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Application of zeolites as an alternative to reduce the effects of salt stress on *Ixora coccinea L.*

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Abstract

Zeolites are crystalline aluminosilicates capable of adsorbing cations in soil and water. Thus, the objective was to evaluate the potential of a synthetic type A zeolite, applied to the substrate, on the growth and physiology of the ornamental species *Ixora coccinea* L., irrigated with saline water. The study was carried out in a greenhouse, in an experimental area at the Federal University of Ceará, municipality of Fortaleza – Ceará, Brazil, between April and June 2018. The design used was in randomized blocks (RBD), in a 4×2 factorial scheme, with four levels of electrical conductivity of irrigation water - ECw (0.6; 2.0; 3.4 and 5.0 dS m^{-1}), with presence and absence of zeolite, totaling 8 treatments, with four replicates. Each block had 24 experimental units, totaling 96 plants. The variables analyzed were: leaf gas exchange, relative chlorophyll index, number of initial flowers, root dry biomass, flower dry biomass, number of open flowers, shoot dry biomass and total dry biomass. Irrigation water salinity caused deleterious effects on the crop, leading to reductions in leaf gas exchange, flower production and plant growth. There was no accumulation of Na^+ in leaf tissues, suggesting that the negative effects of salinity were mainly associated with the osmotic component of salt stress. Interaction between salinity and zeolites was observed for the production of shoot and total biomass. The low accumulation of sodium in *Ixora coccinea* leaves made it impossible to conclude about a beneficial effect of zeolite on the retention of this potentially toxic ion under salt stress.

Keywords: Irrigation, ornamental plants, salinity, sodium

Introduction

High water requirements by crops, or the low quality of water sources for irrigation, may favor the use of alternative sources considered inadequate for agriculture (saline and brackish water). These water resources, however, when used improperly, can cause problems to soil structure and development of agricultural crops (Kiani & Mosavata, 2016; Cavalcante, 2021). In this respect, the use of adequate management strategies and the cultivation of tolerant species can enable the sustainable use of these water sources in irrigation (García-Caparrós & Rao, 2018; Lacerda et al., 2020).

Particularly in northeastern Brazil, characterized by the low availability of water resources, the production of ornamental species can be an alternative for the use of brackish water in irrigation, reducing competition for good quality water (Carter & Grieve, 2010), as has been suggested for other countries (Cassaniti et al., 2013; Niu et al., 2013; García-Caparrós et al., 2016). The cultivation of ornamental plants under irrigation with waters of moderate to high salinity causes restrictions on the growth and commercial quality of plants (Cai et al., 2014; Oliveira et al., 2018; Bezerra et al., 2020), and the intensity of the effect depends on the tolerance of the species to salinity. For *Ixora coccinea*, for example, Oliveira et al. (2018) identified the salinity threshold of 2.98 dS m^{-1}, value from which growth is impacted by excess salts. For these high levels of salt stress, alternatives should be sought to mitigate the deleterious effects, such as zeolites.

Vieira et al. (2014) define zeolites as microporous crystalline aluminosilicates, consisting of a three-dimensional arrangement of TO_4 tetrahedrons (SiO_4 or AlO_4^-) connected to one another to form subunits and, finally, large polymeric networks composed of identical blocks, which are called unit cells. Wen et al. (2018) point out that natural, chemically modified or synthetic zeolites

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have been used to reduce Na^+ in various types of saline water.

Considering that one of the effects of salinity on plants is related to the accumulation of potentially toxic ions, such as Na^+, it is hypothesized that zeolites can minimize this impact by increasing the retention of this ion, especially under irrigation with brackish or saline water. This can be especially relevant in potted crops, where it becomes possible to know the balance of inputs and outputs of salts from the system (Blanc, 1987) and quantify the sodium retained by zeolites and the possible positive consequences for plants.

In view of the above, the objective was to evaluate the potential of a synthetic type A zeolite in reducing the extraction of Na^+ by *Ixora coccinea* plants, mitigating the negative effects of salinity on the physiology and growth of this species.

Material and Methods

The study was carried out in a greenhouse belonging to the Federal University of Ceará (UFC), Fortaleza-CE, Brazil (03° 45' S; 38° 33' W, 20 m), from April to June 2018.

The climate of the region is Aw', tropical rainy, according to Köppen's classification, with two well-defined seasons, a drier one in winter and another with the occurrence of rains, in summer and autumn. The meteorological data inside the greenhouse were obtained with an Onset Hobo® datalogger.

The variables recorded were: average air temperature, average relative air humidity and luminosity (**Figure 1**). The maximum daily temperature inside the greenhouse ranged from 39 to 40 °C, and the relative humidity showed a daily average ranging from 60 to 63 % (Figure 1A). Figure 1B shows the daily average of luminosity data during the experimental period, which ranged from 3,789.72 to 20,207.26 Lux. The photoperiod was approximately 12 hours.

The experiment was carried out in a greenhouse and the design adopted was in randomized blocks, in a 4×2 factorial scheme, corresponding to four levels of electrical conductivity of irrigation water - ECw (0.6; 2.0; 3.4 and 5.0 dS m^{-1}) associated with the presence and absence of zeolite, with four replicates. Each replicate consisted of three pots, totaling 96 plants in the experiment (one plant per pot).

The control treatment used water from the Water and Sewage Company of Ceará – Cagece, which had pH of 6.6, ECw of 0.6 dS m^{-1} and sodium adsorption ratio (SAR) of 1.3. This water is the source for the treatment of ECw 0.6 dS m^{-1}. The saline solutions of the other treatments were prepared using well water, which had pH of 7.7, ECw of 0.96 dS m^{-1} and SAR of 3.10. The levels of electrical conductivity were obtained by the dissolution of NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ salts, in a proportion equivalent to 7:2:1, following the relationship between ECw and their concentrations ($mmol_c$ L^{-1} = ECw x 10), according to Rhoades et al. (2000).

Irrigation management was performed manually by water balance (Equation 1), in which one pot was maintained as a drainage lysimeter for each salinity level. A leaching fraction of 0.15 was added in each irrigation event to avoid excessive accumulation of salts in the root zone (Ayers & Westcot, 1999).

TIR = (VA - VD) / 0.15

Where:

TIR – Total irrigation required in ml;

VA – Volume applied to the lysimeter in ml;

VD – Volume drained in ml; and

0.15 – Leaching fraction

The volume applied was calculated from the volume drained. At the end of the experiment, the volume of water applied in all events for each salinity level was quantified, and it was expected that the volume would tend to decrease with the increase of salinity levels, justifying the difference in volumes at the

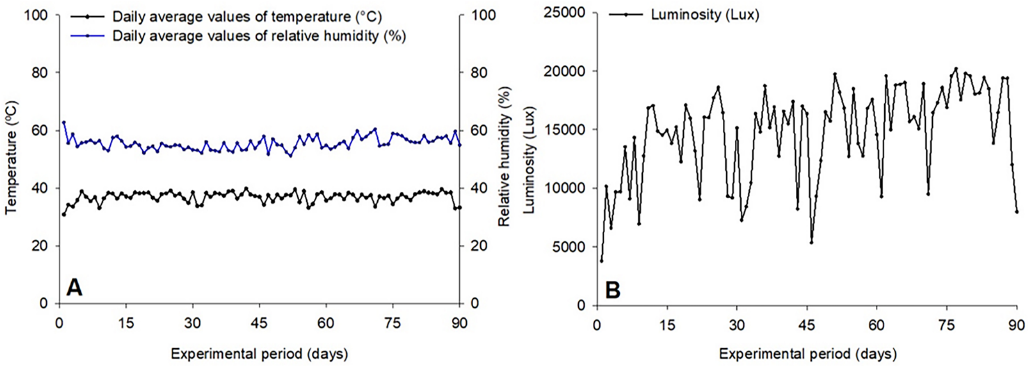

Figure 1. Daily average values of temperature and relative humidity (A), and luminosity (B).

end of the events.

At the end of the experiment, a total volume of water applied at each salinity level (24 pots) was obtained, considering 45 irrigation events, with values of: 641.64, 622.44, 459.42 and 440.40 L for 0.6, 2.0, 3.4 and 5.0 dS m^{-1}, respectively.

The zeolite used was the synthetic type A obtained from kaolin, using the hydrothermal method (Chandrasekhar & Pramada, 2001). The material was subjected to the ion exchange process in a laboratory with sulfuric acid and distilled water and subsequently kept at temperature of 80 °C for 8 hours in the stirring process, using a volumetric flask. In the next step, the solid was washed with deionized water (three times) and subjected to a decanting process. After collecting the supernatant, the material was dried in an oven for 8 hours at 60 °C.

I. coccinea L. seedlings with 30 days after emergence were acquired from a qualified seedling supplier, registered with the Agriculture Ministry. The seedlings were acclimated for ten days in a greenhouse, a definitive site for conducting the experiment with the following characteristics: galvanized metal structure, of trellis arch type, with 3.50 m of ridge height and 2.50 m of ceiling height, 6.40 m wide and 12.50 m long. The cover consists of 0.15-mm-thick low-density polyethylene films, with 80% transparency to solar radiation. The seedlings were irrigated with non-saline water to ensure their establishment. After acclimation, they were transplanted to pots with capacity of 7 liters.

First, the pots were filled with a 2- to 3-cm-thick layer of crushed stone (n. 0), to facilitate drainage. After the crushed stone layer, the substrate composed of a mixture of soil, *arisco*(sandy material with light texture normally used in constructions in Northeast Brazil) and earthworm humus, in the ratio of 6:3:1, respectively, was added.

The proportions were defined from practical observations and by the need for a substrate with more cohesive particles, in order to allow the retention of zeolite and avoid its leaching during irrigation events. The soil used to fill the pots was an *ARGISSOLO AMARELO Eutrocoeso típico* (ULTISOL), characterized mainly by the presence of textural B horizon. This soil was collected in the experimental area of the Hydraulics and Irrigation Laboratory of the Agricultural Engineering Department of UFC, Pici Campus.

The zeolite used was incorporated and mixed into the substrate, using 40 g per pot. This amount was defined as a start due to the scarcity of information on the use of zeolites in ornamental plants.

After transplanting, the plants were irrigated with non-saline water for 10 days to ensure their establishment. After this period, the plants were manually irrigated with the waters of four different salt concentrations. The trial lasted 90 days from the beginning of the application of the treatments.

At 30 and 60 days after application of the treatments, each pot received 1 g of the 10-10-10 (N-P-K) formulation (Simões et al., 2002).

At the end of the experimental trial, evaluations of leaf gas exchange were performed. Photosynthesis, transpiration, stomatal conductance and internal CO_2 concentration readings were performed on fully expanded leaves with a portable infrared gas analyzer (Li-6400XT model, LiCor, USA). Measurements were performed between 08:00 and 10:00 h, under ambient conditions of temperature, relative humidity and CO_2 concentration. The light intensity used in gas exchange measurements was 1,600 μmol m^{-2} s^{-1}.

The chlorophyll index and number of flowers (initial and open) were also obtained at the end of the experiment, determined in the same leaves used for measuring gas exchange, with the aid of a portable meter (SPAD 502, Minolta Co, Ltd, Osaka, Japan), and the results were expressed in the reading unit of the device (SPAD units).

Then, the plants were harvested and, after drying in an oven, the values of dry biomass of branches, leaves and flowers were determined. Samples of mature leaves were dried and ground to determine the Na^+ and K^+ contents and the Na^+/K^+ ratio.

The data were subjected to analysis of variance by the F test ($p < 0.05$). When a significant effect was verified, Tukey test was used to compare the means. In the regression analysis, the equations that best fitted the data were selected, based on the highest coefficient of determination (R^2) and significance. The software programs SISVAR® version 5.3 (Ferreira, 2010) and Microsoft Excel (2016) were used.

Results and Discussion

The variables net photosynthesis (A), stomatal conductance (gs), transpiration (E) and internal CO_2 concentration (Ci) (**Table 1**) were significantly influenced by the irrigation water salinity factor (F_C = 25.70; F_C = 55.37; F_C = 44.91; and F_C = 20.92 with $P < 0.05$, respectively), with no significant single effect of the treatment with zeolites or the interaction between the tested factors.

For the photosynthesis variable, a decreasing linear response was verified, with a reduction of 0.79 μmol

Table 1 – Summary of the analysis of variance for photosynthesis (A), stomatal conductance (gs), transpiration (E) and internal CO_2 concentration (Ci) of the ornamental species, subjected to irrigation with saline water with presence and absence of zeolites

Source of variation	DF	Mean squares			
		A	gs	E	Ci
Blocks	3	2.817^{ns}	0.00026^{ns}	1.446^{**}	2542.93^{**}
Salinity (S)	3	36.30^{**}	0.01341^{**}	2.860^{**}	2364.64^{**}
Zeolite (Z)	1	0.108^{ns}	0.00001^{ns}	0.002^{ns}	118.26^{ns}
Interaction (SxZ)	3	1.041^{ns}	0.0001^{ns}	0.062^{ns}	4.7656^{ns}
Residuals	53	1.4128	0.00024	0.064	113.0439
Total	63	-	-	-	-
CV	-	15.30	15.58	11.41	4.77
With (Z)	31	7.809a	0.099a	2.22a	221.56a
Without (Z)	31	7.727a	0.100a	2.21a	224.28a
Total	62	-	-	-	-

ns, ** and *: not significant and significant at 1 and 5% probability levels by the F test, respectively, C V – coefficient of variation. DF - degrees of freedom. Source: Prepared by the author

of $CO_2 m^{-2} s^{-1}$ per unit increment of electrical conductivity and a total reduction of 37.2%, between the highest and lowest salinity level (**Figure 2**A). The reduction of photosynthetic rates is due to the osmotic effect caused by the concentration of soluble salts in the soil solution, that is, plants tend to reduce water loss by partially closing their stomata, which prevents the reduction of water potential, leading to reduction in the entry of CO_2 in the leaves (Munns & Tester, 2008; Álvarez & Sánchez-Blanco, 2015; Lacerda et al., 2020).

Hirich et al. (2014), working with quinoa, and Neves et al. (2018), working with *Catharanthus roseus, both using* irrigation with saline water, observed significant reductions in the photosynthetic rates of the evaluated crops. As previously stated, salinity can reduce photosynthesis through the osmotic effect, which causes a reduction in water absorption by the plant, leading to stomatal closure and reducing CO_2 fixation, causing excessive reduction in the activity of the photosynthetic electron chain (Munns & Tester, 2008; Taiz et al., 2017; Dias et al., 2016).

Reduction in stomatal conductance (Figure 2B) and lower internal CO_2 concentration (Figure 2D) are in line with the negative effects observed on photosynthetic rates. For stomatal conductance, there was a linear reduction of 0.015 mol $m^{-2} s^{-1}$ per unit increment in ECw and a total reduction of 51.2% at the highest salinity level compared to the control treatment. The results indicate that the plant needed to reduce the loss of water to the atmosphere, hence reducing the flow of water in the system and stomatal opening.

The internal CO_2 concentration decreased by 5.77 µmol mol^{-1} per unit increment in ECw, with a total reduction of 10.87% between the treatments of highest and lowest salinity level. This reduction partially explains the lower values observed for the photosynthetic rate with the increase in irrigation water salinity (Figure 2A), confirming the stomatal limitations induced by salt stress.

The transpiration variable (Figure 2C) showed linear decreases with increasing salinity of irrigation water, with a total reduction of 38.46% at the highest salinity level compared to the treatment of lowest salinity. The observed reduction was 0.21 mmol $m^{-2} s^{-1}$ per unit increment of irrigation water salinity. This behavior results in lower water losses by plants, associated with the decrease in stomatal conductance, resulting from the intensification of osmotic stress.

The limitation of the photosynthetic rate can occur mainly through stomatal closure (Figure 2B), and stomatal closure is the main way for the plant to decrease water loss under salt stress (Munns & Tester, 2008; Lacerda et al., 2020).

The results of total chlorophyll content obtained using the SPAD Index, shown in **Table 2**, indicate that there was no significant effect of the interaction, saline water × zeolite (S × Z), or single effect of zeolite. For the single effect of saline water, it was significantly influenced by the F test ($p \leq 0.05$), since the responses varied according to the increase in salt concentration and intensified with the time of salt stress.

For the RCI, as observed for the other physiological variables, there was a decreasing linear response as a function of the increase in irrigation water salinity, with a reduction of 1.69 per unit increment of salinity and a total reduction, considering the limits of the treatments, of 12.13% (Figure 2E). The deterioration of the membrane due to salt stress can cause a decrease in chlorophyll concentration, and the phenomenon is commonly reported and used as a sensitive indicator of cellular metabolic status (Silveira & Carvalho, 2016).

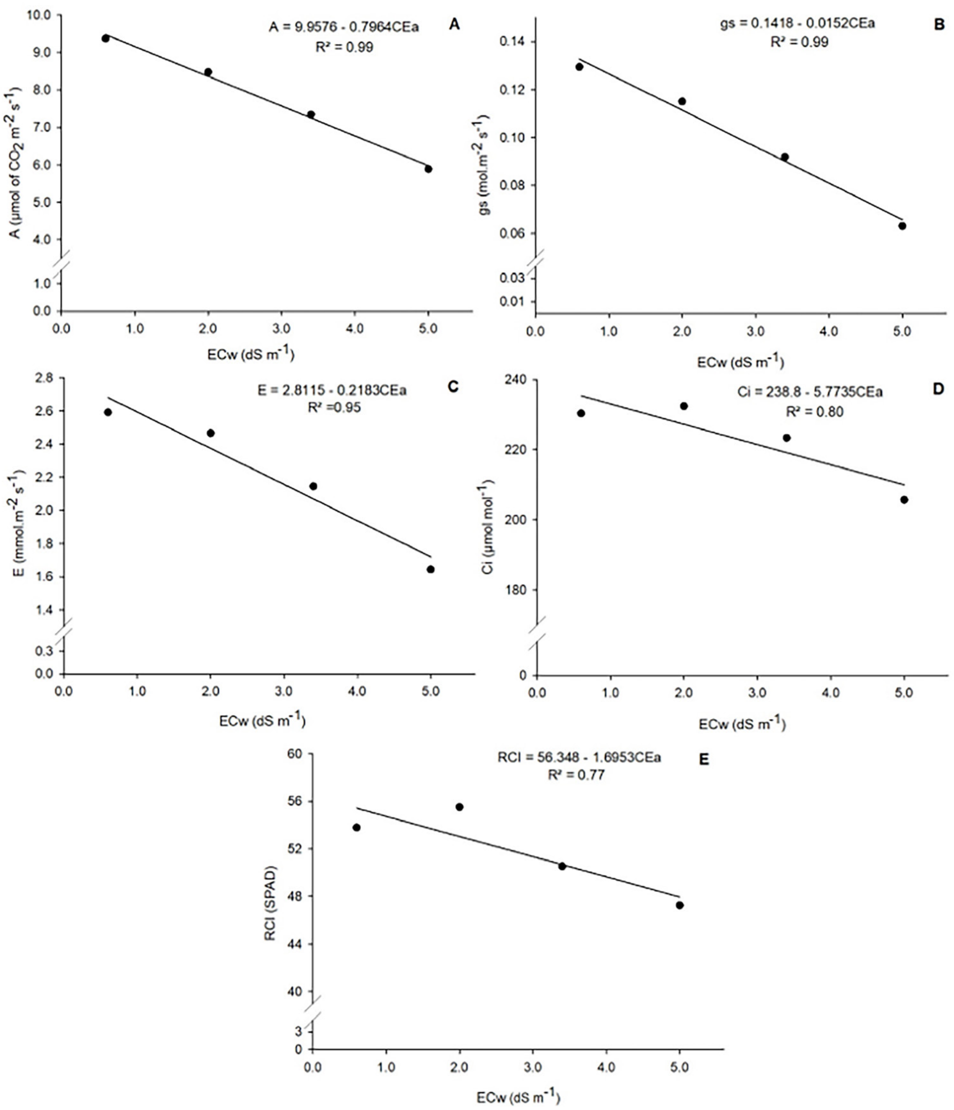

Figure 2. Net photosynthesis (A), stomatal conductance (B), transpiration (C), internal CO_2 concentration (D) and relative chlorophyll index (E) in leaves of *Ixora coccinea* subjected to different levels of irrigation water salinity.

Table 2 – Summary of the analysis of variance for the total chlorophyll values measured through the SPAD index in an ornamental plant species, cultivated with saline water with presence and absence of zeolite

Source of variation	DF	Mean squares
		SPAD index
Blocks	3	90.392^{ns}
Salinity (S)	3	318.889*
Zeolite (Z)	1	10.075^{ns}
Interaction (SxZ)	3	60.982^{ns}
Residuals	85	101.704
Total	95	-
CV	-	19.48
With (Z)	47	51.45^{a}
Without (Z)	47	52.10^{a}
Total	94	-

ns, ** and *: not significant and significant at 1 and 5% probability levels by the F test, respectively, CV – coefficient of variation. DF - degrees of freedom; Source: Prepared by the author.

Flower dry biomass (FDB) and root dry biomass (RDB) responded to the single effects of irrigation water salinity (F_C = 8.51 and F_C = 10.52 with $p < 0.05$, respectively). Regarding the interaction between the tested factors, there was a significant effect for shoot dry biomass (SDB) and total dry biomass (TDB) (Fc = 2.94 and Fc = 4.39 with $p < 0.05$, respectively) (**Table 3**).

The number of initial flowers (**Figure 3**A) and flower dry biomass (Figure 3B) showed linear decreases as salinity increased, with relative losses of 63.9 and 52.2% at the highest salinity level compared to the control treatment, respectively. As discussed earlier, the use of brackish water in plant cultivation can restrict plant growth, amount of biomass and yield, due to the osmotic effects, specific ions and nutritional imbalance (Munns & Tester, 2008; Taiz et al., 2017; Dias et al., 2016).

Table 3 – Summary of the analysis of variance for flower dry biomass (FDB), root dry biomass (RDB), shoot dry biomass (SDB) and total dry biomass (TDB) of an ornamental plant species, cultivates with saline water with the presence and absence of zeolite

Source of variation	DF	Mean squares			
		FDB	RDB	SDB	TDB
Blocks	3	0.8747^{ns}	11.1197^{ns}	80.4620^{**}	146.5542^{**}
Salinity (S)	3	8.5106^{**}	115.8948^{**}	507.7641^{**}	1079.1600^{**}
Zeolite (Z)	1	3.1212^{ns}	184.8705^{**}	31.9358^{ns}	370.4811^{**}
Interaction (SxZ)	3	0.5648^{ns}	26.5007^{ns}	58.3580^{*}	146.3215^{**}
Residual	85	1.6506	11.0223	19.8254	33.3233
Total	95	-	-	-	-
CV	-	88.04	22.40	14.68	12.79
With (Z)	47	1.278a	13.43b	29.75a	43.18b
Without (Z)	47	1.639a	16.21a	30.90a	47.11a
Total	94	-	-	-	-

ns, ** and *: not significant and significant at 1 and 5% probability levels by the F test, respectively, CV – coefficient of variation. DF - degrees of freedom: Source: Prepared by the author.

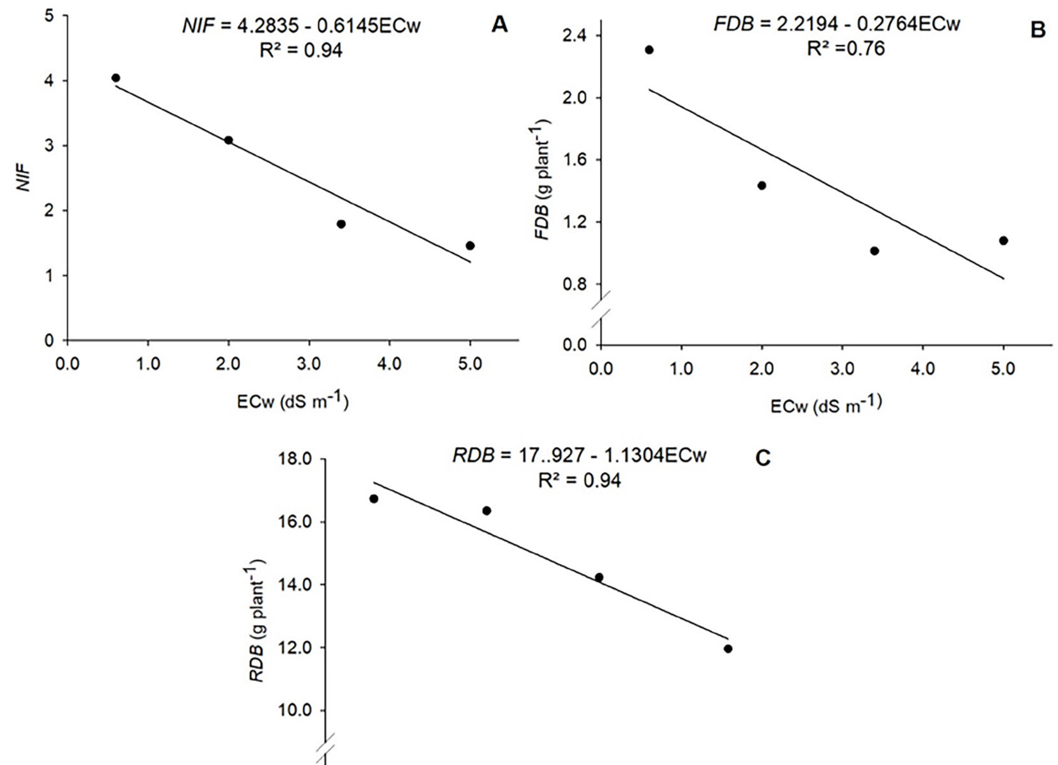

Figure 3. Number of initial flowers (A), flower dry biomass (B) and root dry biomass of *Ixora coccinea* plants subjected to different levels of irrigation water salinity.

Bezerra et al. (2020) also observed a reduction in the number of leaves and shoot dry biomass with the supply of brackish water. However, it must be pointed out that other authors have observed increases in flower production in some ornamental species under moderate levels of salinity (Neves et al., 2018; Oliveira et al., 2018), results that differ from those found in the present study.

Regarding the response of root dry biomass to salt stress (Figure 3C), there was an average reduction of 1.13 g plant^{-1} per unit increment in ECw and a total reduction of 28.5%, comparing the extreme salinity levels. Root growth in general is inhibited by the exposure to high salinity, as a result of the osmotic and toxic effects of salts (Bañón et al., 2012).

Roots are the most vulnerable part of the plant when exposed to salt stress, and as a result, the water absorption capacity and water use efficiency, among other parameters, can be compromised (Sánchez-Blanco et al., 2014).

Significant single effects of the zeolite factor were observed for NOF and RDB. The number of open flowers (NOF) and root dry biomass (RDB) responded individually to the zeolite factor (F_C = 6.30 and F_C = 16.77 with $p <$ 0.05, respectively). It can be noted that the application of zeolites induced a reduction (40.98%) in the values of NOF (**Figure 4**A). Thus, it can be affirmed that the use of zeolite for the studied species caused negative effects on a relevant variable from the commercial point of view.

This result may be related to a change in plant physiology. It is suggested that such alterations may be associated with the attenuation or intensification of water stress, caused by the greater availability of Na^+ in the cultivation medium, which may delay the flowering process. In addition, instead of zeolites adsorbing potentially harmful cation ions, they may have adsorbed other cations, such as K^+ and Ca^{2+} for example, limiting plant development.

Regarding the RDB variable, it was found that the application of zeolites led to the lowest values when compared to the treatment without zeolite, with a reduction of 17.28% in the root system biomass (Figure 4B).

The variables SDB (**Figure 5**A) and TDB (Figure 5B) were significantly influenced by the interaction between salinity and zeolite factors. For the SDB variable, salinity caused linear reductions of 1.69 and 3.15 g plant^{-1} for each increment in electrical conductivity, for treatments with and without zeolites, respectively. Similar results were observed for total dry biomass (Figure 5B).

Although there are no reports in the literature on the interaction between salinity and zeolite application in plants under salt stress, the biomass data of the present study indicate benefits of zeolite, notably with the increment in salinity, as the rates of reduction in the treatment with zeolite were lower and the curves tended to intersect at higher levels of salinity (Figure 5A and 5B).

The benefits of adding zeolites in crops under salt stress are associated with sodium retention and maintenance of low values of the Na^+/K^+ ratio, reducing toxicity caused by high salinity levels. The ratio between sodium and potassium concentration in the cytosol is considered an indicator for plant tolerance to salt stress (Sun et al., 2010).

The reduction in Na^+ absorption associated with low Na^+/K^+ ratio has been used as an important parameter in the selection of salt stress-tolerant wheat genotypes (Munns et at., 2002). In the present study, however, the salinity levels applied did not influence these variables (**Table 4**), and low levels of Na^+ were observed in the leaves. This result may explain the difficulty of observing the beneficial effects of zeolites on *I. coccinea* and suggests the need for other studies with species that are more sensitive and have higher sodium contents in leaf tissues (Lacerda et al., 2020).

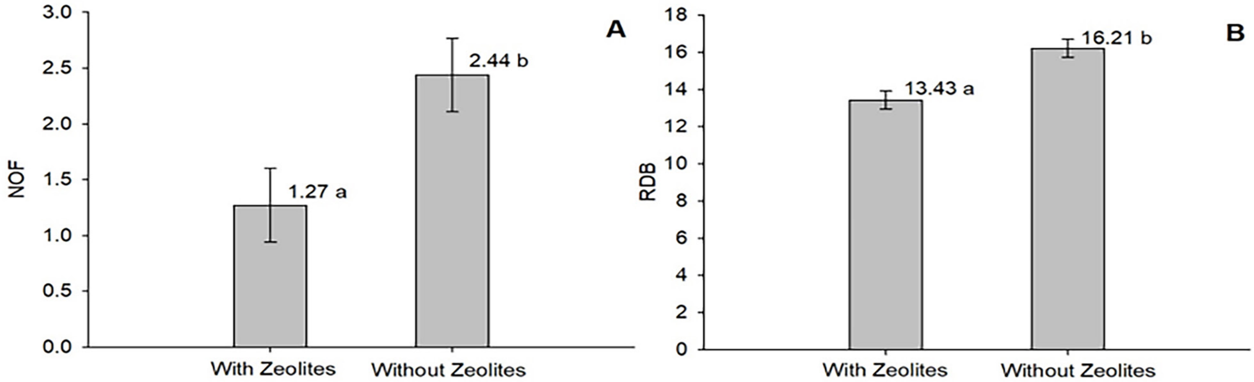

Figure 4. Number of open flowers (A) and root dry biomass (B) in *Ixora coccinea* plants with and without application of zeolites. Standard error for 48 observations.

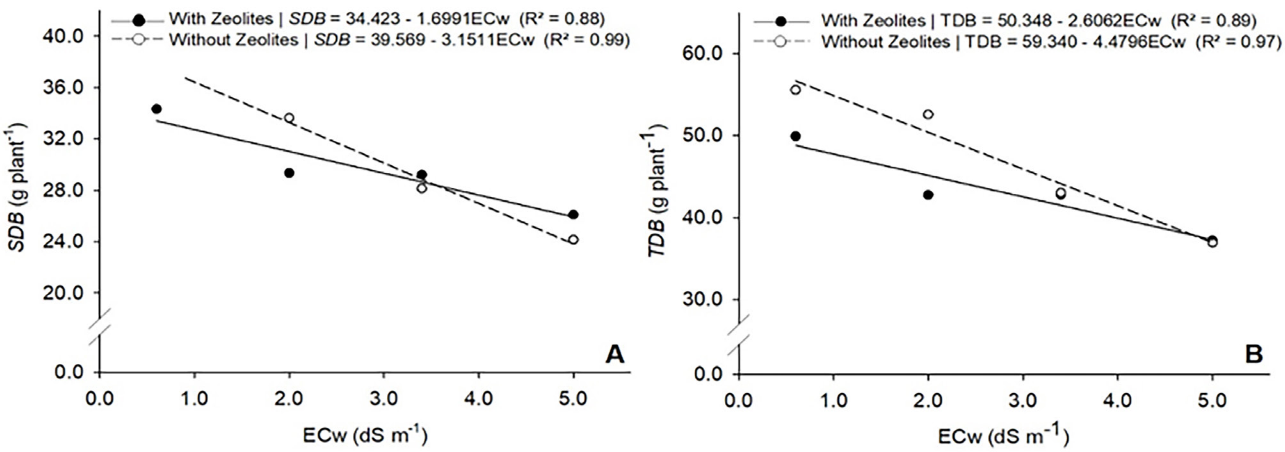

Figure 5. Shoot dry biomass (A) and total dry biomass (B) in *Ixora coccinea* plants under different levels of irrigation water salinity, with and without application of zeolites.

Table 4 – Summary of the analysis of variance for sodium and potassium contents, in g kg $^{-1}$, and Na^+/K^+ ratio in leaves of *Ixora coccinea* cultivated with saline water, in the presence and absence of zeolite

Sources of variation	DF	Mean squares		
		Na^+	K^+	Na^+/K^+
Blocks	3	1.5813^{ns}	0.68714^{ns}	0.0622^{ns}
Salinity (S)	3	0.2801^{ns}	3.22883^{ns}	0.2790^{ns}
Zeolite (Z)	1	0.8005^{ns}	2.40561^{ns}	0.0329^{ns}
Interaction (SxZ)	3	0.3962^{ns}	0.32256^{ns}	0.0723^{ns}
Residuals	21	0.9459	4.02069	0.09977
Total	31	-	-	-
CV	-	23.08	25.89	52.91
With (Z)	15	4.0565a	7.4703a	0.628a
Without (Z)	15	4.3728a	8.0186a	0.564a
Total	30	-	-	-

Conclusions

No interactions were observed between salinity and zeolite application for the physiological and growth variables, except for shoot and total dry biomass production, when better results were observed for treatment with zeolites at the highest salinity levels. However, the application of zeolites reduced root biomass production and the number of open flowers, negatively impacting the visual quality of plants. *Ixora coccinea* plants showed low sodium accumulation in the leaves, which made it impossible to conclude about some beneficial effect of zeolite in the retention of this potentially toxic ion under salt stress.

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