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Accumulation of salts in the soil and growth of cowpea under salinity and phosphorus fertilization[1]

Acúmulo de sais no solo e crescimento do feijão-caupi sob salinidade e adubação fosfatada

Yuri Bezerra de Lima[2], Francisco Vanies da Silva Sá[3]*, Miguel Ferreira Neto[2], Emanoela Pereira de Paiva[4] and Hans Raj Gheyi[3]

ABSTRACT - Cowpea is widely grown in the Brazilian semiarid region, however, due to excess salts in the soil and irrigation water constantly low crop productivity has been observed. Accumulation of salts in the soil, growth and biomass partition of cowpea under salt stress and phosphorus fertilization was evaluated in this study. The assay was conducted in a greenhouse in Mossoró, State of Rio Grande do Norte, Brazil, from September to December 2015, using a Alfissol Latosolic with low phosphorus content. The experiment was carried out in a randomized block design in a factorial 5 x 3 arrangement, consisting of five water salinity levels (0.5, 1.5, 2.5, 3.5 and 4.5 dS m^{-1}) and three doses of superphosphate (60, 100 and 140% of the recommended dose for crop - 60 kg ha^{-1} of P_2O_5), with five repetitions. The plants of cowpea cv. Paulistinha were cultivated for 49 days after sowing in lysimeters with 8 dm^3 capacity. During this period was determined the accumulation of salts in the soil, growth and biomass partition plants. Water with salinity higher than 1.5 dS m^{-1} increased soil salinity above the crop's threshold of 4.9 dS m^{-1}, 21 days after sowing. Increased salinity impaired growth and biomass partition of cowpea plants, with greater accumulation of dry matter in the leaves and stem in comparison to the root. Hinghest dose of superphosphate promoted greater growth of cowpea plants.

Key words: *Vigna unguiculata.* Soil fertility. Irrigation. Growth analysis. Saturation extract.

RESUMO - O feijão-caupi é amplamente cultivado na região semiárida brasileira, todavia, devido ao excesso de sais no solo e na água de irrigação vem sendo constantemente observada baixa produtividade da cultura. Com isso, objetivou-se avaliar o acúmulo de sais no solo, crescimento e a partição de fitomassa do feijão-caupi sob estresse salino e adubação fosfatada. A pesquisa foi realizada em casa de vegetação em Mossoró-RN, no período de setembro a dezembro de 2015, utilizando-se um Argissolo Vermelho-Amarelo latossólico, com baixo teor de fósforo. O delineamento experimental adotado foi o de bloco casualizado em esquema fatorial 5 x 3, constituído de cinco níveis de salinidade da água (0,5; 1,5; 2,5; 3,5 e 4,5 dS m^{-1}) e três doses de superfosfato simples (60; 100 e 140% da dose recomendada para a cultura- 60 kg ha^{-1} de P_2O_5), com cinco repetições. As plantas de feijão-caupi cv. Paulistinha foram cultivadas por 49 dias após a semeadura em lisímetros com capacidade de 8 dm^3. Nesse período determinou-se o acúmulo de sais no solo, o crescimento e a partição de fitomassa das plantas. Águas com salinidade superiores a 1,5 dS m^{-1} elevaram a salinidade do solo acima do limiar da cultura de 4,9 dS m^{-1}, a partir dos 21 dias após a semeadura. O aumento da salinidade prejudicou o crescimento e partição de fitomassa das plantas de feijão-caupi, havendo maior acúmulo de fitomassa nas folhas e caule em relação à raiz. A maior dose de superfosfato simples promoveu maior crescimento das plantas de feijão-caupi.

Palavras-chave: *Vigna unguiculata*. Fertilidade do solo. Irrigação. Análise de crescimento. Extrato de saturação.

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INTRODUCTION

Cowpea (*Vigna unguiculata* L.), a leguminous species with great food potential. Because of its high protein content, cowpea is an important crop, especially for developing countries (MOUSINHO; ANDRADE JUNIOR; FRIZZONE, 2008; RAMOS *et al*., 2012). In Brazil, the cowpea crop occupies an area of approximately 1 million hectares, of which 90% are situated in the Northeast region, and a large part of them are located mainly in the semi-arid region (BEZERRA *et al*., 2010; CARDOSO; RIBEIRO, 2006; MOREIRA *et al*., 2016).

However, the Brazilian semi-arid region is characterized by a low availability of surface water, and the water available for irrigation is mostly of lower quality, mainly due to the high concentration of dissolved salts, commonly reaching levels close to 5.0 dS m^{-1} (CAVALCANTI *et al*., 2005).

Considering that cowpea tolerates electrical conductivity of up to 3.3 dS m^{-1} in the water and 4.9 dS m^{-1} in the soil (AYERS; WESTCOT, 1999), irrigation management strategies for the crop become necessary because of the accumulation of salts in the soil, especially in the form of sodium chloride, which can cause necrosis of leaf tissues and accelerate the senescence of mature leaves, factors that reduce the area available for photosynthesis (ESTEVES; SUZUKI, 2008; MEDEIROS *et al*., 2003; MUNNS; TESTER, 2008; NEVES *et al*., 2009; TAIZ; ZEIGER, 2013).

Salt stress has three crucial effects on plants: osmotic effect, restricting water absorption by the plant, affecting growth and cell elongation; accumulation of potentially toxic ions inside the plant, causing discontinuity in the enzymatic and hormonal activity of the plants; and nutritional disorders, causing deficiency of essential nutrients, affecting the entire physiological functioning of the plant (ESTEVES; SUZUKI, 2008; MUNNS; TESTER, 2008; TAIZ; ZEIGER, 2013).

Numerous strategies to mitigate the deleterious effects of salt stress on plants have been studied, but the management of fertilization favoring the acquisition of nutrients by plants under saline conditions has stood out as the most promising (PRAZERES *et al*., 2015; SILVA *et al*., 2011). For example, phosphorus (P) is an essential element to plants because of its action in root growth, fruit maturation, the formation of grains, fruits and fibers, and in the vigor of the plants. In addition, phosphorus is the most problematic nutrient for Brazilian soils, due to its low availability and mobility in the soil (SANTOS; GATIBONI; KAMINSKI, 2008).

Phosphorus is a crucial nutrient in plant metabolism, acting in the transfer of energy of the cell in the form of adenosine triphosphate (ATP), as well as taking part in respiration and photosynthesis, being a structural component of macromolecules, genes and chromosomes (DIAS *et al*., 2015; TAIZ; ZEIGER, 2013). The P deficiency leads to a low net photosynthetic rates, because P is fundamental for this process to occur, besides reductions in the number and size of leaves, consequently in the photosynthetically active area (CRUZ; SOUZA FILHO; PELACANI, 2015; HENRY *et al*., 2012; ZUCARELI *et al*., 2010).

Here we aimed to evaluate the accumulation of salts in the soil, growth, and phytomass partition of cowpea under salt stress and phosphate fertilization.

MATERIAL AND METHODS

The research was carried out in a greenhouse at the Department of Environmental and Technological Sciences of the Federal Rural University of the Semi-Arid Region (UFERSA), in Mossoró-RN, from September to December 2015. The municipality of Mossoró-RN is located in the semi-arid region of Northeast Brazil, at the geographic coordinates 5°11’ S, 37°20’ W, and altitude of 18 m.

The experiment was conducted in randomized blocks, in a 5 × 3 factorial scheme, composed of five levels of irrigation water salinity (S1 = 0.5; S2 = 1.5; S3 = 2.5 S4 = 3.5; S5 = 4.5 dS m^{-1}) and three doses of P_2O_5 (A1 = 60%; A2 = 100% and A3 = 140% of the dose of 60 kg $P_2O_5^-$ ha^{-1} recommended by CAVALCANTI *et al*., 2008), with 5 replicates, totaling 75 experimental plots.

Phosphorus doses were calculated based on soil analysis, and the soil used in the experiment came from a virgin area of the UFERSA’s experimental farm, Campus of Mossoró, classified as latosolic Red Yellow Argisol, according to Embrapa (2013). Soil samples were collected in the layer of 0.0–30.0 cm and analyzed at the Laboratory of Analysis of Soil, Water, and Plant - LASAP, of the UFERSA, following the methodology of Embrapa (2011) (Table 1).

Fertilization was applied based on soil analysis and the technical bulletin of fertilization recommendation for the Pernambuco state (CAVALCANTI *et al*., 2008). The recommendation for the cowpea crop is 60 kg ha^{-1} of P_2O_5, 20 kg ha^{-1} of K_2O, and 50 kg ha^{-1} of N for one crop cycle. The recommendation of fertilization was used to stipulate the doses of $P_2O_5^-$ (A1 = 36; A2 = 60 and A3 = 84 kg ha^{-1}) applied in the form of single superphosphate (A1 = 0.7; A2 = 1.17 and A3 = 1.64 g pot^{-1} of $P_2O_5^-$), as basal fertilization, and the planting was performed after 20 days (incubation period), in order to release P to the young plants. Since the soil has a sandy texture, nitrogen

Table 1 - Physical and chemical characteristics of the soil collected in the layer of 0–30 cm and of the bovine manure used in the cowpea cultivation

Soil

Clay	Sand	Silt	DS	DP	Porosity	Textural Class
---%---			g cm^{-3}	g cm^{-3}	---%---	
10.0	89.0	1.0	1.57	2.51	37.45	Free Sand

EC 1:2.5	pH	P	K^+	Ca^{+2}	Mg^{+2}	Na^+	Al^{3+}	$H^{+}+Al^{3+}$	SB	T	OM	ESP
dSm^{-1}	H_2O	mg dm^{-3}	---cmol$_c$dm^{-3}---								g kg^{-3}	%
0.16	6.72	1.20	0.20	1.40	0.50	0.05	0.00	0.70	2.15	2.85	13.23	1.75

Bovine manure

N	P	K	Ca	Mg	Na	Zn	Cu	Fe	Mn	pH	C.O.	CTC	C/N	EC
---g kg^{-1}---						---mg kg^{-1}---				H_2O	%	cmol$_c$dm^{-3}		mS/cm
14.85	3.25	1.16	16.11	3.07	0.66	65	15	3.77	121	6.53	10.70	34.24	7.21	2.56

P, K^+, Na^+: extracted by Mehlich 1; Al^{3+}, Ca^{2+}, Mg^{2+}: extracted by 1.0 mol L^{-1} KCl; DS: Soil density; DP: Particle density; EC: Electrical conductivity; SB: Sum of basis; T: Cation exchange capacity; OM: Walkley-Black wet digestion; ESP: Exchangeable sodium percentage

and potassium fertilizations were applied as top-dressing: N was distributed at 14, 21, and 27 days after sowing and K at 28 and 35 days after sowing.

After soil physical and chemical characterization and stipulation of the fertilizations, the soil was placed in pots with capacity for 8 dm^3, with 7 dm^3 filled with soil and 0.5 dm^3 filled with bovine manure, to increase moisture retention and the negative charges of the soil, and 0.5 dm^3 filled with crushed stone at the bottom to facilitate drainage. The lysimeters were filled in the following order: screen; crushed stone; 2 dm^3 of soil; and a mixture of soil (5 dm^3): manure (0.5 dm^3): dose of $P_2O_5^-$, stipulated for each treatment.

After soil preparation, one irrigation was applied, leaving the soil close to maximum water retention capacity. Subsequent irrigations were performed once a day to bring soil moisture close to the maximum retention capacity, based on the drainage lysimeter method. The applied water depth was added to a leaching fraction (LF) of 0.20 of the volume accumulated every seven days. The water volume (Vw) applied per container was obtained by the difference between the previous irrigation depth (Id) minus the mean drainage (D), divided by the number of containers (n), as presented in Equation 1:

$$V_w = \frac{I_d - (D/n)}{1 - LF} \quad (1)$$

The saline solutions of different electrical conductivities were prepared through the addition of sodium chloride (NaCl) salts, which comprise 70% of the salt ions in water sources used for irrigation in small properties of the Brazilian Northeast region (MEDEIROS *et al*., 2003).

The irrigation water with various saline levels was prepared considering the relationship between water electrical conductivity (ECw) and the concentration of salts (10*meq L^{-1} = 1 dS m^{-1} of ECw), according to Rhoades, Kandiah & Mashali (1992), valid for ECw from 0.1 to 5.0 dS m^{-1}, which encompasses the tested levels. The solutions were prepared using water for human supply existing at the site (ECw = 0.53 dS m^{-1}), which was mixed with salts as necessary. For the preparation, the salts were weighed according to the treatment, adding water until reaching the desired level of electrical conductivity (EC). EC values were confirmed using a portable conductivity meter, whose conductivity was adjusted to a temperature of 25 ºC. After preparation, the saline solutions were stored in 150-L plastic containers, one for each studied ECw level, properly protected to avoid evaporation, entry of rainwater and contamination with materials that could compromise quality.

After irrigation, the cowpea cv. ‘Paulistinha’ was sown on October 14, 2015, 20 days after applying the P dose, using 10 seeds per pot. Fifteen days after sowing with the total emergence of the seedlings, thinning was performed, leaving only three plants per pot.

To determine the salt balance of the soil along the experiment, every 7 days during the application of the leaching fraction, the drainage solution was collected below the root zone of each pot and stored in plastic containers. Subsequently, the electrical conductivity of the drained solution (ECds) during the different periods of collection was determined using a conductivity meter. The data of electrical conductivity of the drained water (ECdw) were used to estimate the electrical conductivity of the saturation extract (ECse), as indicated in Equation 2, described by Ayers and Westcot (1999).

$$EC_{se} = \frac{ECds}{2} \quad (2)$$

At 15, 30, and 49 days after sowing (DAS), cowpea plants were evaluated for plant height (PH) (cm), measured using a graduated ruler as the distance between soil and apex of the plants, and stem diameter (SD) (mm), measured using a digital caliper at the base of the plants at the height of 1.0 cm from the soil. These data, according to the methodology proposed by Benincasa (2003), were used to determine the relative growth rates in height (RGRPH) and stem diameter (RGRSD), according to Equation 3:

$$RGR = \frac{\ln X2 - \ln X1}{t2 - t1} \quad (3)$$

Where: RGR = Relative growth rate of the plants (cm cm^{-1} day^{-1}); Ln = natural logarithm; X1 = reading at time t1 (cm); X2 = reading at time t2 (cm).

Also at 49 DAS, plants were collected to obtain the dry matter of leaves (LDM), stem (SDM), and roots (RDM), based on the collection, partition, and drying of the material in a forced-air oven at 65 ºC, until constant weight. After drying, the material was weighed on an analytical scale, with the precision of 0.0001 g. Then, phytomass partition was determined based on the representativeness of each plant part in the phytomass accumulation, by dividing it by the total dry matter of the plant.

The obtained data were subjected to analysis of variance by F test at 0.05 probability level and, in cases of significance, linear or quadratic polynomial regression analyses were applied at 0.05 probability level, for the factor irrigation water salinity, having as parameters the significance of the equation and highest R2. For the factor phosphate fertilization, the Tukey test was applied at 0.05 probability level, using the statistical program SISVAR® (FERREIRA, 2011).

RESULTS AND DISCUSSION

There were variations in the electrical conductivity of the saturation extract as a function of the different periods of collection, fertilization managements, and electrical conductivity of the irrigation water used in the cultivation of cowpea plants (Figure 1A, B, and C). During the first 14 days of cultivation, none of the water salinity levels conditioned the soil to salinity values higher than the threshold of the cowpea crop, which is 4.9 dS m^{-1} for the saturation extract (AYERS; WESTCOT, 1999), regardless of the studied fertilization management. However, from the 21 days of cultivation on, the soil irrigated with saline waters above 2.5 dS m^{-1} reached levels higher than the crop threshold, with values close to 10 dS m^{-1} at the highest saline levels (Figure 1 A, B, and C).

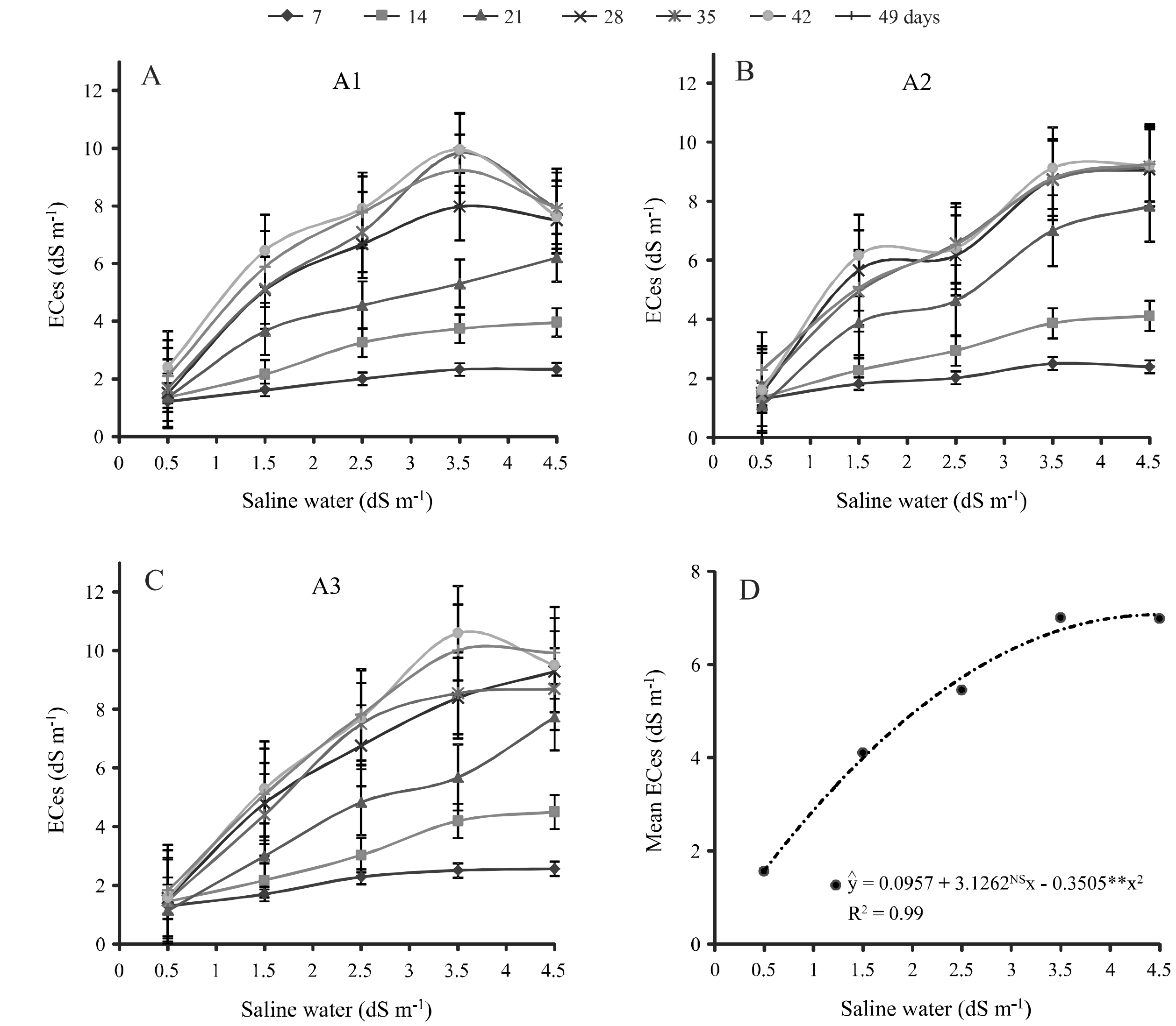
In general, the threshold salinity of the crop was reached with water salinity levels higher than 1.97 dS m^{-1}, regardless of the utilized $P_2O_5^-$ dose. These results indicate the severity of soil salinization due to the irrigation with saline waters rich in NaCl salts, causing the utilized leaching fraction to be inefficient. A similar behavior was observed by Amorim *et al*. (2010), evaluating ECse of soils cultivated with cashew irrigated with saline water for four months. In this study, the authors observed ECse of up to 16.8 dS m^{-1} in the root zone, indicating high soil salinization level and inefficiency of the leaching fraction.

In soils that received fertilization with 140% of the $P_2O_5^-$ recommendation, the threshold salinity of the saturation extract, equal to 4.9 dS m^{-1} (AYERS; WESTCOT, 1999), was only reached at salinity levels above 1.5 dS m^{-1} (Figure 1C). In addition, irrigation with water of 2.5 dS m^{-1} exceeded cowpea threshold salinity at 28 DAS (Figure 1C). The soils fertilized with 60% and 100% of the $P_2O_5^-$ recommendation achieved threshold salinity of the saturation extract when irrigated with water from 1.5 dS m^{-1}, from the first 28 days of the experiment (Figure 1A, B, and C)..

For the relative growth rate in height of the plants cultivated under fertilization with 60% of the $P_2O_5^-$ recommendation, there were linear reductions of growth in all evaluated periods. In plants fertilized with 100% of the $P_2O_5^-$ recommendation, linear reductions occurred in the first 15 days of cultivation; subsequently, plant growth responses were not significant, with mean values of 0.020 and 0.027 (cm cm^{-1} day^{-1}) at 30 and 49 days of cultivation, respectively. The heights of cowpea plants cultivated under fertilization with 140% of the $P_2O_5^-$ recommendation decreased linearly during the first 15 days of cultivation, as a function of the salinity (Figure 2A, C, and E). Additionally, plants fertilized with 140% of the $P_2O_5^-$ recommendation showed a quadratic growth from 30 to 49 days, reaching the maximum growth at the estimated level of 1.65 dS m^{-1} (Figure 2A, C, and E). These results corroborate the data of electrical conductivity of the saturation extract, in which the soils under A3 fertilization irrigated with water of 1.5 dS m^{-1} did not reach the crop threshold salinity during the first 42 days and, when they reached after this period, salinity effects could not reduce plant growth (because the plants of the cv. ‘Paulistinha’ stop growing at the beginning of the reproductive stage) (Figure 1C).

The relative growth rate in stem diameter was not significantly influenced by the fertilization managements,

Figure 1 - Electrical conductivity (EC) A1 (A), A2 (B), A3 (C), mean EC (D) of the saturation extract of the soil cultivated with cowpea, cv. ‘Paulistinha’, under different levels of saline water and phosphate fertilization (A1= 60, A2=100% and A3 140% of the $P_2O_5^-$ recommendation)

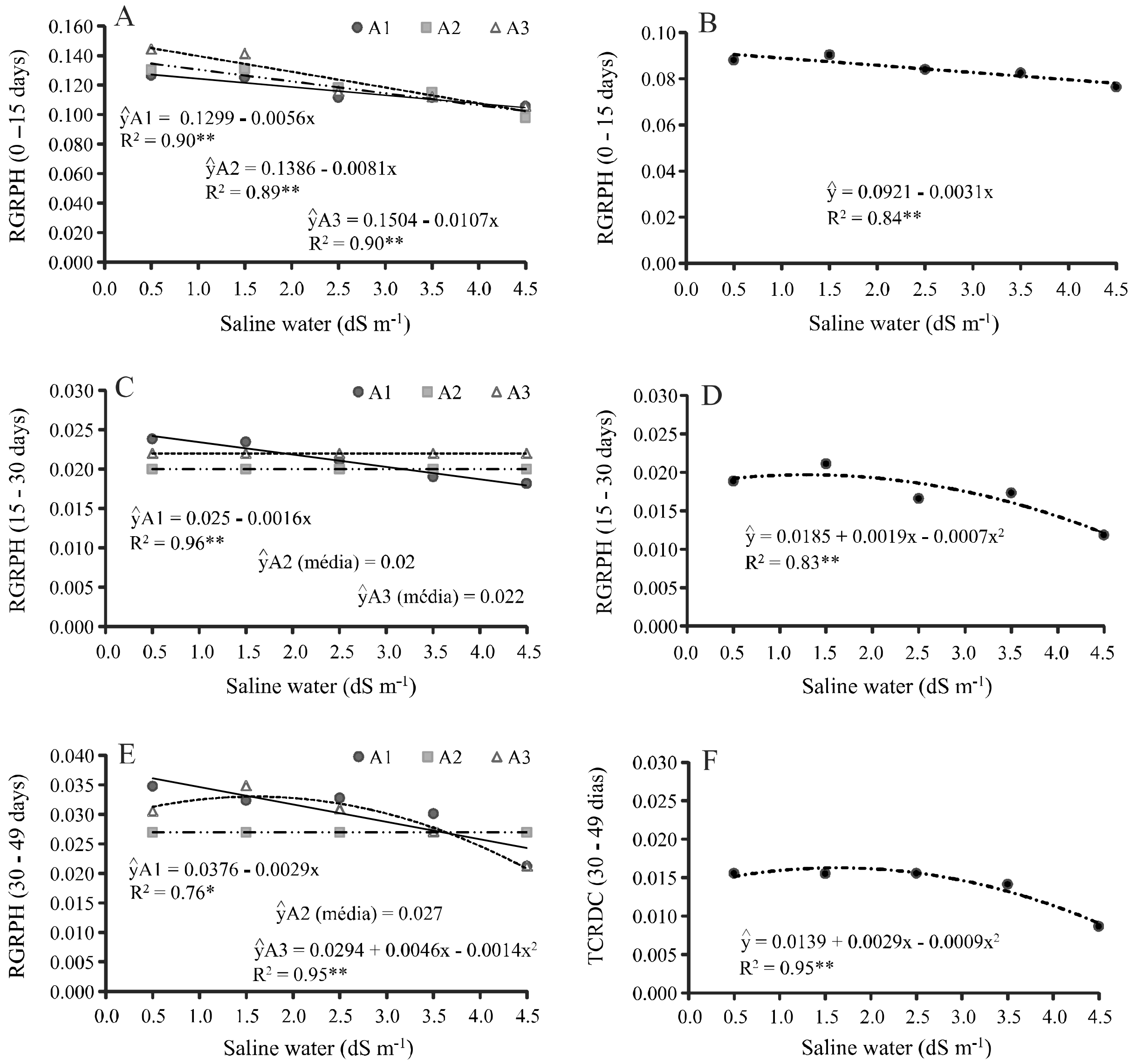


NS and ** = not significant (p>0.05), significant at 0.01 probability (p<0.01)

and there was only influence of the salinity levels (Figure 2B, D, and F). During the first 15 days, RGRSD exhibited a decreasing linear behavior, but from days 15 to 30 and 30 to 49, a quadratic behavior occurred, with maximum growth rates at the levels of 1.4 and 1.6 dS m^{-1}, respectively. In contrast with the results of RGRSD and the ECse, it can be observed that, during the first 15 days, the ECse did not reach the crop threshold salinity at any of the studied levels of salinity; thus, the reductions observed in growth accompanied the linear increase of water and soil salinity, probably due to osmotic limitations (Figure 1D and 2B). Subsequently, from 15 to 49 days, when soil salinity exceeded the threshold salinity of the crop when irrigated with levels above 1.5 dS m^{-1}, there was a progressive reduction of relative growth from this level (Figure 1D and 2D and F).

Salts can affect plant growth because of their concentration in the soil solution, decreasing the osmotic potential and reducing the availability of water to the plants (RICHARDS, 1954). In addition, there may also be a toxic effect of specific ions, due to their excessive accumulation, such as sodium, which can cause damages to the plant (FLOWERS; FLOWERS, 2005; SILVA *et al.*, 2007; WILSON *et al.*, 2006). Thus, the addition of higher P doses reduces the risks of deficiency of the nutrient, guaranteeing the absence of nutritional stress acting

Figure 2 - The relative growth rate in plant height (RGRPH) (A, C, E) and stem diameter (RGRSD) (B, D, F) of cowpea plants, cv. 'Paulistinha', under different levels of saline water and phosphate fertilization (A1= 60, A2=100% and A3 140% of the $P_2O_5^-$ recommendation) at 0-15, 15-30 and 30-49 days after sowing



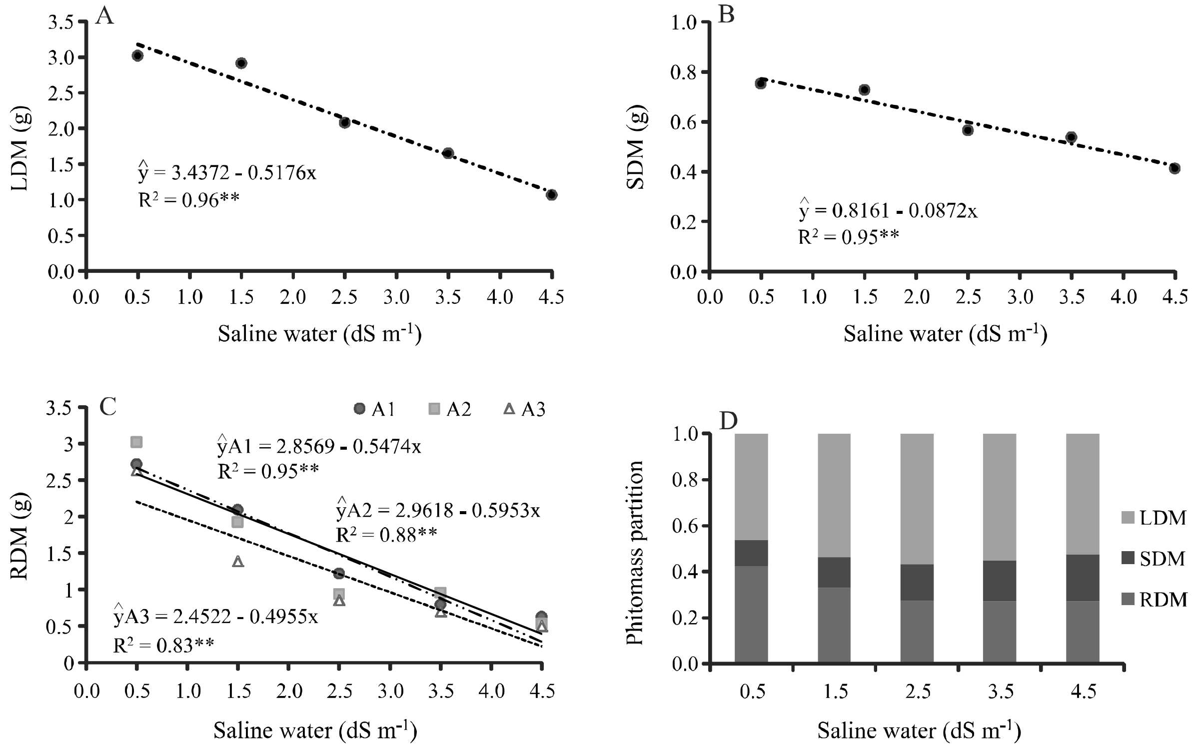
[NS], * and ** = not significant (p>0.05), significant at 0.05 and 0.01 probability levels (p<0.05 and p<0.01)

on leaf production and expansion and, consequently, on the CO_2 assimilation rate (CRUZ; SOUZA FILHO; PELACANI, 2015; HENRY *et al*., 2012; ZUCARELI *et al*., 2010).

It should be highlighted that P is a crucial nutrient in plant metabolism, acting in the transfer of energy of the cell. The increase in P availability can stimulate plant growth, as observed in RGRPH in the present study, possibly by increasing the ATP contents and, consequently, the photosynthetic activity of the plant (DIAS *et al*., 2015; TAIZ; ZEIGER, 2013).

The phytomass accumulation of cowpea plants was drastically reduced with the increase in irrigation water salinity. There were reductions of 15.1% and 10.7% for LDM and SDM due to the unit increase in irrigation water salinity, respectively (Figure 3A and B), corroborating the results of Wilson *et al*. (2006) and Neves *et al*. (2009). The accumulation of salts in the soil, especially in the form of sodium chloride, can cause necrosis in the leaf tissues and accelerate the senescence of mature leaves, factors that reduce the leaf area intended for photosynthesis and, consequently, biomass accumulation

Figure 3 - Leaf dry matter-LDM (A), stem dry matter-SDM (B), root dry matter-RDM (C) and phytomass partition (D) of cowpea plants, cv. ‘Paulistinha’, under different levels of saline water and phosphate fertilization (A1= 60, A2=100% and A3 140% of the $P_2O_5^-$ recommendation), at 49 days after sowing



NS, * and ** = not significant (p>0.05), significant at 0.05 and 0.01 probability levels (p<0.05 and p<0.01)

(AYERS; WESTCOT, 1999; ESTEVES; SUZUKI, 2008; MEDEIROS *et al*., 2003; MUNNS; TESTER, 2008; NEVES *et al*., 2009; TAIZ; ZEIGER, 2013).

For RDM, there was a significant effect (p<0.05) of the interaction salinity vs. phosphate fertilization, with unit reductions of 21.3, 20.1 and 19.67% for the fertilization managements A1, A2, and A3, respectively, per unit increase in irrigation water salinity (Figure 3C). Therefore, under the highest P availability, plants showed lowest reductions in phytomass accumulation, possibly due to the necessity of smaller expansion of the root system, thus minimizing the contact surface with chlorine and sodium ions.

There was a significant influence (p<0.05) of the salinity levels on the phytomass partition of cowpea plants, with an increase in leaf and stem phytomass accumulation and a consequent decrease in root phytomass accumulation, due to the increase in irrigation water salinity (Figure 3D). This behavior indicates that, with the increase in salinity, cowpea plants stop investing in root phytomass and distribute photoassimilates to shoot development. The higher investment in the shoots by cowpea plants, especially in the case of leaves, may be related to its mechanism of tolerance; which aims to increase the number of vacuoles per plant and, thus, reduce the ionic effect of the salt stress, through the compartmentalization of salts in the vacuole, thereby minimizing the toxicity by specific ions (FLOWERS; FLOWERS, 2005; SYVERTSEN; GARCIA-SANCHEZ, 2014).

The increase in stem phytomass accumulation, compared with leaves and roots, at the highest salinity levels, is probably related to the lower production and expansion of leaves, due to the effects of the salt stress (Figure 3D). This is because, at the highest stress levels (3.5 and 4.5 dS m^{-1}), the saturation extract salinity reached the crop threshold from the 21st day after sowing (Figure 1A, B, and C).

CONCLUSIONS

1. Water salinity levels in the soil reached the cowpea threshold (EC_{se} = 4.9 dS m^{-1}) within 26 days of cultivation;

2. Irrigation with high salinity water (4.5 dS m^{-1}) increased the electrical conductivity of the saturation extract by up to 10.6 dS m^{-1} at 49 days after sowing;
3. The increase in salinity compromised the growth and phytomass accumulation of cowpea plants;
4. The highest tested dose of phosphorus led to the greatest growth and phytomass accumulation of cowpea plants;
5. The increase in salinity affected phytomass partition of the cowpea plants, with higher phytomass accumulation in leaves and stem, compared with the roots.

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