

Salinity and salicylic acid on growth, phytomass and gas exchange of *Ocimum basilicum* L.

ABSTRACT

Aim: The aim of this work was to evaluate the attenuating 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 complete 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. **Methodology:** Basil plants were used cv. Cinnamon, where they were planted in pots of 5 dm³. 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, 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: With this, it can be explained the application of salicylic acid to 1.0 mM can be beneficial to the cultivation of basil under salinity.

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 conditions of saline stress, plants can present several physiological disturbances (decrease of the osmotic potential, photosynthetic rate, hormonal synthesis, among others), phenological (reduction in biomass, foliar necrosis, loss of chlorophyll, among others) in more severe cases, in plant senescence [2]. Like most cultivated plants, basil can 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, stomatal and antispasmodic tonic and vermifuge [4,5].

Many strategies are being used to reduce the deleterious effects of salts on plants, among them the use of salicylic acid. 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 production, glycolysis and flowering. In addition to other factors such as ion absorption and transport, photosynthetic rate, stomatal conductance and transpiration [6].

Some studies have been carried out using salicylic acid as a mitigator of the effects of saline stress on basil plants [7,8,9]. However, in the conditions of the Brazilian Northeast these studies are scarce. The objective of this work was to evaluate the attenuating effect of salicylic acid on the gas exchange, growth and phytomass of basil (*Ocimum basilicum* L. cv. Cinnamon) plants submitted to saline stress.

2. MATERIAL AND METHODS

The experiment was carried out in a protected environment (greenhouse) at the Center of Agricultural Sciences of the Federal University of Paraíba, located in the municipal of Areia, PB (6° 51' 47" S; 35° 34' 13" W; 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; apparent 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 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 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 dm³ 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 (H₂O): 6.9; P (mg dm⁻³): 11.71; K⁺ (mg dm⁻³): 87.34; Na⁺ (cmol dm⁻³): 0.24; H⁺ + Al³⁺ (cmol dm⁻³): 1.6; Al³⁺ (cmol dm⁻³): 0.00; Ca²⁺ (cmol dm⁻³): 4.65; Mg²⁺ (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 lisimetry as needed. The water with the desired electrical conductivity (ECw) was prepared by adding a mixture of NaCl, CaCl₂.2H₂O and MgCl₂.6H₂O salts (7:2:1, respectively) in water (0.5 dS m⁻¹) from of the experiment [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 showed below (Table 1).

Table 1. Chemical analysis of salt water used for irrigation

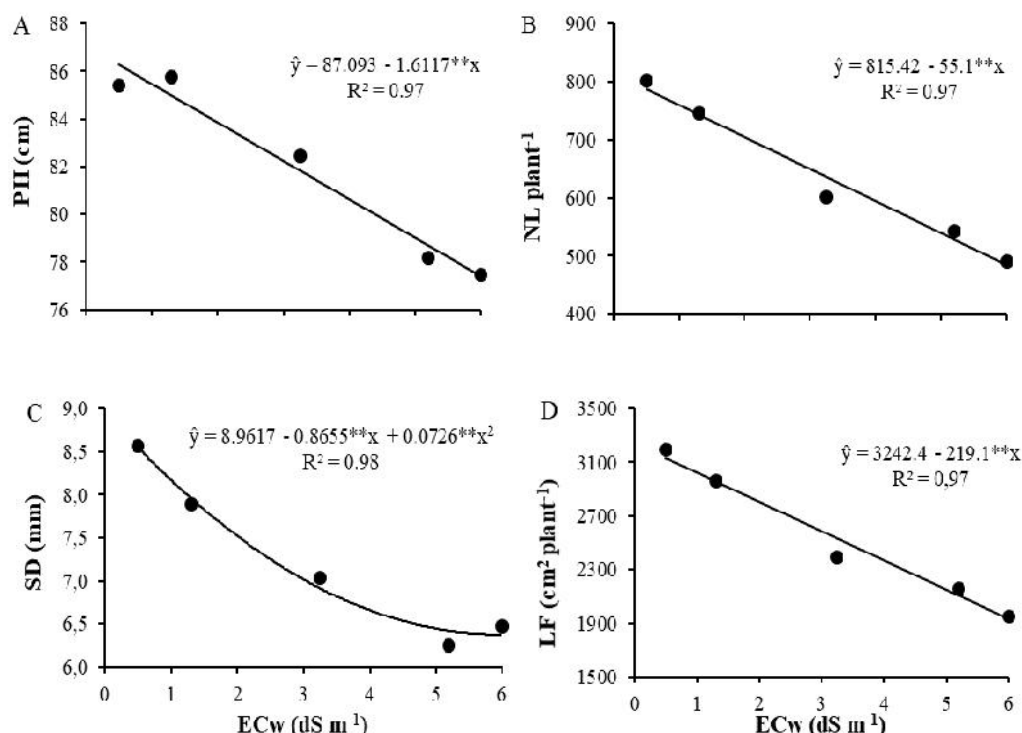
pH	ECw	SO ₄ ⁻²	K ⁺	Na ⁺	Ca ⁺² +Mg ⁺²	Ca ⁺²	Mg ⁺²	CO ₃ ⁻²	HCO ₃ ⁻	Cl ⁻	RAS	PST	Classification	
		mg L ⁻¹			mmol _c dm ⁻³									
7.7	0.50	3.13	0.26	2.28	2.01	1.08	0.93	0.00	2.67	4.17	2.27	2.05	Normal	C ₂ S ₁
7.6	1.30	3.66	0.19	9.37	1.83	0.98	0.85	0.00	2.50	12.50	9.79	11.65	Saline	C ₃ S ₃
7.9	3.25	4.22	0.22	25.44	1.93	0.86	1.07	0.00	2.50	32.83	25.90	26.98	S. sodic	C ₄ S 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 ₄ S 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 ₄ S ₄

The gas exchange evaluations had performed 60 days after the beginning of irrigation with saline water (DAI). Growth, fluorescence and chlorophyll index analyzes were performed at 60 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: liquid photosynthesis rate (A) (μmol of CO₂ m⁻² s⁻¹), stomatal conductance (gs) (mol of H₂O m⁻² s⁻¹), transpiration (E) (mmol de H₂O m⁻² s⁻¹), internal carbon concentration (Ci) (μmol CO₂ 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 DAI, the plants had harvested and separated (root, stem, leaf and inflorescence) and weighed in a semianalian scale (0.01 g). The leaf area was obtained through the average product 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].

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79 3. RESULTS AND DISCUSSION

80 There were no significant interactions between salicylic acid (SA) and the electrical conductivity of
 81 irrigation water (ECw). Salicylic acid had no effect on the analyzed variables. However, irrigation with
 82 saline waters (ECw) had an effect on all variables analyzed. Plant height (PH), number of leaves (NL),
 83 stem diameter (SD) and leaf area (LA) had negatively affected by ECw increase, as shown in Fig 1.



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Fig. 1.
Height of plants (A), number of 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 at 60 days after irrigation with saline water. ** Significant at 1%
**** $P < 0.001$**

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

99 The stem diameter had reduced to ECw at 5.96 dS m⁻¹, having a small increase in the subsequent
 100 ECw (6.0 dS m⁻¹), thus exhibiting quadratic polynomial behavior. However, for the leaf area a
 101 decreasing linear behavior had observed, showing a unit decrease of 6.76% and a mean of 219.1 cm²
 102 per unit of ECw. The reduction of leaf area in plants submitted to saline stress (which may be related
 103 to the decrease in cell division and leaf surface expansion) is possibly an adaptive mechanism,
 104 aiming to reduce the surface of transpiration [15]. In showed in the Fig 2 the fresh masses of the root
 105 (RFM), stem (SFM), leaf (LFM), inflorescence (IFM) and total (TFM). A decrease in these variables
 106 was noted when ECw was increased.

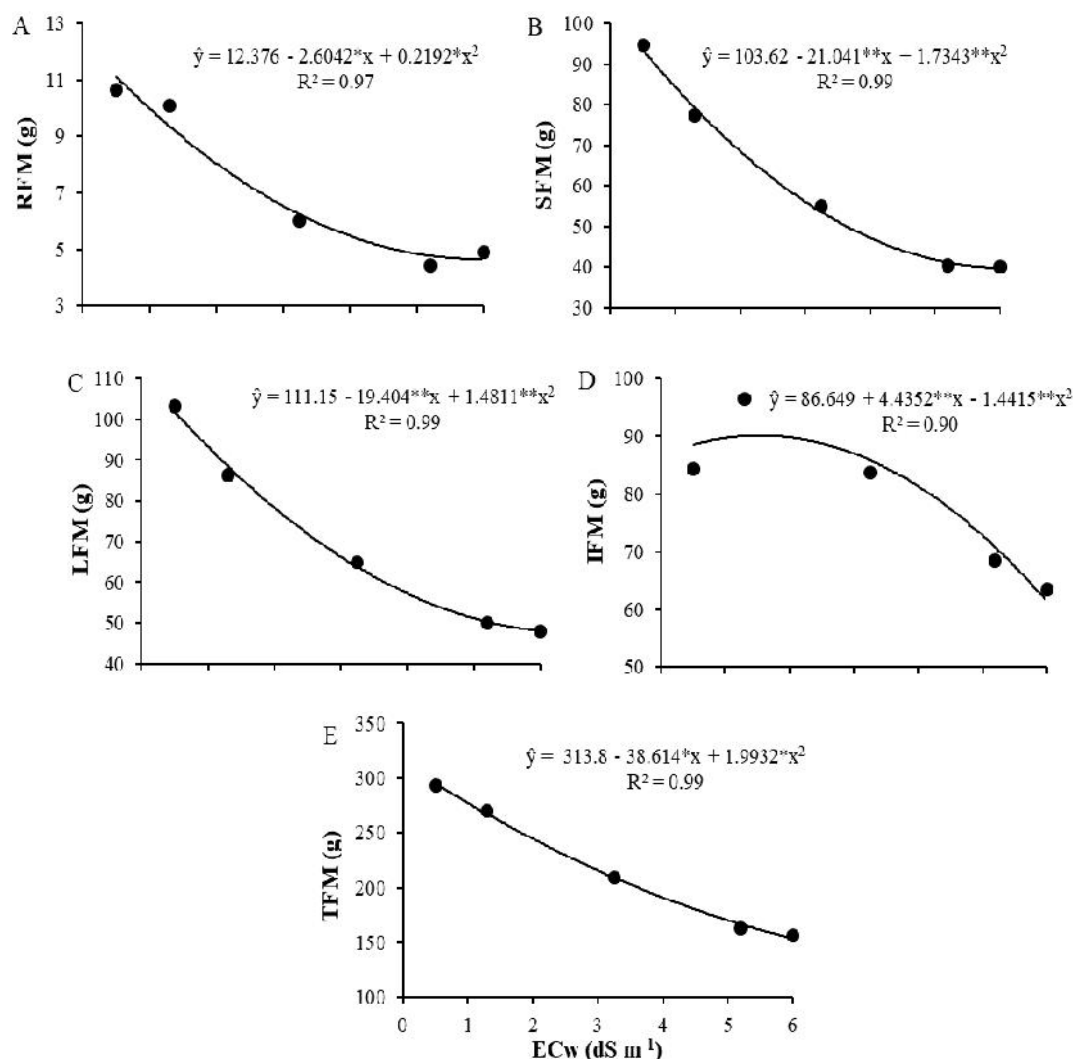


Fig. 2. Fresh root mass (A), fresh stem mass (B), fresh leaf mass (C), fresh mass of inflorescence (D), total fresh mass (E) of basil (*Ocimum basilicum* cv. Cinnamon) as a function of the electrical conductivity of irrigation water (CEa) at 60 days after irrigation with saline water

* $P < 0.05$; ** $P < 0.001$

For the fresh root mass it was noted that there was decrease as the ECw increased to 5.94 dS m⁻¹. At root level, the salts considerably decrease the amount of root hair affecting the absorption of water and nutrients and, consequently, 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 there is the synthesis of abscisic acid (one of the signaling hormones of stress) that can cause changes in the hydraulic conductivity and stomatal opening. Thus, the relationship between salinity and root growth is complex, considering the Na:K ratio affects growth and, consequently, 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]. Regarding the application of SA, growth and fresh phytomass did not show differences, however, their values showed in Table 2.

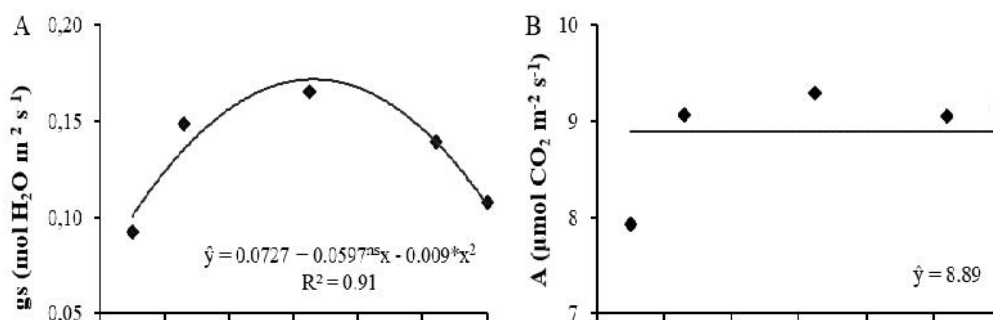
Table 2. Plant height (PH), number leaves (NL), stem diameter (SD), leaf area (LA), fresh root mass (RFM), stem (SFM), leaf (LFM), inflorescence (IFM) and total (TFM) of basil (*Ocimum basilicum* cv. Cinnamon) as a function of salicylic acid (SA) at 64 days after irrigation with saline water

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

When increasing the dose of SA from 0.29 to 1.71 mM. It was noticed that the height of plants was reduced. however, when raising the molarity up to 2.0 mM it was noticed that there was increase for this variable. Regarding the number of leaves, it had observed that up to 1.71 mM of SA there were no large variations between the averages. However, when raising the same to 2.0 Mm, there was a reduction of 16.5%. The same behavior had observed for the leaf area. since this variable is directly related to that one. The diameter of the stem increased up to 1.0 Mm, decreasing in the later molarities. This infers in that the application of SA above 1.71 can influence the plant stand, and consequently, the lower absorption of solar energy. 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. Mohammadzadeh et al. [5] when evaluating the effect of SA on four basil genotypes submitted to salt stress, also did not find significant effects of SA on fresh root and shoot mass.

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 liquid photosynthesis (A), internal carbon concentration (Ci) and intrinsic carboxylation efficiency (EiC) (Fig 3).



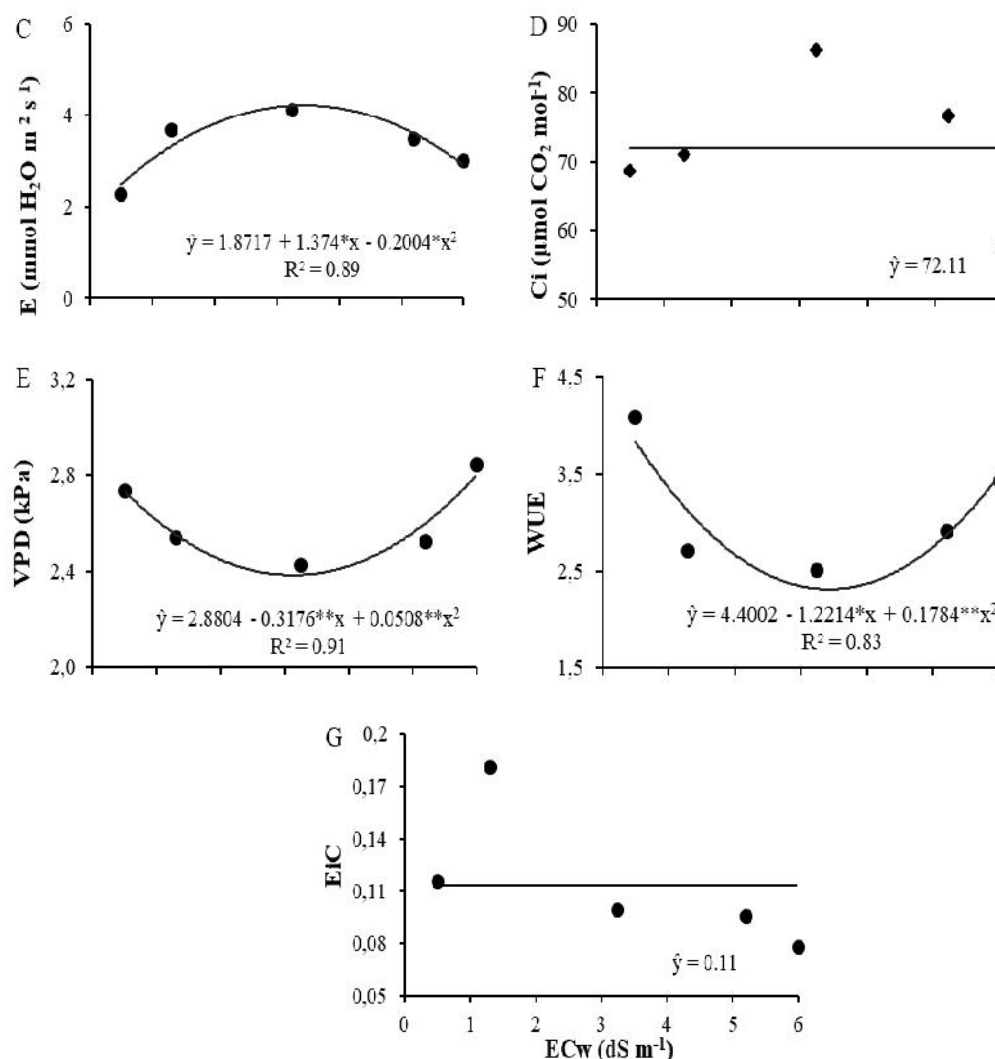


Fig. 3 Stomatal conductance (A), liquid photosynthesis (B), transpiration (C), internal carbon concentration (D), vapor pressure deficit (E), water use efficiency (F) and intrinsic carboxylation efficiency (G) of basil (*Ocimum basilicum* cv. Cinnamon) as a function of the electrical conductivity of the irrigation water (ECw) at 60 days after irrigation with saline water
 $* P < 0.05$; $** P < 0.001$; $^{ns} P > 0.05$

Liquid photosynthesis did not have a significant effect on the increase of ECw, however, it was observed that the highest mean ($9.29 \mu\text{mol mol}^{-1}$) was observed in ECw of 3.25 dS m^{-1} . The same occurred for the internal carbon concentration that presented in this same ECw, average value of $86.3 \mu\text{mol mol}^{-1}$, however, from that ECw this variable suffered a decrease. The intrinsic efficiency of carboxylation showed no differences. however. it was observed that the highest mean (0.18) was observed in ECw 1.3 dS m^{-1} , and there was a decrease. 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].

Table 3. Stomatal conductance (gs), liquid 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 60 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

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. Liquid 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].

4. CONCLUSION

- Growth, phytomass and gaseous basil exchange (*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.

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