Vegetative development and content of calcium, potassium, and sodium in watermelon under salinity stress on organic substrates

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Abstract – The objective of this work was to evaluate the vegetative development and determine the concentration of sodium, potassium, and calcium in watermelon (*Citrullus lanatus*) grown on two organic substrates and under increasing saline concentrations. The substrates were soil + earthworm humus (S_1) and soil + bovine manure (S_2), and the saline treatments consisted of irrigation water with different electrical conductivities: 1.36 (control), 3.56, 5.76, and 7.96 dS m^{-1}. The experimental design used was a randomized complete block in a 2×4 (substrate × conductivity) factorial arrangement with five replicates. Main branch length, root length, stem diameter, leaf number, leaf area, and fresh matter mass of shoots and roots, as well as the sodium, potassium, and calcium contents in the plants, were determined. All phenological parameters interacted with the substrates, and S_1 improved plant performance. Substrates interacted significantly with: leaf area; leaf number; stem diameter; main branch length; root length; fresh matter mass of shoots and roots; and the contents of sodium, potassium, and calcium, in both shoots and roots of watermelon irrigated with saline water for up to 27 days after emergence. There are also interactions between substrates and salinity for stem diameter, main branch length, fresh matter mass of roots, and calcium contents in both shoots and roots, and potassium and sodium contents in the roots.

Index terms: *Citrullus lanatus*, bovine manure, humus of earthworm, salt stress.

Desenvolvimento vegetativo e conteúdo de cálcio, potássio e sódio em melancieira sob estresse salino, em substratos orgânicos

Resumo – O objetivo deste trabalho foi avaliar o desenvolvimento vegetativo e determinar a concentração de sódio, potássio e cálcio em melancieiras (*Citrullus lanatus*), cultivadas em dois substratos orgânicos e concentrações salinas crescentes. Os substratos foram solo + húmus de minhoca (S_1) e solo + esterco bovino (S_2), e os tratamentos salinos consistiram de irrigação com diferentes condutividades elétricas: 1,36 (controle), 3,56, 5,76 e 7,96 dS m^{-1}. Utilizou-se o delineamento experimental de blocos ao acaso, em arranjo fatorial 2×4 (substratos × condutividade), com cinco repetições. Foram determinados comprimento do ramo principal e da raiz, diâmetro do caule, número de folhas, área foliar e massa fresca da parte aérea e da raiz, bem como o conteúdo de sódio, potássio e cálcio nas plantas. Todos os parâmetros fenológicos apresentaram interação com os substratos, e S_1 promoveu melhor desempenho das plantas. Os substratos interagiram significativamente com: área foliar; número de folhas; diâmetro do caule; comprimento do ramo principal; comprimento de raiz; massa de matéria fresca da parte aérea e da raiz; e teores de sódio, potássio e cálcio na parte aérea e nas raízes de melancieiras irrigadas com água salina, até 27 dias após a emergência. Os substratos também interagiram com a salinidade para diâmetro do caule, comprimento do ramo principal, massa de matéria fresca de raiz, e teores de cálcio na parte aérea e raiz, e de potássio e sódio na raiz.

Termos para indexação: *Citrullus lanatus*, esterco bovino, húmus de minhoca, salinidade.

Introduction

Fruits and vegetable producers seek to increase production using alternatives that reduce costs. One of them is the use of substrates composed of organic fertilizers during the initial stage of plant development (Góes et al., 2011; Trani et al., 2013). In many regions,

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organic agricultural products, such as bovine manure and earthworm humus, are easily acquired and often available at the producer's property. Organic fertilization results in the slow release of nutrients that persist longer in the soil. Organic fertilization also presents other advantages, such as improvements to quality, soil aeration, and water drainage (Trani et al., 2013). However, in semiarid regions, poor quality water and degraded soils due to low rainfall and high insolation are often found. These conditions lead to excess salt in irrigation water, which can drastically affect the phenological development and yield of some crops, such as Cucurbitaceae (Farias et al., 2009; Lima Júnior, 2010; Medeiros et al., 2012; Silva Neto et al., 2012).

In plants, salinity exhibits an osmotic and ionic nature and can directly affect cultivation yields by reducing the availability of water in the soil. The magnitude of osmotic stress is proportional to the scarcity of water in the soil for the plants. Also, for crops such as melon and watermelon, the maximum electrical conductivity (EC) tolerance is 2.2 dS m^{-1}; and levels greater than this can be toxic. The effects of salinity on the development of watermelon have been reported by Lucena et al. (2011), Costa et al. (2012), and Martins et al. (2013), among others.

The role of sodium (Na^+) at low concentrations in mineral nutrition of higher plants is essential and can replace potassium (K^+) in some metabolic and osmotic functions (Marschner, 1986). The nutrients K^+ and calcium (Ca^{2+}) positively interact during the development of plants of several species, and a beneficial effect of these ions occurs under the conditions of salt stress (Wu et al., 2014; Diniz Neto et al., 2014; Silva et al., 2017).

The use of organic substrates that have a greater availability of K^+ and Ca^{2+} in their composition can, therefore, aid in the development of plants. However, reports in the literature regarding the effect of substrates naturally rich in these ions on the growth of melons are scarce. Therefore, strategies are needed to produce more vigorous seedlings that can be planted in saline areas of the Brazilian semiarid region.

The objective of this work was to evaluate the vegetative development and to determine the concentration of Na^+, K^+, and Ca^{2+} in watermelon grown on two organic substrates and under increasing saline concentrations.

Materials and Methods

The experiment was carried out in the municipality of Catolé do Rocha (6°20'38"S, 37°44'49"W, at an altitude of 272 m), in the state of Paraíba, Brazil. The seeds of the watermelon (*Citrullus lanatus* L.) cultivar Crimson Sweet were sown in 8-L plastic pots containing substrate. Three seeds were sown per pot at a depth of approximately 3 cm. Seven days after emergence (DAE), seedlings were thinned to only one seedling (the most vigorous one) per pot. For the formulation of substrates used in this experiment, bovine manure, earthworm humus, and soil classified as a fluvial Entisol with a sandy loam texture were used, which were subjected to chemical analysis (Table 1).

Cultivation was conducted using the substrates S_1 (50% soil + 50% earthworm humus [v/v], obtained from composting) and S_2 (50% soil + 50% bovine manure [v/v]), as well as irrigation water with four levels of EC: 1.36 (control), 3.56, 5.76, and 7.96 dS m^{-1}. The experimental design was a randomized complete block in a 2×4 factorial arrangement (two substrates x four levels of EC), with five replicates.

Irrigation was performed with the solutions of different saline concentrations, which were obtained by the addition of NaCl to the irrigation water and monitored using a conductivity meter. Substrate moisture was maintained at 70% of field capacity and monitored by weighing the pots every two days. Water was replenished to return the mass to the desired value.

Data collection began at 15 DAE, when the seedlings had two pairs of fully expanded leaves. The following data were collected: main branch length (MBL), stem diameter (SD), number of leaves (NL), and leaf area (LA, calculated by the width versus length measurement and applying a correction factor of 0.7). At the end of the experiment (30 DAE), the seedlings were separated into shoots and roots and then weighed on a precision balance, from which the fresh mass of the shoots (FMAP) and fresh mass of the roots (FMR) were obtained (gram per plant). Samples were dried in a forced-air oven for 72 hours at 70°C and then ground in a Willey mill to determine the Na^+, K^+, and Ca^{2+} contents.

The Na^+, K^+, and Ca^{2+} ion contents were determined at the laboratory for plant production technologies of Universidade Estadual da Paraíba. The analyses were performed according to Silva et al. (2009) on aqueous extracts obtained by the addition of 50 mg dry matter

(DM) of each vegetative part to 10 mL deionized H_2O. The extracts were heated in a water bath at 100°C for 1 hour in hermetically closed threaded tubes. The obtained extract was filtered, and the content of the ions was determined in a B462 flame photometer (Micronal, Santo Amaro, SP, Brazil).

The data of the phenological and physiological analyses and the coefficients of variation of the treatments were analyzed statistically using the F-test of the analysis of variance. The means of the treatments, when significant, were compared by Tukey's test, at 5 and 1% probability, respectively, using the statistical software Assistat, version 7.7 (Silva & Azevedo, 2016).

Results and Discussion

The evaluated parameters were affected mainly by the substrate, and S_1 promoted better plant vegetative growth than S_2. There was a significant difference for all the phenological parameters observed at 1% probability, with the exception of the K^+ content in the shoots in relation to the EC of the substrate. The evaluated parameters also showed significant interaction between substrate and salinity, with the exceptions of LA, FMAP, root length, K^+, and Na^+ in the shoots. Differences in the MBL of plants subjected to the levels of EC in S_1 were observed (Figure 1 A). Water deficit caused by salinity can limit not only the growth but also the NL by reducing the amount and rate of branch growth (Taiz & Zeiger, 2013).

Watermelons cultured in S_2 showed slower development of MBL in relation to those in S_1 (Figure 1 A and B). This reduction in MBL was directly related to an increase in salinity concentration. Similar results were obtained by Sousa et al. (2014), who reported that growth was negatively affected by increasing salinity in watermelon plants. Under the experimental conditions adopted, the maximum EC value of irrigation water was 3.2 dS m^{-1}.

The observed values for SD in S_1 did not show any differences between the salinity treatments applied during the evaluation period. However, a tendency for greater stem development was verified in plants subjected to S_1 in relation to those under S_2. Similar results were reported by Ferreira et al. (2011), who studied the production of melon seedlings in six types of substrates and reported superior development of plants grown on soil + humus, and by Véras et al. (2014), who also found an increase of stem diameter with the use of humus as a substrate for tamarind (*Tamarindus indica* L.) seedlings.

The NL per plant was affected when the seedlings grown on S_1 were subjected to increasing EC (Figure 2A). The accumulation of salts in the soil by irrigation with high EC negatively contributes to the water absorption by plants, which can be a factor in the reduction of the photosynthetic and metabolic processes of crops, leading to reduced NL (Travassos et al., 2012).

Plants subjected to 1.36 and 3.56 dS m^{-1} in substrate S_2 presented a significant increase in the NL in relation to those at 24 and 27 DAE (Figure 2 B). In S_1, there was a tendency for the superior development of watermelon, when compared with S_2, across all the evaluations performed. The presence of salts in the soil solution increases the water retention force due to the osmotic effect, hindering water absorption by plants (Gheyi et al., 2010).

Regarding S_1, there was a marked increase in leaf area in plants irrigated with water with a EC of 1.36 dS m^{-1} at 27 DAE (Figure 2 C). However, in S_2,

Table 1. Chemical analysis of the soil and of the substrates with humus of earthworm and with bovine manure, used for planting watermelon (*Citrullus lanatus*).

Attribute	Soil	Substrate	
		Earth worm humus	Bovine manure
pH (H_2O)	6.4	7.38	8.47
Ca^{2+} ($cmol_c$ dm^{-3})	5.25	35.4	0.041
Mg^{2+} ($cmol_c$ dm^{-3})	1.15	19.32	3.17
Al^{3+} ($cmol_c$ dm^{-3})	0.0	0.0	-
H^++Al^{3+} ($cmol_c$ dm^{-3})	1.08	0.00	-
K^+ ($cmol_c$ dm^{-3})	0.12	1.41	0.003
Na^+ ($cmol_c$ dm^{-3})	0.2	1.82	14.4
P (mg dm^{-3})	0.003	55.14	924
S (mg dm^{-3})	-	57.95	-
Fe (mg dm^{-3})	59.6	-	-
Zn (mg dm^{-3})	4.05	-	-
Cu (mg dm^{-3})	3.83	-	-
Mn (mg dm^{-3})	53.9	-	-
B (mg dm^{-3})	6.45	-	-
CE (dS m^{-1})	-	2.11	-
Sum of bases ($cmol_c$ dm^{-3})	-	56.13	-
Organic matter (%)	-	-	66.66
C/N ratio	-	-	18.1

a gradual decrease in leaf area was observed as a function of the EC of the irrigation water during the evaluation period, which was more pronounced at 27 DAE (Figure 2 D). The reduction in leaf area may be related to the mechanisms of plant adaptation to salinity stress, in order to reduce the transpirational

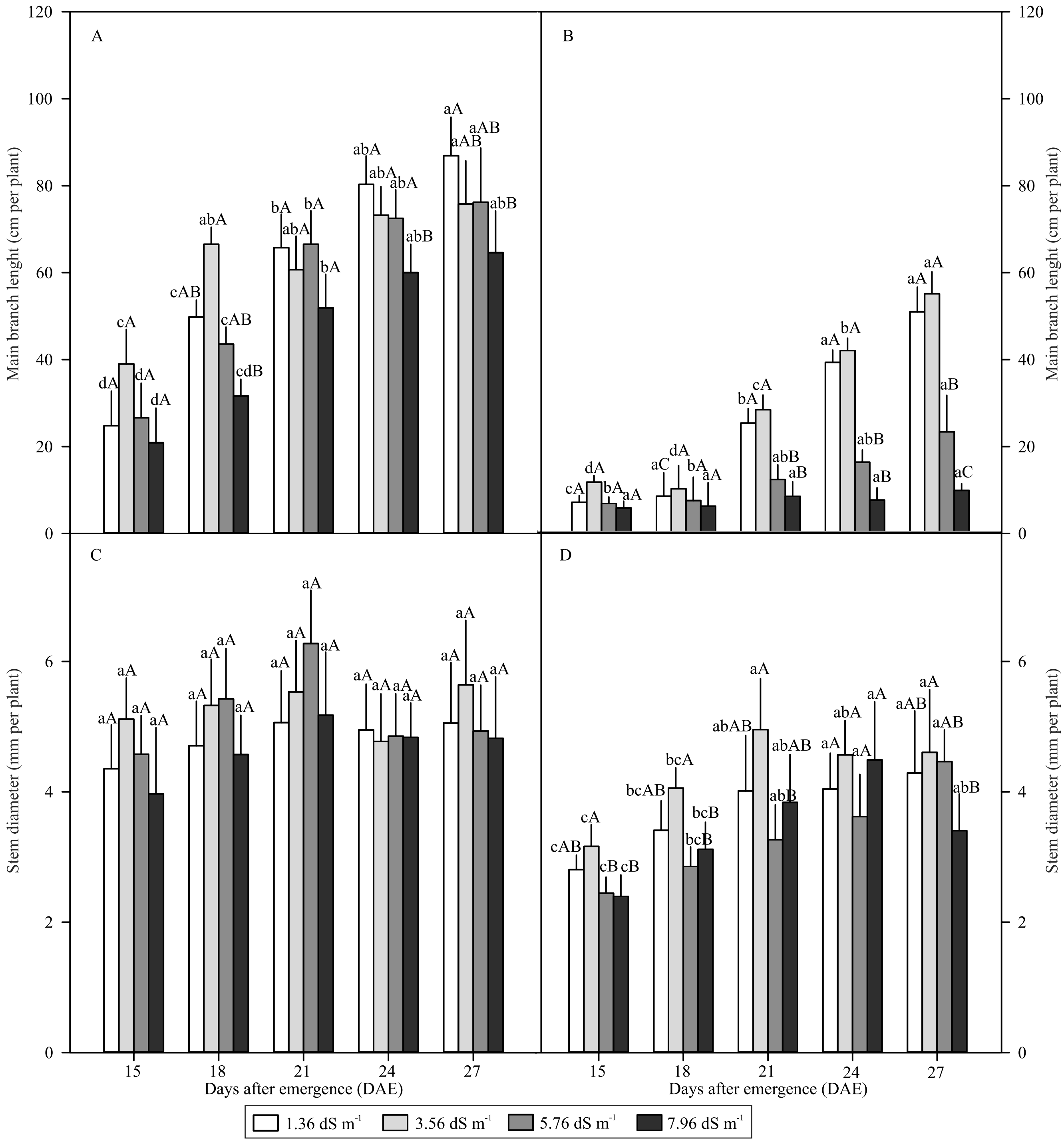

Figure 1. Main branch length of 'Crimson Sweet' watermelon (*Citrullus lanatus*) plants cultivated in soil + earthworm humus (A) and soil + bovine manure (B), and stem diameter of plants cultivated in soil + earthworm humus (C), and soil + bovine manure (D) subjected to different levels of electrical conductivity of the irrigation solution (1.36 - control, 3.56, 5.76, and 7.96 dS m^{-1}). DAE, days after seed emergence. Bars with equal letters do not differ by Tuke's test, at 5% probability: lowercase letters refer to days after emergence and uppercase letters to electrical conductivity.

surface area (Tester & Davenport, 2003). A significant increase in the FMAP in S_1 in relation to that of S_2 was also observed, with the exception of the irrigation water with a EC of 3.56 dS m^{-1} (Figure 3 A). With respect to fresh root mass, the S_1 substrate was superior to S_2 across all salinity concentrations (Figure 3 C).

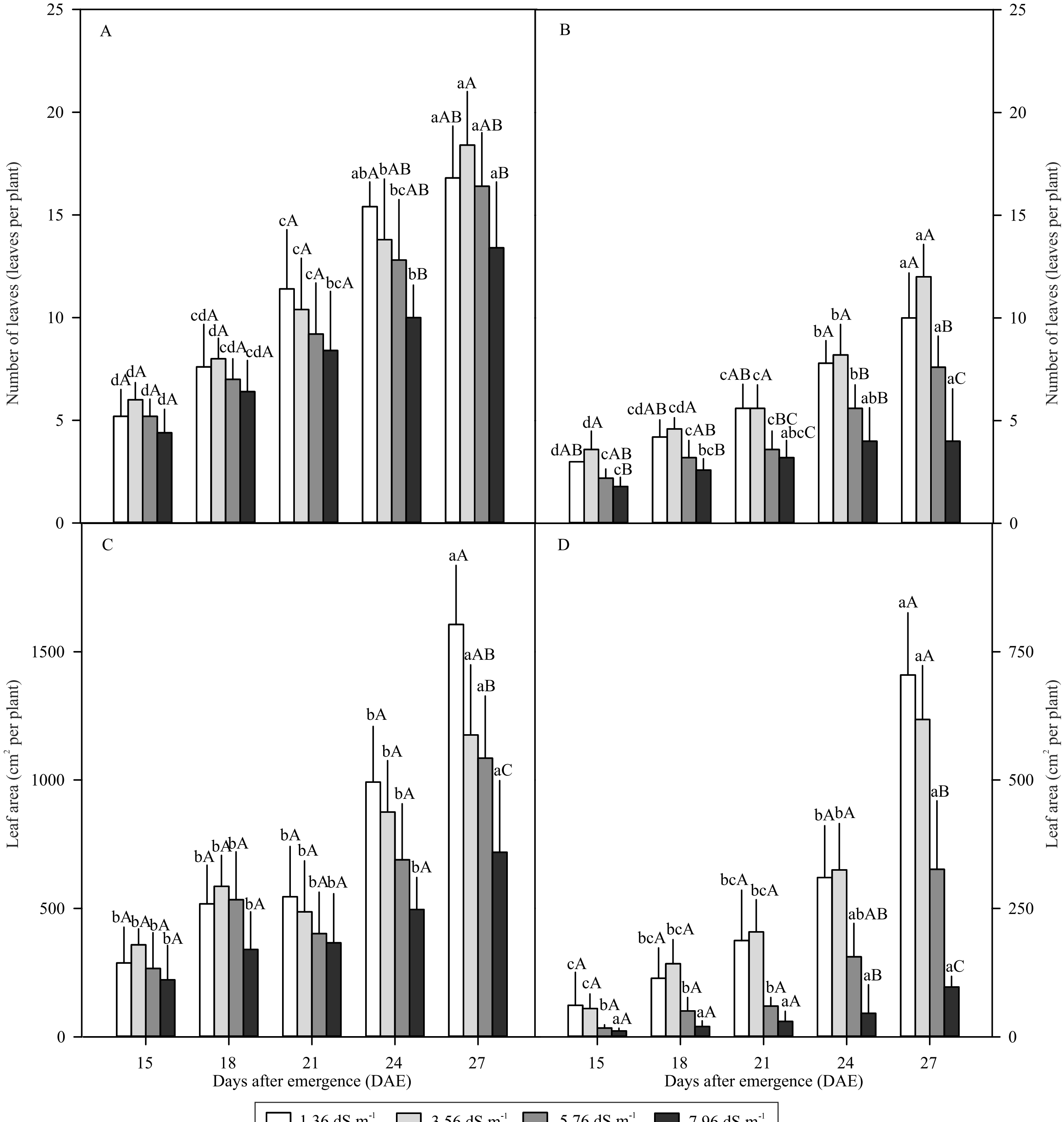

Figure 2. Number of leaves in 'Crimson Sweet' watermelon (*Citrullus lanatus*) plants cultivated in soil + earthworm humus (A) and soil + bovine manure (B), and leaf area of plants cultivated in soil + earthworm humus (C) and soil + bovine manure (D) subjected to different levels of electrical conductivity of the irrigation solution (1.36 – control, 3.56, 5.76, and 7.96 dS m^{-1}). DAE, days after seed emergence. Bars with equal letters do not differ by Tukey's test, at 5% probability: lowercase letters refer to days after emergence and uppercase letters to electrical conductivity.

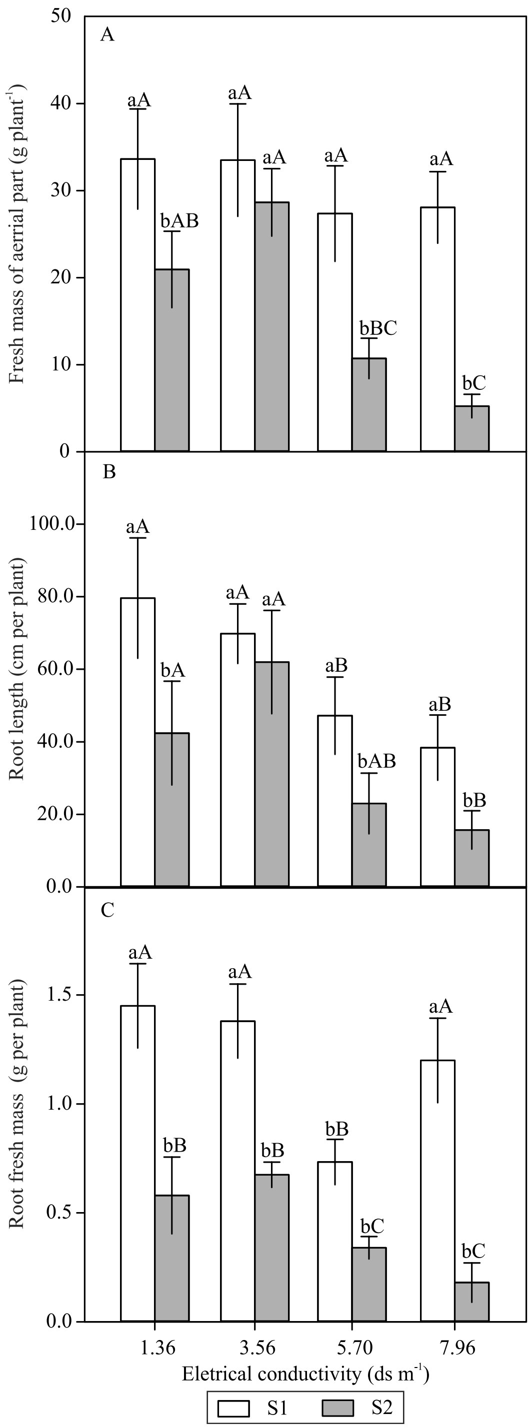

Figure 3. Fresh mass of shoot (A), root lenght (B), and fresh mass of root (C) of 'Crimson Sweet' watermelon (*Citrullus lanatus*) cultivated in soil + earthworm humus (S_1) and soil + bovine manure (S_2) subjected to different levels of electrical conductivity of the irrigation solution (1.36 – control, 3.56, 5.76, and 7.96 dS m^{-1}). Bars with equal letters do not differ by Tukey's test, at 5% probability: lowercase letters refer to days after emergence and uppercase letters to electrical conductivity.

The soil + humus treatment presented high levels of K^+ and Ca^{2+} (Table 1), which contribute to the improved physiological and productive performance of plants under osmotic stress and suggest a tolerance to salinity in the cultivated watermelon plants (Gheyi et al., 2010; Kaddour et al., 2012).

There was a gradual increase of Na^+ in the shoots grown in both substrates in response to the increasing salinity of the irrigation water, but there were no differences between them (Figure 4 A). In S_1 with 5.76 dS m^{-1} irrigation water, there was greater accumulation in the roots than in the shoots.

A high content of Na^+ in tissues is a critical factor of ionic toxicity in plants, since, in addition to interfering with the proper homeostasis of K^+, Na^+ reduces the availability, translocation, and mobilization of Ca^{2+} to the growing regions, which affects vegetative growth and production (Kaddour et al., 2012).

Saline treatments did not affect K^+ levels in the shoots, although the highest values for this ion were found in plants cultivated in soil + manure. A different behavior was verified for this ion in the roots, where a higher concentration was observed under lower levels of NaCl, and a reduced concentration was obtained under higher saline levels (Figure 4 C and D).

Similar results were reported by Souza et al. (2011) and Silva et al. (2015), who studied salinity variation in cowpea bean (*Vigna unguiculata*) and *Jatropha curcas*, and found a decrease of solute concentrations in the roots in response to increasing salinity. These authors attributed the reduction in the root K^+ content to the high concentrations of Na^+ through the antagonism between these ions.

Irrigation with saline water did not result in differences in the Ca^{2+} content in the shoots of the plants grown in S_1. For plants grown in S_2, a gradual increase in Ca^{2+} concentration was observed in response to the higher levels of EC (Figure 4 E). A higher content of this ion was observed in S_1 in relation to S_2 until a EC of 5.76 dS m^{-1}. The presence of Ca^{2+} improved the vegetative growth of the plants, acting directly in the vegetative phase (Figure 1).

The Ca^{2+} content in the roots of plants grown in S_1 increased until the salinity treatment reached 5.76 dS m^{-1}, after which the Ca^{2+} content decreased. No differences were observed in plants cultivated in S_2, with the exception of the EC of 5.76 dS m^{-1}.

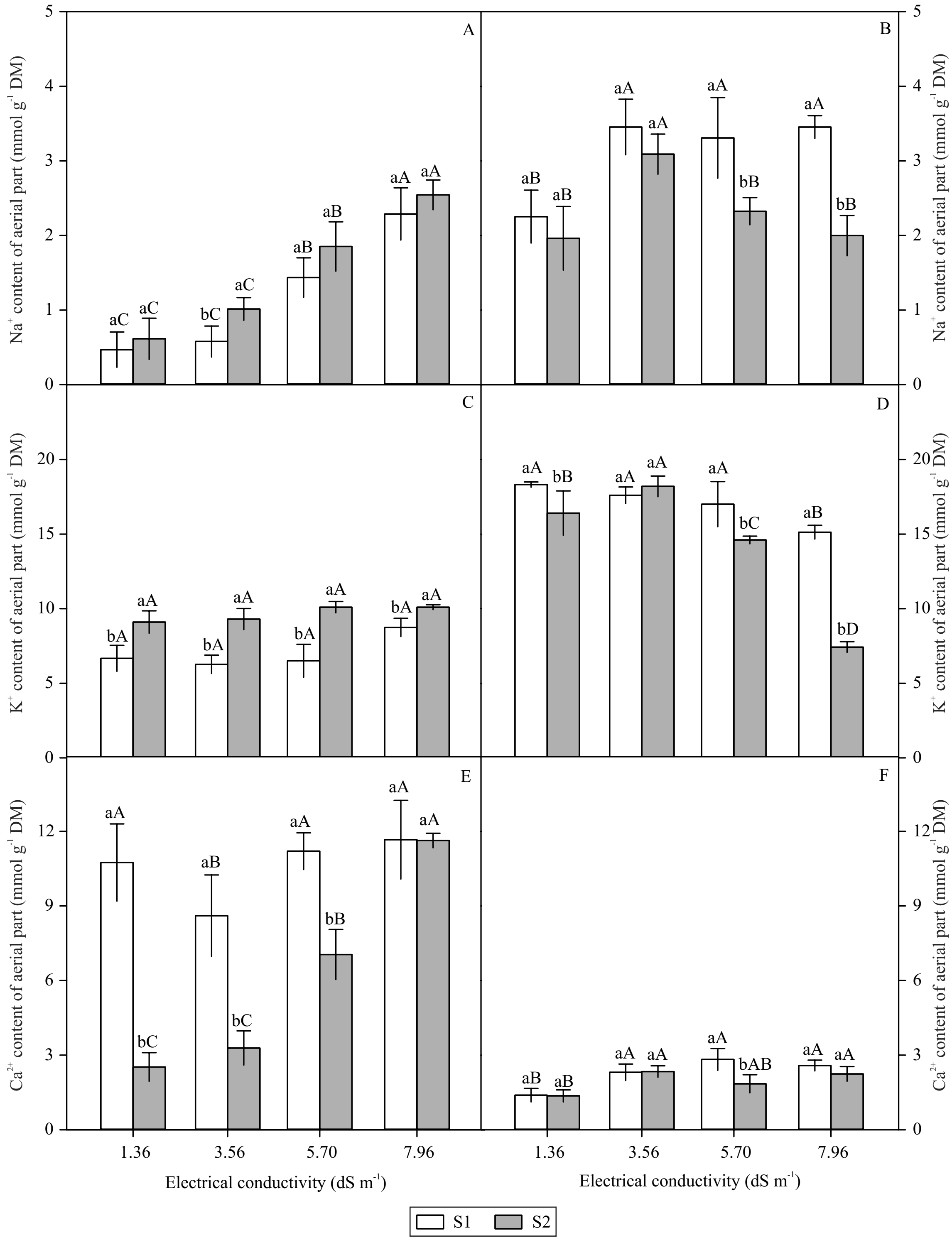

Figure 4. Na^+ content of shoot (A) and root (B), K^+ content of shoot (C) and root (D), and Ca^{2+} content of shoot (E) and root (F) of 'Crimson Sweet' watermelon (*Citrullus lanatus*) cultivated in soil + earthworm humus (S_1) and soil + bovine manure (S_2) subjected to different levels of electrical conductivity of the irrigation solution (1.36 – control, 3.56, 5.76, and 7.96 dS m^{-1}). Bars with equal letters do not differ by Tukey's test, at 5% probability: lowercase letters refer to days after emergence and uppercase letters to electrical conductivity.

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Conclusions

1. The development of watermelon (*Citrullus lanatus*) seedlings irrigated with saline water with electrical conductivities of 1.36 (control), 3.56, 5.76, and 7.96 dS m^{-1} is high for 'Crimson Sweet' plants cultivated in soil + earthworm humus until 27 days after emergence.

2. There is a significant interaction of the soil + earthworm humus and soil + bovine manure substrates with leaf area, leaf number, stem diameter (SD), fresh matter mass of shoots, shoot length, and fresh matter mass of roots (FMR), and also between substrates and Na^+, K^+, and Ca^{2+} contents in shoots and roots.

3. Substrates and increasing salinity (electric conductivity) of the irrigation water interact as to SD, FMR, and Ca^{2+} content in both shoots and roots, and to K^+ and Na^+ in roots.

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