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Organic fertilizers as mitigating effects of water salinity on *Passiflora cincinnata* seedlings

Fertilización orgánica mitigante de los efectos de la salinidad del agua en plantulas de *Passiflora cincinnata*.

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Abstract

Maracujá-do-mato (*Passiflora cincinnata*) is a species adapted to the climatic conditions of the Brazilian semi-arid region and widely used as rootstock, however, studies related to the production of seedlings and their resistance to abiotic stresses are scarce in literature. The objective was to study the production of maracujá-do-mato seedlings under the effect of the electrical conductivity on the irrigation water as a function of the application of organic fertilizers. The experiment was developed at the Paraíba State University, Catolé do Rocha-PB, in a completely randomized experimental design, in a 5 x 3 factorial scheme, with 6 replicates. The factors evaluated were five electrical conductivities of irrigation water (ECw: 1; 2; 3; 4 and 5 dS m^{-1}) and application of three organic fertilizers (bovine urine, bovine biofertilizer and liquid earthworm humus). It was verified that the increase of ECw affected the morphology and the quality of the seedlings negatively, while the bovine biofertilizer presented better efficiency in comparison to the others. It is inferred that the use of organic fertilizers as mitigating effects of salinity may be a strategy for production of maracujá-do-mato seedlings in saline conditions.

Keywords: Maracujá-do-mato, propagation, salts.

Resumen

Maracujá-do-mato (*Passiflora cincinnata*) es una especie adaptada a las condiciones climáticas de la región semiárida brasileña y ampliamente utilizada como portainjertos, sin embargo, los estudios relacionados con la producción de plántulas y su resistencia a estrés abióticos son escasos en la literatura. El objetivo de este estudio fue medir la producción de plántulas de maracuyá bajo el efecto de la conductividad eléctrica en el agua de riego en función de la aplicación de fertilizantes orgánicos. El experimento se desarrolló en la Universidade Estadual da Paraíba, Catolé do Rocha-PB, Brasil, en un diseño experimental completamente aleatorizado, en un esquema factorial de 5 x 3, con seis replicaciones. Los factores evaluados fueron cinco conductividades eléctricas de agua de riego (ECw: 1; 2; 3; 4 y 5 dS m^{-1}) y la aplicación de tres fertilizantes orgánicos (orina de bovino, biofertilizante bovino y humus de lombriz líquida). Se encontró que el aumento de ECw afectó negativamente la morfología y la calidad de las plántulas, mientras que el biofertilizante bovino presentó una mayor eficiencia en comparación con los demás tratamientos. Se concluye que el uso de fertilizantes orgánicos como efectos atenuantes de la salinidad es una estrategia para la producción de plántulas de pasiflora en condiciones de suelos salinos.

Palabras-clave: Maracujá-do-mato, propagación, sales.

Introduction

Maracujá-do-mato (*Passiflora cincinnata* Mast.) is a perennial species, resistant to water deficit, being a species adapted to the semi-arid climate, which may represent a new alternative of cultivation for the small farmers in dry conditions. Despite the efforts to build a knowledge base on this species, little information on its morphology, reproductive biology and physiological aspects is available, thus limiting the creation of management strategies for a future production chain (Santos, Matsumoto, Oliveira, Oliveira, and Silva, 2016). Its uses are varied, being mainly used as root stock for species of the same genus as a source of pathogen resistance, for the production of fruits, as well as ornamental and medicinal purposes (Queiroz, 2011).

In the Northeast region of Brazil the largest cultivation and commercialization refers to yellow passion fruit (*Passiflora edulis* Sims.), passion fruit being marketed in the off season. Besides being well accepted in the market, this plant is native to the region and adapted to the local conditions of cultivation, besides presenting excellent income option for small farmers. In addition, it presents great potential as a root stock for yellow passion fruit (Oliveira Júnior et al., 2010).

In many fruit producing areas, such as the states of Paraíba and Rio Grande do Norte, the rainfall irregularity associated with poor water quality for irrigation induces producers to use water compromised by the salt excess (Nascimento et al., 2015). As it is an area of great fruit production, the irrigated perimeters located in the drought polygon have an important area focused on the cultivation of passion fruit. Many of these productive sectors suffer from the accumulation of salts in their areas, leading to a production decrease and abandonment of the areas (Leite, Santos and Gomes, 2012).

The high concentration of salts is a stress factor for plants, mainly for *Passiflora* spp., because it reduces the osmotic potential and facilitates the action of the ions on the protoplasm. As the salinity increase, resulting from the accumulation by the irrigation water, the osmotic potential of the soil decreases, the water being osmotically retained in the saline solution, which hinders the absorption of water by the roots and consequently, will affect the growth of the plants (Andréo-Souza et al., 2010).

It is highlighted as an alternative used to minimize the deleterious effects of salinity on soil and plants, and the use of liquid bio-fertilizer in the form of microbial ferments. Nobre et al. (2010) state that the bio-fertilizer applied to the soil can induce an increase in the osmotic adjustment of the plants by the accumulation of organic solutes, promoting the absorption of water and nutrients in saline environments.

Some studies have demonstrated the positive action of bio fertilizers as attenuating the effects of irrigation water salinity (Mesquita et al., 2012; Medeiros et al., 2016; Alves, et al., 2016; Alves, et al., 2017; Melo Filho et al., 2017; Véras et al., 2017a; Véras et al., 2017b; Véras et al., 2017c). These effects can be attributed to the presence of humic substances contained in organic inputs that provide a greater osmotic regulation between the root and the soil solution, besides reducing the intensity of the toxic effects of the salts on the plant growth (Aydin, et al., 2012).

One of the most influential phases in fruit production is the propagation and formation of seedlings, since these directly influence the performance and productivity of the crop to be planted. The objective of this study was to evaluate the production of Maracujá-do-mato seedlings (*Passiflora cincinnata*) under the effect of the electric conductivity in the irrigation water due to the application of organic fertilizers.

Material and methods

The experiment was conducted from January to April 2015 in a greenhouse at the Center for Human and Agrarian Sciences of the Universidade Estadual da Paraíba (UEPB) in the municipality of Catolé do Rocha-PB, Brazil, with the following geographic coordinates: 6°20'38"S; 37°44'48"W and 275 meters of altitude. The experimental design was a completely randomized design, in a 5 x 3 factorial scheme, with 6 replicates. The factors evaluated were five electrical conductivities of the irrigation water (ECw: 1; 2; 3; 4 and 5 dS m^{-1}) and application of different organic fertilizers (bovine urine, bovine biofertilizer and liquid earthworm humus). The experimental units were composed of three seedlings grown in polyethylene bags with 2 dm^{-3}.

The maracujá-do-mato seeds were collected in a native area in the city of Catolé do Rocha, PB, pulped and dried in the shade. The sowing was done directly in polyethylene bags with soil, placing five seeds per bag. At 15 days after emergence, thinning was performed, keeping only one (most vigorous) seedling, and during the experiment the monitoring of the phytosanitary management was done in order to avoid problems with pests or diseases.

The maracujá-do-mato seedlings were irrigated daily with each type of water, starting on the fifteenth day after sowing, and irrigation was performed manually by a fine sieve irrigator, providing a sufficient blade to raise the soil moisture at the field capacity level. The different levels of ECw were obtained by the addition of sodium chloride (NaCl) water from the local supply system, according to Rhoades, Kandiah, and Mashali (2000) and the amount of salts (Q) was determined by equation: Q (mg L^{-1}) = ECw x 640 where ECw (dS m^{-1}) represents the desired value of the electrical conductivity of water.

The chemical analysis of the water was performed and presented the following characteristics: pH = 7.53; Ca= 2.31 $cmol_c\ dm^{-3}$; Mg = 1.54 $cmol_c\ dm^{-3}$; Na = 4.00 $cmol_c\ dm^{-3}$; K = 0.03 $cmol_c\ dm^{-3}$; Chloride = 3.91 $cmol_c\ dm^{-3}$; Carbonate = 0.57 $cmol_c\ dm^{-3}$; Bicarbonate = 3.85 $cmol_c\ dm^{-3}$; RAS = 2.88 $(mmol_c\ l^{-1})^{1/2}$.

The bovine urine was collected in the morning from lactating cows, and after collection it was stored in a "PET" bottle. The bovine bio fertilizer was obtained according to Silva et al. (2011). The liquid earthworm humus was prepared with water (without chlorine) and solid earthworm humus in the proportion of 10%.

The treatments with bovine urine, bovine bio fertilizer and liquid earthworm humus were applied 15 days after sowing (DAS) at 8 day intervals, totaling six applications at the 10% dosage of the substrate. For filling the bags, fossil free sandy clay was used, it was collected in the layer of 0 to 20 cm in a native area located on the campus of UEPB.

A sub-sample was removed from the soil to be chemically analyzed (Table 1). Prior to application, bovine urine, bovine bio-fertilizer and liquid earthworm humus were diluted in water (5%), after which the bovine bio fertilizer was submitted to the screen filtration process to reduce the risks of obstruction of the irrigator screen. Said organic fertilizers were analyzed and the results are presented in Table 1.

The growth of the maracujá-do-mato was evaluated at 60 and 90 days after sowing (DAS) through measurements of plant height, number of leaves, stem diameter and leaf area. At the end (90 DAS) of the experiment the root length, dry matter mass of the root, shoot and total were evaluated.

From the mean values of plant height, stem diameter and leaf area, their respective absolute growth rates (AGR) and relative growth rates (RGR) were calculated according to Benincasa (2003).

The root-shoot ratios and the Dickson quality index were obtained according to the Benincasa (2003) methodology (Dickson et al., 1960). The data of total dry matter production were used to calculate the partitioned percentages between the vegetative organs and the index of tolerance to salinity, comparing the data of the saline treatments with those of the control (ECw = 1.0 dS m^{-1}).

The data obtained were evaluated by variance analysis using the 'F' test at the 0.05 and 0.01 probability level and in the cases of significance, linear and quadratic polynomial

Table 1. Chemical attributes of the soil, earthworm humus, bovine biofertilizer and bovine urine used in the experiment.

Characteristics	Soil	Humus	Bovine biofertilizer	Bovine urine
pH ($CaCl_2$)	5.02	7.38	4.68	4.5
EC (dS m^{-1})*	0.60	2.11	4.70	0.4
Ca^{+2} ($cmol_c\ dm^{-3}$)	4.63	3.54	3.75	0.0003
Mg^{+2} ($cmol_c\ dm^{-3}$)	2.39	1.93	3.30	0.00004
Na^{+} ($cmol_c\ dm^{-3}$)	0.30	0.18	1.14	0.00002
K^{+} ($cmol_c\ dm^{-3}$)	0.76	0.14	0.71	1.0
P (mg dm^{3})	0.70	5.51	14.45	0.00048
Al^{3+} ($cmol_c\ dm^{-3}$)	0.00	0.00	0.00	0.00
$H^{+}+Al^{3+}$($cmol_c\ dm^{-3}$)	1.00	0.00	1.00	0.00
SB	7.78	5.61	7.76	0.00
T	8.08	5.79	8.9	0.00
MO (g kg^{-1})	8.05	9.00	8.00	0.00

* Electrical conductivity of the 1:2.5 extract; P, K, Na: Mehlich1 extractor; Al, Ca, Mg: KCl 1.0 mol L^{-1} extractor; SB = $Ca^{+2}+Mg^{+2}+K^{+}+Na^{+}$; $H^{+}+Al^{3+}$: 0.5 mol L^{-1}, pH 7.0 calcium acetate extractor; CTC=SB+H^{+}+Al^{+3}; M.O.: Walkley-Black wet digestion.

regression analysis was performed for the electrical conductivity of the irrigation water and Tukey test for organic fertilizer sources using the SISVAR 5.0 statistical software (Ferreira, 2011).

Results

The electrical conductivity of the irrigation water (ECw) statistically influenced the analyzed variables. The height of the plant showed a significant effect on the two evaluated periods for ECw (Figures 1A and 1B). A linear effect was observed for all the organic fertilizers studied, and the same behavior was observed for the stem diameter (Figures 1C and 1D). Results different to those of this research for plant height were obtained by Dias et al. (2013) when studying the attenuating effect of bovine biofertilizer on saline stress in yellow passion fruit (*Passiflora edulis* L.). According to the authors, the increase in ECw did not significantly influence the aforementioned variable.

At 60 DAS, there was a unit decrease of 2.92, 1.04 and 2.32, while for 90 DAS a decrease of 4.38, 2.79 and 3.79 was observed for bovine urine, bovine biofertilizer and humus of worm, respectively. It was observed that the bovine biofertilizer was the most effective for plant height, since it presented the smallest unit decrease.

Regarding the diameter of the stem, it was observed that 60 DAS bovine urine was 22% more efficient than the earthworm humus, however, it was 58.5% inferior to the bovine biofertilizer. At 90 DAS, behavior was observed close to those found at 60 DAS, where the earthworm humus was 10 and 31% lower than urine and bovine biofertilizer, respectively. Similar results to this research were obtained by Mesquita et al. (2012), where these authors report the attenuating effect of bovine biofertilizer on papaya (*Carica papaya* L.) seedlings under saline stress. While Dias et al. (2013), reported that waters with ECw higher than 2.5 dS m^{-1} adversely affected the stem diameter in yellow passion fruit plants.

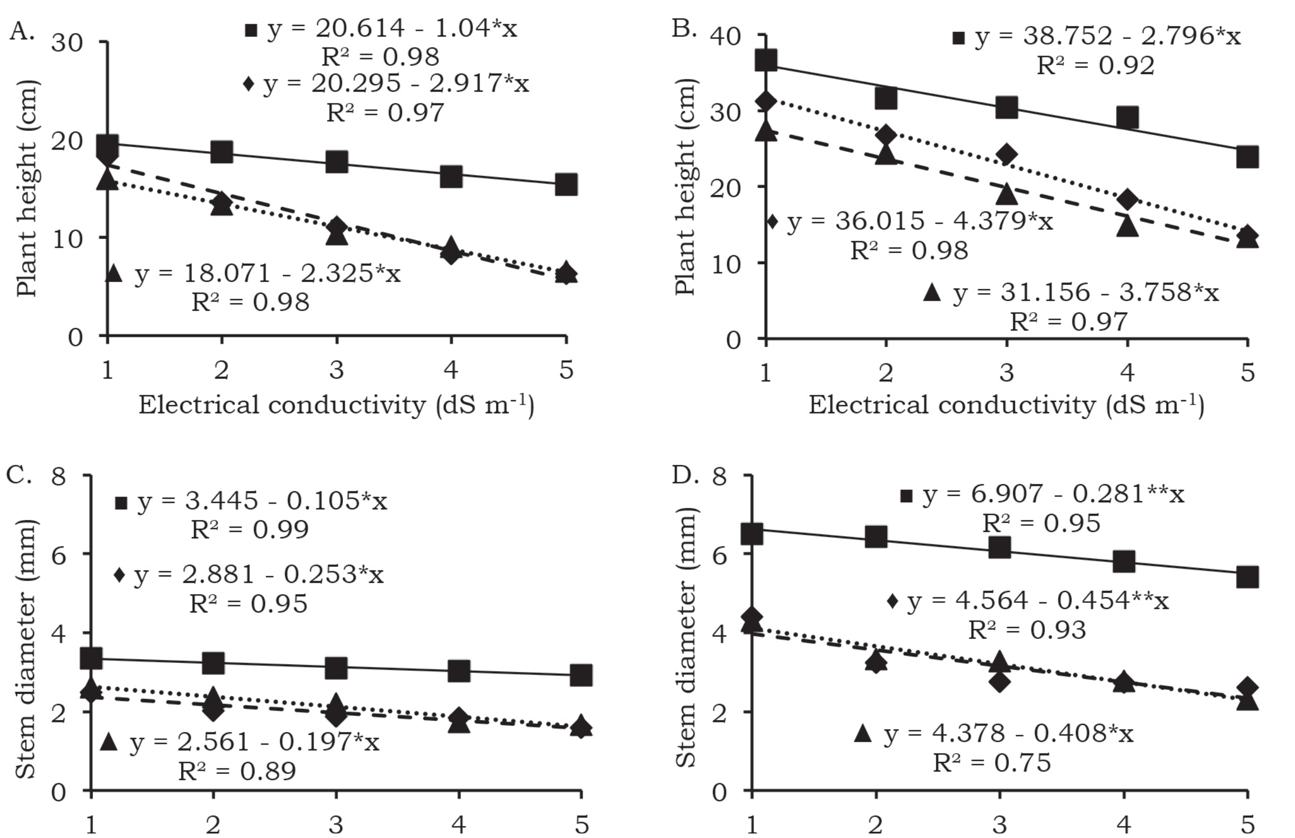

Figure 1. Plant height at 60 (A) and 90 (B) and stem diameter at 60 (C) and 90 (D) days after sowing of *Passiflora cincinnata* seedlings under the effect of ECw as a function of the application of organic fertilizers: (♦) bovine urine, (■) bovine biofertilizer and (▲) liquid earthworm humus.

The number of leaves did not present significant differences for the organic fertilizers used, as demonstrated in Figure 2A. However, there is a linear decrease with the increase of ECw. The same behavior was observed for leaf area at 60 DAS, occurring opposite effect to 90 DAS (Figures 2B and 2C).

The effect of ECw on the number of leaves did not present differences regarding the linear decreases in the two evaluated periods, however, a reduction was observed. This was also observed in other fruit trees irrigated with saline water, such as guava (*Psidium guajava* L.) (Cavalcante et al., 2009) and papaya (Sá et al., 2013) seedlings. Mesquita et al. (2012) observed that the increase of water salinity reduced the number of leaves of yellow passion fruit, however, in the seedlings that received application of bovine biofertilizer there was an increase in the number of leaves.

At 90 DAS, the seedlings of maracujá-do-mato showed a unit decrease of 55.2, 20.27 and 70 cm^2 due to bovine urine, bovine biofertilizer and earthworm humus, respectively. It was noticed that the earthworm humus was the least efficient, being 71.1% inferior to the bovine biofertilizer, the most efficient fertilizer in this variable. Similar results were observed by Mesquita et al. (2012) and Souza et al. (2008), where they observed that the leaf area of the yellow passion fruit plants that received the application of bovine biofertilizer stood out to those that were dod not receive the application.

It was observed that the absolute height growth rate of the plant showed a decrease when ECw was increased (Figure 3A), and the earthworm humus showed to be the least efficient when compared to the other fertilizers studied. The absolute and relative growth rates of stem diameter were influenced by ECw and organic fertilizers (Figures 3B and 3C), with linear decreases noted as ECw was increased. The absolute and relative growth rate of the leaf area were affected by ECw, and linear behaviors were observed regarding the attenuating effects of the organic fertilizers used (Figures 3D and 3E).

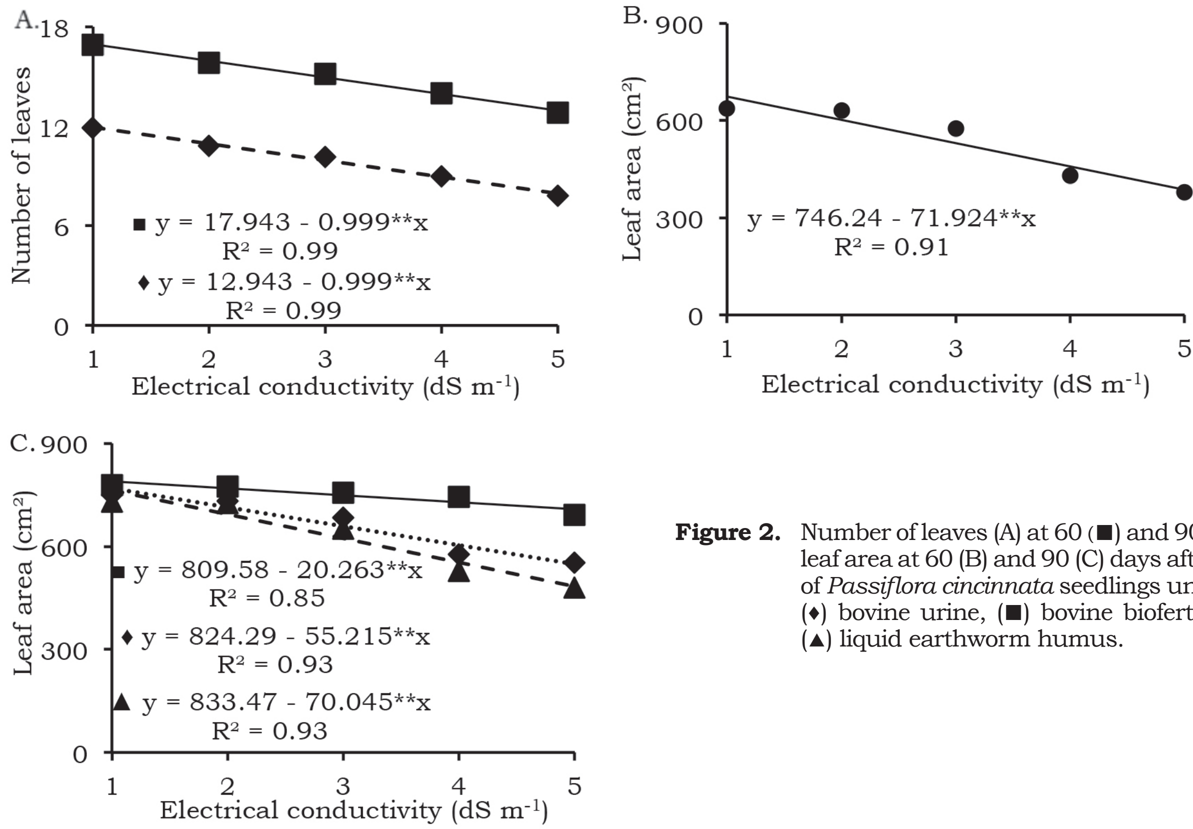

Figure 2. Number of leaves (A) at 60 (■) and 90 (♦) days, leaf area at 60 (B) and 90 (C) days after sowing of *Passiflora cincinnata* seedlings under ECw. (♦) bovine urine, (■) bovine biofertilizer and (▲) liquid earthworm humus.

The bovine biofertilizer was the one that presented the best effect when compared to the others for the AGRap, being superior in 75 and 85% in relation to the bovine urine and the earthworm humus, respectively. Similar results were observed by Mesquita et al. (2012), where they observed that the AGRap of yellow passion fruit seedlings had a decrease with the salinity increase with values up to 0.08 and 0.083 cm day^{-1}, in both situations in the soil without and with biofertilizer, respectively.

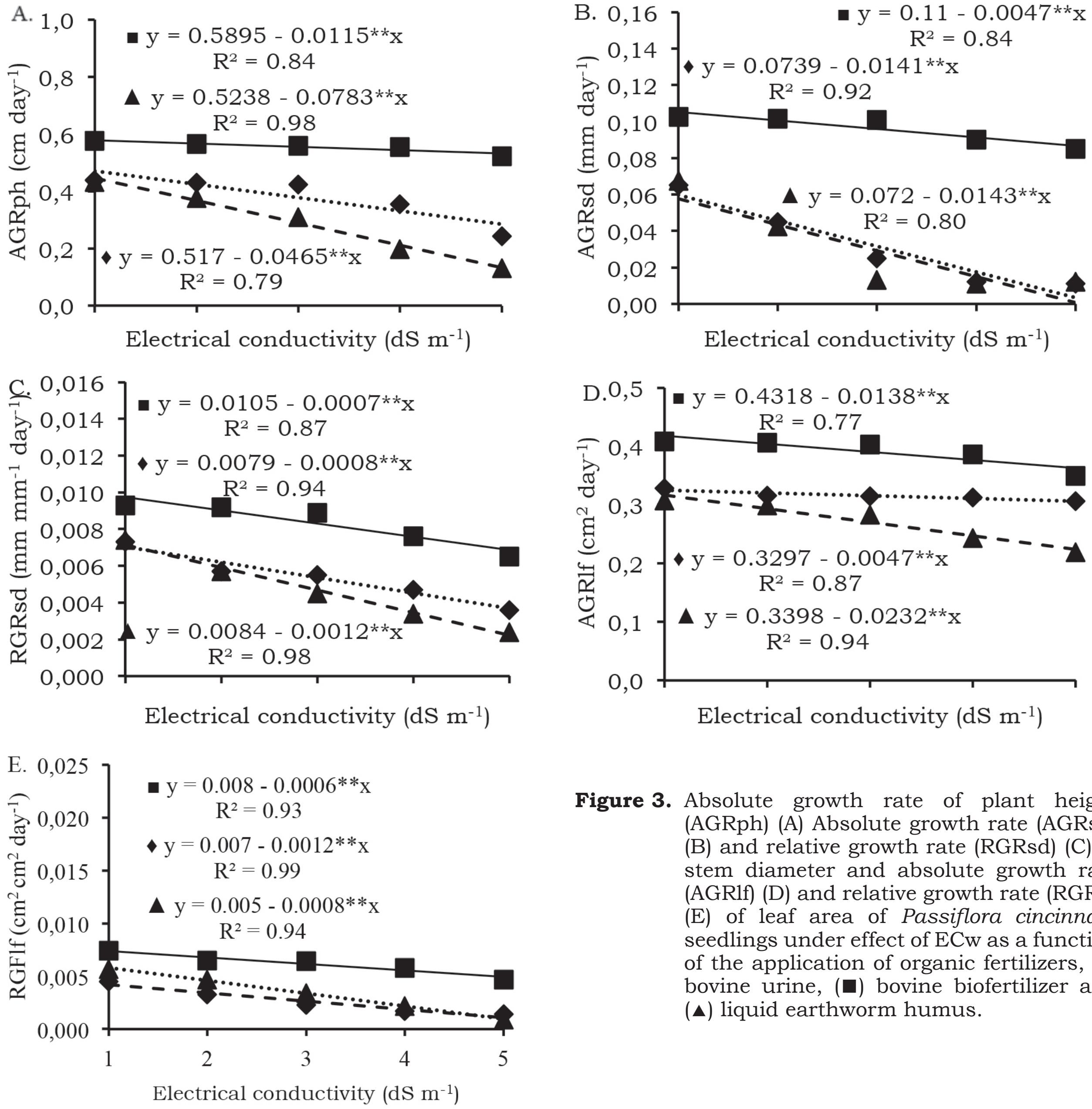

Figure 3. Absolute growth rate of plant height (AGRph) (A) Absolute growth rate (AGRsd) (B) and relative growth rate (RGRsd) (C) of stem diameter and absolute growth rate (AGRlf) (D) and relative growth rate (RGRlf) (E) of leaf area of *Passiflora cincinnata* seedlings under effect of ECw as a function of the application of organic fertilizers, (♦) bovine urine, (■) bovine biofertilizer and (▲) liquid earthworm humus.

For the absolute growth rate of stem diameter (AGRdc), bovine urine presented a unit decrease of 0.0143 mm day^{-1}, while bovine biofertilizer and earthworm humus presented 0.0141 and 0.0047 mm day^{-1}, respectively. It was found that the bovine biofertilizer was superior at 66.6 and 67.1% in relation to bovine urine and earthworm humus, respectively. Regarding the AGRRdc, the bovine biofertilizer presented better results, being higher at 12.5 and 41.6% in relation to bovine urine and earthworm humus, respectively. Similar results were observed by Dias et al. (2013) and Mesquita et al. (2012), where they observed a reduction in the absolute growth rate of yellow passion fruit stem diameter as the ECw increase, however, there was not significance observed for the relative growth rate of stem diameter.

The bovine biofertilizer presented better results when compared to the others for the RGRaF and RGRaf. However they were affected by the increase in ECw. This may be related to the lower availability of water to the plants exerted by the increase of the concentration of salts, since the plant spends more energy expenditure to absorb water.

The root dry mass had a linear decreasing effect as ECw was increased, with fertilizers having significant effects (Figure 4A), the same was observed for the total dry mass (Figure 4D). As for the dry mass of the stem, there were only isolated effects for each factor (Figures 4B and 4C).

The increase of ECw had a negative influence on the stem dry mass, the same was observed by Sá et al. (2012) in papaya seedlings and by Gurgel et al. (2007) in guava seedlings. Regarding organic fertilizers, it was observed that the bovine biofertilizer differed statistically from the others, the same was observed in papaya seedlings (Mesquita et al., 2012).

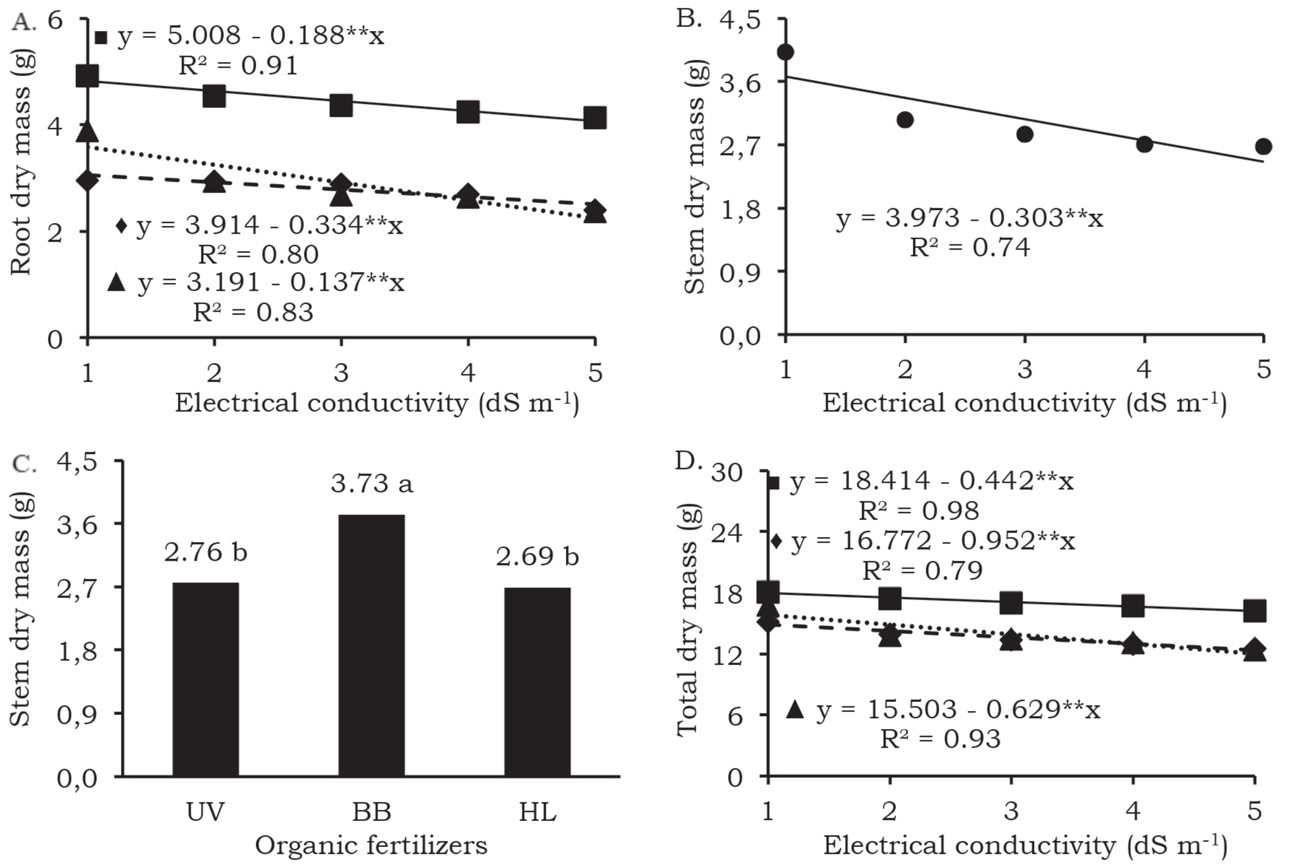

Figure 4. Root dry mass (A), stem dry mass under the effect of ECw (B) as a function of the application of organic fertilizers (C) and total dry mass (D) of *Passiflora cincinnata* seedlings as a function of the application of organic fertilizers, (♦) bovine urine, (■) bovine biofertilizer and (▲) liquid earthworm humus. UV: bovine urine, BB: bovine biofertilizer and HL: liquid earthworm humus.

*Means followed by the same letter do not differ statistically by the Tukey test at 5% probability.

The earthworm humus showed the lowest unit decrease of 0.137 g, 27 and 59% higher than the biofertilizer and the bovine urine, respectively, for the root dry mass variable. In papaya seedlings, Mesquita et al. (2012), observed that the total dry mass was reduced as ECw was increased, however, the seedlings treated with bovine biofertilizer presented higher results than those without the addition of the input. The same was observed in cashew (Sousa et al., 2011), guava (Cavalcante et al., 2010) and imbu seedlings (*Spondias tuberosa* Arruda) (Silva and Amorim, 2009).

For the total dry mass there was a unit increment of 0.95, 0.44 and 0.63 g for bovine urine, bovine biofertilizer and earthworm humus, respectively. It was observed that he bovine biofertilizer had a better attenuating effect, aiming to present the smallest unit increment.

The influence of organic fertilizers on ECw in the variables total dry mass and root-shoot ratio is shown in Figure 5A. For Dickson's quality index there was no significant interaction between the factors, however there was significance for the factors isolated (Figures 5B and 5C). A linear behavior is observed for all evaluated fertilizers and decreases as ECw increases. The same is observed for the tolerance index (Figure 5D).

For the root-shoot ratio, it was observed that the bovine biofertilizer showed the best effect, presenting the lowest unit increment (0.21 g). The reduction in the root-shoot ratio is related to the greater reduction of root growth in relation to the shoot, aiming to reduce the absorption of salts from the environment, especially in environments with higher levels of salinity (Sá et al., 2013). Since the dissolved salts in the soil solution cause changes in the physiological processes of the crops, with consequent reduction in their root growth and their production of photoassimilates (Mesquita et al., 2012). This fact was confirmed in view of the drastic reductions observed in root dry mass accumulation.

A unit increment of 0.04 was observed in the Dickson quality index, where a reduction of the same was observed when ECw was elevated. Similar results were observed in neem seedlings (*Azadirachta indica* A. Juss.) by Mesquita et al. (2015) and in Oiticica (*Licania rigida* Benth.) by Diniz Neto et al. (2014).

For the tolerance index, a unit increase of 10.6, 6.2 and 11.75% was observed for bovine urine, bovine biofertilizer and earthworm humus, respectively. It was noticed that the bovine biofertilizer presented the best results, where it showed the smallest unit increment. The same was 41.2 and 47% higher than bovine urine and earthworm humus, respectively.

Discussion

The highest plant height values with the use of bovine biofertilizer can be explained due to the benefits that the organic input provides. Since it acts on soil improvement [on chemical attribu-

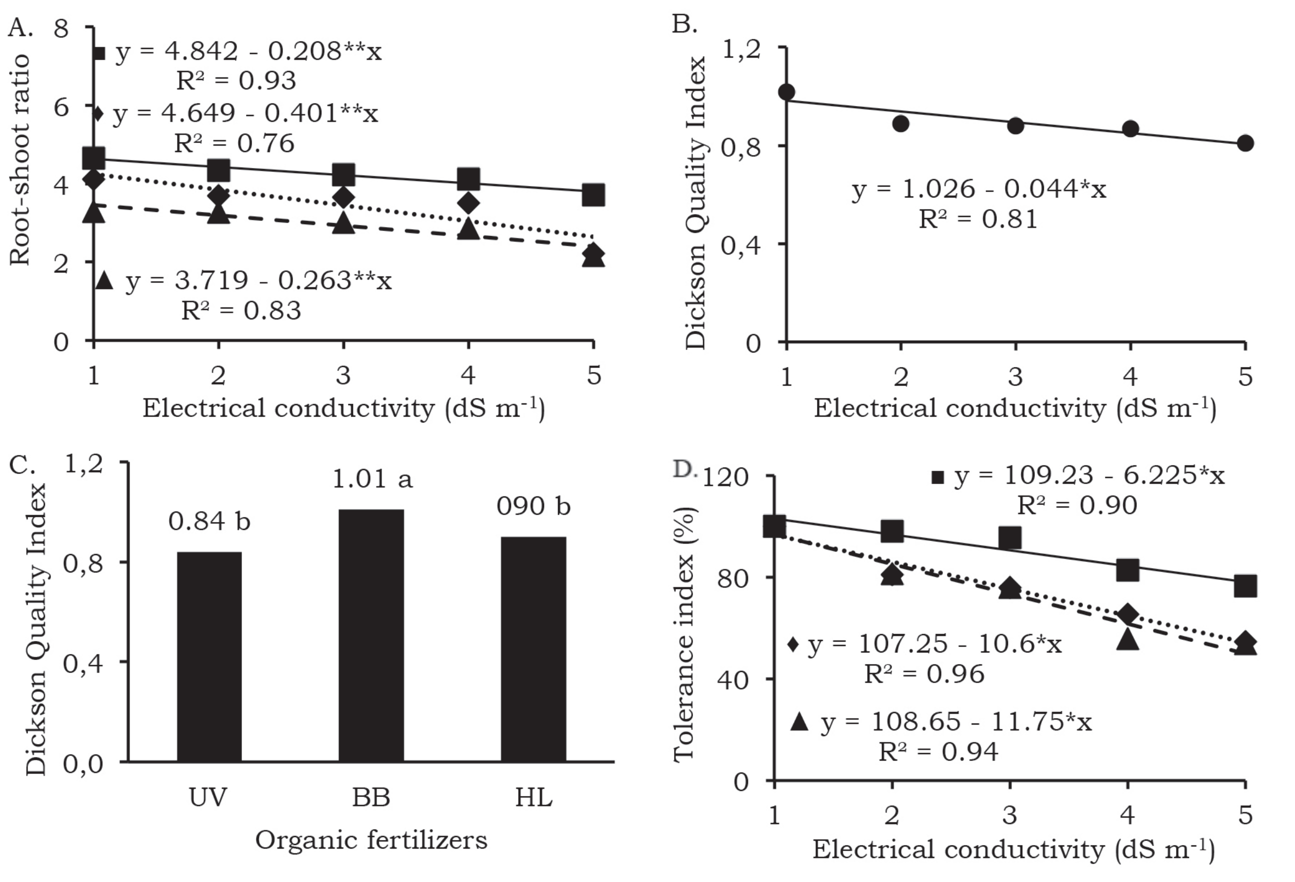

Figure 5. Root-shoot ratio (A), Dickson Quality Index under ECw effect (B) and according to the application of organic fertilizers (C) and tolerance index (D) of *Passiflora cincinnata* seedlings under the effect of ECw as a function of application of organic fertilizers. UV: bovine urine, BB: bovine biofertilizer and HM: liquid earthworm humus. *Means followed by the same letter do not differ statistically by the Tukey test at 5% probability.

tes (increasing nutrient availability), on physical attributes (increasing water retention capacity)], as well as favoring the microbiota and reducing the action of toxic ions on plants (Boraste et al., 2009; Patil, 2010).

The reduction of stem diameter is due to the stress caused by the accumulation of salts applied to the soil due to the irrigation with saline water. The salts cause the reduction of the photosynthetic rate and the stomatal conductance, making it difficult to assimilate CO_2, compromising the assimilation of photoassimilates and, consequently, the growth of plants (Fernández-García et al., 2014). Increased salinity causes phytotoxicity in plants, causing a series of ionic and hormonal changes directly affecting plant growth (Sá et al., 2013).

When they are in saline environments, the plants reduce the leaf area as a way to adapt to saline stress, in addition, there is a decrease in the transpiratory process and, as a consequence, reduction of the Na^+ and Cl^- loading in the xylem and water conservation in the tissues of plants (Sucre and Suáres, 2011). The effect of osmotic stress on plants may decrease the speed of leaf expansion, leading to a decrease in growth rates (Munns and Tester, 2008), which was confirmed by the results obtained in this work.

As for the attenuating use of fertilizers, it was verified that the bovine biofertilizer presented the best results, differing statistically from the others. The biofertilizer assists in the improvement of soil fertility, since it even helps the adsorption of exchangeable bases through the formation of organic complexes, in addition to developing negative charges. Such characteristics may favor the reduction of deleterious effects on plants of water with high concentrations of salts (Silva et al., 2011).

The tolerance to salinity in some crops may be associated with their ability to decrease their water potential and, consequently, to reduce water use (Borde et al., 2011). By decreasing water consumption, which may be affected by salts, the plants decrease the assimilation of the toxic ions present in the soil solution. However, it is important to note that the organic matter does not eliminate the degenerative effects of toxic ions on the plants. However, it reduces these effects, leading to better seedling growth (Lima Neto et al., 2015). Thus, the study of the degree of tolerance of species to the stress caused by the salts is of great importance, considering the use of saline soils and water in the affected regions (Freitas et al., 2014).

Conclusions

Irrigation with saline water adversely affects the morphology and quality of maracujá-do-mato seedlings; the bovine biofertilizer minimizes the deleterious effects of saline water on seedlings.

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