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Scientific Article

Pyroligneous solution as a salt stress attenuator in BRS 323 sunflower

Extrato pirolenhoso como atenuador do estresse salino em girassol BRS 323

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ABSTRACT - Proper salinity concentration can positively impact the growth of sunflower plants. From this perspective, this study aimed to evaluate the sunflower BRS 323 with regard to the effects of salinity and the use of pyroligneous solution as a salt stress mitigator. The experiment was conducted in a plant nursery. The experimental design was completely randomized, in 5 x 2 factorial arrangement with four replications, with one plant per plot. The treatments corresponded to five electrical conductivity levels of irrigation water: 0.75 dS m^{-1} (control); 1.65; 1.95; 2.65 and 2.85 dS m^{-1}, obtained by dissolving sodium chloride, and two conditions of pyroligneous solution application , with and without. Weekly, the plants were evaluated for growth in height, diameter, and leaf area. Also, the values of the indices of chlorophyll *a*, chlorophyll *b*, total chlorophyll, shoot and root fresh and dry mass, and flower bud fresh and dry mass were evaluated 50 days after sowing, corresponding to the end of the experimental phase. The results suggest that the pyroligneous solution, at the concentration tested, can be recommended to increase the levels of chlorophyll *a*, chlorophyll *b* and total chlorophyll. Moderate salt stress of 1.65 dS m^{-1} promoted an increase in the growth parameters of sunflower plants.

Keywords: Irrigation. *Helianthus annuus*. Plant growth. Chlorophyll indices.

RESUMO - A concentração adequada de salinidade pode impactar positivamente o crescimento das plantas de girassol. Nessa perspectiva, este trabalho teve como objetivo avaliar o girassol. BRS 323 no que diz respeito aos efeitos da salinidade e ao uso de solução pirolenhosa como mitigante. O experimento foi conduzido em viveiro de plantas. O delineamento experimental foi inteiramente casualizado, em arranjo fatorial 5 x 2 com quatro repetições, uma planta por parcela. Os tratamentos corresponderam a cinco níveis de condutividade elétrica da água de irrigação: 0.75 dS m^{-1} (controle); 1.65; 1.95; 2.65 e 2.85 dS m^{-1}, obtidos pela dissolução de cloreto de sódio, e duas condições de aplicação de solução pirolenhosa, com e sem. Semanalmente, as plantas foram avaliadas quanto ao crescimento em altura, diâmetro e área foliar. Além disso, foram avaliados os valores de índices de clorofila *a*, clorofila *b*, clorofila total, matéria fresca e seca da parte aérea e radicular e matéria fresca e seca dos botões florais aos 50 dias após a semeadura, correspondente ao final da fase experimental. Os resultados sugerem que a solução pirolenhosa, na concentração testada, pode ser recomendada para aumentar os índices de clorofila *a*, *b* e total. O estresse salino moderado de 1.65 dS m^{-1} promoveu o aumento nos parâmetros de crescimento em plantas de girassol.

Palavras-chave: Irrigação. *Helianthus annuus*. Crescimento de plantas. Índices de clorofila.

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a plant species belonging to the family Asteraceae and can be intercropped with other crops and integrated with beekeeping and livestock farming (NGUYEN et al., 2021). Furthermore, sunflower finds wide application in various industries, including pharmaceuticals, food and biofuels (BRITO et al., 2022; RODRIGUES et al., 2022).

Sunflower production in northeastern Brazil plays a crucial role in the economic development of the region as this crop is characterized by its ability to withstand the stress imposed by the lack of water and high temperatures (CARVALHO et al., 2018). However, although sunflower plants are moderately tolerant to salinity, the presence of high salt levels can reduce plant growth and negatively affect stress markers, with variations among different sunflower genotypes (PEREIRA et al., 2016).

Excessive accumulation of sodium and chloride ions can induce toxicity in plants, since chloride has the ability to compete with the absorption of essential ions such as nitrate, phosphate and potassium, affecting the nutritional balance of plants (MUNNS et al., 2020). The osmotic effect and the impacts of ionic toxicity caused by sodium ions present in the soil lead to a rapid reduction in water availability, whereas the gradual accumulation of sodium in the shoot parts inhibits photosynthesis, suppressing plant growth (ZELM; ZHANG; TESTERINK, 2020).

The aqueous acidic liquid obtained through the distillation of smoke generated during the anaerobic process of charcoal production, known as pyroligneous solution or wood vinegar, exhibits properties with the potential to positively influence the antioxidant defense system against oxidative stress induced by abiotic agents in plants (OFOE et al., 2022). It stands out for showing significant activity in the elimination of free radicals, reaching 78.52% against the free radical 1.1-diphenyl-2-picrylhydrazyl, as well as a high accumulation of total phenolics (95.81 ± 1.45 gallic acid equivalents (GAE)/mL) and flavonoid content (49.46 µg of quercetin/mL) (OFOE; GUNUPURU; ABBEY, 2022).

Pyroligneous solution has shown significant potential as a driving agent for increasing wheat production in salt-affected areas in Central China (LASHARI et al., 2015). In tomato seedlings, pyroligneous acid demonstrated efficacy in germination and growth, exerting a positive influence on the antioxidant defense system against aluminum-induced oxidative stress (OFOE et al., 2022). Furthermore, combined application of soluble calcium and wood vinegar solution promoted growth and improved cotton yield in areas impacted by salinity (ZENG et al., 2022).

In this scenario, although the positive effects of pyroligneous solution in agriculture are recognized, there are still few studies addressing the effects of irrigation with saline water on the sunflower BRS 323. Therefore, this study aimed to evaluate the growth parameters and leaf chlorophyll indices of the sunflower BRS 323 in response to salinity and the use of pyroligneous solution as a salt stress-mitigating agent.

MATERIAL AND METHODS

The study was carried out from September to November 2022 in a protected environment at the Department of Agronomic and Forestry Sciences of the Federal University of the Semi-arid Region (UFERSA), Campus Mossoró, RN, in northeastern Brazil (5° 11′ 31″ S and 37° 20′ 40″ W), with an average altitude of 18 meters. According to Köppen's classification, the climate of Mossoró is classified as *BSwh*, i.e., a very hot, dry, and semi-arid tropical climate during most of the year (ALVARES et al., 2013). During the experiment, climate data, including temperature and relative humidity (minimum and maximum), were also recorded at the Jerônimo Rosado Meteorological Station at UFERSA, with values of 35.96 and 23.56 °C for maximum and minimum temperature and 88.51 and 39.03% for maximum and minimum relative humidity, respectively (Figure 1).

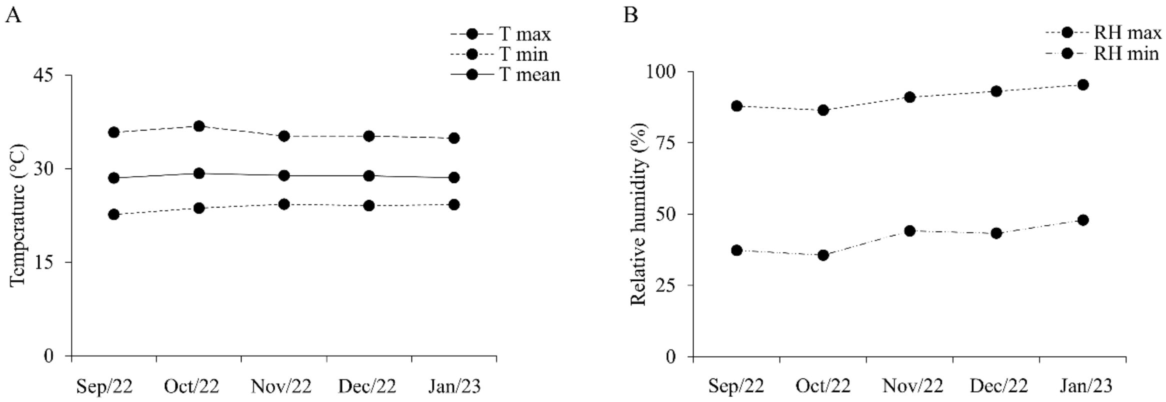

Figure 1. Climatic data on temperature (A) and relative humidity (B) in 2022. UFERSA, Mossoró, RN, Brazil.

The experimental design was completely randomized, in a 5 x 2 factorial arrangement with four replications, each plot containing one plant, referring to five salinity levels (0.75 dS m^{-1} (control); 1.65; 1.95; 2.35 and 2.85 dS m^{-1}) and two conditions of pyroligneous solution application, i.e., with and without.

For seedling formation, three seeds of *H. annuus* BRS 323 were sown at a depth of approximately 3 cm in each polyethylene bag measuring 28 x 20 cm, with a total volume of 2.5 dm^3. The substrate consisted of a mixture of soil and commercial substrate at a ratio of 2:1 (v/v). Prior to the application of treatments, the substrate was chemically characterized for fertility, showing the following characteristics: pH = 6.72; Ca = 12.2 $cmol_c$ dm^{-3}; Mg = 2.5 $cmol_c$ dm^{-3}; Na = 307.31 $cmol_c$ dm^{-3}; K = 321.5 $cmol_c$ dm^{-3}; N = 1.53 g kg^{-1}; P = 594.1 mg dm^{-3}; electric conductivity = 0.67 dS m^{-1}; and exchangeable sodium percentage = 8.0%.

Watering was performed manually once a day, after saturation and free drainage for 24 hours. A uniform daily volume was applied to all plots. The plants were thinned to one plant per container fifteen days after sowing (DAS). The irrigation water with different electrical conductivity levels was applied at 20 DAS. These levels were obtained by following the methodology proposed by Richards (1954), through which the desired water electrical conductivity (ECw) was multiplied by 640 in order to obtain the amount of sodium chloride (NaCl) (mg L^{-1}). The respective water samples were kept in 60-L plastic containers, and the ECw was measured with a digital conductivity meter (MCA150 -Tecnopon).

The 1% concentration of pyroligneous solution (PS) was used to promote plant growth, following the recommendations of the manufacturer. The dilution method adopted followed the methodology of Schnitzer et al. (2015). The 1% concentration of PS corresponding to 10 mL L^{-1} was added to 990 mL of tap water and applied. Subsequently, five applications of the diluted solution were carried out, always in the morning, between 7:00 and 8:00 am, at intervals of seven days on plants subjected to EC levels and the treatment with PS. On the other hand, in the treatments without PS, the plants were only exposed to the ECw levels.

One sample of concentrated pyroligneous solution was sent to chemical analysis, revealing the following characteristics: pH = 2.6; ECw = 1.15 dS m^{-1}; OM = 8.07 g L^{-1}; C = 4.68 g L^{-1}; N = 1.45 g L^{-1}; P = 0.06 g L^{-1}; K = 2.5 Mg = 0.20 g L^{-1}; Ca = 1.20 g L^{-1}; Na = 0.30 g L^{-1}, C/N = 3.23 g L^{-1}; Fe = 52.0 mg L^{-1}; Cu = 5.0 mg L^{-1}; Mn = 4.0 mg L^{-1}; B = 91.0 mg L^{-1}; Zn = 2.0 mg L^{-1}.

Daily irrigation was controlled by following the methodology of Sá et al. (2021), in order to maintain soil moisture close to the maximum retention capacity (drainage lysimeter) using a leaching fraction (LF) of 20%, in addition to the water volume applied (Va) per container, obtained by the difference between the previous water depth (La) minus the mean drainage (D), divided by the number of containers (n) (Equation 1).

$$Va = \frac{(La - D)}{n(1 - LF)} \quad (1)$$

The plants were evaluated at 27, 34, 41, and 48 days after sowing through non-destructive determinations (plant height, measured with a tape measure; base diameter, measured with a digital caliper; and leaf area (LA), calculated by the non-destructive method, a potentially interesting method for sunflower given the maximum width measurements of this organ (W - cm), according to the model suggested by Aquino et al. (2011) (Equation 2).

$$LA\ (cm^2) = 1.6329 * W^{1.7164} \quad (2)$$

The indices of chlorophyll *a*, chlorophyll *b*, and total chlorophyll (*a*+*b*) were determined at the end of the experimental phase, from 7:00 a.m. to 9:00 am., through four readings performed per leaf, two in each point, avoiding the midrib region. These analyses were performed with a portable chlorophyll meter (Falker, ChlorofiLOG® 1030), and the result of the measurements was expressed in dimensionless units (Falker Chlorophyll Index - FCI).

The values of dry shoot and root biomass and dry and fresh flower bud biomass were obtained after the plant material was oven-dried to constant weight in a forced air oven at 65 °C for 72 h. Then, weighing was performed on an analytical balance accurate to 0.0001 grams.

The data were subjected to analysis of variance (ANOVA) by the F-test. In the cases of significance, Tukey's test was performed at 0.05 probability level to compare the means. The statistical analyses were performed in the statistical software SISVAR® (FERREIRA, 2019).

RESULTS AND DISCUSSION

Increased salinity in irrigation water (ECw) resulted in adverse impacts on plant growth, while pyroligneous solution (PS) more effectively preserved the green color of leaves (chlorophyll indices) compared to plants without PS (Table 1).

Table 1. Summary of the analysis of variance using mean square values for morphological and chlorophyll variables in sunflower plants, subjected to irrigation with saline water and salinity reduction with pyroligneous solution.

SV	Saline	Attenuator	S x A	Residual	CV (%)	Mean
DF	4	1	4	30		
PH	525.96**	4.60^{ns}	22.89^{ns}		13.64	33.05
SD	1.70*	2.20*	0.20^{ns}		11.26	5.80
LA	760707.61**	69190.78^{ns}	5304.42^{ns}		40.57	652.22
FCI a	52.13**	120.33**	13.93^{ns}		19.07	16.42
FCI b	21.86**	36.08**	3.98^{ns}		22.91	7.12
FCI t	117.99**	288.31**	24.02^{ns}		15.92	23.54

SV - source of variation; DF – degrees of freedom; CV - coefficient of variation; ^{ns}, *, ** - not significant, significant at $p \leq 0.05$ and $p \leq 0.01$ by F test, respectively.

The main restrictions on the growth of sunflower plants were evident when electrical conductivity reached 2.85 dS m^{-1} (Figure 2). Under these conditions, there were significant reductions of 47.39% in plant height compared to the control treatment (0.75 dS m^{-1}) (Figure 2A) and 70.27% in leaf area (Figure 2B). Additionally, under high salinity and without the pyroligneous solution, there were reductions of 15.87 and 7.79%, respectively, in stem diameter (Figures 2C and 2D). There were also decreases of 35.39 and 19.11% in chlorophyll *a* contents (Figures 2E and 2F), 25.67 and 23.54% in chlorophyll *b* contents (Figures 2G and 2H), and 32.72 and 20.47% in total chlorophyll contents (Figures 2I and 2J).

The increase in salinity causes a high osmotic potential of the soil, which makes it difficult for the roots to absorb water, gradually affecting leaf cells, which face transport problems due to the accumulation of salt (YASMEEN et al., 2020). Plants exposed to salinity can manifest morphological changes such as reduction of leaves and roots and lower leaf density, impacting their ability to acquire new resources and carry out metabolic processes (ZELM; ZHANG; TESTERINK, 2020). Likewise, changes in leaf color may occur due to salinity, resulting in a decrease in photosynthetic activity (GUIMARÃES et al., 2021).

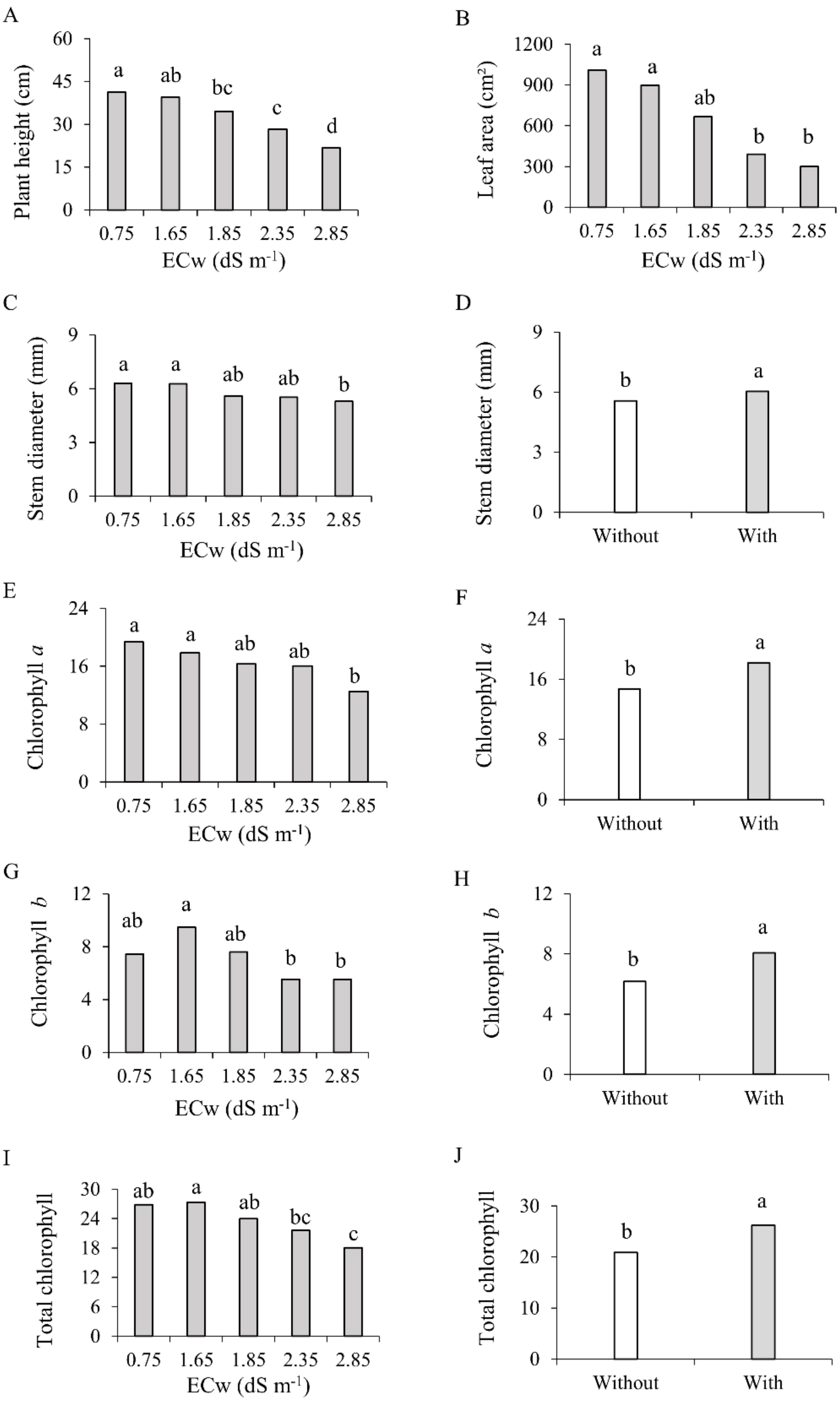

Figure 2. Plant height (A), leaf area (B), stem diameter (C and D), chlorophyll *a* (E and F), chlorophyll *b* (G and H) and total chlorophyll (I and J) in sunflower plants evaluated under different salinity levels without and with application of pyroligneous solution in irrigation water. Distinct lowercase letters indicate significant differences between irrigation conditions.

Analysis of data on plant height (Figure 2A) and leaf area (Figure 2B) showed that the average values, when subjected to salinity of 1.65 dS m^{-1}, were statistically similar to the average values of the control treatment (41.21 cm and 1009.12 cm^2, respectively). This represents a percentage difference of only 4.02% in plant growth and 11.14% in leaf size under salinity, highlighting the potential advantages associated with the exclusive use of moderately saline water in sunflower cultivation.

The results obtained in this study using sunflower plants (BRS 323) under increasing salinity conditions showed a similar pattern to those described by Rodrigues et al. (2022). When studying the cultivar Catissol, these authors observed that salinity levels of irrigation water ranging from 2.1 to 5.1 dS m^{-1} had harmful effects on several plant parameters, including plant height, leaf area, stem diameter, and the number of leaves. These negative effects occurred both with and without the application of mineral and organic fertilizers.

The excess uptake of sodium and chloride ions by plant tissues can cause significant physiological disorders, compromising the uptake of essential ions such as potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and nitrate (NO^{3-}), one of the ways through which nitrogen appears in the environment (MUNNS et al., 2020).

Stem diameter (SD) was proportional at all salinities evaluated (2.85; 2.35; 1.95 and 1.65 dS m^{-1}) under the different irrigation conditions (Figure 2C), exhibiting average values of 5.3, 5.5, 5.6 and 6.2 mm, respectively. Despite that, it is relevant to note that larger diameters were identified at salinity of 1.65 dS m^{-1}, with no statistically significant difference by the means test compared to the control treatment (6.3 mm).

Simultaneously and based on the stem diameter (SD) results with the pyroligneous solution, plants subjected to treatment with this solution showed a 7.8% increase in SD, compared to plants that did not receive the pyroligneous solution (Figure 2D). Recent studies conducted by Ofoe et al. (2022), testing different concentrations of pyroligneous acid (0; 0.25, 0.5; 1.0 and 2% PA/ddH$_2$O, v/v) on tomato plants, highlighted the positive biostimulatory effect on plant growth and fruit quality. However, it was found that the highest concentration of pyroligneous acid, 2%, caused a significant reduction in plant growth.

In the analysis of the chlorophyll *a* index values (Figure 2E), there was a decrease as the salinity of the irrigation water increased, from 1.65 to 1.85, 2.35 and 2.85 dS m^{-1}, with decreases of 8.02, 15.73, 17.18 and 35.39%, respectively, compared to the control. On the other hand, the application of the pyroligneous solution, at a concentration of 1% of the diluted extract, promoted an increase of 19.11% in chlorophyll *a* compared to the group of plants that did not receive the application of the solution (Figure 2F).

As observed for the chlorophyll *b* (FCI b) and total chlorophyll (FCI t) indices under low saline water application conditions (Figures 2G and 2I), plants subjected to treatment with 1.65 dS m^{-1} showed a significant increase, with average values of 9.48 in FCI *b* and 27.3 in FCI t. This increase corresponds to an increase of 23.54 and 2% in FCI *b* and FCI t, respectively, compared to the control treatment.

As for the results after application of the pyroligneous solution (PS), in the chlorophyll *b* (Figure 2H) and total chlorophyll (Figure 2J) indices, a positive effect on plants stands out, with increases of 23.54 and 20.47%, respectively, compared to the absence of PS application.

Understanding the efficacy of photochemical reactions in plants involves the analysis of the contribution of chlorophyll *b* in the energy transfer mechanism to chlorophyll *a*. Chlorophyll *b* absorbs light at wavelengths that are now efficiently absorbed by chlorophyll *a*, thus widening the light spectrum that plants can use for photosynthesis (MAGNEY; BARNES; YANG, 2020).

Benzon and Lee (2017) conducted studies to remediate polluted environments in South Korea using methods that involved the application of pyroligneous acids. During the research, they found that the biweekly application of pyroligneous acid, diluted 250 times, resulted in significant increases in the levels of chlorophyll *a*, chlorophyll *b* and total chlorophyll in Indian mustard plants, recording percentage increases compared to the control group of 21.38, 18.26 and 20.42%, respectively.

Brito et al. (2022) found the highest total chlorophyll values in sunflower (cv. BRS 323) in the groups with organic fertilizers produced with mixed algae, achieving mean values close to 35 (SPAD index). Adequate fertilization minimizes the harmful effects of salinity, promoting better photosynthetic activity and the movement of water and substances within the plant (SÁ et al., 2021).

In the analysis of variance (Table 2), only the concentrations of salts in the irrigation water had a statistically significant influence on the results of the variables shoot dry mass, root dry mass, flower bud fresh mass and flower bud dry mass.

Table 2. Analysis of variance (mean squares) of shoot dry mass (SDM), root dry mass (RDM), floral bud fresh mass (FBFM) and floral bud dry mass (FBDM) data, highlighting the specific effects of each source of variation.

SV	Saline	Attenuator	S x A	Residual	CV (%)	Mean
	4	1	4	30		
SDM	1.41**	0.27^{ns}	0.03^{ns}		14.78	2.89
RDM	0.40**	0.04^{ns}	0.006^{ns}		12.17	1.11
FBFM	0.93**	0.17^{ns}	0.05^{ns}		17.20	1.21
FBDM	1.00**	0.04^{ns}	0.06^{ns}		24.69	0.73

Data transformed by extracting the square root of (x+0). SV - source of variation; DF – degrees of freedom; CV - coefficient of variation; **, * and ^{ns} significant at 0.01, significant at 0.05 and not significant by F test, respectively.

At the salt stress level of 2.85 dS m^{-1}, a greater susceptibility to NaCl stress was evident, resulting in lower biomasses compared to those irrigated with water of 0.75 dS m^{-1} (control). There were reductions of approximately 25% in shoot dry mass (SDM) (Figure 3A), 30% in root dry mass (RDM) (Figure 3B), 44% in flower bud fresh mass (FBFM) (Figure 3C) and 63% in flower bud dry mass (FBDM) (Figure 3D). Despite possible variations in sensitivity to soil salinity among different plants, most of them experience negative effects on their growth and yield characteristics due to disturbances in respiration, photosynthesis and mineral absorption (MAJEED; MUHAMMAD, 2019).

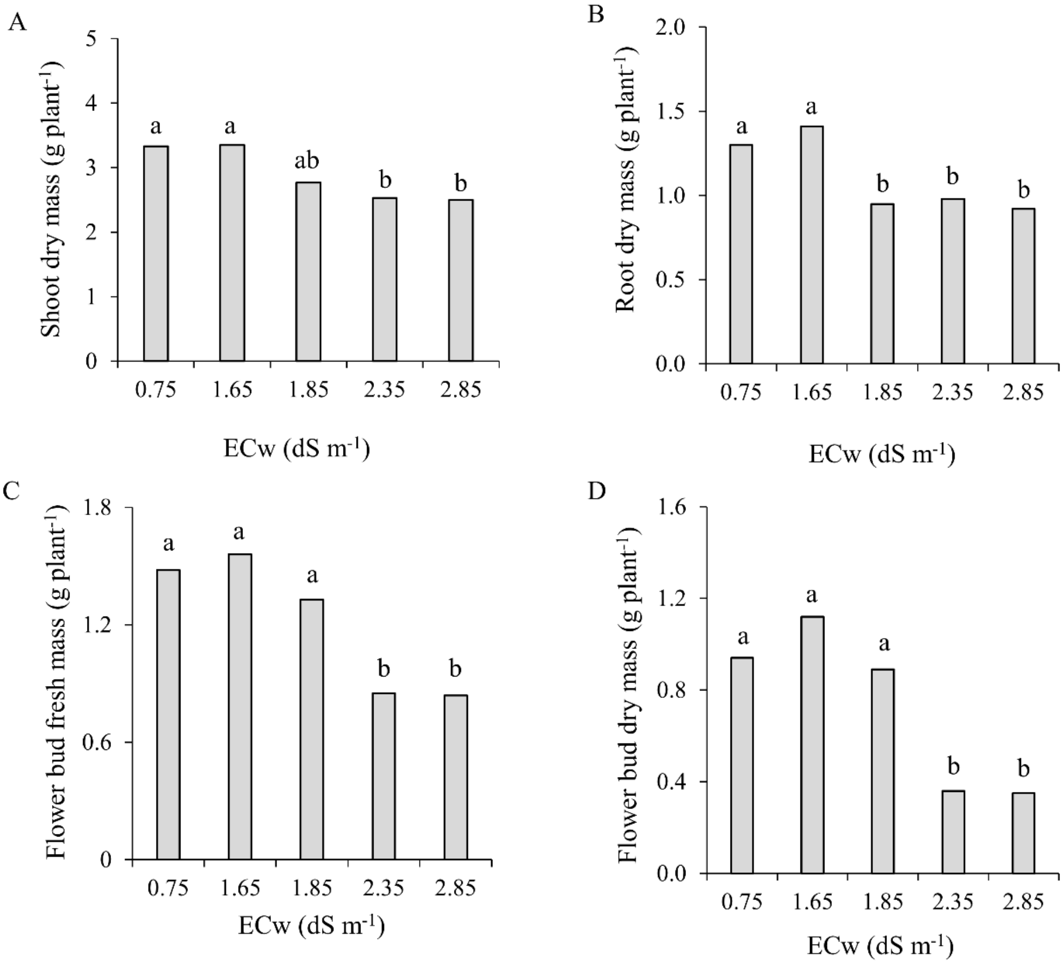

Figure 3. Shoot dry mass (A), root dry mass (B), flower bud fresh mass (C) and flower bud dry mass (D) in sunflower plants evaluated under different salinity levels. Different lowercase letters do not differ according to the Tukey test at 5% probability level.

While one group of sunflower plants was significantly impacted by maximum salt stress, others, irrigated with less saline water (1.65 dS m^{-1}), experienced increases in biomass production over a 50-day stress period. There was an increase of approximately 1% in SDM (Figure 3A), 7.8% in RDM (Figure 3B), 5.1% in FBFM (Figure 3C) and 14.5% in FBDM (Figure 3D) compared to the control group.

Silva and Nascimento (2020) carried out studies to evaluate the effects of salinity from different water sources on sunflower development and soil. The sources analyzed included rainwater (0.20 dS m^{-1}), well water (1.50 dS m^{-1}), septic tank effluent (3.50 dS m^{-1}) and brackish water (5.00 dS m^{-1}). The results indicated that rainwater, well water and septic tank effluent are suitable for sunflower irrigation. However, it was observed that the septic tank effluent caused soil sodification, and the brackish water resulted in reductions in biomass.

The increase in excessive use of chemical fertilizers in agriculture is generating a demand for innovative products that can improve the quality of crops in a more sustainable way (CARRIL et al., 2023).

CONCLUSIONS

The ability to tolerate salt stress was improved in sunflower plants exposed to salinity of 1.65 dS m^{-1}, resulting in improvements in shoot growth, plant height, stem diameter and leaf area. Furthermore, significant increases were observed in chlorophyll contents (*a*, *b* and total), as well as in the dry mass of shoots, roots and the development of flower buds.

The pyroligneous solution promoted the best development of the crop in relation to the growth parameters of stem diameter and the chlorophyll *a*, chlorophyll *b* and total chlorophyll indices.

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