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Morphophysiological responses of table beet irrigated with saline water under application of humic substances

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Abstract

The use of saline water is an alternative for irrigating agricultural crops, especially in the Brazilian Northeastern semi-arid region, where water quality is limited in most cases. Thus, the objective was to evaluate the morphophysiological responses of table beet cv. "Wonder" irrigated with saline waters under the application of humic substances. The experiment was conducted under a randomized block design, with six replications in a 6 x 4 x 5 factorial scheme, referring to six electrical conductivities of irrigation water (ECw): 0.5; 1.5; 2.5; 3.5; 4.5 and 5.5 dS m^{-1}, four humic substances rates (HS) (0; 10; 20 and 30 ml per plant), and five stages of assessment (23, 38, 53, 68 and 83 days after emergency). The characteristics evaluated were: plant height, number of leaves, leaf area, chlorophyll content (a, b and total), stomatal conductance, transpiration, net photosynthesis and CO_2 internal concentration, and in the soil, the soil pH and the electrical conductivity of saturated paste extract. The increase of salinity reduced growth, chlorophyll a content, and the stomatal conductance of beet plants. The application rate of 30 ml per plant of humic substances promotes an increase in stomatal conductance. The application of humic substances raises the pH in sandy acidic soils. It is recommended to irrigate table beet plants with water of 0.5 dS m^{-1} associated with the application of 30 mL per plant of humic substances.

Keywords: *Beta vulgaris L.*; humic acids, salinity; gas exchange.
Abbreviations: HS_humic substances; ECw_electrical conductivity of irrigation water; DAE_days after emergence; Cl a_ chlorophyll a; Cl b_ chlorophyll b; Cl total_total chlorophyll; A_net photosynthesis; Gs_stomatal conductance; Ci_CO_2 internal concentration; E_transpiration rate; ECspe_electrical conductivity of saturated paste extract.

Introduction

Salinity is one of the factors that cause the most limitation in crop production, and causes severe restriction in physiological activities and in the productive potential of cultivated plants. (Taiz et al., 2017). This occurs mainly in prone areas of drought, rainfall irregularities and high temperatures. Due to these factors, the low availability of good quality water is the main limiting factor for agriculture in the Brazilian semi-arid region, with negative impacts on production (Freire et al., 2014). Thus, the use of saline water for irrigation has been a challenge for rural producers and researchers, who are constantly developing studies to enable the use.

One way to mitigate the harmful effects of saline water on agriculture is the use of organic inputs, such as humic substances. Studies have shown that the use of humic acids has provided several benefits in the chemical and biochemical processes of the soil, such as in the plant's nutrient absorption capacity, in cations complexing and transporting, and in physiological reactions in microorganisms and plants (Mora et al., 2010; Zandonadi et al., 2014). In addition to positive effects on the growth and development of some crops such as beans (Aydin et al., 2012), cucumber (Karakurt et al., 2015), maize (Kaya et al., 2018), almond (Hatami et al., 2018), and sorghum (Ali et al., 2019), and beet cultivation, as observed in studies carried out by Rassam et al., (2015) and Wilczewski et al., (2018). Some studies indicate that humic substances have auxin-like activity (Canellas et al., 2002; Nardi et al., 2002; Zandonadi et al., 2007), as well as in mitigating the effects of salinity (Khaled and Fawy, 2011; Jarošová et al., 2016; Saidimoradi et al., 2019).

In the face of few studies showing the effects of humic substances in vegetables grown in saline conditions, the relevance for irrigated agriculture is observed, especially for semi-arid regions, in order to develop strategies to mitigate the effects of excess salts and increase the efficiency and preservation of the production system. Thus, the objective of this study was to evaluate the effect of humic substances on the development and physiology of table beet, and on the soil, under irrigation with saline water.

Results and discussion

The growth parameters of plant height, number of leaves and leaf area were significant for the stage of assessments (SoA) at 5% probability. ($p<0,05$) (Table 3). There was an effect of the stage of assessments (SoA) ($p <0.05$) for the levels of chlorophylls a (Cl a), b (Cl b) and total (Total Cl), and there was interaction between the humic substances rates (HS) and the stage of assessments (SoA) for chlorophyll content a ($p <0.05$) (Table 4).
The humic substances rates (HS) significantly influenced the stomatal conductance (Gs) ($p <0.05$), while there was an effect of the stages of assessment (SoA) for the stomatal conductance (Gs), CO_2 internal concentration (Ci), and the transpiration rate (E) (Table 5), with interaction HS x SoA for Ci. The levels of electrical conductivity in irrigation water (ECw) significantly influenced ($p <0.05$) the electrical conductivity of the saturation paste extract (ECspe), while the HS rates influenced the pH ($p <0.05$), and there was no interaction between the factors for ECspe and pH. (Table 6).

Effect of humic substances rates, saline water and stages of assessment on growth parameters
The height of the beet plant increased as a result of the evaluation stages, with growth up to 64 days after emergency (DAE), with a value of 38.74 cm, remaining constant until 83 DAE (Figure 1A). This observed behavior occurs because the beet plants continues to emit leaves until the end of the cycle, and the older leaves enter the senescence process, which is related to the physiological cycle of the crop. The result came close to what was found by Alves et al. (2008), that evaluating the beet in hydroponic cultivation, verified the height of 40.5 cm at 63 days after sowing, and were lower than that found by Gondim et al. (2010), who also working with hydroponic beet cultivation, observed that the plants reached 49.58 cm in height at 60 DAE. Humic substances reduce the damage caused by salinity in fennel, as observed by Zulfiqar et al., (2019), who observed significantly taller plants using humic substances, with an increase of 23% compared to the control. In research of Sani (2014), it was observed that leaf application of humic acid promoted positive results in the height of the plant and in the number of leaves of canola plants.
The number of leaves increased with a quadratic trend up to 53 DAE, with a maximum value observed at 38 DAE, of 7.7 leaves per plant (Figure 1B). At 38 DAE, the beet plants begin to emit the tuber, where nutrients are being accumulated, thus decreasing the translocation of nutrients to the aerial part of the plant, and consequently restricts the production of new leaves. Results differ from those found by Gondim et al. (2010), that evaluating the growth of beet throughout the cultivation, verified a linear increase in the number of leaves, presenting an average value of 7.67 leaves per plant at 65 days after transplanting.
There was an increase in the leaf area up to 65 DAE, and stabilized until 83 DAE (Figure 1C), with an approximate value of 140 cm^2. Corroborating the results found by Gondim et al. (2010), who observed that the indicative variables of plant development, maintained the same behavior, with growth until the end of the crop cycle.

Effect of humic substances rates, saline water and stages of assessment on physiological parameters
There was a linear increase in the values of chlorophyll a, b and total, up to 83 DAE (Figure 2A, 2B e 2C), obtaining the highest values of chlorophyll a, chlorophyll b and total of 27.81; 9.06 and 36.87 ICF, respectively, indicating that with the advancing age of the plants there was an increase in the index of chlorophyll a, b and total, that is, the leaves changed their color from green to intense green. Chlorophyll is the pigment that absorbs light, and will be used in photosynthesis, these pigments being essential in the transformation of light radiation into chemical energy (Taiz et al., 2017).
Stomatal conductance reduced from 0.26 mol H_2O m^{-2} s^{-1} at 36 DAE to 0.12 mol H_2O m^{-2} s^{-1} at 57 DAE (Figure. 3A). Possibly, the decrease in stomatal conductance has occurred, as a strategy of the plant to minimize water loss due to high temperature stress (Taiz et al., 2017), inside the greenhouse. The CO_2 internal concentration also decreased from 307.90 μmol m^{-2} s^{-1} at 36 DAE to 237.24 μmol m^{-2} s^{-1} at 57 DAE (Figure 3B). CO_2 internal concentration decreased as transpiration rate increased (Figure 3C), since both are directly interconnected. According to Taiz et al. (2017) daily carbon gain in photosynthesis is lost by breathing, a loss that tends to double in older plants.
The transpiration rate increased from 1.3 mmol H_2O m^{-2} s^{-1} at 36 DAE to 2.1 mmol H_2O m^{-2} s^{-1} at 57 DAE (Figure 3C). Environmental factors, such as high temperatures in the greenhouse, may have contributed to this increase in the transpiration rate over time. According to Taiz et al. (2017), transpiration increases according to temperature, responding to short-term changes as a function of temperature, varying with plant development and external factors.

Effect of humic substances rates and saline water on soil pH and electrical conductivity of the saturation paste extract
Soil pH was influenced by the application of humic substances, with a linear increase as the HS rates increased, increasing from 6.03 in the control to 6.69 in the 30 mL L^{-1} HS rate (Figure 4). The presence of negative charges on the surface of HS from carboxylic and phenolic groups contributes to the increase of the cation exchange capacity, which provides more charge exchange sites to adsorb H^+, which raises the pH (Sposito, 1984). The magnitude of this effect may have been greater due to the sandy texture of this soil, which causes less buffering for pH variations, as also observed by Yang et al. (2013) in sandy soil.
Irrigation with saline waters did not influence the soil pH, and presented values in agreement with those observed by Garcia et al. (2008), that did not verify changes in the pH values in an Oxisol, as function of electrical conductivity of irrigation water from 0.5 to 10 dS m^{-1} in greenhouse.
Irrigation with saline water promoted an increase in ECspe, with the values of 0.68 dS m^{-1} elevated to 4.10 dS m^{-1} between the treatments irrigated with CEw of 0.5 and 5.5 dS m^{-1} waters, respectively (Figure 5), going from non-saline to moderately saline (Richards, 1954). The increase in ECspe is related to the increase in ions activities from saline solutions, such as Na^+, Ca^{2+}, Mg^{2+}, and Cl^-. The increase in HS rates did not have significant effects on ECspe, obtaining an average of 2.33 dS m^{-1}.

Table 1. Physical and chemical characteristics of the substrate used in the experiment.

Physical			
Attributes	0 - 20 cm		20 - 40 cm
Sand (g kg^{-1})	607		534
Silt (g kg^{-1})	56		82
Clay (g kg^{-1})	337		384
Textural class	Sandy clay loam		Sandy clay loam
Chemical			
Attributes	0 - 20 cm	20 - 40 cm	Composite sample
pH (H_2O: 1:2,5)	5.81	5.17	6.40
P (mg dm^{-3})	18.72	8.50	26.25
K^+ (mg dm^{-3})	62.61	44.94	63.03
Na^+ ($cmol_c$ dm^{-3})	0.07	0.05	0.19
Ca^{2+} ($cmol_c$ dm^{-3})	2.05	1.55	3.80
Mg^{2+} ($cmol_c$ dm^{-3})	1.85	0.70	1.91
Al^{3+} ($cmol_c$ dm^{-3})	0.00	1.00	0.00
H+Al ($cmol_c$ dm^{-3})	3.55	5.69	2.81
Effective CTC ($cmol_c$ dm^{-3})	4.13	5.60	6.06
Potential CTC ($cmol_c$ dm^{-3})	7.68	10.29	8.87
SB ($cmol_c$ dm^{-3})	4.13	4.60	6.06
m (%)	0.0	17.86	0.00
V (%)	46.22	44.70	31.68
Organic matter (g kg^{-1})	20.84	14.80	20.53

P, K^+, Na^+ = Mehlich-1 extractor; H + Al = calcium acetate 0.5 M at pH 7.0 extractor; Al^{3+}, Ca^{2+}, Mg^{2+} = KCl 1 M extractor; CTC = cation exchange capacity; m% = exchangeable Al saturation; SB = sum of bases; V% = bases saturation.

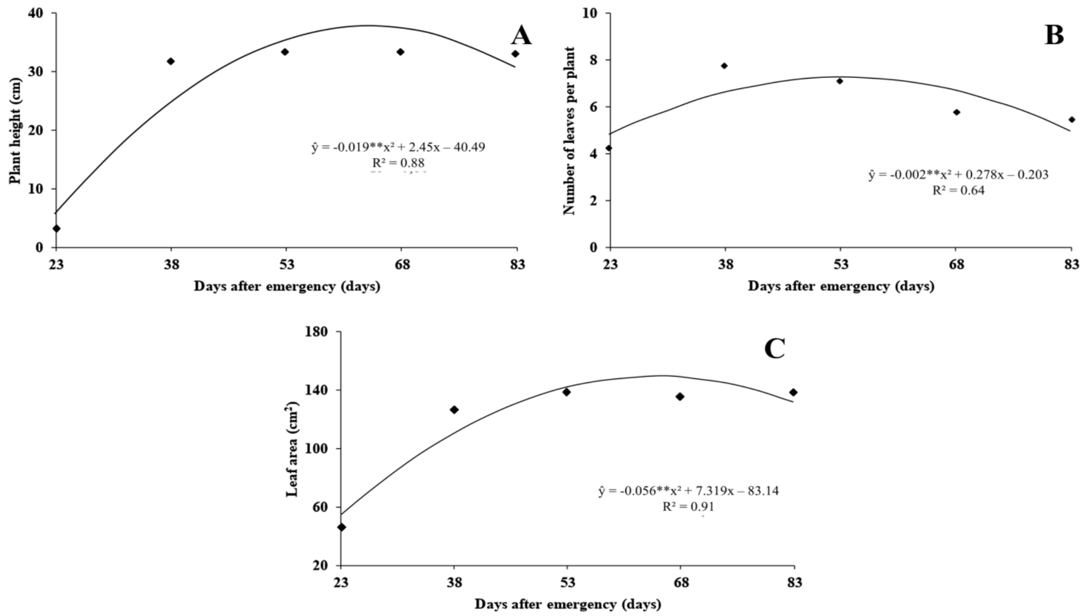

Figure 1. Plant height (A), number of leaves per plant (B), and leaf area (C) of beet plants as a function of stages of assessment (days after emergency).

Table 2. Chemical characterization of non-saline (A1) and saline waters (A2, A3, A4, A5 and A6) used in irrigation.

Attributes	Waters samples					
	A1	A2	A3	A4	A5	A6
pH	6.4	6.89	7.27	7.5	7.81	7.5
ECw	≤0.50	1.5	2.5	3.5	4.5	5.5
Na^+ ($mmol_c$ L^{-1})	1.13	6.48	8.79	14.84	19.1	23.02
K^+ ($mmol_c$ L^{-1})	0.06	0.06	0.07	0.07	0.07	0.08
Ca^{2+} ($mmol_c$ L^{-1})	0.1	0.11	0.11	0.11	0.12	0.11
Mg^{2+} ($mmol_c$ L^{-1})	0.11	0.17	0.11	0.14	0.13	0.18
Cl^- ($mmol_c$ L^{-1})	2.8	12.3	21.2	30	40.9	41.7
SO_4^{2-} ($mmol_c$ L^{-1})	2.5	3.01	3.23	3.8	3.8	6.13
CO_3^{2-} ($mmol_c$ L^{-1})	0.0	0.0	0.0	0.0	0.0	0.0
HCO_3^- ($mmol_c$ L^{-1})	0.9	1.2	1.1	1.0	1.0	1.1
SAR	3.48	17.32	26.55	42.03	54.1	60.57

ECw = Electrical conductivity at 25°C; RAS = sodium adsorption ratio: Na/ $[(Ca + Mg)/2]^{1/2}$; PST = Percentage of exchangeable sodium.

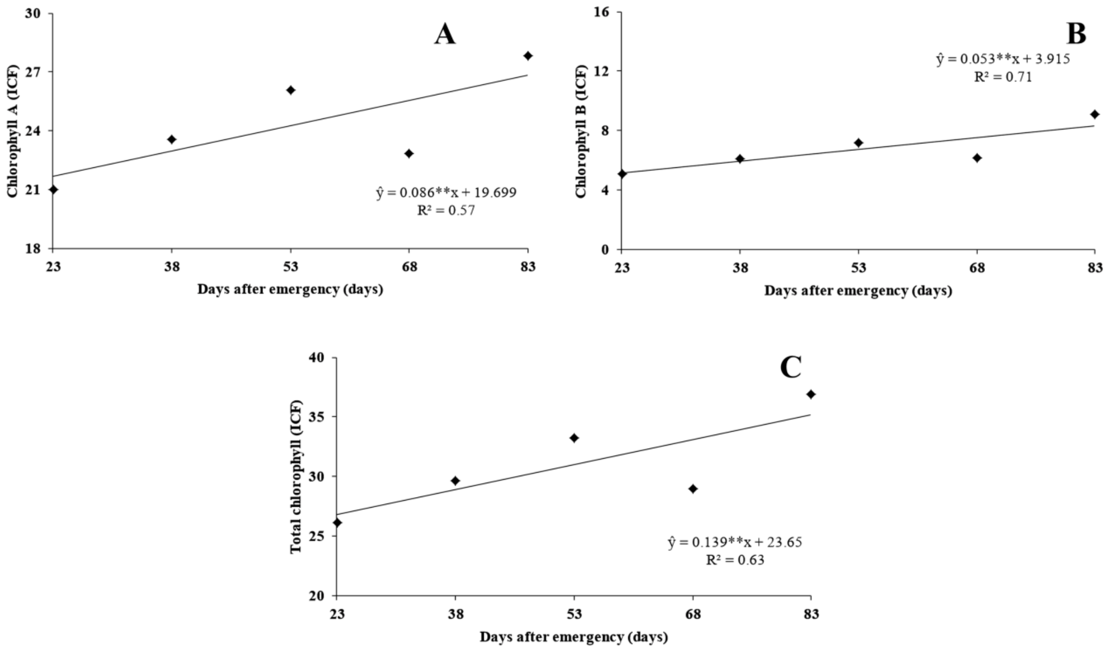

Fig 2. Chlorophyll a (A), chlorophyll b (B) and total chlorophyll (C) of beet plants as a function of stages of assessment (days after emergency).

Table 3. Valores de F para altura de planta, número de folhas e área foliar sob níveis de condutividade elétrica da água de irrigação (ECw), doses de substâncias húmicas (HS), e estágios de avaliação (SoA)

Sources of variation	DF	Plant height	Number of leaves	Leaf area
Blocks	2	2.03	1.00	10.87
ECw	5	0.15^{ns}	1.11^{ns}	0.13^{ns}
HS	3	0.66^{ns}	0.14^{ns}	1.44^{ns}
SoA	4	3304.08^{**}	128.98^{**}	640.85^{**}
ECw x HS	15	0.87^{ns}	0.95^{ns}	1.04^{ns}
ECw x SoA	20	0.70^{ns}	1.02^{ns}	0.86^{ns}
SH x SoA	12	0.70^{ns}	0.84^{ns}	1.78^{ns}
ECw x HS x SoA	60	0.78^{ns}	0.85^{ns}	0.76^{ns}
Error	238	6.94	1.36	270.39
CV (%)		9.80	19.29	13.63

ECw: eletrical condutivity of irrigation water; HS: humic substances rates; SoA: stages of assessment; DF: degree of freedom; CV: coefficient of variation.

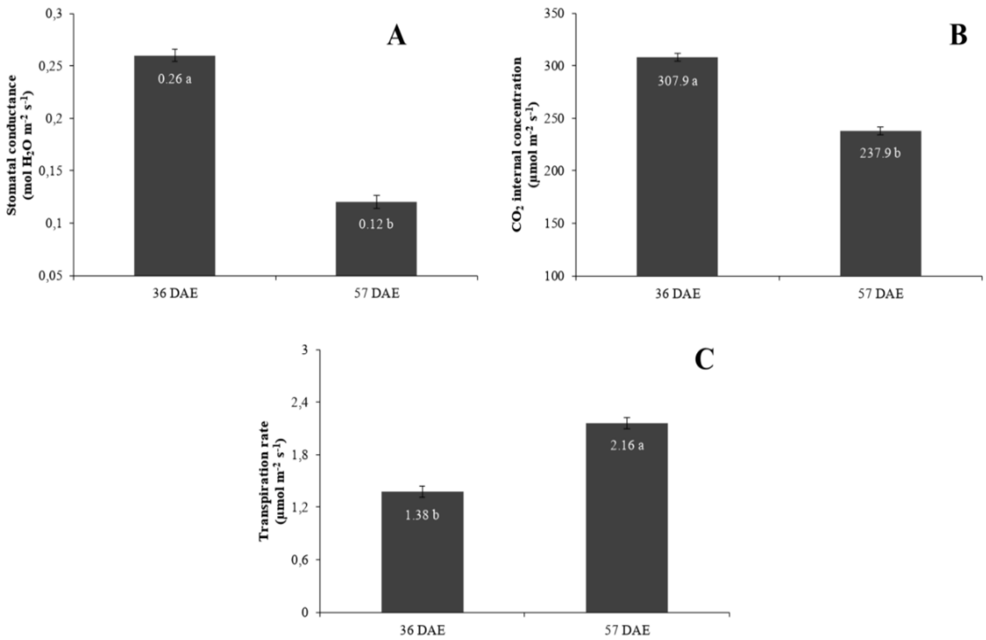

Figure 3. Mean values and standard errors for stomatal conductance (A), CO_2 internal concentration (B), and transpiration rate (C) of beet plants at 36 and 57 days after emergency.

Table 4. Valores de F para os teores de clorofila a (Clo a), clorofila b (Clo b) e clorofila total (Clo total) sob níveis de condutividade elétrica da água de irrigação (ECw), doses de substâncias húmicas (HS), e estágios de avaliação (SoA).

Sources of variation	DF	Clo *a*	Clo *b*	Total Clo
Blocks	2	4.93	1.11	3.24
ECw	5	0.67^{ns}	0.31^{ns}	0.52^{ns}
HS	3	1.20^{ns}	0.90^{ns}	1.12^{ns}
SoA	4	121.75**	90.02**	114.42**
ECw x HS	15	0.71^{ns}	0.43^{ns}	0.56^{ns}
ECw x SoA	20	0.96^{ns}	0.71^{ns}	0.84^{ns}
HS x SoA	12	1.90*	0.66^{ns}	1.41^{ns}
ECw x SH x SoA	60	0.86^{ns}	0.75^{ns}	0.81^{ns}
Residue	238	5.52	2.13	13.58
CV(%)		9.68	21.71	11.88

ECw: eletrical condutivity of irrigation water; HS: humic substances rates; SoA: stages of assessment; Clo a: Chlorophyll a; Clo b: chlorophyll b; Total clo: total chlorophyll; DF: degree of freedom; CV: coefficient of variation.

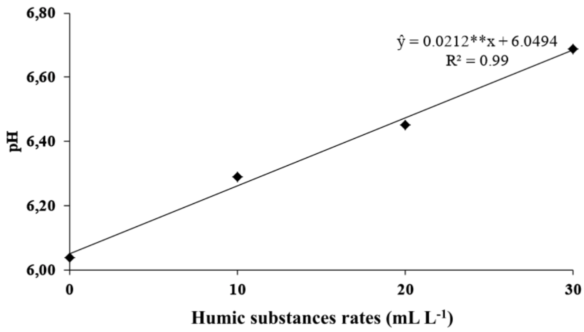

Figure 4. Soil pH as function of humic substance rates.

Table 5. Valores de F para taxa fotossintética (A), condutância estomática (Gs), conteúdo intercelular de CO_2 (Ci), e taxa de transpiração (E) sob níveis de condutividade elétrica da água de irrigação (ECw), doses de substâncias húmicas (HS), e estágios de avaliação (SoA)

Sources of variation	DF	A	gs	Ci	E
Blocks	2	20.89	12.80	44.34	31.88
ECw	5	1.14^{ns}	1.08^{ns}	0.68^{ns}	1.08^{ns}
HS	3	1.27^{ns}	3.39*	1.31^{ns}	2.27^{ns}
SoA	1	0.01^{ns}	293.45**	120.67**	58.35**
ECw x HS	15	0.90^{ns}	1.13^{ns}	2.03*	0.78^{ns}
ECw x SoA	5	0.62^{ns}	1.26^{ns}	0.11^{ns}	0.83^{ns}
HS x SoA	3	0.65^{ns}	0.25^{ns}	0.27^{ns}	0.36^{ns}
ECw x HS x SoA	15	0.60^{ns}	0.48^{ns}	0.53^{ns}	0.62^{ns}
Error	94	3.80	0.002	148.50	0.37
CV(%)		27.80	26.24	14.15	34.44

ECw: eletrical condutivity of irrigation water; HS: humic substances rates; SoA: stages of assessment; A: net photosynthesis; Gs: stomatal conductance; Ci: CO_2 internal concentration; E: transpiration rate; DF: degree of freedom; CV: coefficient of variation.

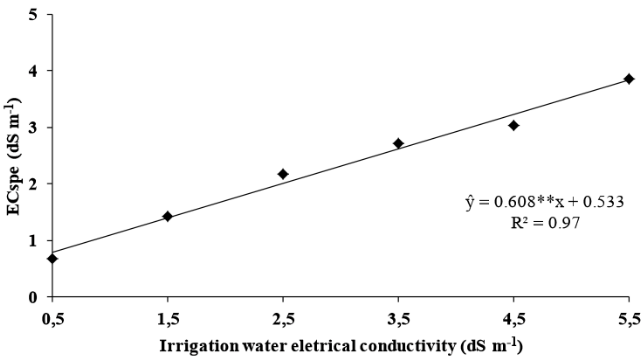

Figure 5. Electrical conductivity of saturated paste extract as irrigation water electrical conductivity.

Table 6. Valores de F para o pH do solo (pH) e a condutividade elétrica na pasta de saturação (ECspe) sob níveis de condutividade elétrica da água de irrigação (ECw) e doses de substâncias húmicas (HS).

Sources of variation	DF	pH	ECspe
Blocos	2	3.10^{ns}	1.20^{ns}
ECw	5	0.58^{ns}	50.76^{**}
HS	3	10.41^{**}	1.04^{ns}
ECw x HS	15	0.82^{ns}	0.57^{ns}
Error	46	0.09	0.30
CV (%)		4.8	23.5

ECw: eletrical condutivity of irrigation water; HS: humic substances rates; ECspe: electrical conductivity of saturated paste extract; DF: degree of freedom; CV: coefficient of variation.

Material and methods

Location of experimental area

The experiment was conducted in a greenhouse located at the Center for Human and Agricultural Sciences (CCHSA), Campus III of the Federal University of Paraíba (UFPB), in Bananeiras - PB, located at the geographical coordinates 6°45'S and 35°37'W and 520 m altitude. The average temperature inside the greenhouse was 34°C, and the air humidity ranged from 29 to 59%.

Experiment installation and conduction

The experimental design adopted was in randomized blocks, in six repetitions in a 6 x 4 x 5 factorial scheme, referring to six electrical conductivities of irrigation water (ECw): 0.5; 1.5; 2.5; 3.5; 4.5 and 5.5 dS m^{-1}, four humic substances rates (HS) (0; 10; 20 and 30 ml per plant), and five stages of assessment (23, 38, 53, 68 and 83 days after emergency).

The experimental units were composed of pots with a volumetric capacity of 5 dm^3. The pots were arranged in a spatial arrangement of 30 cm between rows and 15 cm between rows, on a black plastic canvas, so that they were not in direct contact with the soil of the greenhouse.

The soil was collected in the 0 - 20 and 20 - 40 cm layers in the Agriculture Sector of CCHSA-UFPB, and were analyzed chemically and physically according to Donagema et al. (2011) (Table 1), and the pots was filled with the mixture of soil from the two layers. The soil was classified as Oxisol (IUSS Working Group WRB, 2014; Santos et al., 2018).

Beet seeds cv. 'Maravilha' were sown directly in the pots at a depth of 2 cm, placing three seeds in each pot, and at 15 days after emergence thinning was carried out, leaving only the most vigorous seedling.

Irrigation was carried out daily in the early morning, in order to maintain the soil in the field capacity, in order to ensure the emergence and development of seedlings. The applied water level was calculated using the values of crop evapotranspiration, estimated for each stage of plant development from the reference evapotranspiration (ETo) obtained from the agrometeorological station near the experimental area, and the culture coefficient (Kc) adapted for environments protected.

The preparation of the waters with each electrical conductivity was performed weekly by the addition of NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$, in the proportion of 7:2:1 (Rhoades et al., 2000). From this solution, the solutions with the proposed ECw were prepared: 1.5; 2.5; 3.5; 4.5 and 5.5 dS m^{-1} using a portable conductivimeter to measure values. The waters used for irrigation were analyzed for salinity (Tabela 2).

The source of humic substances was the commercial product Humitec®, without granulometric specifications, soluble in water with solubility of 100 g l^{-1}, containing 68% (w:w) of total humic extract; 31% (w:w) organic carbon; 52% (w:w) humic acids; 16% (w:w) fulvic acids; and 17% (w:w) of water-soluble potassium (K_2O). The humic substances rates were fractionated and applied manually at 7, 26 and 45 days after transplanting at rates of 10, 20 and 30 ml/plant, adding 10 ml of 100 mmol citric acid for each 1 liter of the solution.

Fertilization with NPK was carried out at rates of 40, 180 and 90 kg ha^{-1} of N, P_2O_5 and K_2O, respectively, according to the "Recommendations for fertilizing for the State of Pernambuco" (Cavalcanti, 2008), using urea, simple superphosphate and potassium chloride as sources of fertilization. The NPK rates were diluted and applied as a solution, divided into three times, on the foundation, at 28 and 56 DAE.

Evaluated characteristics

The effects of treatments on the beet culture were evaluated through growth analysis: plant height (using a ruler graduated in cm), number of leaves (manual counting) and leaf area, using the model proposed for the cultivation of beet: LA = 0.5083 * LW + 31.928 (Tsialtas e Maslaris, 2008), with LA = leaf area (cm^2), LW = product of the length (cm) and width (cm) of the leaf); and physiological analyzes: chlorophyll index (a, b and total), stomatal conductance, transpiration rate, net photosynthesis and CO_2 internal concentration.

The determination of chlorophyll index a, b and total was carried out by the non-destructive method at 23; 38; 53; 68 and 83 days after emergency, using a portable chlorophyll meter (ClorfiLOG®, model CFL 1030) (Falker, 2008). Readings were performed on the middle part of the fully expanded leaf of each plant and on all plants per plot, between 9:30 and 10 a.m.

Stomatal conductance (mol of H_2O m^{-2} s^{-1}), transpiration rate (mmol of H_2O m^{-2} s^{-1}), net photosynthesis (μmol m^{-2} s^{-1}) and CO_2 internal concentration (μmol m^{-2} s^{-1}) were obtained through readings at 36 and 57 DAE using the infrared gas analyzer (IRGA) model LCpro + System.

At 83 DAE, after the last determination of growth and physiological analyzes of beet plants, soil samples were collected from each experimental unit to assess the electrical conductivity of the saturation paste extract and the soil pH (Richards, 1954).

Data analysis

The data were submitted to analysis of variance by the F test at 5% probability. For measurements with a quantitative nature (electrical conductivity of irrigation water and humic substances rates) polynomial regression analysis was performed, and the best fit was considered to be the one with the highest R^2 value and the lowest residual square sum value. For measurements with a qualitative nature, as days after emergency t test was performed. All analyzes were performed using the software SAS University Edition (Cody, 2015).

Conclusions

Salinity in irrigation water reduces growth, chlorophyll a levels and stomatal opening of beet plants.
The application of humic substances above 30 ml / plant promotes a decrease in the fresh matter of the beet root.
The application of humic substances reduces the pH in sandy acidic soils.

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