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Physiological responses of sugar-apple seedlings under saline wastewater irrigation and NPK doses[1]

Respostas fisiológicas de mudas de pinheira irrigadas com águas residuárias salinas e doses de NPK

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ABSTRACT - Salt stress stands out as one of the main limiting factors in agricultural production in arid and semi-arid regions, due to its osmotic and ionic effects on plants. In this context, the objective was to evaluate the physiological responses of sugar-apple seedlings irrigated with saline wastewater under different doses of NPK. The experiment was carried out in a greenhouse, in a randomized block design, in a 3 x 5 factorial scheme, with four replicates. Three irrigation waters were tested: local-supply water (control), reject brine and fish farming effluent, and five doses of NPK, referring to the proportions of 25, 50, 75, 100, and 125% of the N:P:K fertilizer recommendation of 100:300:150 mg dm^{-3}. Seedlings were cultivated in 2-dm^3 containers filled with sandy soil for 90 days after sowing. At the end of this period, gas exchange, chlorophyll fluorescence, and biomass accumulation were evaluated. The use of reject brine and fish farming effluent to irrigate sugar-apple seedlings reduced photosynthetic activity and biomass accumulation. The best physiological responses and biomass accumulation occur at NPK doses of 75% (75:225:112.5 mg dm^{-3} of N:P:K) for seedlings irrigated with local-supply water, 60% (60:180: 90 mg dm^{-3} of N:P:K) for seedlings irrigated with reject brine, and 40% (40:120:60 mg dm^{-3} of N:P:K) for seedlings irrigated with fish farming effluent.

Key words: *Annona squamosa* L. Salt stress. Leaf gas exchange. Photochemical efficiency. Photochemical quenching.

RESUMO - O estresse salino destaca-se como um dos principais fatores limitantes na produção agrícola nas regiões áridas e semiáridas, devido os efeitos osmótico e iônico sobre as plantas. Neste contexto, objetivou-se avaliar as respostas fisiológicas de mudas de pinheira irrigadas com águas residuárias salinas sob diferentes doses de NPK. Para isso, o experimento foi conduzido em casa de vegetação, em delineamento de blocos casualizado, em esquema fatorial 3 x 5, com quatro repetições. Foram testadas três águas de irrigação, sendo elas água de abastecimento local (controle), rejeito de dessalinizadores e efluente da piscicultura. E cinco doses de NPK, referentes as proporções de 25, 50, 75, 100 e 125% da recomendação de adubação de 100:300:150 mg dm^{-3} de N:P:K. As mudas foram conduzidas em recipientes com capacidade de 2 dm^3 preenchido com solo de textura arenosa por 90 dias após a semeadura. No final desse período foram avaliadas as trocas gasosas, fluorescência da clorofila e o acúmulo de biomassa. O uso do rejeito salino e efluente da piscicultura na irrigação de mudas de pinha reduziu a atividade fotossintética e o acúmulo de biomassa. As melhores respostas fisiológicas e acúmulo de biomassa ocorrem nas doses de NPK de 75% (75:225:112,5 mg dm^{-3} de N:P:K) para mudas irrigadas com água de abastecimento, 60% (60:180:90 mg dm^{-3} de N:P:K) para irrigadas com rejeito salino e 40% (40:120:60 mg dm^{-3} de NPK), para irrigadas com efluente da piscicultura.

Palavras-chave: *Annona squamosa* L. Estresse salino. Trocas gasosas. Eficiência fotoquímica. Quenching fotoquímico.

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INTRODUCTION

Sugar-apple (*Annona squamosa* L.) is one of the main species of the family Annonaceae, native to Central America and adapted to the tropical climate of Brazil. Fruits are mainly used for fresh consumption since they have medicinal and nutritional properties, especially vitamins A, B, C, E, and K1, antioxidants, polyunsaturated fatty acids, and essential minerals (LIU *et al*., 2015; LIU; YUAN; JING, 2013). In addition, the cultivation of this species in the Brazilian semi-arid region has become an important agroeconomic alternative (LEMOS, 2014).

In the Brazilian semi-arid region, the main commercial orchards are irrigated, but the water available for irrigation is restricted and usually contains a high concentration of soluble salts, which reduces crop yield (SÁ *et al.*, 2019, 2021). In addition, alternative sources of water for irrigation, such as desalination reject brine and effluents from fish farming and shrimp farming, have been increasingly used and studied for irrigation in the semi-arid region (DIAS *et al.*, 2021).

Young sugar-apple plants are moderately sensitive to salinity, but under salt stress conditions they reduce growth, photosynthesis, stomatal conductance, photochemical efficiency, and the absorption of water and nutrients (ANDRADE *et al*., 2018; MARLER; ZOZOR, 1996; SÁ *et al*., 2015).

Salt stress causes osmotic and ionic restrictions in plants, because the high concentrations of soluble salts cause changes in the osmotic potential of the soil, preventing plants from absorbing water, and due to the accumulation of toxic ions, such as Na^+ and Cl^-, causing an imbalance in their nutrition, due to ionic competition with other essential ions (GUPTA; HUANG, 2014; VOLKOV; BEILBY, 2017; WAN *et al*., 2017).

Some studies have been conducted to improve the response of sugar-apple seedlings to irrigation water salinity, for instance with irrigation frequency and use of polymers (SILVA *et al*., 2018), phosphate fertilization (ANDRADE *et al*., 2018), nitrogen fertilization (FIGUEIREDO *et al*., 2019) and organic fertilization (SÁ *et al*., 2015). However, saline water from 1.1 dS m^{-1} is restrictive to the development of sugar-apple seedlings. Sá *et al*. (2021) evaluated fertilization management in sugar-apple plants irrigated with saline water until the production phase and found that adequate fertilization mitigates the effects of salt stress on growth, photosynthesis, and production of sugar-apple irrigated with water of 3.0 dS m^{-1}. However, little is known about the fertilization management of sugar-apple seedlings irrigated with saline water.

The objective of this study was to evaluate the physiological responses of sugar-apple seedlings irrigated with saline wastewater under different doses of NPK.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Federal Rural University of the Semi-Arid Region (UFERSA), in Mossoró-RN, Brazil, from May to August 2019. During the experimental period, the maximum and minimum temperatures recorded were 44.2 and 20.4 ºC, with maximum and minimum humidity of 86 and 22%, respectively.

The study was conducted in a randomized block design (RBD) in a 3 x 5 factorial scheme, corresponding to three types of irrigation water (local-supply water (control), desalination reject brine, and fish farming effluent) and five proportions of NPK (25, 50, 75, 100, and 125% of the fertilizer recommendation), with four replicates and two seedlings per replicate, totaling 120 plants.

The sugar-apple seedlings were produced through the seminiferous propagation method. The seeds were obtained from ripe and healthy fruits acquired in a local supermarket chain. Then, the seeds were extracted manually, washed in running water, arranged on a paper towel in the shade for drying for one week, and then the dormancy breaking process was performed according to the methodology of the Rules for Seed Analysis (BRASIL, 2009).

Sowing was performed in 2 dm^3 bags, with three seeds at 1.5 cm depth; after emergence, thinning was performed to maintain only one plant per bag. The emergence of pests and/or diseases in the seedlings was preventively evaluated every day, and no occurrence was observed during the experiment.

The soil used (Oxisol) was collected from a virgin area of the Rafael Fernandes Experimental Farm of UFERSA, District of Alagoinha, Mossoró, RN. Soil samples were collected in the 0.0-30.0 cm layer, pounded to break up clods, sifted (4 mm), and characterized according to their physical and chemical attributes following the methodology of Teixeira *et al*. (2017) (Table 1).

After physical and chemical characterization of the soil, its acidity was corrected using hydrated lime, with CaO and MgO contents of 48% and 24%, raising base saturation to 90%. After 15 days, the soil was fertilized according to the proportions recommended by Novais, Neves, and Barros (1991). For the dose of 100% NPK, 300 mg of $P_2O_5^-$, 150 mg of K_2O, and 100 mg of N, per dm^3 of soil, were added by fertigation, using urea (45% N), potassium chloride (KCl = 60% K_2O) and monoammonium phosphate (MAP = 12% N and 50% $P_2O_5^-$). Fertilization with micronutrients was foliar applied using the fertilizer Liqui-Plex Fruit®, in the proportion of 3 ml L^{-1} of the solution, following the manufacturer's recommendation (Table 2).

About the irrigation waters, local-supply water (ECw = 0.55 dS m^{-1}), fish farming effluent, from the cultivation of tilapia in the fish farming sector of UFERSA, and reject brine from reverse osmosis desalinators of rural communities in the municipality of Mossoró were collected and stored in 150 L plastic containers. The electrical conductivity (EC) of desalination reject brine was equaled to the EC of the fish farming effluent by dilutions, with local-supply water up to EC = 3.5 dS m^{-1}.

After soil tillage, an irrigation was carried out to leave it close to its maximum water retention capacity, and subsequent irrigations were performed once a day to leave the soil with moisture close to the maximum retention capacity, based on the drainage lysimeter method, with a leaching fraction (LF) of 15% added to the applied depth every 30 days. The volume applied (Va) per container was obtained by the difference between the previous depth (Lp) applied minus the mean drainage (D), divided by the number of containers (n), as indicated in Equation 1:

$$V\alpha = \frac{Lp - D}{n(1 - LF)} \quad (1)$$

The total volume of water applied per plant was 3.68 L, corresponding to an application of 1.18 g of salts in plants irrigated with local-supply water (0.5 dS m^{-1}) and 8.24 g of salts in plants irrigated with reject brine and fish farming effluent (3.5 dS m^{-1}). At 90 days after sowing, another leaching depth was applied (15%), and the drained volume was collected, in which the electrical conductivity of the drainage water (ECd) was measured using a benchtop conductivity meter with the data expressed in dS m^{-1} adjusted to a temperature of 25 ºC and pH. The electrical conductivity of the saturation extract (ECse) and the pH (Table 4) were determined using Equation 2 (Eq.2), proposed by Ayers and Westcot (1999) for medium-textured soils.

Table 1 - Chemical and physical analysis of the soil used in the experiment

pH	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	T	CEC	V	ESP
	(%)	-------- (mg dm^{-3}) -------			--------------------------- (cmolc dm^{-3}) -------------------------							------- % -------	
5.30	1.67	2.1	54.2	21.6	2.70	0.90	0.05	1.82	3.83	3.88	5.65	68	2.0
ECse dS m^{-1}	BD kg dm^{-3}	Sand				Silt				Clay			
		--- (g kg^{-1}) ---											
0.58	1.60	820				30				150			

OM - Organic matter; SB - sum of bases; CEC - cation exchange capacity; ESP - exchangeable sodium percentage; ECse - electrical conductivity of soil saturation extract; BD - Bulk density

Table 2 - Chemical characterization of the foliar fertilizer Liqui-Plex Fruit®

Parameters								
N	Ca	S	B	Cu	Mn	Mo	Zn^-	OC
-- g L^{-1} --								%
73.50	14.70	78.63	14.17	0.74	73.50	1.47	73.50	2.45

N – Nitrogen; Ca - Calcium; S – Sulphur; B – Boron; Cu – Copper; Mn – Manganese; Mo – Molybdenum; Zn – Zinc; OC – organic carbon

Table 3 - Analysis of water sources of the treatments used in the irrigation of sugar-apple seedlings

Water sources	pH	EC	K^+	Na^+	Mg^{2+}	Ca^{2+}	Cl^-	CO_3^{2-}	HCO_3^-	SAR
	H_2O	dS m^{-1}	------------------------------------- $mmol_c$ L^{-1} -------------------------------------							$(mmol_c\ L^{-1})^{0.5}$
1	7.80	0.53	0.31	6.64	0.30	1.10	2.60	0.20	2.80	7.9
2	8.10	3.50	0.35	19.37	5.70	8.80	30.80	0.60	2.30	7.2
3	8.20	3.50	0.66	16.34	8.90	12.20	22.60	1.20	3.40	5.0

Water source 1 - local-supply water; Water source 2 - desalination reject brine; Water source 3 - fish farming effluent; SAR - Sodium adsorption ratio

Table 4 - Electrical conductivity (ECse) and pH (pHse) of the soil saturation extract under irrigation with saline waters and different doses of NPK

NPK fertilizer recommendation (%)	ECse (dS m^{-1})			pHse		
	LSW	RBR	FFE	LSW	RBR	FFE
25	1.12	4.64	4.94	6.64	6.28	6.77
50	1.86	5.98	4.50	6.35	5.96	6.85
75	2.10	4.65	4.99	6.37	5.96	6.6
100	2.75	5.34	6.19	6.34	5.89	6.19
125	2.93	4.78	5.62	5.56	5.39	5.81

LSW – local-supply water; RBR – reject brine; FFE - fish farming effluent

$$ECse = \frac{ECd}{2} \quad (2)$$

At 90 days after sowing, the seedlings were evaluated for gas exchange, from 7 to 9 a.m. The evaluations were performed on the fully expanded leaves located in the upper third of each plant, with a portable infrared carbon dioxide analyzer (IRGA), Portable Photosynthesis System® LCPro+ model (ADC BioScientific Limited, UK), with temperature control at 25 °C, irradiation of 1200 μmol photons m^{-2} s^{-1} and airflow of 200 mL min^{-1}, to obtain net photosynthesis (A_N) in μmol CO_2 m^{-2} s^{-1}, transpiration (E) in mmol H_2O m^{-2} s^{-1}, stomatal conductance (gs) in mol H_2O m^{-2} s^{-1}, and internal CO_2 concentration (Ci) in mol m^{-2} s^{-1}, as well as leaf temperature (Tl) in ºC. These data were then used to quantify the instantaneous water use efficiency in ($WUEi = A_N/E$) (μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} $s^{-1})^{-1}$ and instantaneous carboxylation efficiency in (A_N/Ci) (μmol CO_2 m^{-2} s^{-1}/mol CO_2 m^{-2} $s^{-1})^{-1}$ (SÁ *et al*., 2019).

Immediately after gas exchange analysis, chlorophyll *a* fluorescence was evaluated using an Opti Science OS5P pulse-modulated fluorometer, and the Fv/Fm protocol was used for evaluations under dark conditions. Under these conditions, the maximum quantum efficiency of PSII (Fv/Fm) was estimated (SÁ *et al*., 2019). The pulse-modulated fluorometer was also used for evaluations under light conditions, using the Yield protocol. To obtain the initial fluorescence before the saturation pulse (F'), maximum fluorescence after adaptation to saturating light (Fm'), electron transport rate (ETR), and actual quantum efficiency of photosystem II (PS II) ($Y_{(II)}$).

With these data, the following parameters were determined: minimal fluorescence of the illuminated plant tissue (Fo') (OXBOROUGH; BAKER, 1997), photochemical quenching coefficient by the "lake" model (qL) (KRAMER *et al*., 2004), quantum yield of regulated photochemical quenching ($Y_{(NPQ)}$) (KRAMER *et al*., 2004), and quantum yield of non-regulated photochemical quenching ($Y_{(NO)}$) (KRAMER *et al*., 2004).

After physiological analyses, the seedlings were collected, sectioned into shoots and roots, packed in Kraft paper bags, dried in a forced air circulation oven at 65 °C until reaching constant weight, and weighed on the analytical scale (0.0001 g) to obtain shoot dry mass (SDM) and root dry mass (RDM), with results expressed in g per plant.

The data obtained were subjected to analysis of variance by the F test and, in cases of significance, a test of means was performed for qualitative factors (saline waters) and regression was applied for the quantitative factor (NPK doses), at 5% significance level, in the statistical software SISVAR® (FERREIRA, 2014).

RESULTS AND DISCUSSION

There was significant interaction ($p < 0.001$) between the factors irrigation water and NPK doses on transpiration rate (E), stomatal conductance (gs), net photosynthesis (A_N), carboxylation efficiency (A_N/Ci), and leaf temperature (Tl). There was a significant effect ($p < 0.001$) of the waters on internal CO_2 concentration (Ci) and instantaneous water use efficiency (*WUEi)*. There was a significant effect of NPK doses on Ci ($p < 0.001$) and *WUEi* ($p < 0.01$) (Table 5).

The *Ci* values of seedlings irrigated with reject brine and fish farming effluent were reduced by 6.90 and 15.12% compared to those of seedlings irrigated with local-supply water, respectively (Table 5). *Ci* was reduced with the increase in NPK dose by up to 12.61% when comparing the doses of 25 and 125% (Figure 1A).

The tested models failed to the data of E, gs, A_N, and A_N/Ci of sugar-apple seedlings irrigated with local-supply water, with means of 1.23 (mmol H_2O m^{-2} s^{-1}), 0.055

Table 5 - F test and means comparison test for internal CO_2 concentration (*Ci*, in mol CO_2 mol^{-1}), transpiration (*E*, in mmol H_2O m^{-2} s^{-1}), stomatal conductance (*gs*, in mol H_2O m^{-2} s^{-1}), net photosynthesis (A_N, in μmol CO_2 m^{-2} s^{-1}), instantaneous water use efficiency (*WUEi*, in μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}), carboxylation efficiency (A_N/Ci, in μmol CO_2 m^{-2} s^{-1}/ mol CO_2 m^{-2} s^{-1}), and leaf temperature (*Tl*, in °C) of sugar-apple seedlings under irrigation with saline waters and NPK doses, at 90 days after sowing

F test (p-value)							
Sources of variation	*Ci*	*E*	*gs*	A_N	*WUEi*	A_N/Ci	*Tl*
Block	0.714	0.075	0.001	0.263	0.528	0.841	0.000
Waters	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NPK doses	0.000	0.000	0.000	0.000	0.000	0.001	0.000
Waters x NPK doses	0.164	0.000	0.000	0.000	0.282	0.000	0.000
CV (%)	8.26	13.30	14.04	14.91	8.64	22.29	0.49
Tukey test ($p < 0.05$)							
Waters	*Ci*	*E*	*gs*	A_N	*WUEi*	A_N/Ci	*Tl*
LSW	207.25 a	1.24 a	0.056 a	5.75 a	4.66 a	0.028 a	32.74 c
RBR	192.95 b	1.19 a	0.047 b	4.99 b	4.23 b	0.026 a	34.18 b
FFE	175.90 c	0.90 b	0.030 c	3.57 c	3.92 c	0.021 b	35.60 a
LSD	12.19	0.11	0.047	0.546	0.283	0.004	0.128

LSW - local-supply water; RBR - reject brine; FFE - fish farming effluent. CV = coefficient of variation; LSD = least significant difference. Means followed by equal letters in the column do not differ by the Tukey test at 0.05 probability level

(mol H_2O m^{-2} s^{-1}), 5.75 (μmol CO_2 m^{-2} s^{-1}), and 0.027 (μmol CO_2 m^{-2} s^{-1}/mol CO_2 m^{-2} s^{-1}), respectively (Figures 1B, C, D, E). For seedlings irrigated with reject brine, the best values of *E*, *gs*, A_N, and A_N/Ci were obtained at the NPK doses of 60, 62, 61, and 64%, with means of 1.61 (mmol H_2O m^{-2} s^{-1}), 0.07 (mol H_2O m^{-2} s^{-1}), 6.82 (μmol CO_2 m^{-2} s^{-1}), and 0.036 (μmol CO_2 m^{-2} s^{-1}/ mol CO_2 m^{-2} s^{-1}), respectively (Figures 1B, C, D, E). In sugar-apple seedlings irrigated with fish farming effluent, *E*, *gs*, A_N, and A_N/Ci were linearly reduced with the increase in NPK doses, with reductions of up to 59.22, 64.10, 58.64, and 72.20% when comparing the doses of 25 and 125%, respectively (Figures 1B, C, D, E).

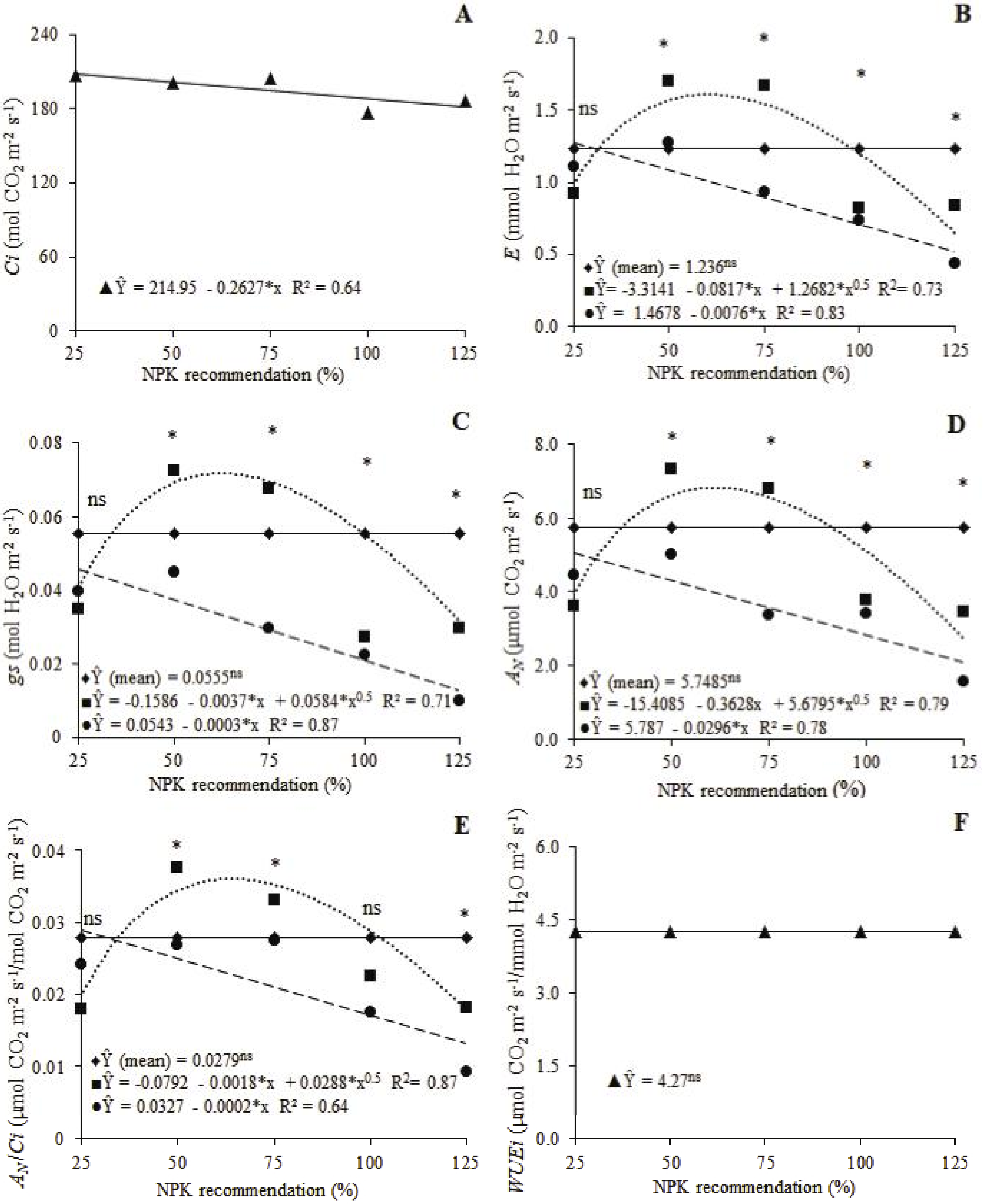
At the NPK dose of 25%, the highest means of *E*, *gs*, A_N, and A_N/Ci were obtained in sugar-apple irrigated with local-supply water, and there was no difference between the means of seedlings irrigated with reject brine and fish farming effluent (Figures 1B, C, D, E). At the NPK dose of 50%, the highest means of *E*, *gs*, A_N, and A_N/Ci were obtained in sugar-apple irrigated with reject brine (Figures 1B, C, D, E). For *E*, A_N, and A_N/Ci, similar means were observed for local-supply water and fish farming effluent (Figures 1B, D, E). The lowest *gs* values were obtained with fish farming effluent (Figures 1C). At the NPK dose of 75%, the highest means of *E*, *gs*, A_N, and A_N/Ci were obtained in seedlings irrigated with reject brine (Figures 1B, C, D, E). For *E* and A_N/Ci, similar means were observed for local-supply water and fish farming effluent (Figures 1B, D). The lowest values of *gs* and A_N were obtained with fish farming effluent (Figures 1C, E).

At the NPK dose of 100%, there was no difference between the irrigation waters for A_N/Ci (Figures 1E). For *E*, *gs*, and A_N, the highest values were obtained in seedlings irrigated with local-supply water, and there was no difference between the means of seedlings irrigated with reject brine and fish farming effluent (Figures 1B, C, D). At the NPK dose of 125%, seedlings irrigated with local-supply water obtained the highest means of *E*, *gs*, and A_N, followed by those irrigated with reject brine and fish farming effluent, respectively (Figures 1B, C, D). At this dose, seedlings irrigated with local-supply water obtained the highest means of A_N/Ci, and there was no difference between reject brine and fish farming effluent (Figure 1E).

The *WUEi* values of sugar-apple seedlings irrigated with reject brine and fish farming effluent were reduced by 9.22 and 15.87% compared to those of seedlings irrigated with local-supply water, respectively (Table 5). For fertilization with NPK, there was no significant fit of the regression, with a mean of 4.27 (μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}) (Figure 1F).

The gas exchange of sugar-apple seedlings irrigated with local-supply water (0.5 dS m^{-1}) was not affected by NPK doses. Under this condition, mean values of 1.24 mmol H_2O m^{-2} s^{-1} for *E*, 0.056 mol H_2O m^{-2} s^{-1} for *gs*, 5.75 μmol CO_2 m^{-2} s^{-1} for A_N, and 0.028 (μmol CO_2 m^{-2} s^{-1}/ mol CO_2 m^{-2} s^{-1}) for A_N/Ci were observed. The results observed are within the appropriate range

Figure 1 - Regression analysis and Tukey test for internal CO_2 concentration, *Ci* (A), transpiration, *E* (B), stomatal conductance, *gs* (C), CO_2 assimilation rate, *A* (D), instantaneous carboxylation efficiency, A_N/Ci (E), and instantaneous water use efficiency, *WUEi* (F) of sugar-apple seedlings under irrigation with saline waters and NPK doses, at 90 days after sowing



♦ local-supply water, ■ reject brine, and ● fish farming effluent. * and ns = significant at a 5% probability level and not significant for fitting parameters in the regression analysis and Tukey test between means for each NPK dose, respectively reported in the literature for young sugar-apple plants (FIGUEIREDO *et al*., 2019; SÁ *et al*., 2021). Although there is no difference in photosynthetic activity between NPK doses, the best shoot biomass accumulation was found with the dose of 75% of NPK recommendation. This reduction is explained by the increase in the electrical conductivity of the soil saturation extract (ECse) as a function of NPK fertilization (Table 4). The osmotic restriction imposed by the increase of ECse may not reduce A_N, but reduces the number of leaves and the photosynthetically active area of the plant, restricting biomass accumulation (SILVA *et al*., 2021).

In the analysis of the gas exchange of seedlings irrigated with reject brine (3.5 dS m^{-1}), the best values of *E, gs*, A_N, and A_N/Ci were obtained with doses from 60 to 64% of the NPK recommendation, but seedlings fertilized with doses from 50 to 100% of the NPK recommendation obtained *E*, *gs*, A_N, and A_N/Ci higher than or equal to those of seedlings irrigated with local-supply water (control) and higher than those of seedlings irrigated with fish farming effluent.

In seedlings irrigated with reject brine (3.5 dS m^{-1}), the behavior of *E*, A_N, and A_N/Ci corroborated with the behavior of stomatal conductance (*gs*), indicating that stomatal restriction, besides regulating water loss by transpiration, was effective in controlling photosynthesis. It should be noted that the reject brine contained more Na^+ than the fish farming effluent (Table 3), disfavoring the K^+/Na^+ ratio in the soil near the root zone, requiring greater transpiration flow (*E*) to absorb water and K^+ by the roots, thus forcing the opening of the stomata (*gs*). The opening of the stomata favors the influx of CO_2 (*Ci*) to the substomatal chamber (Table 5), so there was an increase in the substrate (CO_2) for ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), consequently increasing net photosynthesis (A_N) and carboxylation efficiency (A_N/Ci). However, this mechanism lost efficiency with the increase in soil salinity, due to irrigation with saline water and high doses of NPK (Table 4).

Silva *et al*. (2014) found that some genotypes have the capacity to increase the photosynthetic rate as a mechanism of salinity tolerance. In sugar-apple seedlings irrigated with reject brine, the increase in A_N coincided with the increase in SDM, with the highest values of these variables being observed at doses of 61 and 69.5% of the NPK recommendation. However, the dry mass accumulations of shoots and roots of seedlings irrigated with reject brine were low, below those of seedlings irrigated with low-salinity water by up to 59% for SDM and 67% for RDM.

In seedlings irrigated with fish farming effluent, there were linear reductions in *E*, *gs*, A_N, and A_N/Ci, with the increase in NPK doses. In this treatment, the reductions in stomatal conductance as a function of increased NPK doses coincide with the reductions in *Ci*, A_N, and *E*. Irrigation with fish farming effluent led to the lowest *Ci*, A_N, and *E*, and consequently the lowest *WUEi* and A_N/Ci. Under salt stress conditions, plants close their stomata as a defense mechanism, no longer capturing CO_2 (HUSSAIN *et al*., 2012), and CO_2 restrictions in mesophyll cells reduce the efficiency of the RuBisCO enzyme, consequently reducing CO_2 assimilation (TAIZ *et al*., 2017). Under these conditions, the reductions in A_N were greater than the reductions in *E*, reducing *WUEi*. According to Silva *et al*. (2014), the decrease in A_N/Ci is indicative of malfunction of photosystem II, and consequently low production of adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) (HUSSAIN *et al*., 2012).

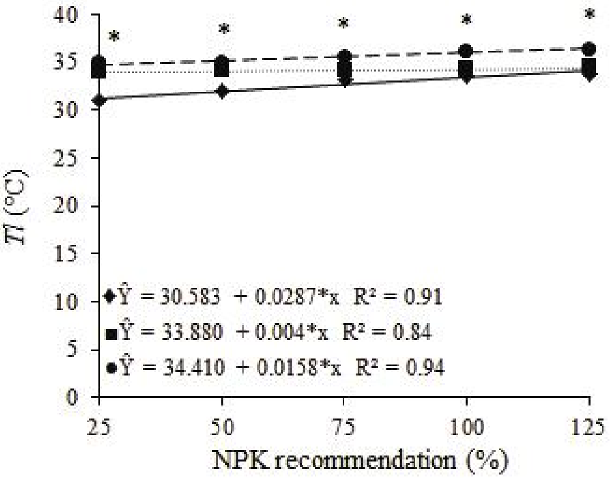
The *Tl* values of sugar-apple seedlings irrigated with local-supply water, reject brine, and fish farming effluent was increased by 9.16, 2.99, and 4.54%, between the NPK doses of 25 and 125%, respectively (Figure 2). Seedlings irrigated with fish farming effluent obtained the highest means of *Tl*, followed by seedlings irrigated with reject brine and local-supply water at all doses of NPK, respectively (Figure 2). Transpiration is the main mechanism involved in *Tl* regulation; however, due to the smaller stomatal openings, there is a decrease in transpiration, leading to an increase in leaf temperature (FIGUEIREDO *et al*., 2019). In sugar-apple seedlings, there was an increase in leaf temperature with the increase in NPK doses, regardless of irrigation water, but with fish farming effluent and reject brine the average leaf temperatures were 8.7 and 4.4% higher in seedlings irrigated with local-supply water. The increase in leaf temperature is indicative of energy dissipation through heat irradiation (TAIZ *et al*., 2017).

There was no significant interaction ($p > 0.05$) between irrigation waters and NPK doses for chlorophyll a fluorescence variable (Table 6). However, there was a significant effect of the irrigation water factor on the maximum quantum efficiency of PSII (Fv/Fm) ($p < 0.01$), the actual quantum efficiency of PSII ($Y_{(II)}$) ($p < 0.001$), electron transport rate (ETR) ($p < 0.05$), minimal fluorescence of illuminated plant tissue (Fo') ($p < 0.001$), and quantum yield of regulated photochemical quenching (Y_{NPQ}) ($p < 0.001$) (Table 6). NPK doses failed for the photochemical efficiency and photochemical quenching of sugar-apple seedlings (Table 6).

The Fv/Fm, $Y_{(II)}$, and ETR of sugar-apple seedlings were reduced by 2.6, 35.7, and 23.44% under irrigation with reject brine and by 2.3, 35.7, and 23.3% under irrigation with fish farming effluent, compared to seedlings irrigated with local-supply water, respectively (Table 6). Fo' and Y_{NPQ} of sugar-apple seedlings were increased by 51.34 and 27.97% under irrigation with reject brine and by 47.79 and 28.16% under irrigation with fish farming effluent, compared to seedlings irrigated with local-supply water, respectively (Table 6).

The use of reject brine and fish farming effluent in irrigation reduced the efficiency of PSII (Fv/Fm and $Y_{(II)}$).

Figure 2 - Regression analysis and Tukey test (p < 0.05) for leaf temperature (Tl) of sugar-apple seedlings under irrigation with saline waters and NPK doses, at 90 days after sowing



♦ local-supply water, ■ reject brine, and ● fish farming effluent. * and ns = significant at a 5% probability level and not significant for fitting parameters in the regression analysis and Tukey test between means for each NPK dose, respectively

These results corroborate the decrease in A_N and A_N/Ci, indicating that, in addition to stomatal closure, the lack of ATP and NADPH compromised photosynthetic activity. This fact was also verified by Sá *et al*. (2021) in sugar-apple plants irrigated with saline water in the vegetative and reproductive stages.

The reductions in Fv/Fm and $Y_{(II)}$ of seedlings irrigated with reject brine and fish farming effluent can be explained by the reduction of ETR. Under this condition, the energy that reaches the PSII is not following in the electron transport chain and accumulates in the PSII, and the dissipation of this energy can occur in two ways, with increased flowering or increased heat irradiation (TAIZ *et al*., 2017). In the present study, there was an increase in Tl, and consequently, part of the accumulated energy was dispersed in the form of heat. Another part was dissipated by regulated photochemical quenching ($Y(_{NPQ})$) and by energy losses due to damage to the photosynthetic apparatus (Fo') (KRAMER *et al*., 2004; OXBOROUGH; BAKER, 1997; SANTOS *et al*., 2020; SILVA *et al*., 2021).

The interaction between irrigation waters and NPK doses was significant for shoot dry mass (SDM) (*p* > 0.01) and root dry mass (RDM) (*p* > 0.001) (Table 7).

The SDM of sugar-apple seedlings irrigated with local-supply water, reject brine, and fish farming effluent showed quadratic behavior as a function of NPK doses, with the highest values at doses of 75.0, 69.5, and 43% of NPK recommendation, equal to 2.24, 1.11, and 0.92 g per plant, respectively (Figure 3A).

Table 6 - Summary of F test and means test for the actual quantum efficiency of PSII ($Y_{(II)}$), electron transport rate (ETR, in µmol (photons) m^{-2} s^{-1}), minimal fluorescence of illuminated plant tissue (Fo', in µmol (photons) m^{-2} s^{-1}), photochemical quenching coefficient (qL), the quantum yield of regulated photochemical quenching ($Y(_{NPQ})$), and quantum yield of unregulated photochemical quenching (Y_{NO}) of sugar-apple seedlings under irrigation with saline waters and NPK doses, at 90 days after sowing

F test (p-value)							
Sources of variation	Fv/Fm	$Y_{(II)}$	ETR	Fo'	qL	$Y_{(NPQ)}$	$Y_{(NO)}$
Block	0.671	0.155	0.721	0.896	0.524	0.164	0.531
Waters	0.005	0.000	0.044	0.000	0.541	0.000	0.717
NPK doses	0.952	0.334	0.946	0.095	0.767	0.314	0.685
Waters x NPK doses	0.832	0.141	0.559	0.054	0.905	0.127	0.688
CV(%)	2.66		28.31	15.68	39.01	32.37	14.78
Tukey test (p < 0.05)							
Waters	Fv/Fm	Y	ETR	Fo'	qL	Y_{NPQ}	Y_{NO}
LSW	0.732 a	0.42 a	52.12 a	5.59 b	0.015 a	0.529 b	0.048 a
RBR	0.713 b	0.27 b	39.90 b	8.46 a	0.015 a	0.677 a	0.050 a
FFE	0.715 b	0.27 b	39.99 b	7.87 a	0.014 a	0.678 a	0.050 a
LSD	0.147	0.07	11.45	0.88	0.003	0.07	0.007

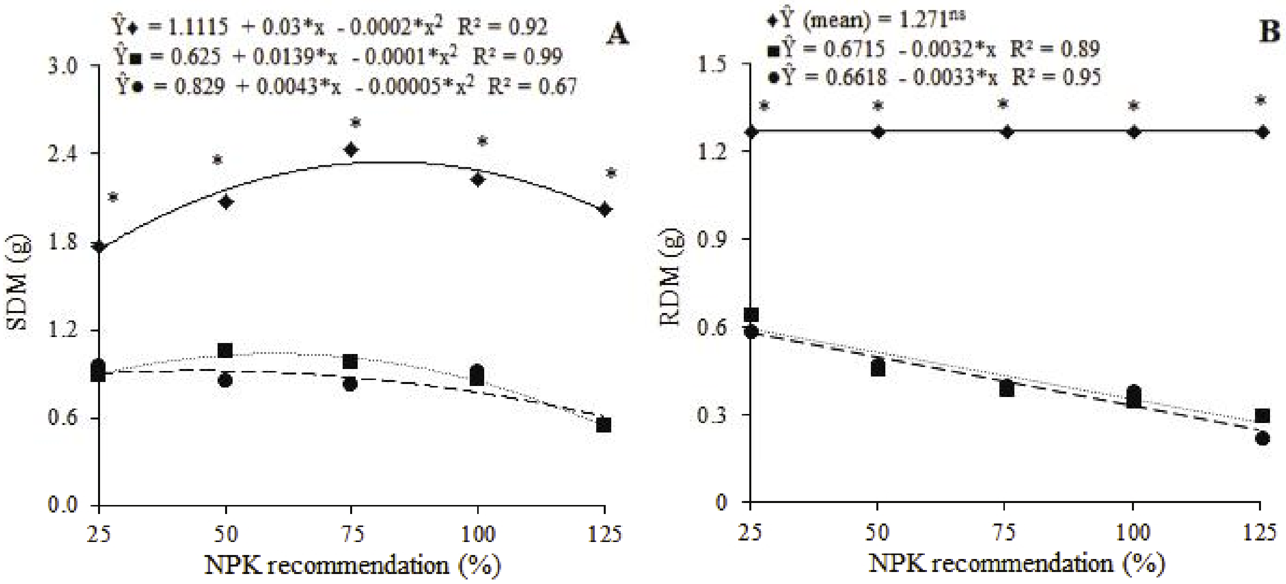
LSW - local-supply water; RBR - reject brine; FFE - fish farming effluent. CV = coefficient of variation; LSD = least significant difference. Means followed by equal letters in the column do not differ by the Tukey test at a 0.05 probability level

Table 7 - F test and means test for shoot dry mass (SDM, in g) and root dry mass (RDM, in g) of sugar-apple seedlings under irrigation with saline waters and NPK doses, at 90 days after sowing

	F test (p-value)	
Sources of variation	SDM	RDM
Block	0.525	0.015
Waters	0.000	0.000
NPK doses	0.000	0.000
Waters x NPK doses	0.001	0.000
CV(%)	13.14	21.22
	Tukey test ($p < 0.05$)	
Waters	SDM	RDM
1 - LSW	2.11 a	1.30 a
2 - RBR	0.87 b	0.43 b
3 - FFE	0.82 b	0.41 b
LSD	0.13	0.12

LSW - local-supply water; RBR - reject brine; FFE - effluent from fish farming. CV = coefficient of variation; LSD = least significant difference. Means followed by equal letters in the column do not differ by the Tukey test at a 0.05 probability level

Figure 3 - Shoot dry mass, SDM (A) and root dry mass, RDM (B) of sugar-apple seedlings under irrigation with saline water and NPK doses, at 90 days after sowing



♦ local-supply water, ■ reject brine, and ● fish farming effluent. * and ns = significant at a 5% probability level and not significant for fitting parameters in the regression analysis and Tukey test between means for each NPK dose, respectively

For RDM, there was no fit of the models tested in irrigation with local-supply water, with a mean of 1.271 g per plant (Figure 3B). In seedlings irrigated with reject brine and fish farming effluent, there were reductions of 56 and 57% in RDM with the increase in NPK dose from 25 to 125% of the recommendation, respectively (Figure 3B).

The SDM and RDM of sugar-apple seedlings irrigated with local-supply water were higher than those obtained in seedlings irrigated with reject brine and fish farming effluent at all NPK doses (Figures 3A and 3B). There were reductions of up to 59 and 61% in the SDM and up to 67 and 68% in the RDM of seedlings irrigated with reject brine and fish farming effluent, compared to those irrigated with local-supply water, respectively (Figures 4A and 4B). There was no difference in the SDM and RDM accumulations of seedlings irrigated with reject brine and fish farming effluent at the NPK doses (Figures 3A and 4B).

Although seedlings irrigated with fish farming effluent showed lower photosynthetic activity than that of seedlings irrigated with reject brine, there was no difference in SDM and RDM accumulations as a function of irrigation with these types of wastewater and, with both, biomass accumulation was lower than that of seedlings irrigated with local-supply water. Salt stress causes osmotic and ionic restrictions in plants, as the high concentrations of soluble salts cause changes in the osmotic potential of the soil, preventing the plant from absorbing water, and due to the accumulation of toxic ions, such as Na^+ and Cl^-, causing an imbalance in plant nutrition, resulting from ionic competition with other essential ions (GUPTA; HUANG, 2014; VOLKOV; BEILBY, 2017; WAN *et al*., 2017).

The efficiency losses of PSII associated with stomatal closure contributed to a reduction in the photosynthetic rate, and consequently to the lower accumulations of shoot and root dry mass in sugar-apple seedlings irrigated with reject brine and fish farming effluent.

CONCLUSIONS

1. The use of reject brine and fish farming effluent in the irrigation of sugar-apple seedlings reduces photosynthetic activity and biomass accumulation;
2. The best physiological responses and biomass accumulation occur at NPK doses of 75% (75:225:112.5 g N:P:K dm^{-3}) for seedlings irrigated with local-supply water, 60% (60:180:90 g dm^{-3} of N:P:K) for seedlings irrigated with reject brine and 40% (40:120:60 g dm^{-3} of NPK), for seedlings irrigated with fish farming effluent.

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