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Morphological and physiological changes in papaya seedlings "Hawaii" irrigated with saline water and application of humic substances

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Abstract

In the northeast semiarid region water with high salt content is very common, which may negatively affect crops growth and development. Thus, using possible salt stress attenuators is extremely important because it allows the use of saline waters for agricultural purposes. Among the possible attenuators of salt stress, humic substances stand out. This work was driven in order to evaluate the effect of the application of humic substances as a possible attenuator of salt stress from papaya seedlings irrigation. The experiment was conducted under entirely randomized design with five replications in a 4 x 4 factorial, regarding the four doses of humic substances (5; 10; 15 and 20 g), and the electrical conductivity of the irrigation water (ECw 1.5; 3.0; 4.5 and 6 dS m^{-1}). Were evaluated: the plant height, stem diameter, leaf number, carbon internal concentration, stomatal conductance, transpiration, net photosynthetic rate, instantaneous water use efficiency and the instantaneous efficiency carboxylation, chlorophyll indices a, b, and total ratio of chlorophyll a/chlorophyll b at 70 days of emergence. When it irrigates the papaya seedlings with water of 6.0 dS m^{-1} is recommended dose of 20 g of humic substances, which provided greater growth. Irrigation of papaya seedlings with high salinity (3.0 and 4.5 dS m^{-1}) allied with application of 20 g of humic substances provide increased CO_2 concentration, transpiration rate, instantaneous water use efficiency, carboxylation efficiency and chlorophyll b content, however, stomatal conductance, net photosynthesis and chlorophyll a content are reduced with increase of the *ECw*.

Keywords: *Carica papaya* L., physiology, salt stress, humic acid

Introduction

Papaya (*Carica papaya* L.) is a crop of tropical and subtropical climate, adapted to the Brazilian Northeast, it presents great importance in the regional economy, which contributes to social and economic aspects, such as providing employment and income (Diniz et al., 2018; Sá et al., 2013). In the state of Paraíba, seedling production of this species stands out among the fruits, being the 4th largest producer in the northeast (Carvalho, 2017).

However, in this region the water and soil salinity is one of the major obstacles to the production system, therefore increasing the salt concentration in the irrigation water inhibits the germination of seeds, and delay the initial plant growth, thereby it compromises the seedlings formation (Sá et al., 2013; Coelho et al., 2015). The harmful effect of salinity on the initial growth of fruit plants were noted by several authors, as one example, Sá et al. (2013), Mesquita et al. (2014), Mesquita et al. (2015), Santos et al. (2015) and Diniz et al. (2018) in papaya seedlings and in yellow passion fruit plants (Bezerra et al., 2016; Ribeiro et al., 2016).

The adverse effects of salinity on the plants can be mitigated by the use of organic inputs applied to the soil, such as humic substances. These can have direct and indirect effects on plant growth. The indirect effects involve improvements in soil properties, such as aggregation, aeration, permeability, water holding capacity, micronutrients transport and availability. Various studies point that humic substances could present effects against stress, caused mainly by salinity through increasing nutrients absorption and reducing some toxic elements absorption. There are several studies about the application of humic acid and their effect on salinity conditions (Caron et al., 2015).

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soils is due to the presence of Ca, Mg and K in their composition. The mere presence of these salts could turn other elements more available (Ouni et al., 2014). The mitigating effects of organic inputs occur due to the presence of humic substances in their composition which promote improvements in the soil attributes physical, chemical and biological. Which are reflected in root architecture, the formation of new roots and increased absorption of water and nutrients by plants (Canellas & Olivares, 2014).

However, there are few studies on the use of attenuators of salt stress in fruit seedlings, especially in papaya seedlings. In this sense, the objective was to evaluate the effect of the application of humic substances as a possible attenuator of salt stress in papaya seedlings "Hawaii" under saline irrigation conditions.

Material and Methods

The experiment was conducted in greenhouse belonging to the Science Humanities Center, Social and Agricultural – CCHSA of the Federal University of Paraíba - UFPB, located in the municipality of Bananeiras-PB, Brazil, located in the geographic coordinates Latitude: -6.75105 Longitude: 35.6334 6° 45' 4 ''South, 35 ° 38' 0'' West.

The climate by Köppen classification is the type - Tropical rainy, with dry summer. According to the Executive Agency for the Waters Management of the State of Paraíba - AESA, the municipality of Bananeiras-PB, Brazil, presented during months from January to July 2017, the maximum temperature of 28.4 and 20.2 minimum, while the relative humidity was maximum 93.2 and 92.2 minimum, with cumulative rainfall of 472.7 mm.

Was adopted a completely randomized design with five replications in a factorial 4 x 4. The treatments consisted of doses of humic substances (5, 10, 15 and 20 g), and the electrical conductivity of the irrigation water (ECw 1.5; 3.0; 4.5 and 6 dS m^{-1}). The doses used were based on the work of Cavalcante et al. (2011) and Mesquita et al. (2012) who tested the use of humic substances (humine, fulvic acid and humic acids; Humitec) and biofertilizer chemically enriched in the papaya cultivar 'Formosa' and 'Sunrise Solo', associated with the observations made by Asik et al. (2009) and Canellas & Olivares, (2014). The experimental units were comprised of three seedlings cultivated in polyethylene pots with a capacity of 5 dm^3.

Five Papaya seeds of "Hawaii" type were sown directly in the pots at a depth of 1.5 cm and after 8 days of emergence (DAS) was carried out the thinning, leaving only one seedling per pot. During the experiment, conduction weeding was made as needed.

Pots were filled with soil collected from a depth of 0-20 cm and classified as oxisol. Soil samples were collected for chemical analysis, which presented the following characteristics (Table 1).

The electrical conductivity of the irrigation water (ECw) were obtained by adding saline complex (NaCl) to a non-saline water (0.5 dS m^{-1}) from the local supply system, being determined by a digital conductivity meter until the conductivity desired to be reached (ECw 1.5, 3.0, 4.5 and 6.0 dS m^{-1}).

Table 1. Chemical attributes of the soil used before the experiment.

Attributes	Values
pH (water 1:2.5)	5.20
Al^{+3} ($cmol_c$ dm^{-3})	0.10
Ca^{+2} ($cmol_c$ dm^{-3})	0.50
Mg^{2+} ($cmol_c$ dm^{-3})	0.45
P (mg. dm^{-3})	9.82
K ($cmol_c$ dm^{-3})	0.03
Na^+ ($cmol_c$ dm^{-3})	0.13
H^++Al ($cmol_c$ dm^{-3})	0.10
O.C. (g kg^{-1})	2.35
SB ($cmol_c$ dm^{-3})	1.21
CEC ($cmol_c$ dm^{-3})	1.11
V (%)	91.7

SB (sum of basis) = Na^+ + K^+ + Ca^{2+} + Mg^{2+}; CEC (Cation-exchange capacity in pH 7.0) = SB + (H^+ + Al^{3+}); V (saturation for basis) = (100 x SB/CEC); O.C = organic carbon.

The humic substances were obtained from the commercial product and doses were prepared by diluting it to the irrigation water supplied manually and 4 dm^3 of each mixture to the seedlings being applied 20 days after seedling emergence (DAE) in the interval of 5 in 5 days. The papaya seedlings were irrigated daily, rising at the beginning soil moisture about 80% of field capacity (Fc), determined by the weighing method and the soil saturation curve.

The source of humic substances was a commercial product Humitec®, liquid black coloring soluble in water, containing 16.5% p/v (15% p/p) of the total humic extract, 11.2% p/v (10% p/p) organic carbon, 13.2% p/v (12% p/p) of humic acid, 3.3% p/v (3% p/p) of fulvic acid, 9.0% p/v (8.0 p/p) of nitrogen soluble in water and 4.5% p/v (4% p/p) of potassium (K_2O).

The growth of papaya seedlings was evaluated from the plant height measurements (measurement with a ruler graduated in cm), stem diameter (measured using a digital caliper model Starret® 799 with with 0.01mm accuracy, and the measurement made on the stem base approximately 2 mm above the ground) and leaf number at 70 days after emergence (DAE).

The effect of different treatments on the physiology of papaya seedlings was evaluated at 70 days after emergence (DAE) by determining the carbon internal concentration (μmol CO_2 mol air^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}), transpiration (mmol H_2O m^{-2} s^{-1}), net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), instantaneous water use efficiency and the instantaneous efficiency carboxylation using the infrared gas analyzer (IRGA) model LCpro+Sistem with air flow of 300 ml min^{-1} and the light source 1000 μmol m^{-2} s^{-1} and chlorophyll indices a, b, and total ratio of chlorophyll a/chlorophyll b using a model chlorophyll meter CFL 1030, which provides measurements of the indeces of chlorophylls a, b and total (a + b), expressed in units of Falker Chlorophyll Index (FCI), during the day (from 8:00 am to 11:00 am), on the third leaf.

The treatment effects were determined by analysis of variance using the F-test, with scrolling the quantitative treatments in polynomial regression components. The choice of model was based on the significance of the coefficients, using the F test, up to 5% probability. Statistical analyses were performed using the SAEG software, version 9.1.

Results and Discussion

There was a significant effect on the interaction between the electrical conductivity in the irrigation water (ECw) x humic substances for seedling height, leaf number and for stem diameter. Still were recorded interactions to the internal concentration of CO_2, carboxylation efficiency and chlorophyll b index, trans*piration rate and* instantaneous water use efficiency.

For isolated effect, it was observed that the ECw significantly affects the stomatal conductance, net photosynthesis and chlorophyll a being influenced. The application humic substances influenced significantly the stomatal conductance and CO_2 internal concentration, respectively. Chlorophyll a/b ratio chlorophyll a/ chlorophyll b and total chlorophyll were not influenced by variation tested factors.

It was observed that papaya seedlings subjected to higher electrical conductivity in the irrigation water with the application of 5 g of humic substances have restricted the growth in height, with only 8.1 cm (Figure 1A). However, maintaining the same ECw associated with the application of 20 g of humic substances, was registered the greatest height in the seedlings, reaching up to 24 cm, that is, comparatively there was a reduction of 15.9 cm (33.35% reduction).

Reduction in height of papaya seedlings with increment of ECw was also observed by Sá et al. (2013) when they found decreases of 29.4; 49.2 and 54.2% for the electrical conductivities of 2.4; 3.6 and 4.8 dS m^{-1}, in this order, when compared with water 1.2 dS m^{-1}, which did not exceed 15 cm. These results are lower than those obtained in this study, which was obtained 24 cm in seedling irrigated with water of 6.0 dS m^{-1} and application of 20 g of humic substances (Figure 1A).

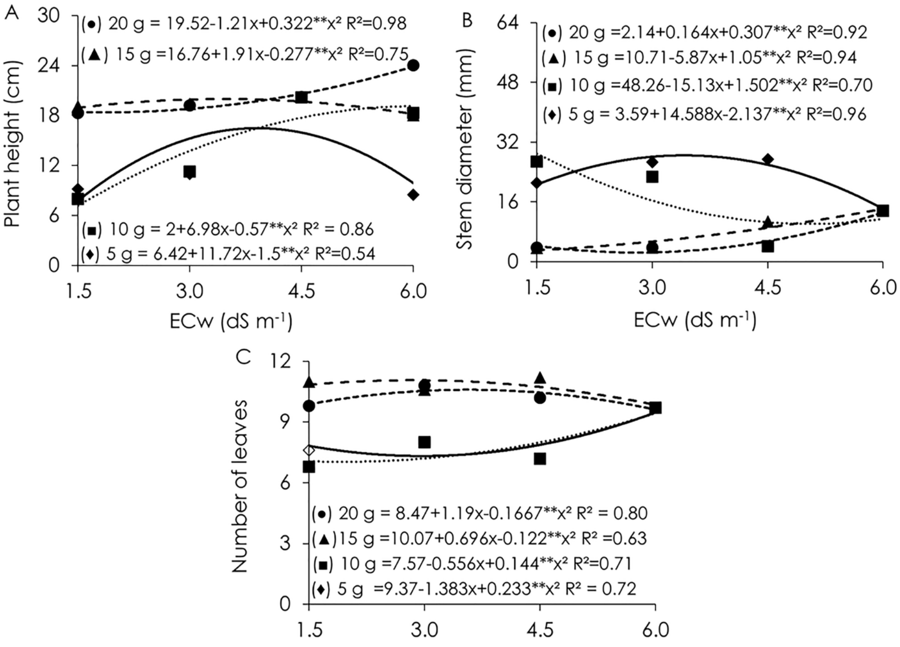

Figure 1. Height (A), stem diameter (B) and number of leaves (C) of papaya seedlings irrigated with saline water and humic substances doses at 70 DAE.

Corroborating to these results, Diniz et al. (2018) observed that increasing the ECw have reduced the height of papaya seedlings, which had a maximum height of 13 cm with irrigation water 0.3 dS m^{-1} and minimum ECw of 4.3 dS m^{-1} (8 cm), below those obtained in Figure 1A.

Furthermore, it can be seen from the results presented in Figure 1, that the irrigation water of 6.0 dS m^{-1} reduced the height of papaya seedlings even with the application of humic substances, except for using 20 g, in which the seedlings presented 24 cm of the height that is, increment of 23.75% in seedling compared to those with the application of 5 g. The greatest height of papaya seedlings with the highest doses of humic substances is due to the larger supply of Ca, Mg and K present. These salts maintain the sites of cation exchange active, aggregating with other elements. To some extent, the Na becomes more diluted and can be lost by leaching (Ouni et al., 2014).

Similar behavior was observed for the stem diameter, in which the seedlings irrigated with water of 6.0 dS m^{-1} presented 13.68 mm stem diameter at all doses of humic substances (Figure 1B). However, it was observed that irrigating papaya seedlings with water of 4.5 dS m^{-1} and with the application of 3.41 g of humic substances was recorded the largest diameter of the stem (28.48 mm). Conversely, the smaller stem diameter obtained under ECw of 4.5 dS m^{-1} and the dose of 15 g of humic substances, yielding 2.48 mm at both doses, that is, mean decrease of 84% compared with the application of 5 g (Figure 1B).

Diniz et al. (2018) reported that ECw adversely affected stem diameter of papaya seedlings, it was observed reduction until 28.91% for water up to 4.3 dS m^{-1} having the largest stem diameter (6.2 mm) in plants irrigated with water of 1.3 dS m^{-1}, lower than that obtained in the present study, which have stem diameter of 7.1 mm under irrigation of ECw 6.0 dS m^{-1} associated with the application of 20 g of humic substances. Sá et al. (2013) also found to stem diameter reduction in papaya seedlings of 0.12 mm in each increase in the ECw, yielding the greatest diameter of 4.5 mm, at 45 DAS under ECw of 1.2 dS m^{-1}, that is, lower than those presented in Figure 1B.

The number of leaves of papaya seedlings, it was found to the most ECw that the dose of 15 g of humic substances promoted higher values, obtaining 11; 10.6 and 11.2 seedling leaves irrigated with water of 1.5; 3.0 and 4.5 dS m^{-1}, respectively, and the smaller numbers of leaves in plants irrigated with measured water at 4.5 dS m^{-1} with applications of 5 and 10 g of humic substances, (Figure 1C). These values are superior to those observed by Diniz et al. (2018) with 9.8 leaves in plants irrigated with water of 0.3 dS m^{-1} and 7.7 leaves with irrigation water of 4.3 dS m^{-1}. In this same comparison, Sá et al. (2013) obtained 9 leaves in the seedlings ECw of 1.2 dS m^{-1} and 5 leaves with irrigation water of 4.8 dS m^{-1}.

It is important to emphasize that in salinity conditions the reduction in plant growth is due to nutritional imbalance by increasing the absorption of Na^+ and Cl^- and decrease of K^+ and Ca^{2+}, which are essential for the growth and development of plants (Sá et al, 2013; Coelho et al., 2015).

Thus, the applications of humic substances promote changes in growth, pattern formation and differentiation of plant organs (Canellas & Olivares, 2014). This stimulating action is attributed, in general, to a direct effect of plant hormones or in hormonal behavior of plants (Boyhan et al., 2001). Particularly the auxin hormone, which can be stimulated in the presence of humic acids, resulting in growth of the plants root system (Baldotto et al., 2017).

It was found that the greater stomatal conductance (0.12675 mol de H_2O m^{-2} s^{-1}) was obtained when papaya seedlings were subjected to water with salinity of 1.5 dS m^{-1}, and from this conductivity there was decrease, yielding in the seedlings under higher ECw (6.0 dS m^{-1}) stomatal conductance of 0.065375 mol de H_2O m^{-2} s^{-1}, a reduction of 0.061375 mol de H_2O m^{-2} s^{-1} (51.57% decrease) comparable between seedlings under ECw 6.0 dS m^{-1} compared to the control (Figure 2A).

Regarding stomatal conductance of papaya seedlings under application of humic substances, was verified dissimilar behavior, that is, the increase in the humic substances doses up to 10 g provided increase in stomatal conductance. Thus, the seedlings presented the highest value in this dose (0.246792 mol de H_2O m^{-2} s^{-1}) and lower stomatal conductance (0.178833 mol de H_2O m^{-2} s^{-1}) in the seedlings which received 5 g of humic substances, a reduction of 27.53% (Figure 2B).

The reduction in stomatal of papaya seedlings conductance occurs mainly due to the greater difficulty of seedlings in absorbing water from the soil, and thus, in order to reduce the loss of water, there are stomatal closure, resulting in decreases in important processes for plants, such as net photosynthesis and water use efficiency changes (Oliveira et al., 2017).

The combination of 4.5 dS m^{-1} and 15 g of humic substances promoted the highest concentration of CO_2 with 250,4 μmol m^{-2} s^{-1}. However, papaya seedlings irrigated with water of 6.0 dS m^{-1} and doses application of 10 and 20 g of humic substances had the lowest concentrations of CO_2, obtaining 166 and

162 μmol m^{-2} s^{-1}, respectively (Figure 2C).

The decrease in internal CO_2 concentration in papaya seedlings may have been caused due to the closure of stomata, since the plants close their stomata in order to reduce water loss. Similar behavior was observed by Sá et al. (2018) and Sá et al. (2015) in citrus rootstocks genotypes about saline waters above 0.3 dS m^{-1} reducing the internal CO_2 concentration.

The increase of the salt concentration in the irrigation water and doses of humic substances affected negatively net photosynthesis of papaya seedlings, yielding the net photosynthesis (4.4 μmol CO_2 m^{-2} s^{-1}) in seedlings irrigated with water of 6 dS m^{-1} and applying 20 g of humic substance. However, irrigating the papaya seedlings with low salinity (4.5 dS m^{-1}) at a dose of 3.88 g of humic substances led to the hightest net photosynthesis (17.28 μmol CO_2 m^{-2} s^{-1}) (Figure 2D).

The transpiration rate of papaya seedlings decreased to ECw of 4.5 dS m^{-1}, with a raising trend when irrigated with water of 6.0 dS m^{-1}. The higthest transpiration rate was observed in papaya seedlings irrigated with water of 1.52 dS m^{-1}, yielding 10.24 mmol of H_2O m^{-2} s^{-1} and the lowest transpiration rate of 7.27 in the seedlings under 4.94 dS m^{-1}, that is, comparing there is reduction of 31.25% (Figure 2E).

The decrease in photosynthetic activity may have been caused due to the stomata restrictions, since the highest concentration of CO_2 according Campos et al. (2014), occurred in the papaya seedlings under ECw of 4.5 dS m^{-1} (Figure 2C), thus it is possible that other factors may have affected the photosynthetic activity, such as inhibiting the activity of rubisco and ATP synthesis (Sabra et al., 2012; Medeiros, 2017). What may have occurred in papaya plants is that if the CO_2 internal concentration is below the optimum range the plant uses CO_2 respiration for maintaining a minimum level of photosynthetic rate, making it limited (Melo et al., 2017).

Increasing water salinity up to 3.0 dS m^{-1} and doses

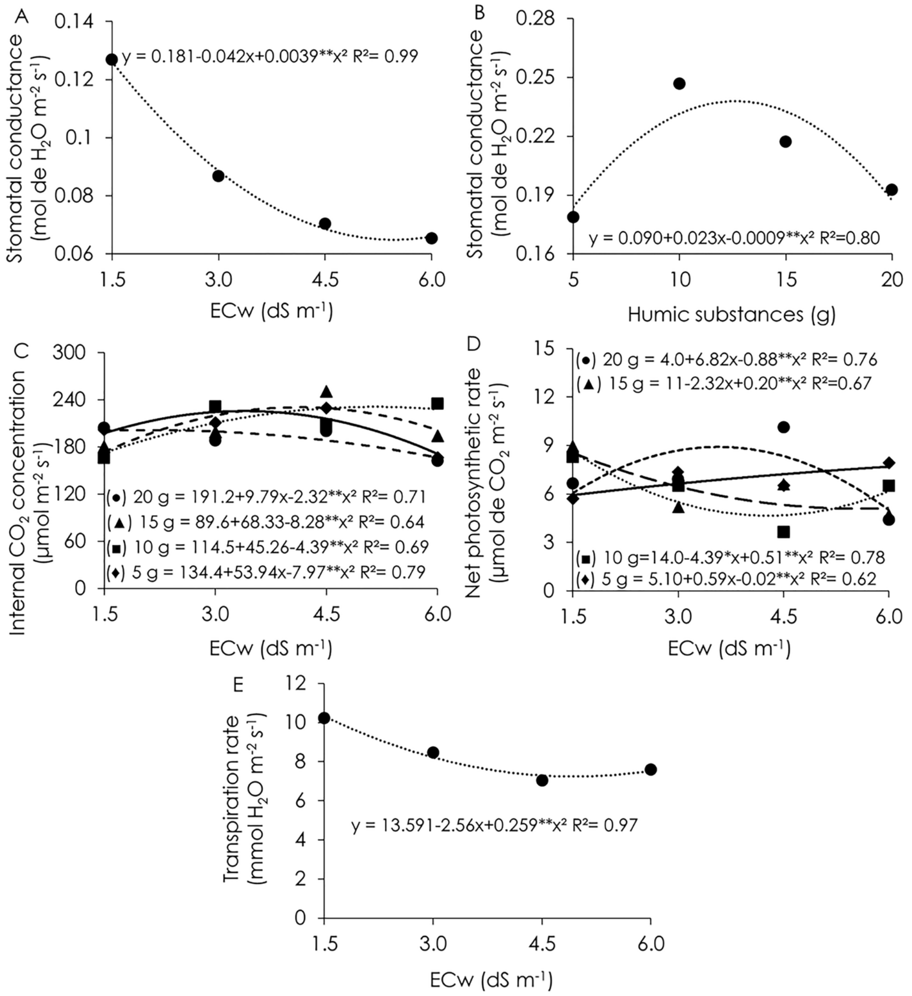

Figure 2. Stomatal conductance (A and B), internal CO_2 concentration (C), net photosynthetic rate (D) and transpiration rate (E) of papaya seedlings irrigated with saline water and doses of humic substance at 70 DAE.

of humic substances promoted instantaneous increase in water use efficiency of papaya seedlings, getting the highest efficiency (5.06) in plants irrigated with water of 3.0 dS m^{-1} and application of 23.33 g of humic substances, and the lowest instantaneous water use efficiency (2.03) in the managed with ECw of 1.5 dS m^{-1} and application of 0.04 g of humic substances (Figure 3A).

The same behavior was observed for the instantaneous efficiency of carboxylation, which was higher in plants irrigated with water of 3.0 dS m^{-1} and 20 g of humic substances application, obtaining these treatments 0.1048 instantaneous efficiency of carboxylation. Also was observed that the ECw above 4.5 dS m^{-1} reduced the instantaneous efficiency of carboxylation (Figure 3B).

The reduction in the efficiency of carboxylation in papaya seedlings under ECw 6.0 dS m^{-1} can be related to a decrease in stomatal conductance as related to the internal CO_2 concentration, due to osmotic effects of salt stress. However, other cofactors may be involved beyond the stomatal conductance, like chlorophyll a fluorescence (Silva et al., 2014).

In the other hand, the seedlings irrigated with water of 4.5 dS m^{-1} presented the highest efficiency in water use and efficiency of carboxylation, which may be related to the increased internal concentration of CO_2. Although it has presented low internal CO_2 concentration, papaya seedlings had a higher instantaneous efficiency of carboxylation, indicating that the ribulose 1.5-bisphosphate carboxylase-/oxygenase (Rubisco) has higher activity (Silva et al. 2014). This may be occurred due to the expression of salt tolerance mechanisms, which may have increased photosynthetic efficiency, favoring the solute flow in the plant and enabling higher dilution and translocation of ions within the plant, consequently, accelerating its compartmentalization in the vacuole (Sá et al., 2015).

It was observed that the chlorophyll a content of the papaya seedlings decreased as the increased salts contents in irrigation water, with higher chlorophyll an index (300.79) with the irrigation water of 1.5 dS m^{-1}; a limitation was being verified about 10.23% compared to seedlings under ECw of 6.0 dS m^{-1} (Figure 3C). Similar behavior was observed for chlorophyll b content, which decreased as the increase of ECw and humic substances doses, getting 98.14 with irrigation 3.0 dS m^{-1} and 5 g of humic substance (Figure 3D).

The decrease in chlorophyll a and b content

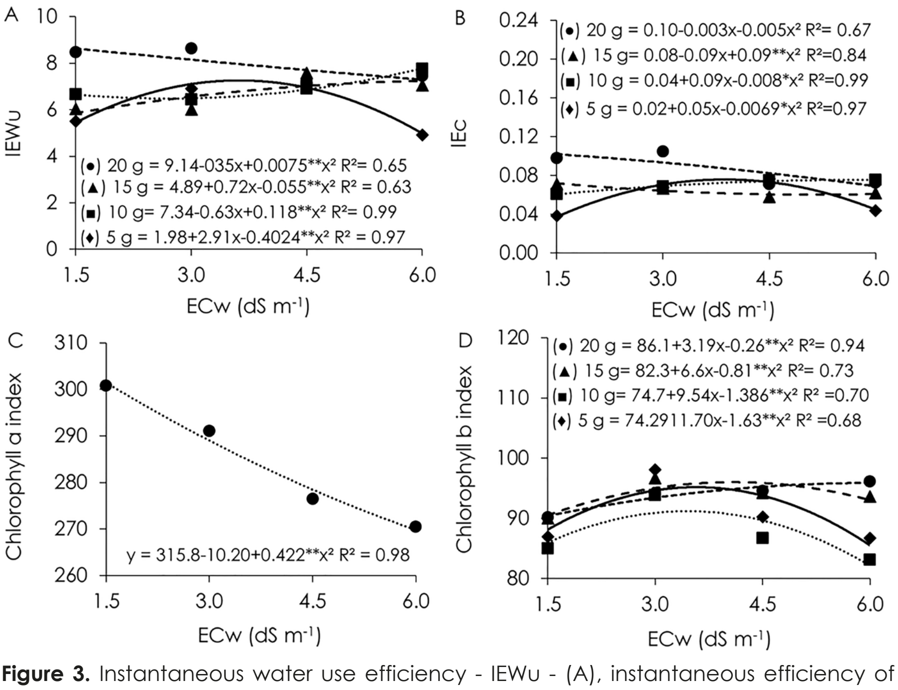

Figure 3. Instantaneous water use efficiency - IEWu - (A), instantaneous efficiency of carboxylation - IEc - (B), chlorophyll an index (C) and chlorophyll b (D) papaya seedlings irrigated with saline water humic substance and dose at 70 DAE.

under conditions of water irrigation with high salinity was also checked by Silva et al. (2014) in citrus when realized that the increase in ECw decreased 31.05% of the chlorophyll a content reduced 37.86% in the chlorophyll b, in the seedlings irrigated with saline water in greater concentration in relation to the controls. One of the most remarkable effects of salt stress is the change of the biosynthesis of photosynthetic pigments, since the pigment content of pigments, like chlorophyll, is reduced and as the activity of photosynthetic enzymes also decreases, limiting the transport of electrons in chloroplasts, with consequent reduction in the photochemical efficiency of photosystem II (Huang et al., 2012).

According to Ertani et al. (2011) humic substances provide the increase of chlorophyll content also the activity of Rubisco enzyme. The increased chlorophyll content confers to the plant higher light absorption ability, which stimulates the photosynthetic machinery. This was not observed in this study since the higher salinity of the irrigation water (ECw 6.0 dS m^{-1}), despite occurring increase levels of chlorophyll b, there was a reduction in chlorophyll a content, as well, there was a reduction in net photosynthetic rate (Figure 2E), probably due to damage in the photosynthetic apparatus through salts toxicity, especially sodium and chlorine.

In general, the results obtained in growth variables evidenced that the papaya plant is slightly tolerable to salinity associated with humic substances, supporting electrical conductivity of extract of soil saturation between 1.5 to 6 dS m^{-1} as observed in the present work and also to Lima-Neto et al. (2016). This tolerance is reflected in the plant survivability under stress conditions without inhibition of growth (Figures 1, 2 and 3), higher instantaneous efficiency of water usage (Figure 3A), carboxylation efficiency (Figure 3B) and its productive ability as also demonstrated the benefits of the application of humic substances in mitigation of salt stress.

Conclusions

When the papaya seedlings are irrigated with water of 6.0 dS m^{-1}, is recommended dose of 20 g of humic substances, which promoted a higher growth;

Irrigating papaya seedlings with water with salinity from 3.0 to 4.5 dS m^{-1} combined with application of 20 g of humic substances provided higher CO_2 concentration, transpiration rate, instantaneous water use efficiency, the efficiency of carboxylation and chlorophyll b index, however, stomatal conductance, net photosynthesis and chlorophyll content is reduced with increment of salinity of the irrigation water.

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