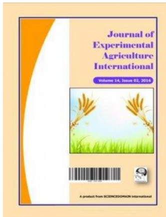

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Salicylic Acid Effect on *Ocimum basilicum* L. during Growth in Salt Stress and Its Relationship between Phytomass and Gas Exchange

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Authors' contributions

This work was carried out in collaboration between all authors. Author TIS designed the study, performed the statistical analysis and wrote part of the manuscript. Authors JSMF, ACMG, LVS and JGM collaborated in the implementation and evaluation of the data. Authors TJD, JCAP, WEP and RMNM collaborated in the development of the study and made the corrections of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this work was to evaluate the effect of salicylic acid on gaseous changes, growth and basil biomass (*Ocimum basilicum* L. cv. Cinnamon) submitted to saline stress.
Study Design: The experiment had conducted in a randomized incomplete block design (5x5-Compound Central Box) with five replicates.
Place and Duration of Study: The experiment had carried out at the Agrarian Sciences Center of the Federal University of Paraíba, Areia, PB, between May and August 2017.

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Methodology: Basil plants were used cv. Cinnamon, where they were planted in pots of 5 dm^3. Five electrical conductivities of the irrigation water (0.5, 1.3, 3.25, 5.2, 6.0 dS m^{-1}) and five doses of salicylic acid (0.0, 0.29, 1.0, 0.71, 2.0 and 2.0 mM). The following variables had evaluated: gas exchange, fluorescence and chlorophyll index, growth and fresh basil phytomass.
Results: The increase of electrical conductivity of irrigation water negatively affected the growth (height of plants, number of leaves, stem diameter and leaf area), phytomass and gas exchange of basil. The application of salicylic acid had no significant effect, however, the application of this acid up to 1.0 mM positively affected most of the variables analyzed.
Conclusion: Our results show that 1 mM of salicylic acid applied during the basil plants growth in saline stress conditions, affected positively to biomass and gas exchange.

Keywords: Saline water; attenuation of salinity; basil.

1. INTRODUCTION

Salinity is one of the most critical environmental factors limiting plant growth. About 900 million hectares of world agricultural land are affected by salinity [1]. Under saline conditions plants fills various several physiological disturbances. The major inhibitory effect of salinity on plant growth has been osmotic effect and ion toxicity. It also negatively influence photosynthetic rate, gases exchange parameters, fluorescences, hormonal synthesis etc.; phenological (reduction in biomass, foliar necrosis, loss of chlorophyll content, among others) in more severe cases, in plant senescence [2]. Like most cultivated plants, basil cab be affected by soil salinity and irrigation water [3].

Ocimum basilicum L. is commonly known as basil, being an aromatic and medicinal species known worldwide. Its essential oil is widely used by the perfumery, cosmetics, flavoring and medicine industries. It is known in folk medicine as carminative, used as stomatal and antispasmodic tonic and vermifuge [4,5].

Many strategies used to reduce the deleterious effects of salts on plants; one of them is salicylic acid, a phenolic derivative. This acid is a phenolic derivative, distributed over a wide range of plant species. It is considered a phytohormone, playing an important role in the regulation of plant growth and development, seed germination, fruit yield, glycolysis and flowering. In addition to other factors such as ion absorption and transport, photosynthetic rate, stomatal conductance and transpiration rate [6]. This hormone has an effect on several abiotic stresses, among them saline stress.

Some studies have been carried out using salicylic acid as a mitigator of the effects of saline stress on basil plants [7,8,5,9]. However, in the conditions of the Brazilian Northeast these studies are scarce. In view this, our aim of this investigation was to study the effect of salicylic acid on basil (*Ocimum basilicum* L. cv. Cinnamon) plants when growth stressed by salts, and to quantify its effect on gas exchange and phytomass.

2. MATERIALS AND METHODS

The experiment was carried out in a protected environment (greenhouse) at the Center of Agricultural Sciences of the Federal University of Paraiba, located in the municipal of Areia, PB (6º 51 '47 "S; 35º 34'13"; 575 m), from May to August 2017. The soil used was classified as Planosol [10], sandy loam texture, with the following physical characteristics: sand (g kg^{-1}): 756.9; silt (g kg^{-1}): 59.1; clay (g kg^{-1}): 184.0; apparently density (kg dm^{-3}): 1.38; density of particle (kg dm^{-3}): 2.67; total porosity (%): 48; field capacity (g kg^{-1}): 78; permanent wilting point (g kg^{-1}): 43.

The experimental design was in randomized blocks, in an incomplete factorial scheme through the central composite box matrix. Five level of electrical conductivities of irrigation water (ECw - 0.5, 1.3, 3.25, 5.2 and 6.0 dS m^{-1}) and five doses of salicylic acid (SA - 0.0 0.29, 1.0, 1.71 and 2.0 mM), with five replicates and two plants per replicate.

The used basil seeds were of the Cinnamon cultivar. The seeds were purchased from the Breeding Sector of the Federal University of Alagoas, through the researcher Arie Fitzgerald Blank. The seedlings had produced in polyethylene trays of 162 cells. Soil and commercial organic compost (1:1 v/v) were used as the substrate for their production. Twenty-five days after planting (DAP), they were transplanted to the pots with soil previously moistened to their field capacity. The experimental units were composed of polyethylene pots with volume capacity of 5.0 dm^{-3} filled with soil and 20 g dm^{-3} of poultry manure. A sample of the soil and manure mixture had collected for fertility analysis. The analysis had the following characteristics: pH (H_2O): 6.9; P (mg dm^{-3}): 11.71; K^+ (mg dm^{-3}): 87.34; Na^+ (cmol dm^{-3}): 0.24; H^++ Al^{+3} (cmol dm^{-3}): 1.6; Al^{+3} (cmol dm^{-3}): 0.00; Ca^{+2} (cmol dm^{-3}): 4.65; Mg^{+2} (cmol dm^{-3}): 0.39; SB (cmol dm^{-3}): 7.52; CTC (cmol dm^{-3}): 9.12; V (%): 82.45; M.O (g dm^{-3}): 22.73.

Irrigation had performed by drainage lysimetry as needed. The water with the desired electrical conductivity (ECw) was prepared by adding a mixture of NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ salts (7:2:1, respectively) in water (0.5 dS m^{-1}) from of the experiment place [11]. They had performed weekly, and a portable conductivity meter had used for verification. Four additional plants had used to check the water depth to be applied. They had irrigated with ECw of 0.5 dS m^{-1} and applied 0.0 mM of SA. For the preparation of the doses of salicylic acid with distilled water was used and 0.05% Tween 80 added as surfactant to improve the absorption by the plants. The control treatment for this factor was prepared with distilled water and Tween 80. The plants had sprayed until completely wetted with the solutions described above weekly for a period of twenty-one days. The water salinity analysis is shown below (Table 1).

The gas exchange evaluations had performed 60^{th} days after the application of irrigation with saline water (DAI). Growth (height of plants, number of leaves, stem diameter and leaf area) analyze was performed at 60^{th} DAI. The determinations of the gas exchanges were performed using an infrared gas analyzer (LI-COR®-model LI-6400XT, Nebraska, USA), and the measurements were measured between 9 and 10h00. The variables: net photosynthesis rate (A) (μmol of CO_2 m^{-2} s^{-1}), stomatal conductance (gs) (mol of H_2O m^{-2} s^{-1}), transpiration (E) (mmol de H_2O m^{-2} s^{-1}), internal carbon concentration (Ci) (μmol CO_2 m^{-2} s^{-1}), vapor pressure deficit (VPD) (VPD leaf-air), water use efficiency (WUE = A/E) and intrinsic carboxylation efficiency (EiC = A/Ci). At 64^{th} DAI, the plants had harvested and separated (root, stem, leaf and inflorescence) and weighed in a semianalitic scale (0.01 g). The leaf area was obtained through the average product of the length and width of 20 leaves and the leaf area form factor (0.6775 undimensioned). The data had submitted to the analysis of variance and the polynomial regression analysis had performed using the statistical program SAS University [12].

3. RESULTS AND DISCUSSION

There were no significant interaction found significant interactions between salicylic acid (SA) and the electrical conductivity of irrigation water (ECw). Salicylic acid had no effect on the analyzed variables. The results show that when basil plants were irrigated with saline waters (ECw) had an effect on all variables analyzed. Fig. 1 show that plant height (PH), number leaves (NL), stem diameter (SD) and leaf area (LA) up to leave area decreases proportional as the electrical conductivity of irrigation water increases.

The plant height decreased linearly 1.85%, per unit increase in salinity (dS m-1), that is as ECw increases 0.5 – 6.0 dS m^{-1}. Bione et al. [13] studied the effect of saline stress on basil, found that after 49 days of transplanting, there was a reduction of 0.117 mm in plant height. In the present study, a reduction of 0.1612 mm had observed at 84 days after planting. Regarding the number of leaves, there was a similar behavior, with a unit decrease of 6.76% and a mean of 55.1 leaves per unit of ECw. For saline conditions, there is a restriction on the growth of plants, especially in the height, due to the low osmotic and water potential exerted by the ions, as well as their interference in the assimilation of many essential nutrients. Since growth is the result of nutrient assimilation and cell division and stretching [14].

The stem diameter of basil plant from control 8.6 mm was reduced to 6.4 mm when it treated with increasing value of ECw from 0 to 5.96 dS m^{-1}, thus exhibiting quadratic polynomial behavior. However, for the leaf area a decreasing linear behavior had observed, showing a unit decrease of 6.76% and a mean of 219.1 cm^2 per unit of ECw. For the fresh root mass, it was noted that there was decrease as the ECw increased to 5.94 dS m^{-1}.The reduction of leaf area in plants submitted to saline stress (which may be related to the decrease in cell division and leaf surface expansion) is possibly an adaptive mechanism, aiming to reduce the surface of transpiration [15]. The Fig. 2 showed that fresh masses of the following parameters from basil plant decreases: root length, stem length, leaf size, inflorescence

Table 1. Chemical analysis of salt 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	ESP	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

ECw = Electrical conductivities of irrigation water; SAR = Sodium adsorption ratio; ESP = Exchangeable sodium percentage

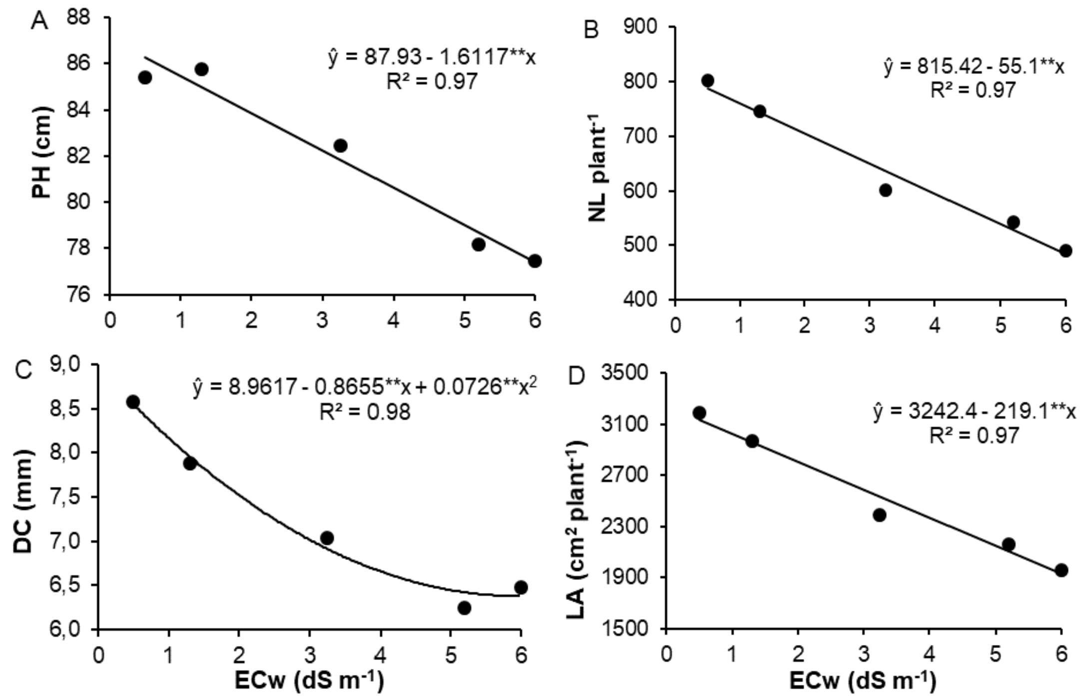

Fig. 1. Plant height (A), number leaves (B), stem diameter (C) and leaf area (D) of basil (*Ocimum basilicum* cv. Cinnamon) as a function of the electrical conductivity of irrigation water (ECw) at 60th days after irrigation with saline water

*** $P < 0.01$*

and the total fresh mass of them as ECw increased from 1 to 6 dS m^{-1}. A decrease in these variables was noted when ECw was increased.

For the fresh root mass, it was noted that there was decrease as the ECw increased to 5.94 dS m^{-1}. It is known that the salts decreases the growth of roots of the plants, decreases drastically the amount of root hair, affecting the absorption of water and nutrients; consequently, affecting the growth of plants [14]. The root, as an organ of absorption, has great importance in the responses to the saline stress, in physiological and metabolic terms. In this organ is synthesized abscisic acid (one of the signaling hormones of stress) that cause changes in the hydraulic conductivity and stomatal opening. Thus, the relationship between salinity and root growth is complex. Other important stressor to plants is the ratio of Na:Ca that affect growth of plants through inhibiting root biomass [16].

The fresh stem, leaf and total masses presented quadratic behavior, decreasing as ECw increased, presenting the maximum physical efficiency at 6.07, 6.55 and 9.69 dS m^{-1}, respectively. The same behavior occurred for the fresh mass of inflorescence, however, for this variable, ECw of 1.54 dS m^{-1} was the one that presented the best result. Changes in morphology influence the ability of plants to assimilate nutrients, water and light and when under stress, they may undergo changes that may influence morphological responses and, consequently, their growth and survival [17].

The disturbance caused by this stress can lead to reduced plant growth, affecting its metabolism and, consequently, the production of organic matter [18]. Bione et al. [13] report that the fresh basil phytomass had linearly reduced by increasing ECw. The same had observed by Mohammadzadeh et al. [5]. Table 2 show that when salicylic acid was applied of basil plants, during growth, in stressed conditions. The following parameters: number leaves, stem diameter, leaf area, root fresh mass, stem fresh mass, leaf fresh mass, inflorescence fresh mass and total fresh mass of basil plants, all of them increases as SA concentrations increases up to 1 mM. In addition, higher concentration of SA had adverse effect. However, basil plant height show a minor positive response to SA applied almost at all concentration.

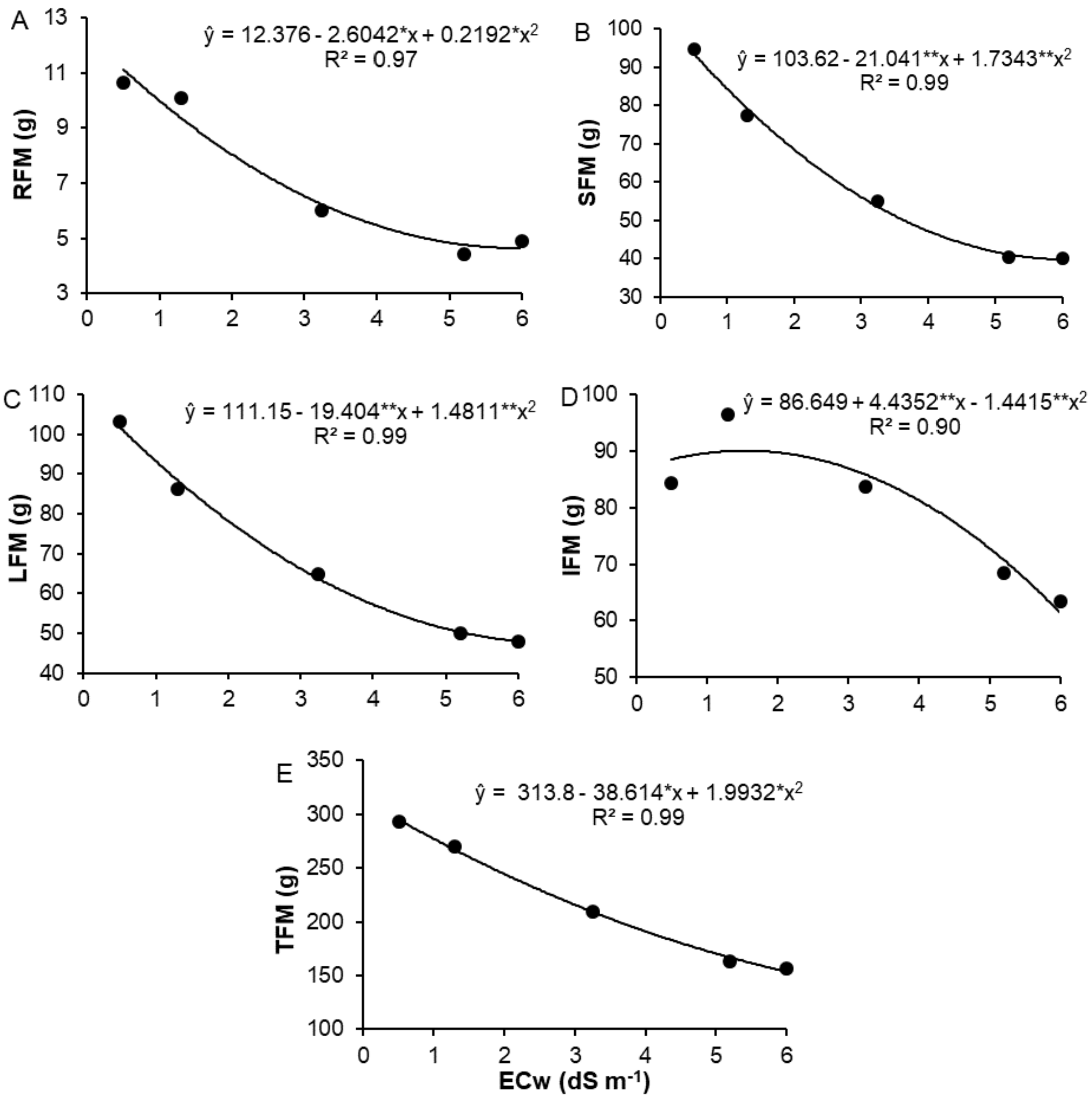

Fig. 2. Fresh root mass (A), fresh stem mass (B), fresh leaf mass (C), fresh inflorescence mass (D), total fresh mass (E) of basil (*Ocimum basilicum* cv. Cinnamon) as a function of the electrical conductivity of irrigation water (ECw) at 60th days after irrigation with saline water
$* P < 0.05$; $** P < 0.01$

Salicylic acid and other salicylates are known to affect various physiological and biochemical activities of plants, acting as an endogenous regulator that potentially affects plant growth and productivity [19]. However, when applied too heavily it may have adverse effects.

The application of SA up to 0.29 mM increased the fresh mass of the root and from this molarity there is reduction in this variable. The fresh stem and leaf mass presented a mean increase until the application of 1.0 Mm, whereas the fresh mass of the inflorescence had negatively affected until this variable, increasing its average values when the molarity was increased. As for the total dry mass, it was observed that there was increase up to 0.29 mM, decreasing up to 1.71 mM, however, there was a small increase when 2.0 mM of SA was applied, as shown here in Table 2. Contrary to our results, Mohammadzadeh et al. [5] found no-significant effect when SA was applied on four basil genotypes growing in salt stress environment, evaluated on fresh mass of root and shoot.

The increase in electrical conductivity of irrigation water significantly affected stomatal conductance (gs), transpiration (E), vapor pressure deficit (VPD) and water use efficiency (WUE). However, it did not affect net photosynthesis (A), internal carbon concentration (Ci) and intrinsic carboxylation efficiency (EiC) (Fig. 3).

Fig. 3 show that when the basil plants grown in salt stress condition, and then irrigated with water as increasing in electrical conductivity; the stomatal conductance (gs), the transpiration rate, the net photosynthesis rate, and internal carbon concentration, increases in a parabolic way when ECw increases from 1 to 6 dS m^{-1}. In addition, vapor pressure deficit and water use efficiency decreases as ECw increases from 1 to 3 dS m^{-1} and then increases again. However, the intrinsic carboxylation efficiency suffer minor changes when ECw increases. The decrease in photosynthetic processes, caused by saline stress, causes excessive energy excitation, which when not dissipated properly can cause damage to the photosynthetic system. In addition, the production of reactive oxygen species (ROS) has increased by excess energy, which can inhibit D1 protein synthesis and directly damage PSII by oxidation [20].

Stomatal conductance and transpiration presented similar behaviors, where there was an increase in the mean values of these variables up to ECw 3.25 dS m^{-1}. From this, there was a decrease in values, which implies the aforementioned ECw affects these variables. Regarding the vapor pressure deficit and the water use efficiency, the behaviors were similar, where there was a decrease up to the ECw 3.25 dS m^{-1}, and in the upper ECw there was an increase for the same ones. Saline stress affects stomatal conductance immediately, first and transitory due to disturbed relations with water and shortly thereafter due to the local synthesis of abscisic acid [21].

It is difficult to explain the relationship between photosynthesis and its cause and effects on plant growth. It is difficult to say that the decrease in photosynthesis can be attributed to the cause of reduced plant growth. When submitted to salinity, initially the plant reduces its photosynthetic rate, however, this is not the only cause of the reduction of growth, since there is a rapid change in the rate of leaf expansion and increase in the content of stored carbohydrates. However, as this stress is prolonged, the responses from the drains to the source can adjust the photosynthetic rate to match the reduced demand from inhibition of growth [21].

Salicylic acid did not interfere with gas exchange, however, its mean values are shown below (Table 3). It is a fact that salicylic acid potentially entails a wide range of metabolic responses in plants affecting photosynthetic parameters [19].

The stomatal conductance had decreased by 1.0 mM, however, as molarity increased, there was an increase in this variable. The same behavior can be observed for transpiration and internal carbon concentration. Net photosynthesis had decreased to 0.29 mM, increasing to 1.71 mM, therefore decaying. The vapor pressure deficit and water use efficiency had raised to 1.0 mM and had negatively affected as the molarity increased. On the other hand, the intrinsic efficiency of carboxylation decreased from 1.0 mM SA. The functional mechanism of SA against stress is related to its role in the regulation of enzymes (such as catalases and peroxidases) antioxidants and other compounds with active oxygen in plants. This acid acts directly or indirectly in the production or activation of these antioxidants [9].

Table 2. Plant height (PH), number leaves (NL), stem diameter (SD), leaf area (LA), fresh root mass (RFM), stem (SFM), leaf (LFM), inflorescence (IFM) and total (TFM) of basil (*Ocimum basilicum* cv. Cinnamon) as a function of salicylic acid (SA) at 64th days after irrigation with saline water

SA	Averages								
	PH	NL	SD	LA	RFM	SFM	LFM	IFM	TFM
0.00	81.55	635.40	7.23	2526.59	5.71	52.72	64.40	81.68	204.51
0.29	83.13	640.25	7.23	2545.87	7.54	60.96	69.30	82.70	220.50
1.00	81.85	640.23	7.35	2545.81	7.21	63.25	71.95	76.96	219.37
1.71	80.10	646.75	6.91	2571.72	6.98	56.70	67.05	82.18	212.91
2.00	83.85	540.10	6.86	2147.64	6.32	56.83	65.59	86.51	215.25

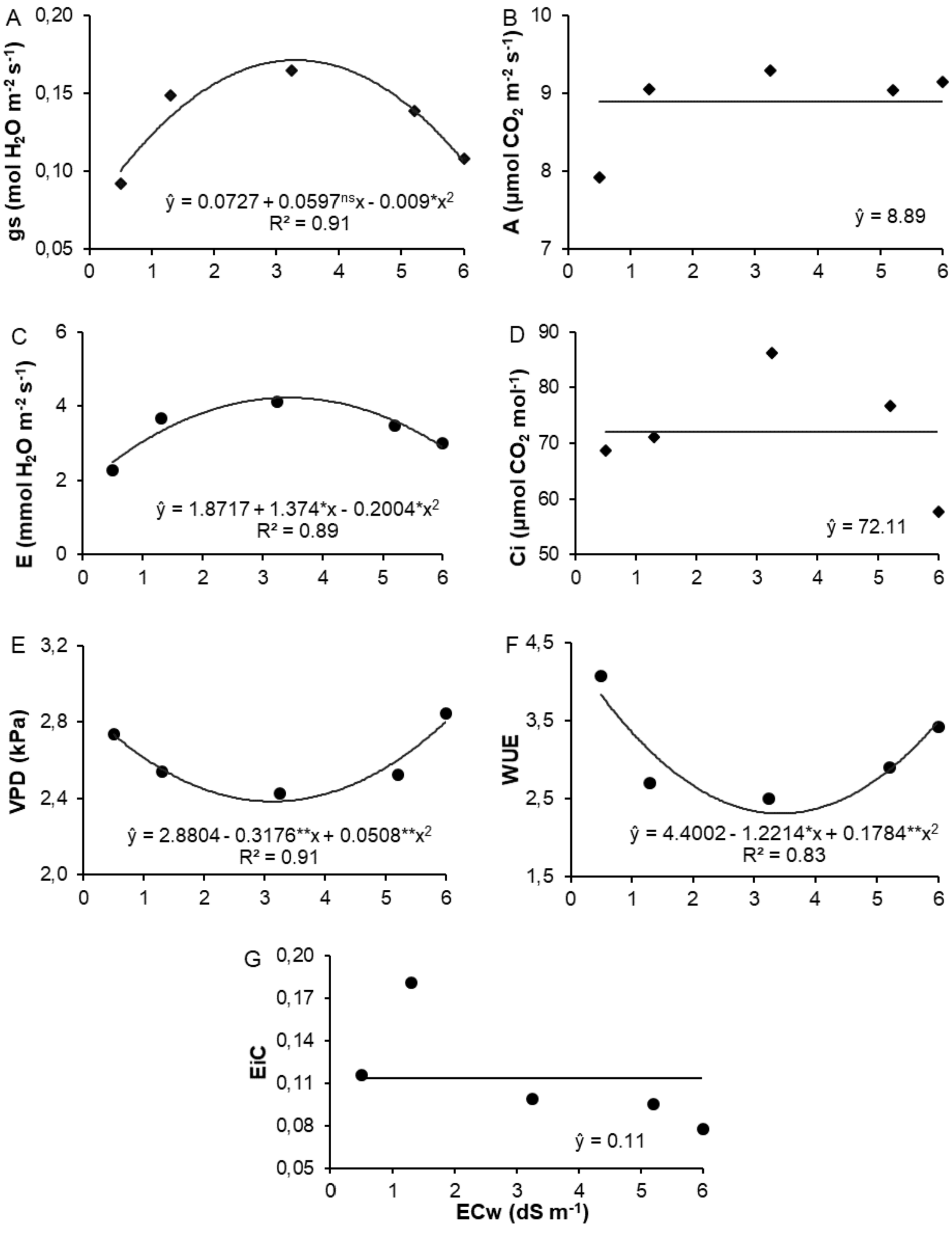

Fig. 3. Stomatal conductance (A), net photosynthesis (B), transpiration (C), internal carbon concentration (D), vapor pressure deficit (E), water use efficiency (F) and intrinsic carboxylation efficiency (G) of basil (*Ocimum basilicum* cv. Cinnamon) as a function of the electrical conductivity of the irrigation water (ECw) at 60^{th} days after irrigation with saline water

* $P < 0.05$; ** $P < 0.01$; ^{ns} $P > 0.05$

Table 3. Stomatal conductance (gs), net photosynthesis (A), transpiration (E), internal carbon concentration (Ci), vapor pressure deficit (VPD), water use efficiency (WUE) and intrinsic carboxylation efficiency (EiC) in basil plants as a function of the application of salicylic acid (SA) at 60th days after irrigation with saline water

SA	Averages						
	Gs	A	E	Ci	VPD	WUE	EiC
0.00	0.16	9.37	4.06	80.24	2.47	2.62	0.19
0.29	0.15	8.89	3.57	73.75	2.51	2.7	0.16
1.00	0.12	8.94	3.03	66.29	2.7	3.44	0.18
1.71	0.14	9.22	3.57	74.21	2.56	2.92	0.15
2.00	0.17	8.74	4.24	100.37	2.37	2.25	0.09

4. CONCLUSION

- Growth, phytomass and gaseous exchange parameters of basil (*Ocimum basilicum* cv. Cinnamon) has negatively affected by the increase in the electrical conductivity of the irrigation water.
- The application of salicylic acid up to 1.71 mM favors the number of leaves and leaf area, as for stem diameter and phytomass, up to 1.0 mM. Even this molarity favors also the vapor pressure deficit, the water use efficiency and the intrinsic carboxylation efficiency. While stomatal conductance, transpiration and internal carbon concentration is positively affected above this molarity. Net photosynthesis increases, while transpiration decreases as ECw rises.
- For a better growth of basil (*Ocimum basilicum* cv. Cinnamon) plants in salinity land, we postulate to use our results found here.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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