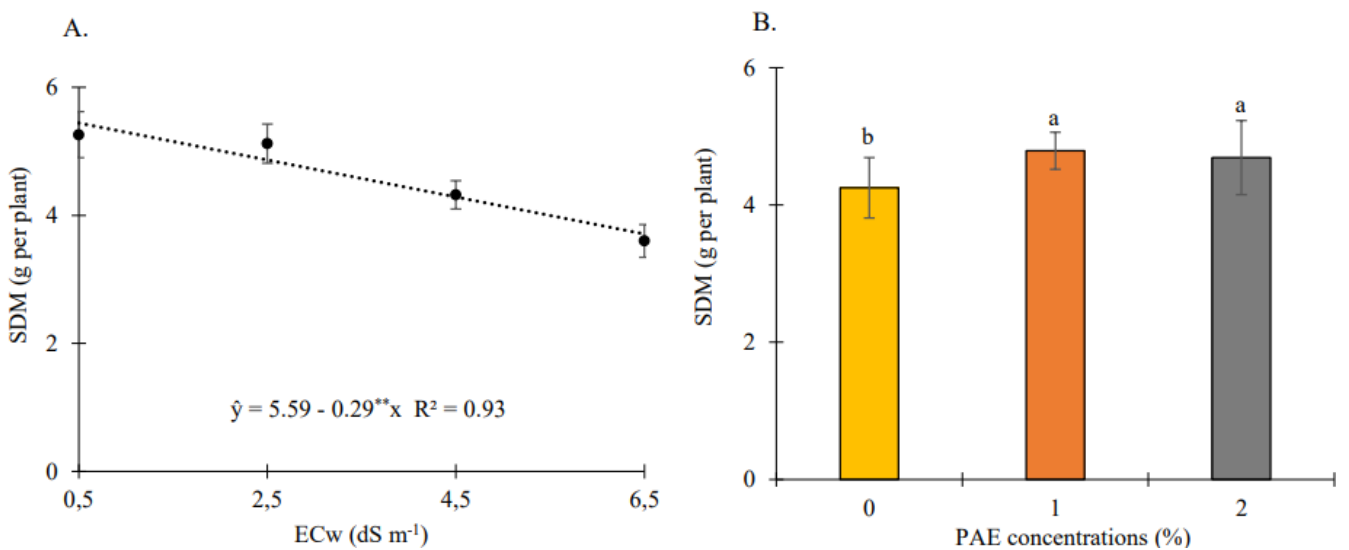
\*Same letters do not differ for different concentrations of pyroligneous acid extract within each salinity level by Tukey's test ( $p \leq 0.05$ ). Vertical bars represent the standard error ( $n = 4$ ). **Figure 4.** Leaf proline content (A) and total soluble sugar content in Surinam cherry seedlings irrigated with saline water and under application of pyroligneous acid extract concentrations at 80 days after the application of the treatments

irrigation with saline water, with conductivities of 3 and 4 dS m<sup>-1</sup>, proved viable for cultivating these plants. In *E. uniflora* plants, the highest levels of total soluble sugars were obtained in treatments with saline water at a concentration of 2.0 g L<sup>-1</sup>, suggesting a strategy of osmotic adjustment in the face of salt stress (Nofal et al., 2024).

The decrease in shoot dry mass (Figure 5A) was observed with the increase in salinity, with the linear model having the best fit to the data. Under irrigation with water at 0.5 (control) and 2.5 dS m<sup>-1</sup>, the highest estimated values of shoot dry mass were 5.44 and 4.87 g per plant, respectively. Plants under salinity with an electrical conductivity (EC) of 6.5 dS m<sup>-1</sup> showed the lowest biomass, with 3.70 g per plant. Even under saline conditions, the Surinam cherry remains conditioned to absorb water, allowing for growth within acceptable levels.

Nofal et al. (2024) investigated the tolerance of *E. uniflora* to the salinity of irrigation water and found that the application of 2.0 g L<sup>-1</sup> of natural Siwa halite resulted in a significant increase in the dry weight of leaves, stems, and roots compared to other concentrations. The authors emphasize that, although high salt concentrations can compromise water absorption and essential physiological processes, it is feasible to use saline water with up to 6.0 g L<sup>-1</sup> to maintain the quality and aesthetic value of the shrubs under the studied conditions.

The plants were treated with the application of pyroligneous acid extract at concentrations of 1 and 2%, which resulted in significant increases in SDM, with increments of 11.27 and 9.38%, respectively, compared to the control treatment (Figure 5B). The increase in dry mass was not dependent on a higher or lower concentration, which implies that the effect of the extract,



Vertical bars represent the standard error ( $n = 4$ ). Distinct lowercase letters indicate differences between the means of the PAE concentrations factor by Tukey's test at 5% probability level **Figure 5.** Shoot dry mass in Surinam cherry seedlings irrigated with saline water (A) and under pyroligneous acid extract (PAE) concentrations (B) at 80 days after the application of the treatments

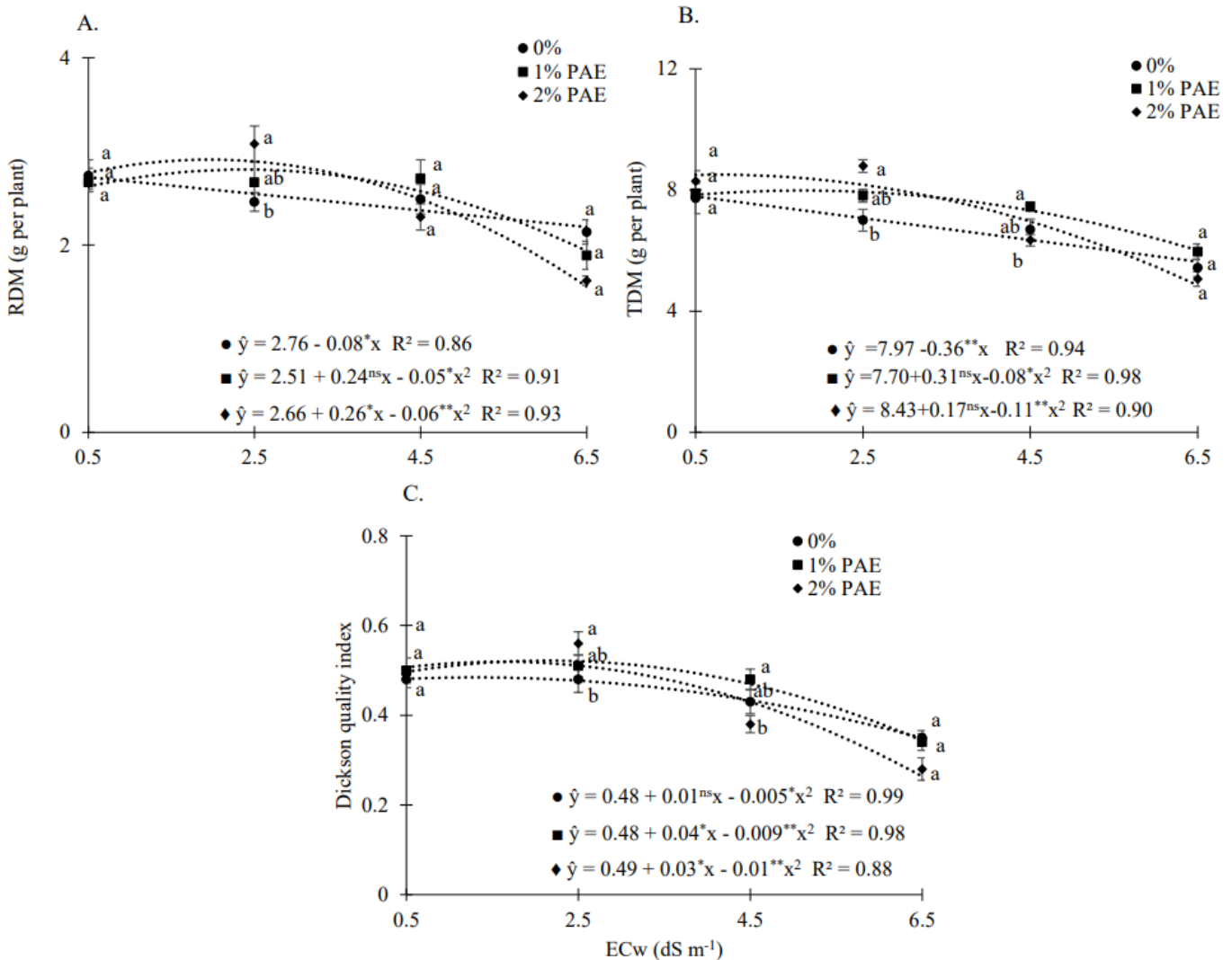
within the range of concentrations tested, was equivalent. This is partly due to pyroligneous acid, which contains a mixture rich in carbon molecules essential for forming biomass and organic compounds (Grewal et al., 2018).

The root dry mass of Surinam cherry (Figure 6A) showed a linear reduction with the increase in EC<sub>w</sub>. In the absence of the extract, the effects of salinity were less intense at 2.5 dS m<sup>-1</sup>, with RDM values (2.56 g per plant) close to those found in the control (2.72 g per plant). It is observed that, for the biomass variables, under salinity conditions and extract application, the quadratic model had the best fit to the data. This resulted in estimated RDM values of 2.80 g per plant for EC of 2.4 dS m<sup>-1</sup> with 1% PAE, suggesting a more efficient adaptation of the plants to salinity. In contrast, the application of 2% PAE at EC of 2.1 dS m<sup>-1</sup>, with 1.84 g per plant, suggests that higher concentrations of the extract may be less beneficial or even detrimental to root growth.

For total dry mass (Figure 6B), in the absence of the extract, irrigation water at 2.5 dS m<sup>-1</sup> resulted in TDM of 7.07 g per plant, showing a difference of 0.72 g per plant compared to the control (0.5 dS m<sup>-1</sup>). The quadratic behavior analysis revealed that the estimated TDM value of 8.0 g per plant at an EC of

1.94 dS m<sup>-1</sup> with 1% of pyroligneous acid extract suggests that the extract may have positively contributed to plant growth, improving the response to moderate salinity. On the other hand, the application of 2% of PAE at an EC of 0.7 dS m<sup>-1</sup>, with 8.49 g per plant, suggests that the mitigation of negative effects is not directly related to the concentration of PAE but rather to the lower salinity, which favored plant growth.

The Dickson quality index (Figure 6C) shows a negative quadratic response to increasing salinity, regardless of the application of extract concentrations. According to the regression equation, for untreated plants, the maximum DQI value was 0.48, achieved without the salinity effect. For the 1% PAE concentration, the maximum DQI value was 0.52, recorded at an EC of 2.22 dS m<sup>-1</sup>, while for the 2% PAE concentration, the maximum value was 0.51, observed at an EC of 1.5 dS m<sup>-1</sup>. These results suggest that higher concentrations of PAE are less effective than 1% in improving seedling quality under salinity. Additionally, the maximum DQI value for untreated plants (0.48) was achieved without salinity, emphasizing the importance of evaluating the ideal salinity conditions and PAE concentrations to optimize plant growth and quality.



\*Same letters do not differ for different concentrations of pyroligneous acid extract within each salinity level by Tukey's test ( $p \leq 0.05$ ). Vertical bars represent the standard error ( $n = 4$ )

**Figure 6.** Root dry mass (A), total dry mass (B) and Dickson quality index (C) of Surinam cherry seedlings irrigated with saline water and under application of pyroligneous acid extract concentrations at 80 days after the application of the treatments



The results are consistent with those of Yang et al. (2024), who observed that the application of wood vinegar (WV) and biochar (BC), either separately or in combination, significantly increased plant height, stem thickness, and dry weight of cotton plants (*Gossypium hirsutum* L.), enhancing tolerance to salt stress.

## CONCLUSIONS

1. The 2% extract concentration was beneficial for increasing the number of leaves in Surinam cherry plants under low salinity conditions (1.41 dS m<sup>-1</sup>).

2. Pyroligneous acid extract favored the accumulation of proline in response to salinity; this increase was accompanied by a reduction in the number of leaves, demonstrating that, under salinity stress, the balance between tolerance and growth may be impaired.

3. The 1% concentration of pyroligneous acid extract promoted root growth and total dry mass, demonstrating a more efficient adaptation of plants to moderate salinity. These findings indicate that using the extract at 1% is an effective strategy to improve seedling quality under saline conditions.

**Contribution of authors:** Ferreira, A. dos Santos, Souto, A. G. L. and Sá, F. V. da S. de: worked on research, data acquisition, data analysis, implementation of computational simulations, and manuscript writing. Mendonça, V. de: served as a research advisor and worked on the conceptualization of the problem and literature review. Souto, A. G. de L. and Sá, F. V. da S.: worked on data acquisition, manuscript improvements, and corrections. Ribeiro, J. E. da S.: advised and worked on data analysis, corrections, and improvements to the simulation models.

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