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Basil (*Ocimum basilicum*) growth under saline stress and salicylic acid

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Basil (*Ocimum basilicum* L.) is an aromatic and spice plant used around the world in cooking, pharmaceutical, cosmetic and flavoring industries. Salicylic acid has been used to mitigate the deleterious effects of salinity on plants. This work aimed to evaluate the growth of basil under saline stress and salicylic acid. A randomized block design was used. Five electrical conductivities of irrigation water (ECw - 0.5, 1.3; 3.25; 5.2 and 6.0 dS m^{-1}) and five salicylic acid doses (SA - 0.0, 0.29, 1.0, 1.71 and 2.0 mM) was used. Plant height, number of leaves, stem diameter, leaf area, specific leaf area, relative growth rate, leaf area ratio, leaf mass ratio, relative leaf growth rate and chlorophyll a, b and total indices were evaluated at 32, 39, 46, 53 and 60 days after irrigation with saline water (DAI). The increase in the electrical conductivity of irrigation water (ECw) negatively affected growth and increased the basil chlorophyll index. The application of salicylic acid attenuated the negative effects of salt stress on the number of leaves and leaf area and had negative effects on the stem diameter, but did not affect the other growth variables and chlorophyll indices.

Keywords: Abiotic stress, Chlorophyll indices, Phytohormone, Plant physiology, Salinity

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Basil (*Ocimum basilicum* L. - Lamiaceae) is an aromatic plant, native to southwest Asia, being cultivated around the world and has great economic importance[1]. This species has medicinal properties[2] and can be used as flavorings in foods and fragrances. Its essential oil, rich in linalool, is highly valued in the international market, used in the pharmaceutical, fragrance and cosmetics industries[3]. Basil is a plant widely used by traditional knowledge in Brazil. This plant is widely used by Brazilians as tea, infusions and macerates to cure various diseases. Basil is used in spiritual rituals, as the aroma of this plant is related to the opening of the spiritual door that connects these people with the spirits/heaven. In addition, basil is used symbolically as a spirit reliever, dissolving heavy energies and emotional upsets.

Basil is widely grown in Brazil, especially in the Northeast. However, its production is affected by several abiotic factors, including saline stress from irrigation water[3]. Soil salinity can also occur through the application of fertilizers that contain various salts. The high content of salts, both in the soil and in irrigation water, limits plant development, causing damage to the photosynthetic apparatus and interfering with plant nutrition[4]. The accumulation of Na^+ and Cl^- causes toxicity, favors the accumulation of reactive oxygen species, leading to chlorosis and necrosis and, consequently, affects the physiological and biochemical processes of plants[5,6]. The use of salicylic acid can be used to minimize the negative effects of salts on plants.

Salicylic acid is a plant growth regulator and can be a promising alternative to minimize the deleterious effects of salinity, generating metabolic and physiological responses in plants[7]. The effects of this phytohormone on plants under salt stress are associated with its ability to prevent the accumulation of reactive oxygen species, through activation of the antioxidant system, favoring the absorption of nutrients, as well as inducing photosynthetic gains[8,9].

The effect of salicylic acid in attenuating the deleterious effects of salt stress has been studied in artichokes (*Cynara scolymus* L.) and basil[10,11] and other plants. The transition from subsistence

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agriculture to commercial agriculture (with sophisticated machines and implements) accelerated environmental degradation[12], therefore, information on the use of salicylic acid in basil is essential to understand the acclimatization mechanisms of this plant under conditions of saline stress. Thus, the aim of this work was to evaluate the growth of basil (*O. basilicum*) under saline stress and salicylic acid.

Material and Methods

The experiment was carried out in a greenhouse of the Universidade Federal daParaíba, Areia, Paraíba, Brazil, between June and August, 2017. The soil used was classified as Planossoil, with a sandy-loam texture, with the physical characteristics: clay (g kg^{-1}): 184.0; sand (g kg^{-1}): 756.9; silt (g kg^{-1}): 59.1; particle density (kg dm^{-3}): 2.67; total porosity (%): 48; apparent density (kg dm^{-3}): 1.38; permanent wilt point (g kg^{-1}): 43; field capacity (g kg^{-1}): 78.

The basil seedlings were produced in 162-cell polyethylene trays and, 25 days after planting, transplanted to 5.0 dm^3 pots with 100 g poultry manure. The chemical analysis of this mixture was: pH: 6.9; P: 11.71 mg dm^{-3}; K^+: 873.43 mg dm^{-3}; Na^+: 0.24 $cmol_c$ dm^{-3}; H^++Al^{+3}: 1.6 $cmol_c$ dm^{-3}; Al^{+3}: 0.00 $cmol_c$ dm^{-3}; Ca^{+2}: 4.65 $cmol_c$ dm^{-3}; Mg^{+2}: 0.39 $cmol_c$ dm^{-3}; SB: 7.52 $cmol_c$ dm^{-3}; CEC: 9.12 $cmol_c$ dm^{-3}; BS: 82.45%; OM: 22.73 g dm^{-3}.

The basil plants were irrigated with saline water after transplantation. The saline water (ECw) was made from a mixture of NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ (7:2:1, v/v) salts in non-chlorinated water (0.5 dS m^{-1}). An analysis of the saline water was carried out (Table 1). Four plants irrigated with ECw of 0.5 dS m^{-1}were used to determine the amount of water applied to the other plants, using the drainage lysimeter method. Distilled water was used to prepare the salicylic acid doses and Tween 80 (0.05%) was added. The control was distilled water and Tween 80 (0.05%). The plants were sprayed (100 mL salicylic acid, approximately) weekly, during three weeks. Weeds and pests (caterpillars, mainly) were controlled by manual picking.

The growth assessments and the chlorophyll indices were carried out at 32, 39, 46, 53 and 60 days after saline water irrigation (DAI). Number of leaves, plant height, stem diameter, leaf area, specific leaf area, leaf area ratio, leaf mass ratio, relative growth rate and relative leaf growth rate were evaluated. The leaf area was calculated from non-destructive method using the length and width of 20 leaves. The leaf area form factor (0.6775, dimensionless)[13] was used. The chlorophyll a, b and total indices were determined by the non-destructive method, using a chlorophyll meter (ClorofiLOG®, model CFL 1030, Porto Alegre, RS).

Anrandomized block design was used with an incomplete factorial scheme (Central Composite Design – CCD). The formula for calculating the minimum number of treatments (MNT) was: 2^k + 2.k + 1, where k = number of factors evaluated. Five electrical conductivities of irrigation water (ECw - 0.5, 1.3, 3.25, 5.2 and 6.0dS m^{-1}) and five salicylic acid doses (SA - 0.0, 0.29, 1.0, 1.71 and 2.0 mM), with five blocks and two plants per block were used. The data were subjected to analysis of variance and polynomial regression. The R statistical program[14] was used. Response surfaces were made when there was an interaction between the evaluated factors (ECw and SA).

Results and Discussion

The plant height had an isolated effect on the salinity (ECw) and days after irrigation with saline water (DAI) (Fig. 1). The plant height was reduced linearly with the increase in ECw, with a reduction of 10.9% when comparing the lowest (71.5 cm) and highest (64.4 cm) salinity, with a 1.77% reduction by the unit value of ECw. The highest plant height, as a function of time, was at 60 DAI, with a unit increment of 4.69% per unit day. The decrease in plant height under saline stress is related to the damage caused by stress in the physiological processes that result in growth losses. The physiological and metabolic

Table 1 — Chemical analysis of saline water used for irrigation

pH	ECw	SO_4^{-2}	K^+	Na^+	Ca^{+2}+Mg^{+2}	Ca^{+2}	Mg^{+2}	CO_3^{-2}	HCO_3^-	Cl^-	SAR	PES	Classification	
		mg L⁻¹		......................................$mmol_c dm^{-3}$..										
7.7	0.50	3.13	0.26	2.28	2.01	1.08	0.93	0.00	2.67	4.17	2.27	2.05	Normal	C_2S_1
7.6	1.30	3.66	0.19	9.37	1.83	0.98	0.85	0.00	2.50	12.50	9.79	11.65	Saline	C_3S_3
7.9	3.25	4.22	0.22	25.44	1.93	0.86	1.07	0.00	2.50	32.83	25.90	26.98	S. sodic	C_4S_4
6.3	5.20	4.26	0.20	40.62	1.99	0.84	1.15	0.00	2.83	54.00	40.72	37.03	S. sodic	C_4S_4
7.8	6.00	4.60	0.20	49.56	2.00	0.90	1.10	0.00	2.67	63.83	49.56	41.81	S. sodic	C_4S_4

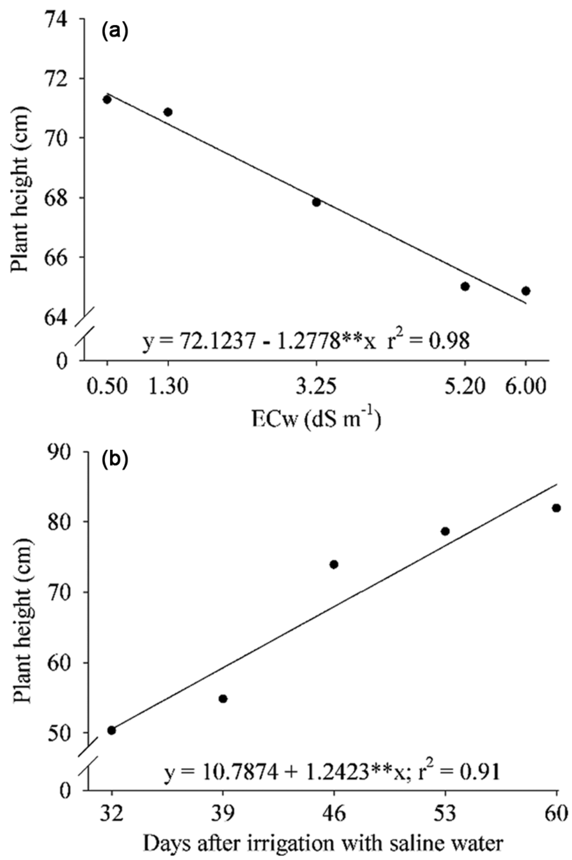

Fig. 1 — Plant height of basil (*Ocimum basilicum*) under saline stress (ECw – a) and days after irrigation with saline water (b).

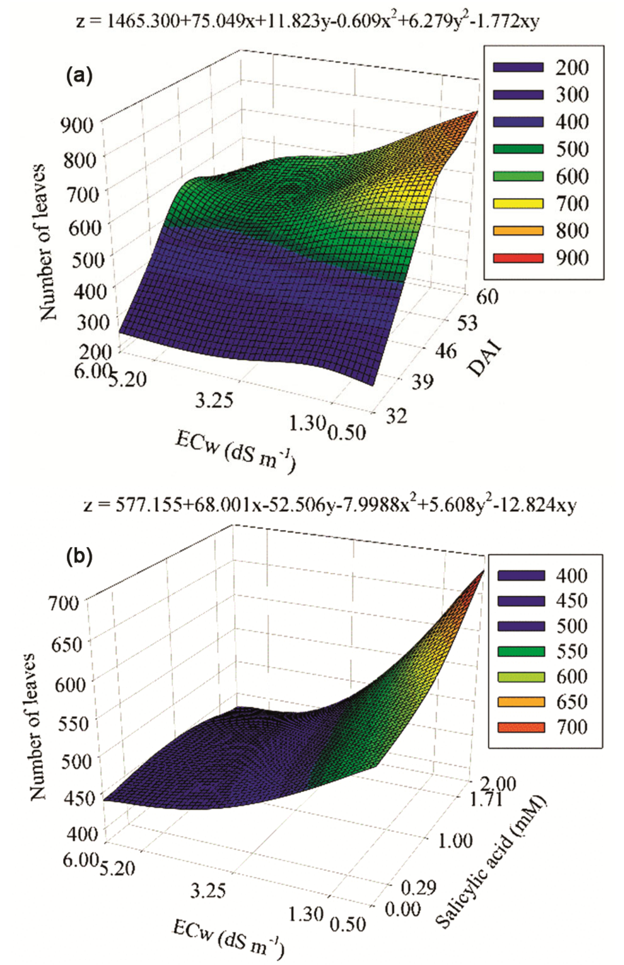

Fig. 2 — Number of leaves (a and b) of basil (*Ocimum basilicum*) under saline stress (ECw), days after irrigation with saline water (DAI) and salicylic acid.

changes promoted by saline stress, limit the processes of elongation, expansion and elasticity of the cell wall[15], consequently, the development of the plant is compromised.

The number of leaves had an interaction between the ECw and DAI factors and between ECw and SA (Fig. 2). The largest number of leaves (798 leaves) was observed at 60 DAI in plants irrigated with ECw of 0.52 dS m^{-1}, the number of leaves was reduced as the salinity increased. The decrease in the number of leaves promoted by the increase in ECw occurs due to the morphophysiological changes exerted by the plant to reduce the damage caused by saline stress, caused by nutritional imbalance, the toxicity of specific ions and changes in metabolic activities[16,17].

The salicylic acid application attenuated the harmful effect of saline stress on the number of leaves, with a greater increase (455 leaves) in plants submitted to ECw of 4.98 dS m^{-1} and 0.26 mM of SA (Fig. 2b).This increase in leaf emission, even under saline conditions, is related to the effect of salicylic acid in the regulation of defense mechanisms against stresses in plants[18], acting in several processes involved in plant growth, such as division, cell stretching and death[19].

The leaf area had an interaction between ECw and DAI, with the maximum increase (3175.21 cm^2) at 60 DAI and lower ECw, showing that the saline stress reduces leaf emission and, consequently, the leaf area of basil plants (Fig. 3). The decrease in leaf area with the increase in ECw is related to the responses of defense mechanisms, since the accumulation of specific ions (Na^+ and Cl^-), reduces physiological processes, such as photosynthesis, compromising leaf expansion[20].

The specific leaf area had an interaction between ECw and SA, with the largest increase (316.18 cm^2 g^{-1}) in the dose of 0.0092 mM of SA and ECw of 5.72 dS m^{-1} (Fig. 3b). This response is associated with the induction that this phytohormone induces the

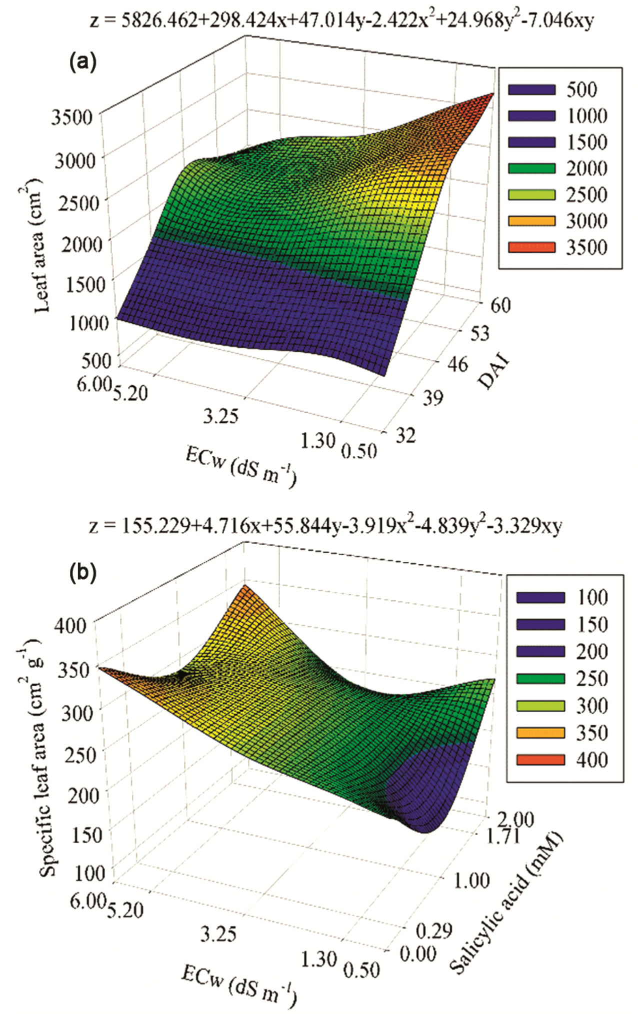

Fig. 3 — Leaf area (a) and specific leaf area (b) of basil (*Ocimum basilicum*) under saline stress (ECw), days after irrigation with saline water (DAI) and salicylic acid.

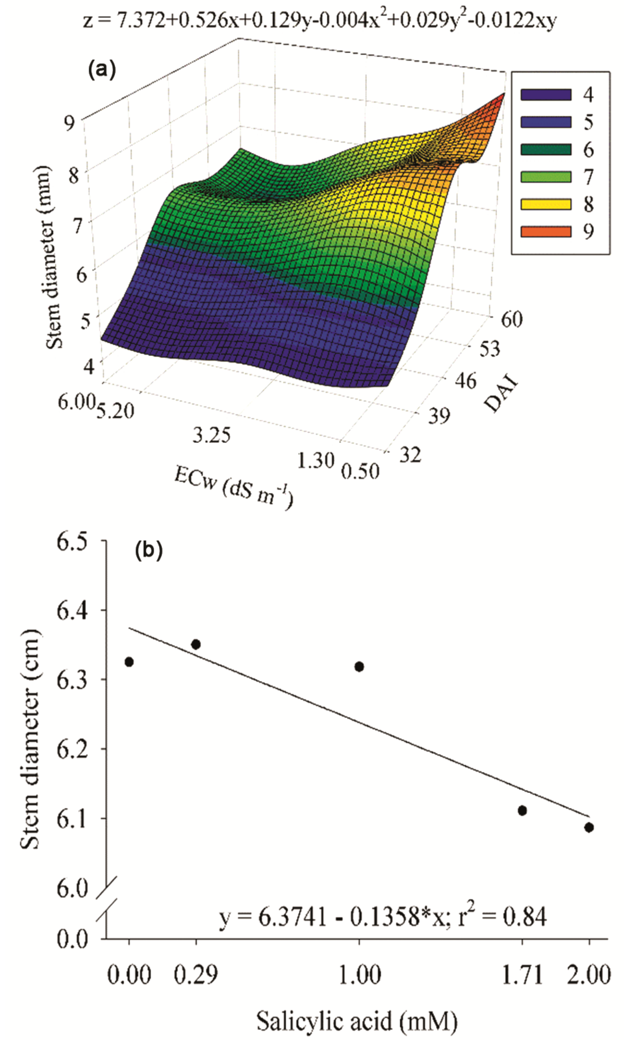

Fig. 4 — Stem diameter (a and b) of basil (*Ocimum basilicum*) under saline stress (ECw), days after irrigation with saline water (DAI) and salicylic acid.

plant's antioxidant system to exercise defense mechanisms against stress conditions. These changes promoted by SA in the expression and signaling of genes trigger a series of reactions in secondary metabolism[21], such as increased osmoprotective substances accumulation, synthesis of amino acids, enzymes and nucleic acids[22,23], reducing the damage caused by ROS produced by saline stress[5].

The largest stem diameter (8.29 mm)was observed at 60 days after irrigation with saline water in the ECw of 0.52 dS m^{-1} (Fig. 4). The stem diameter was reduced linearly by the concentrations of SA, with a reduction of 2.13% of unit increment per unit of SA.

The decrease in stem diameter with the increase in ECw is related to the higher energy expenditure by plants under saline stress, due to less water availability, resulting in less biomass production[24]. Also, the decrease in stem diameter with the increase in ECw may be associated with hormonal dysregulation, providing a negative regulation between division and cell increase[25].

The relative growth rate of the leaf was reduced by an 12.5% unit increment per unit of ECw and an 11.4% unit increment per unit days (Fig. 5). This effect may be related to the cell wall turgor reduction, consequently, with the decrease of plasticity and increase in the concentration of the solutes, causing a reduction in cell division and expansion, providing decreases in the growth rate of leaves, mainly with the increase in ECw and the days when plants were irrigated with saline water[26].

There was no interaction between the factors for the chlorophyll variables. Chlorophyll a, b and total

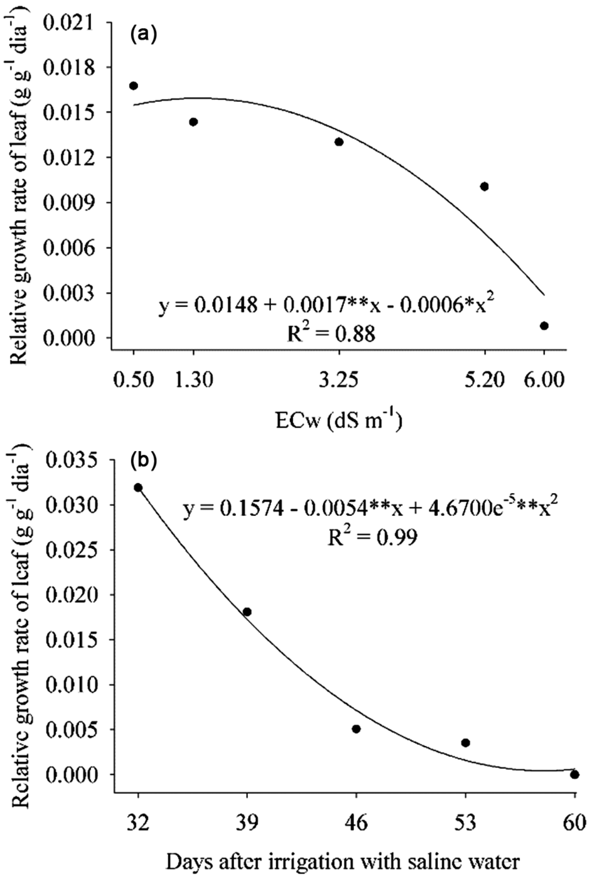

Fig. 5 — Relative growth rate of leaf of basil (*Ocimum basilicum*) under saline stress (ECw – a) and days after irrigation with saline water (b).

indices were higher at 46 DAI with saline water, with 36.2, 14.7 and 50.8 ICF, respectively, decreasing in the other days (Fig. 6).

The highest values of chlorophyll up to 46 DAI can be associated with the nutritional status of plants, where during this period there is a greater absorption of nutrients such as nitrogen and magnesium, favoring the chlorophyll synthesis. However, reductions in chlorophyll indices at the end of the cycle occur due to the accumulation of salts in the leaf tissue that stimulates the activity of the chlorophyllase enzyme that degrades chlorophylls and chloroplasts, causing losses in the photosynthetic activity of the plant and of pigmentation proteins[27]. Chlorophyll a, b and total indices increased (4.1, 13.4 and 6.6%, respectively) with the increase in ECw (Fig. 7).

The increase in chlorophyll indexes with the increase in salinity levels suggests that basil can adjust osmotically when subjected to saline stress (up to 6 dS m^{-1}), accumulating substances, such as proline and other osmoregulators, and which acts by

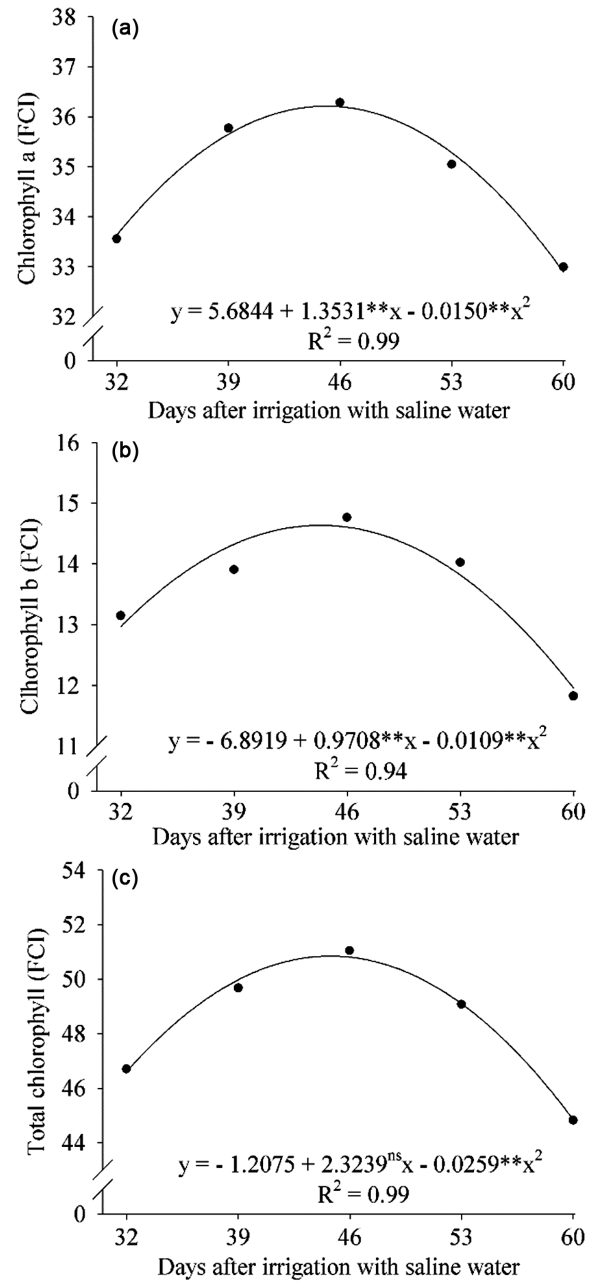

Fig. 6 — Chlorophyll a (a), chlorophyll b (b) and total chlorophyll (c) of basil (*Ocimum basilicum*) under days after irrigation with saline water.

decreasing the NH_4^+ accumulation and provides osmotic adjustment[28]. The salt increase in the irrigation water reduced the amount of chlorophyll in *Tagetes erecta* L., indicating that these variables may vary between species when subjected to saline stress[29]. Accumulation of inorganic and organic osmolytes in plants under salt stress maintains leaf turgor and creates a water absorption gradient, preventing further damage to their metabolism[11].

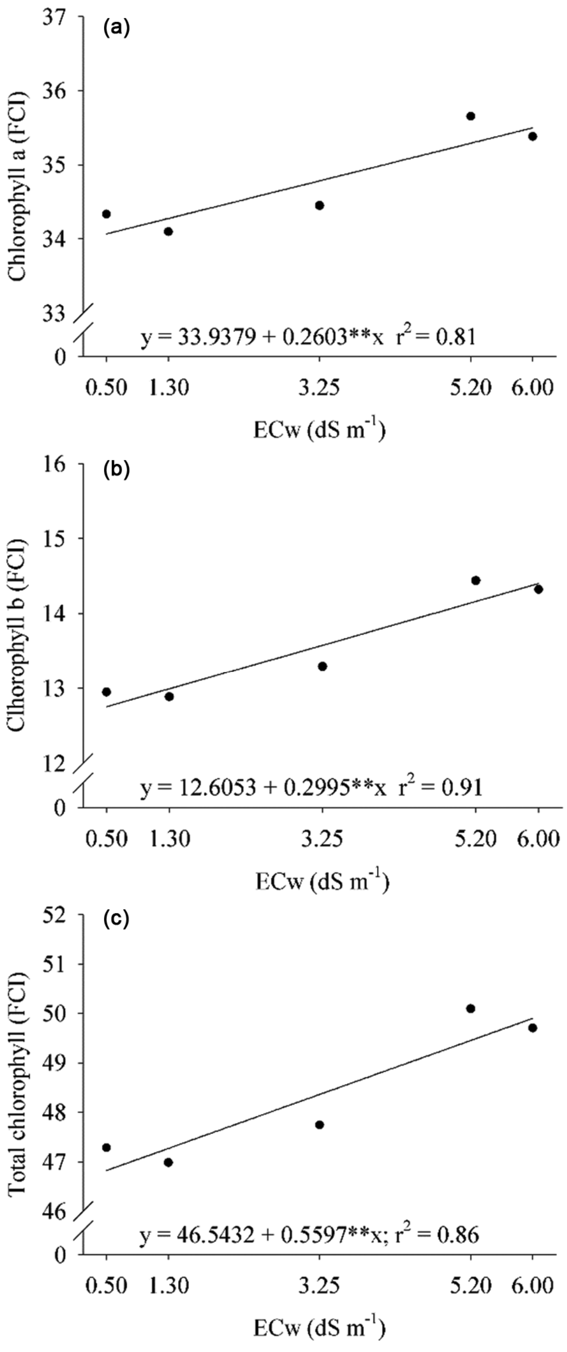

Fig. 7 — Chlorophyll a (a), chlorophyll b (b) and total chlorophyll (c) of basil (*Ocimum basilicum*) under saline stress (ECw).

Conclusion

The increase of saline stress (ECw) negatively affected growth and increased the chlorophyll indices of basil. The salicylic acid application attenuated the harmful effects of saline stress on the leaf area and number of leaves and had negative effects on stem diameter, but did not affect the other growth variables and chlorophyll indices.

Conflict of Interest

The authors declare that there has been no conflict of interest.

Authors' Contributions

All authors contributed equally during the research work and writing of this manuscript.

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