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# **Growth and ionic homeostasis** of custard apple seedlings irrigated with saline wastewater<sup>1</sup>

## Crescimento e homeostase iônica de mudas de pinha irrigadas com águas residuais salinas

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#### HIGHLIGHTS:

Custard apple seedlings irrigated with saline wastewater achieve better ion homeostasis and save fertilizers. Independent of the NPK amount applied, irrigation with saline wastewater limits the development of custard apple seedlings. Under irrigation with local-supply water, custard apple seedlings require 75% of the NPK recommendation.

**ABSTRACT:** Custard apple (*Annona squamosa* L.) is a sensitive fruit crop to salinity. One of the alternatives to minimize the deleterious effects of salt stress is adopting mineral fertilization management practices, promoting increased crop tolerance to salts. Therefore, the objective of the present study was to evaluate NPK doses to mitigate salt stress in custard apple seedlings irrigated with saline wastewater. The experiment was performed in a greenhouse, adopting a randomized block design in a  $3 \times 5$  factorial scheme, corresponding to three irrigation waters (local-supply water of 0.53 dS m<sup>-1</sup> electrical conductivity (control); desalination reject of 3.5 dS m<sup>-1</sup>; fish farming effluent of 3.5 dS m<sup>-1</sup>) and five proportions of the NPK recommendation (25, 50, 75, 100, and 125% of the fertilization with desalination reject, and fish farming effluent restricted the growth of custard apple seedlings, regardless of the NPK dose used. The ionic homeostasis of seedlings irrigated with desalination reject and fish farming effluent is optimized by fertilization with 50% of the NPK recommendation corresponding to 50, 150, and 75 mg of N, P,O, and K<sub>2</sub>O dm<sup>-3</sup>, respectively. For the production of seedlings irrigated with local-supply water, the dose of 75% NPK, corresponding to 75, 225, and 112.5 mg of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O dm<sup>-3</sup>, respectively, is recommended.

Key words: Annona squamosa L., irrigation, salt stress, fertilization, plant nutrition

**RESUMO:** A pinheira (*Annona squamosa* L.) é uma frutífera sensível à salinidade. Uma das alternativas para minimizar os efeitos deletérios do estresse salino é a adoção de práticas de manejo da fertilização mineral, promovendo o aumento da tolerância da cultura aos sais. Portanto, o objetivo foi avaliar as doses de NPK para mitigar o estresse salino em mudas de pinheira irrigadas com água residual salina. O experimento foi realizado em casa de vegetação, em delineamento de blocos casualizados, em esquema fatorial 3 × 5, correspondendo a três águas de irrigação (água de abastecimento local de 0,53 dS m<sup>-1</sup> de condutividade elétrica (controle); rejeito salino da dessalinização de 3,50 dS m<sup>-1</sup>; efluente de piscicultura de 3,50 dS m<sup>-1</sup>) e cinco proporções da recomendação de NPK (25, 50, 75, 100 e 125% da recomendação de fertilização), com quatro repetições. O crescimento, acúmulo de biomassa e nutrientes foram avaliados. A irrigação com rejeito salino e efluente de piscicultura é dase de NPK utilizada. A homeostase iônica das mudas irrigadas com rejeito salino e efluente de piscicultura é o timizada pela fertilização com 50% da recomendação de NPK correspondente a 50, 150 e 75 mg de N, P<sub>2</sub>O<sub>5</sub> e K<sub>2</sub>O dm<sup>-3</sup>, respectivamente. Para a produção de mudas irrigadas com água de abastecimento local, recomenda-se a dose de NPK, correspondente a 75, 225 e 112,5 mg de N, P<sub>2</sub>O<sub>5</sub> e K<sub>2</sub>O dm<sup>-3</sup>, respectivamente.

Palavras-chave: Annona squamosa L., irrigação, estresse salino, fertilização, nutrição de plantas

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#### INTRODUCTION

Custard apple (*Annona squamosa* L.) is a fruit crop from the Annonaceae family of dry tropical climates (Pereira & Kavati, 2011). Within the family of Annonaceae plants, this species holds excellent economic significance and is popular in Brazil, where it is frequently used in traditional medicine to treat a range of illnesses, such as cancer and parasite infections (Cascaes et al., 2021; Ferreira et al., 2022).

The cultivation of custard apple fruit has increased due to recent interest. However, the expansion of this crop faces several agronomic limitations, as the recommendations of fertilization are carried out empirically and because of the noticeable scarcity of experimental studies on nutritional requirements, especially under semi-arid conditions, where the nutritional imbalance associated with salt stress can cause drastic effects on the custard apple establishment and production (Sá et al., 2015; Figueiredo et al., 2019; Fernandes et al., 2021; Sá et al., 2021). The excess of soluble salts, commonly found in water sources in northeastern Brazil (Silva Junior, 1999), can inhibit seedling growth and make seedling production and, consequently, agricultural yield unfeasible (Cheng et al., 2021).

Thus, it is necessary to seek soil and water management strategies, such as the management of organic and mineral fertilizers and irrigation management, to reduce the accumulation of salts in the soil and their adverse effects on plants (Sá et al., 2015). According to Lacerda et al. (2006), the optimal nutrient level for a species without salts can be toxic to some plants when grown in saline conditions. For Sá et al. (2019; 2021), the beneficial effect of increasing fertilization on plant growth, physiology, and production depends on the salinity condition under which the plant grows. Therefore, this study aimed to evaluate NPK doses to mitigate salt stress in custard apple seedlings irrigated with saline wastewater.

#### MATERIAL AND METHODS

The experiment was conducted from May to August 2019 in a greenhouse belonging to the Center of Agrarian Sciences, located on the East campus of the Federal Rural University of the Semi-Arid Region - UFERSA, in Mossoró-RN, Brazil (5° 12' S and 37° 19' W, with an average altitude of 18 m). The predominant climate in the municipality where the study was carried out is BSh, a hot semiarid climate, according to Köppen's classification (Alvarez et al., 2013). In the greenhouse, the average temperature and daily relative humidity of air during the experimental period were 33.8 °C and 49.0%, respectively. The greenhouse consists of an arch-shaped cover, with a width of 6.4 m, length of 18.0 m, and ceiling height of 3.0 m, covered with low-density polyethylene film with anti-ultraviolet additive and thickness of  $150~\mu\text{m},$  protected on the sides with 50% black net.

The experimental design used was randomized blocks in a 3 x 5 factorial scheme; the first factor consisted of three irrigation waters (local-supply water of 0.53 dS m<sup>-1</sup> (control); desalination reject of electrical conductivity of 3.50 dS m<sup>-1</sup>; fish farming effluent of 3.50 dS m<sup>-1</sup>), while the second factor corresponded to five proportions of NPK (25, 50, 75, 100, and 125% of the fertilization recommendation), totaling 15 treatments with four replications and two seedlings per replication, in a total 120 plants.

Seeds of custard apples acquired in a local supermarket network were extracted to produce seedlings. In the removal of the seeds, the procedure of washing them in running water to remove pulp remnants was adopted. Then they were dried in the shade on sheet of newspaper. Mechanical scarification was used to break dormancy by cutting the seed tegument opposite the embryo axis before being sown (BRASIL, 2009). Sowing was carried out in polyethylene bags with a capacity of 2 dm<sup>3</sup>. Initially, three seeds were sown at 1.5 cm depth, and after emergence, thinning was performed to maintain only one plant per bag. To prevent diseases and pest attack the seedlings were monitored daily.

In the greenhouse cultivation of custard apple seedlings, the soil used came from an uncultivated area of the experimental farm of UFERSA, Campus of Mossoró, classified as an Ultisol (USDA, 2014) equivalent to an Argissolo Vermelho-Amarelo latossólico (EMBRAPA, 2018). Soil samples were collected in the 0-30 cm layer and taken to the Soil and Plant Nutrition Laboratory of UFERSA, where physical and chemical characteristics were evaluated following the methodology of Silva (2009). The results are presented in Table 1.

After physical and chemical characterization of the soil, soil acidity was corrected with hydrated lime, with CaO and MgO contents of 48 and 24%, base saturation was increased to 90% and, after 15 days, the soil was fertilized according to the recommendations of Novais et al. (1991) for pot experiment in protected cultivation, applying 100 mg of N per dm<sup>3</sup> of soil, 300 mg of P<sub>2</sub>O<sub>5</sub> and 150 mg of K<sub>2</sub>O through fertigation, using as source urea (45% N), and monoammonium phosphate (MAP = 12% N and 50% P<sub>2</sub>O<sub>5</sub>), and potassium chloride (KCl = 60% K<sub>2</sub>O). Fertilization with micronutrients was performed by foliar application with the fertilizer Liqui-Plex Fruit<sup>\*</sup>, in the proportion of 3 mL L<sup>-1</sup> of solution, following the manufacturer's recommendation (Table 2).

Regarding irrigation waters, local-supply water (electrical conductivity -  $EC = 0.53 \text{ dS m}^{-1}$ ), fish farming effluent from the tilapia cultivation of the fish farming sector of UFERSA, and reverse osmosis desalination reject from rural communities in the municipality of Mossoró were collected and stored in 150-L

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

nU	OM	P	<b>K</b> +	Na+	Ca <sup>2+</sup>	Mg <sup>2+</sup>	<b>Al</b> <sup>3+</sup>	H+AI	SB	t	CEC	BS	ESP
рН	(%)		(mg dm <sup>-3</sup> )					(cmol <sub>c</sub> dm <sup>-3</sup> )	)			(9	%)
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
	Density			Sar	ıd			Silt				Clay	
	(kg dm <sup>-3</sup> )							(g kg <sup>.1</sup> )					
	1.60			820				30				150	

pH - Soil potential hydrogen in water; OM - Organic matter; SB - Sum of exchangeable bases; t - Effective cation exchange capacity; CEC - cation exchange capacity at pH 7.0; BS - Base saturation; ESP - Exchangeable sodium percentage

Table 2. Chemical characterization of Liqui-	i-Plex Fruit <sup>®</sup> foli	ar fertilizer
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				Nutrients				00	
N	Ca	S	В	Cu	Mn	Мо	Zn	- OC - (%)	
(g L <sup>-1</sup> )									
73.50	14.70	78.63	14.17	0.74	73.50	1.47	73.50	2.45	
N - Nitrogen; Ca - Ca	- Nitrogen; Ca - Calcium; S - Sulfur; B - Boron; Cu - Copper; Mn - Manganese; Mo - Molybdenum; Zn - Zinc; OC - Organic carbon								

plastic containers. The EC of desalination reject was equal to the EC of the fish farming effluent  $(3.5 \text{ dS m}^{-1})$  by diluting the latter with local-supply water (Table 3). The treatments began to be applied on the first day, shortly after sowing.

After soil preparation, irrigation was carried out to elevate soil moisture content close to its maximum water retention capacity. Subsequent irrigations were performed once a day in order to leave the soil with moisture close to the maximum retention capacity, based on the drainage lysimetry method, applying a leaching fraction (LF) of 15% every 30 days along with the applied depth. The volume applied (Va) per container was obtained by the difference between the previous depth applied (La) minus the mean drainage (D), divided by the number of containers (n), as indicated in Eq. 1:

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

where:

Va - volume of water to be applied (L);

La - previous depth applied (L);

D - drainage (L);

n - number of containers; and,

LF - leaching fraction (0.15).

The total volume of water applied per plant was 3.68 L, corresponding to applications of 1.18 g of salts in plants irrigated with local-supply water (0.5 dS m<sup>-1</sup>) and 8.24 g of salts in plants irrigated with desalination reject and fish farming effluent. At 90 days after sowing, another leaching fraction was applied (15%), the drained volume was collected, and the electrical conductivity of the drainage water (ECd) was measured using a benchtop conductivity meter, with data expressed in dS m<sup>-1</sup> adjusted to a temperature of 25 °C. The electrical conductivity of the saturation extract (ECse) was estimated according to the methodology of Ayers & Westcot (1985) for medium-textured soils (Table 4), using Eq. 2:

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

where:

ECse - electrical conductivity of the saturation extract (dS m<sup>-1</sup>); and,

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

NPK fertilization	EC	se (dS n	1 <sup>-1</sup> )	pHse			
recommendation (%)	LSW	DR	FFE	LSW	DR	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	6.60	
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; DR - Desalination reject; FFE - Fish farming effluent

ECd - electrical conductivity of the drainage water (dS m<sup>-1</sup>).

At 90 days after sowing, the seedlings were evaluated for height, stem diameter, main root length, and number of leaves. Height was measured using a graduated ruler from the soil to the apical meristem insertion, with data expressed in cm. Stem diameter was measured using a digital caliper at 1 cm from the soil surface, with readings expressed in mm. The number of leaves was determined by simply counting each plant's fully expanded green leaves.

After growth analysis, the seedlings were partitioned into leaves, stem, and root, packed in Kraft paper bags, kept in an oven with forced air circulation at 65 °C until reaching constant weight and weighed on the analytical scale (0.0001 g), and the results were expressed in g. These data were then used to calculate the total dry mass (TDM, g).

The dry matter of the leaves was crushed in a stainlesssteel Wiley mill, stored in labeled plastic bags, and sent for analysis. In the laboratory, the material underwent wet digestion (98%  $H_2SO_4$  - A.R. [Analytical Regent] + 98%  $H_2O_2$  - A.R.) in an open system, to determine the total leaf concentrations of nitrogen (N) by the Kjeldahl method and digestion in nitric acid (98% HNO<sub>3</sub> - A.R.) in a microwave oven to obtain the extract used to read the total leaf concentrations of phosphorus (P), potassium (K<sup>+</sup>), calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>), and sodium (Na<sup>+</sup>) according to the procedures described in Silva (2009), with readings performed in Inductively Coupled Plasma (ICP). These data were used to estimate the amount of each nutrient per plant and to determine the Na<sup>+</sup>/K<sup>+</sup>, Na<sup>+</sup>/Ca<sup>2+</sup>, and Na<sup>+</sup>/Mg<sup>2+</sup> ratios.

The data obtained were subjected to analysis of variance (F test). In cases of significance, Tukey's test was performed for the qualitative factor (saline waters), and regression was performed

Table 3. Analysis of the water used in the irrigation of custard apple seedlings

			8			arameters				
Water sources	nU	EC	<b>K</b> +	Na+	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl	<b>CO</b> <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> -	SAR
	рН	(dS m <sup>-1</sup> )		(mmol <sub>c</sub> L <sup>-1</sup> )						
1 LSW	7.80	0.53	0.31	6.64	0.30	1.10	2.60	0.20	2.80	7.9
2 DR	8.10	3.50	0.35	19.37	5.70	8.80	30.80	0.60	2.30	7.2
3 FFE	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; Water source 3 - Fish farming effluent; pH - Hydrogen potential; EC - Electrical conductivity; K<sup>+</sup> - Potassium; Na<sup>+</sup> - Sodium; Mg<sup>2+</sup> - Magnesium; Ca<sup>2+</sup> - Calcium; Cl<sup>-</sup> - Chlorine; CO<sub>3</sub><sup>2-</sup> - Carbonate; HCO<sub>3</sub><sup>-</sup> - Bicarbonate; SAR - Sodium adsorption ratio

for the quantitative factor (NPK doses) at 0.05 probability level, using SISVAR statistical software (Ferreira, 2019).

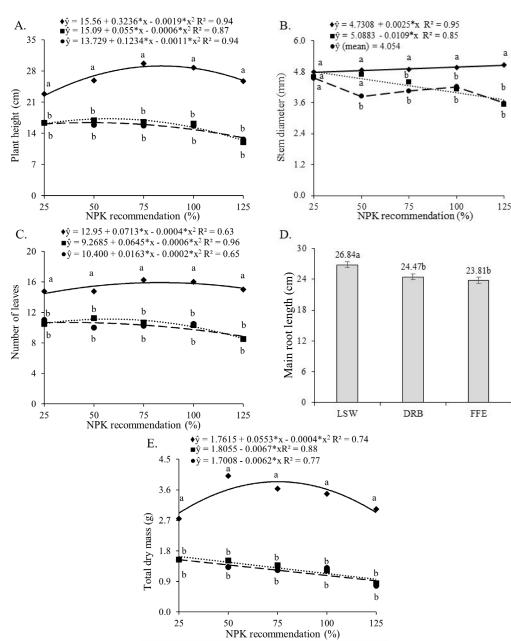
### **Results and Discussion**

There was a significant interaction between factors water and NPK doses for plant height (p<0.01), stem diameter (p<0.01), number of leaves (p<0.01), and total dry mass (p<0.001) (Table 5). For the main root length, there was a significant effect (p<0.001) of the irrigation waters (Table 5).

The highest plant height and number of leaves of custard apple seedlings irrigated with local-supply water were obtained at 85.15 and 89.12% NPK doses, corresponding to 29.33 cm and 16 leaves, respectively (Figures 1A, C). In seedlings irrigated with reject of desalination, the highest plant height (PH) and number of leaves (NL) were obtained at doses of 45.83 **Table 5.** Summary of the F test for plant height (PH), stem diameter (SD), number of leaves (NL), main root length (MRL), and total dry mass (TDM) of custard apple seedlings under irrigation with saline waters and NPK doses, at 90 days after sowing

Sources of variation	DF	F test (p-value)							
Sources of variation	UF	PH	SD	NL	MRL	TDM			
Block	3	0.152	0.893	0.067	0.875	0.028			
Waters	2	0.000	0.000	0.000	0.000	0.000			
NPK doses	4	0.000	0.003	0.000	0.417	0.000			
Waters $\times$ NPK doses	8	0.001	0.002	0.005	0.053	0.000			
Error	42								
CV (%)		9.35	7.79	6.66	9.24	11.73			
CV (%)     9.35     7.79     6.66     9.24     11.7       DE     Descrete of freedom: CV     Coefficient of writetion     CV     COEfficient of writetion     CV     COEfficient of writetion     CV     COEfficient of writetion     COEfficient of writetion     COEfficient of writetion     CV     COEfficient of writetion     COEffic									

DF - Degrees of freedom; CV - Coefficient of variation



\*, \*\* - Significant at p<0.05 and not significant, respectively, by the F-test. Means in the same NPK recommendation with same letters do not differ by the Tukey's test (p<0.05). **Figure 1.** Plant height (A), stem diameter (B), number of leaves (C), and total dry mass (E) of custard apple seedlings under irrigation with saline water ( $\blacklozenge$  local-supply water - LSW, • desalination reject - DRB, and • fish farming effluent - FFE) and NPK doses and main root length (D) of sugar apple as a function of type of irrigation water, 90 days after sowing

and 40.75% NPK, corresponding to 16.35 cm and 10 leaves, respectively (Figures 1A, C). Already in seedlings irrigated with fish farming effluent, the highest PH and NL were obtained at

doses of 56.09 and 53.75% NPK, corresponding to 17 cm and 11 leaves, respectively (Figures 1A, C).

At all NPK doses, according to the mean values of each treatment, seedlings irrigated with desalination reject and fish farming effluent obtained values that were up to 41.3 and 42.5% lower for PH, 33.2 and 34.5% lower for NL, and 61.9 and 63.9% lower for total dry mass (TDM), compared to local-supply water, respectively (Figures 1A, C, E). There was no significant difference between the means of the desalination reject and fish farming effluent for the NPK doses (Figures 1A, C, E).

In the stem diameter (SD) of custard apple seedlings irrigated with local-supply water, there was an increase of 5.21% at the dose of 125% NPK compared to 25% NPK with the highest SD obtained at a dose of 125% NPK, equivalent to 5.04 mm (Figure 1B). For seedlings irrigated with desalination reject, there was a reduction of 22.6% at the dose of 125% NPK compared to 25% NPK (Figure 1B). When seedlings were irrigated with fish farming effluent, there was no satisfactory fit of NPK doses, and a mean value of 4.05 mm was obtained (Figure 1B). The main root length of custard apple seedlings was reduced by 8.8 and 11.3% with desalination reject and fish farming effluent in irrigation, compared to seedlings irrigated with local-supply water (control) (Figure 1D).

The highest total dry mass of custard apple seedlings irrigated with local-supply water was obtained at a dose of 69.12% NPK, equivalent to 3.67 g (Figure 1E). The TDM of seedlings irrigated with desalination reject and fish farming effluent were reduced by 40.9 and 40.1% at a dose of 125% NPK compared to 25% NPK (Figure 1D).

The highest growth and biomass accumulation of custard apple seedlings under low salinity conditions were obtained between doses of 69 and 89% NPK and the dose of 75%, corresponding to 75, 225, and 112.5 mg of N,  $P_2O_5$ , and  $K_2O$  dm<sup>-3</sup>, respectively, was recommended. There was a reduction in seedling growth at doses higher than this (75%) because the NPK doses increased the ECse (Table 4). Sá et al. (2019; 2021) verified that the beneficial effect of increased fertilization on West Indian cherry and custard apple plant growth, physiology, and production depends on the salinity condition to which the plant is subjected. The plants showed no symptoms of toxicity in response to NPK doses, so the restrictions in growth are attributed to the increase of ECse and, consequently, reduction in the osmotic potential of the soil.

Irrigation with high-salinity water reduced the growth of custard apple seedlings, regardless of the wastewater source used. The ionic effect leads to the nutritional imbalance caused by changes in the processes of absorption, transport, assimilation, and distribution of nutrients in the plant; for example, the excess of Na<sup>+</sup> in the medium inhibits the absorption of K<sup>+</sup> and Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Cl<sup>-</sup> inhibits the absorption of NO<sub>3</sub><sup>-</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-</sup> (Zelm et al., 2020; Zhao et al., 2020; Sá et al., 2020; Fernandes et al., 2022).

Reductions in the growth rates of custard apples under conditions of salt stress have also been verified by Passos et al. (2005), Sá et al. (2015), and Silva et al. (2018a,b). The responses of the seedlings to NPK fertilization were similar to those irrigated with desalination reject and fish farming effluent. The highest growth of seedlings under high salinity conditions was obtained between the doses of 25.00 and 53.75% of the NPK recommendation and the dose of 50%, which corresponds to 50, 150, and 75 mg of N,  $P_2O_5$ , and  $K_2O$  dm<sup>-3</sup>, respectively, was recommended. The increase in NPK dose in seedlings irrigated with saline water linearly reduced their growth, and these results indicate that the optimal level of the nutrient for custard apple in the absence of salt stress is toxic when grown in a saline condition. This behavior was observed in custard apple (Figure 1). Growth and biomass accumulation response to fertilization decreased when plants were irrigated with desalination reject and fish farming effluent. This decrease in the NPK recommendation was equal to 35% and represented a lower NPK requirement, about 35, 120, and 60 mg of N,  $P_2O_5$ , and  $K_2O$  dm<sup>-3</sup>, respectively.

There was significant interaction (p<0.01) between waters and NPK doses for nitrogen (N), phosphorus (P), potassium (K<sup>+</sup>), calcium (Ca<sup>2+</sup>), and magnesium (Mg<sup>2+</sup>) accumulation (Table 6).

The most significant accumulations of N, P, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> in custard apple seedlings irrigated with local-supply water were obtained at doses of 106.5, 105.1, 83.3, 80.0, and 74.1% NPK, equivalent to 89.00, 11.81, 43.40, 49.83, and 36.79 mg per plant, respectively (Figures 2A, B, C, D, E).

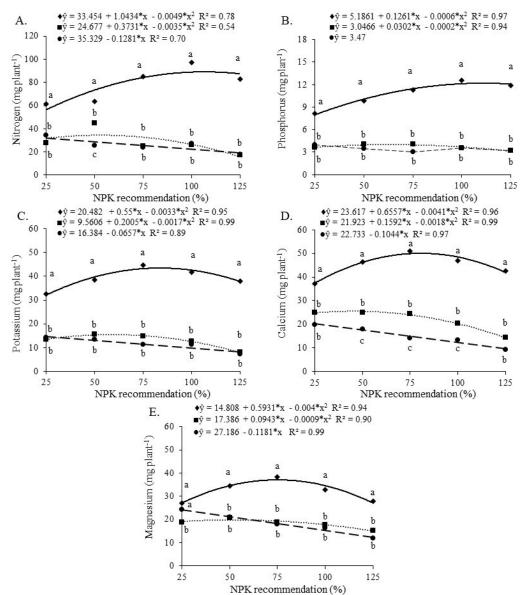
Seedlings irrigated with desalination reject obtained the highest accumulations of N, P, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> at doses of 53.3, 75.5, 59.0, 74.1, and 52.4% NPK, equivalent to the values of 34.60, 4.18, 15.47, 36.80, and 19.85 mg plant<sup>-1</sup>, respectively (Figures 2A, B, C, D, E). On the other hand, when seedlings were irrigated with fish farming effluent, there were reductions of 39.88, 3.47, 15.47, 10.44, and 11.81 mg plant<sup>-1</sup> in the accumulations of N, P, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> at the 125% NPK dose compared to the 25% NPK dose (Figures 2A, B, C, D, E).

At all NPK doses, seedlings irrigated with desalination reject and fish farming effluent obtained nutrient accumulations lower than those of plants irrigated with local-supply water, with reductions in nutrient accumulation of up to 67.05 and 62.83% for N, 64.48 and 67.69% for P, 65.52 and 70.73% for K<sup>+</sup>, 51.41 and 67.08% for Ca<sup>2+</sup>, and 43.16 and 43.48% for Mg<sup>2+</sup>, compared to local-supply water, respectively (Figures 2A, B, C, D, and E). The accumulations of N, P, K<sup>+</sup>, and Mg<sup>2+</sup> were similar in seedlings irrigated with desalination reject and fish farming effluent at different NPK doses, and Ca<sup>2+</sup> contents were more than 6.20 mg plant<sup>-1</sup> higher when irrigation was performed with desalination reject compared to fish farming effluent at doses 50, 75, and 100% NPK (Figures 2A, B, C). At the dose of

**Table 6.** Summary of the F test for nitrogen (N), phosphorus (P), potassium ( $K^+$ ), calcium ( $Ca^{2+}$ ), and magnesium ( $Mg^{2+}$ ) accumulation in the shoot of custard apple seedlings under irrigation with saline water and NPK doses, at 90 days after sowing

Sources of variation	DF	F test ( <i>p</i> -value)							
		N	P	K+	Ca <sup>2+</sup>	Mg <sup>2+</sup>			
Block	3	0.504	0.683	0.445	0.728	0.662			
Waters	2	0.000	0.000	0.000	0.000	0.000			
NPK doses	4	0.177	0.019	0.000	0.000	0.000			
Waters $\times$ NPK doses	8	0.000	0.000	0.000	0.000	0.000			
Error	42								
CV (%)		22.91	15.66	13.14	13.32	13.89			

DF - Degrees of freedom; CV - Coefficient of variation



\*, \*\* - Significant at p<0.05 and not significant, respectively, by the F-test. Means in the same NPK recommendation with same letters do not differ by the Tukey test (p<0.05) **Figure 2.** Accumulation of nitrogen(A), phosphorus (B), potassium (C), calcium (D), and magnesium(E) of custard apple seedlings under irrigation with saline water ( $\blacklozenge$  local-supply water, • desalination reject, and • fish farming effluent) and NPK doses, at 90 days after sowing

25% NPK, the soil irrigated with high-salinity water obtained ECse 4.1 and 4.4 times higher for desalination reject and fish farming effluent, respectively, compared to the soil at the same dose irrigated with low-salinity water (Table 4). This high ECse caused restrictions in the growth of roots and shoots, as well as reductions in the accumulations of N, P, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>, compared to plants irrigated with low-salinity water.

There was significant interaction (p<0.01) between the factors waters and NPK doses for sodium (Na) accumulation, sodium/potassium ratio (Na<sup>+</sup>/K<sup>+</sup>), sodium/calcium ratio (Na<sup>+</sup>/Ca<sup>2+</sup>), and sodium/magnesium ratio (Na<sup>+</sup>/Mg<sup>2+</sup>) in the shoot of custard apple seedlings (Table 7).

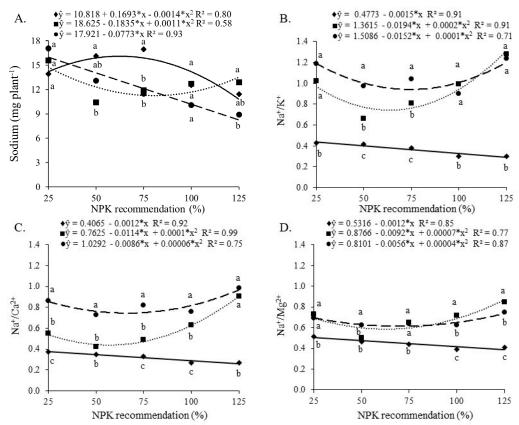
The highest accumulation of Na<sup>+</sup> in custard apple seedlings irrigated with local-supply water was obtained at 60.5% NPK, equivalent to 15.93 mg per plant, respectively (Figure 3A). Seedlings irrigated with desalination reject obtained the minimum accumulations of Na<sup>+</sup> under the dose of 83.4% NPK, equivalent to 10.97 mg per plant (Figure 3A). When the seedlings were irrigated with fish farming effluent, 48.37% **Table 7.** Summary of the F test for sodium (Na<sup>+</sup>) accumulation and sodium/potassium (Na<sup>+</sup>/K<sup>+</sup>), sodium/calcium (Na<sup>+</sup>/Ca<sup>2+</sup>), and sodium/magnesium (Na<sup>+</sup>/Mg<sup>2+</sup>) ratios in the shoot of custard apple seedlings under irrigation with saline water and NPK doses, at 90 days after sowing

Sources of variation	DF	F test (p-value)							
Sources of variation	DF	Na+	Na <sup>+</sup> /K <sup>+</sup>	Na+/Ca <sup>2+</sup>	Na <sup>+</sup> /Mg <sup>2+</sup>				
Block	3	0.932	0.536	0.934	0.979				
Waters	2	0.002	0.000	0.000	0.000				
NPK doses	4	0.000	0.000	0.000	0.000				
Waters $\times$ NPK doses	8	0.000	0.000	0.000	0.000				
Error	42								
CV (%)		15.07	15.50	9.68	7.10				

DF - Degrees of freedom; CV - Coefficient of variation

Na<sup>+</sup> accumulation was reduced between 25% and 125% NPK (Figure 3A).

At the dose of 100% NPK, there was no difference between irrigation waters for Na<sup>+</sup> accumulation (Figure 3A). At the dose of 25% NPK, the Na<sup>+</sup> accumulations of seedlings irrigated



\* - Significant at p<0.05, by the F-test. Means in the same NPK recommendation with same letters do not differ by the Tukey test (p<0.05)</li>
Figure 3. Sodium (Na<sup>+</sup>) accumulation (A), sodium/potassium ratio, Na<sup>+</sup>/K<sup>+</sup> (B), sodium/calcium ratio, Na<sup>+</sup>/Ca<sup>2+</sup> (C), and sodium/ magnesium ratio, Na<sup>+</sup>/Mg<sup>2+</sup> (D) in the shoot of custard apple seedlings under irrigation with saline water (♦ local-supply water,
testination reject, and ● fish farming effluent) and NPK doses, at 90 days after sowing

with desalination reject and fish farming effluent were 11.9 and 22.6% higher than those observed in seedlings irrigated with local supply water (Figure 3A). At the doses of 50 and 75% NPK, the Na<sup>+</sup> accumulations of seedlings irrigated with local-supply water were 54.5 and 42.2% higher than those of seedlings irrigated with desalination reject and 23.2 and 47.5% higher than those of seedlings irrigated with fish farming effluent (Figure 3A). At the dose of 125% NPK, the Na<sup>+</sup> accumulations of seedlings irrigated with desalination reject were 41.1 and 12.9% higher than those observed in seedlings irrigated with fish farming effluent and local-supply water, respectively (Figure 3A).

The Na<sup>+</sup> content in plants irrigated with low-salinity water was also higher than that obtained in plants irrigated with high-salinity water at 50 and 75% NPK doses. This lower accumulation of nutrients and Na<sup>+</sup> is explained by the fact that the biomass accumulation obtained in plants under low salinity was twice as high as that obtained by plants under high salinity, especially at the doses of 50 and 75% NPK, which approached the optimal dose for production of custard apple seedlings under low salinity.

The lowest Na<sup>+</sup>/K<sup>+</sup>, Na<sup>+</sup>/Ca<sup>2+</sup>, and Na<sup>+</sup>/Mg<sup>2+</sup> ratios in custard apple seedlings irrigated with desalination reject were obtained at doses of 48.5, 57.0, and 65.7% NPK, being equal to 0.89, 0.44, and 0.57 (Figures 3B, C, D). In custard apple seedlings irrigated with fish farming effluent, the lowest Na<sup>+</sup>/K<sup>+</sup>, Na<sup>+</sup>/Ca<sup>2+</sup>, and Na<sup>+</sup>/Mg<sup>2+</sup> ratios were obtained at doses of 76.0, 71.7, and 70.0% NPK and were equal to 0.93, 0.72, and 0.61 (Figures 3B, C, D).

When seedlings were irrigated with local-supply water, there were reductions of 34.1, 31.9, and 23.9% in the Na<sup>+</sup>/K<sup>+</sup>, Na<sup>+</sup>/ Ca<sup>2+</sup>, and Na<sup>+</sup>/Mg<sup>2+</sup> ratios between the doses of 125 and 25% NPK (Figures 3B, C, D).

The Na<sup>+</sup>/K<sup>+</sup>, Na<sup>+</sup>/Ca<sup>2+</sup>, and Na<sup>+</sup>/Mg<sup>2+</sup> ratios of seedlings irrigated with local-supply water were up to 2.67, 1.88, and 1.57 times lower than the ratios obtained in seedlings irrigated with desalination reject and 3.00, 2.63, and 1.52 times lower than the ratios obtained in seedlings irrigated with fish farming effluent (Figure 3B, C, D). The Na<sup>+</sup>/K<sup>+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> ratios of seedlings irrigated with fish farming effluent were 16.7 and 56.4% higher for the dose of 25% NPK, 48.5 and 73.8% higher for the dose of 50% NPK and 28.4 and 67.3% higher for the dose of 75% NPK, compared to those of seedlings irrigated with desalination reject, respectively (Figures 3B, C). There was no difference in the Na<sup>+</sup>/Mg<sup>2+</sup> ratio between seedlings irrigated with fish farming effluent and desalination reject (Figure 3D).

As the dose of NPK increased above 75%, the seedlings irrigated with local-supply water reduced their accumulation of cations K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Na<sup>+</sup> in the shoot, and there were also reductions with doses above 25% NPK for Na<sup>+</sup>/K<sup>+</sup>, Na<sup>+</sup>/Ca<sup>2+</sup>, and Na<sup>+</sup>/Mg<sup>2+</sup> ratios. Decreases in the accumulation of nutrients in the shoot may be due to the increase in salinity caused by fertilization, with values above 2.10 to 2.93 dS m<sup>-1</sup> with doses above 75% NPK. This behavior also occurred with the above 2.1 dS m<sup>-1</sup> growth variables of salinity. Above this salinity, the accumulation of salts in the root zone of custard apple seedlings may have

reduced the osmotic potential and, consequently, the soil's water potential to the point of restricting water availability for the plant (Munns et al., 2019).

At the doses of 50, 75, and 100% NPK, the values of  $Na^+/K^+$ of seedlings irrigated with desalination reject and fish farming effluent were lower than those obtained at doses of 25 and 125% NPK. This result coincides with lower accumulations of Na<sup>+</sup> in seedlings irrigated with desalination reject and fish farming effluent and with higher accumulations of K<sup>+</sup> and Ca<sup>2+</sup> in seedlings irrigated with desalination reject, indicating that these NPK doses contributed to the ionic homeostasis of custard apple seedlings. However, the Na<sup>+</sup>/K<sup>+</sup> ratios of seedlings irrigated with desalination reject and fish farming effluent and Na<sup>+</sup>/Ca<sup>2+</sup> ratios in seedlings irrigated with fish farming effluent indicate that the seedlings absorbed twice as much Na<sup>+</sup> than K<sup>+</sup> and Ca<sup>2+</sup> under the condition of low salinity, and at doses of 25 and 125% NPK, indicating that, in addition to the osmotic effect, the ionic effect was also responsible for restricting the growth and biomass accumulation of custard apple seedlings.

The ionic effect refers to the accumulation of specific ions -Na<sup>+</sup> and Cl<sup>-</sup> - that cause toxicity (Zelm et al., 2020; Zhao et al., 2020). Under the condition of low salinity, the accumulation of nutrients followed the order of N, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, and P; however, under high salinity, the order changed to N, Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, and P. Thus, it can be inferred that the restriction of K<sup>+</sup> absorption and accumulation due to competition with Na<sup>+</sup> was the main responsible for the reduction in the growth and biomass accumulation of custard apple seedlings. Restriction in K<sup>+</sup> absorption due to the increase in Na<sup>+</sup> in custard apple seedlings under salt stress conditions was observed by Andrade et al. (2018) when evaluating phosphorus doses to reduce salt stress; however, the isolated increase in phosphorus did not mitigate salt stress in custard apple seedlings, with a Na<sup>+</sup>/K<sup>+</sup> ratio of up to 1.48. In the present study, a Na<sup>+</sup>/K<sup>+</sup> ratio of 0.89 was observed at a dose of 48.5% NPK, indicating that adequate NPK fertilization can improve K<sup>+</sup> absorption and mitigate salt stress in custard apple seedlings.

#### **CONCLUSIONS**

1. Irrigation with desalinization reject and fish farming effluent restricts the growth of custard apple seedlings, regardless of the NPK dose used.

2. The ionic homeostasis of custard apple seedlings irrigated with desalinization reject and fish farming effluent is optimized by fertilization with 50% of the NPK recommendation corresponding. 50, 150, and 75 mg of N,  $P_2O_5$ , and  $K_2O \text{ dm}^{-3}$ , respectively.

3. The dose of 75% NPK is recommended for producing custard apple seedlings irrigated with local-supply water, corresponding to 75, 225, and 112.5 mg of N,  $P_2O_5$ , and  $K_2O$  dm<sup>-3</sup>, respectively.

4. For producing custard apple seedlings irrigated with desalination reject and fish farming effluent, the average dose of 50% NPK is recommended, corresponding to 50, 150, and 75 mg of N,  $P_2O_5$ , and  $K_2O \text{ dm}^{-3}$ , respectively.

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