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SILICON IMPROVES SEEDLING PRODUCTION OF *MORINGA OLEIFERA* LAM. UNDER SALINE STRESS

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Abstract

Salinity is one of the most common environmental stresses, particularly in agricultural areas. This condition affects growth and development of crops, since it causes physiological, biochemical and morphological changes. Different techniques have been proposed to mitigate crops salinity damage, including application of silicon (Si). The objective of the present work was to evaluate the effect of salinity, Si and its interaction, in emergence, growth, biomass and ion concentration of moringa seedlings (*Moringa oleifera* Lam.). The experiment was set up in a completely randomized design with a factorial arrangement 3*3 and three replications per treatment. Seedlings were developed in nine nutritive solutions derived from combination of three electric conductivities (2.5, 6.0 and 9.5 dS/m by adding sodium chloride) and three Si concentrations (0, 50 and 100 mg/L by adding potassium silicate). Results showed that salinity reduced emergence, growth, biomass and concentration of K and Mg, and increased concentration of Zn, Cu and Mn in root, similarly reduced concentration of Mg and increased concentration of Na in stem and leaves. Si increased growth of aerial part, biomass and Ca concentration in root, meanwhile increased Mg concentration and reduced Na concentration in leaves. Salinity-Si interaction was found in root were Si reduced Na concentration and increased Fe concentration, both in high salinity. Similarly, Si increased K, Ca, Cu and Fe leaves concentrations when EC was high. These results suggest that Si should be considered as an alternative to reduce the effects of salinity in plants.

Key words: Emergence, Growth, Nutrients, Potassium silicate, Sodium chloride.

Introduction

Salinity affects the productivity of soils since the beginning of agriculture. In the world, more than 7% of the land and more than 20% of the available agricultural areas are affected by salinity, with arid and semi-arid zones being the most disturbed (Gupta & Huang, 2014; Rasool *et al.,* 2013). The term salinity refers to the presence of high salt concentration in soil that harms plants due to its osmotic and toxic effects. This condition modifies the osmotic process and cellular hydration. High salt concentrations in the growth medium solution limit the water available to the plant (Yin *et al.,* 2013, Chen *et al.,* 2014). In the process of ionic absorption, the high concentration of some ions hinders the absorption of essential nutrients for the plant, because in active absorption they interfere with the transporters of the plasma membrane of the root, decreasing the levels of nutrients such as potassium (K), magnesium (Mg), zinc (Zn), and iron (Fe), in addition to the high ionic concentration also hinders passive absorption, affecting the absorption of calcium (Ca), which entails a negative impact on plant growth and physiological activity (Batool *et al.,* 2015). These modifications cause a negative impact on the growth and physiological activity of the plants.

Silicon (Si) is one of the most abundant elements in nature. Si is found in nature as silicon dioxide (SiO_2) or silicates that chemically bond with various metals due to its strong affinity with oxygen. This element is beneficial for most of the plants and only essential for some plants (Artyszak *et al.,* 2014; Savvas & Ntatsi, 2015). Si has a beneficial effect on plants under biotic and abiotic stress, since it generates a significant increase in growth, suggesting a potential use in agriculture (Balakhnina & Borkowska, 2013; Guntzer *et al.,* 2012; Imtiaz *et al.,* 2016; Yan *et al.,* 2018).

Moringa (*Moringa oleifera* Lam.) is the most widespread species of the Moringaceae family. The origin of this specie is located in Pakistan and India and its exploitation has extended to most tropical countries. Among the many uses of this plant are food, medicines, fodder and biodiesel production (Goss, 2012; Castillo-Lopez *et al.,* 2017). Moringa can be produced in different soils, including those that contain moderate amounts of soluble salts, because it is considered to be moderately tolerant to salinity (Oliveira *et al.,* 2009).

The objective of the present work was to evaluate the effect of salinity, Si and the interaction between them, in the emergence, growth, biomass and nutritional concentration of moringa seedlings.

Material and Methods

Plant material, growth conditions and treatments: Seeds of *Moringa oleifera* Lam. were planted on polystyrene propagation trays, using peat moss as substrate. The trial was conducted during the summer of 2016, in a greenhouse at the Universidad Autonoma de Nuevo Leon, Mexico. A completely randomized design was used, with a treatment factorial arrangement (3*3) and three replications with thirty-five seedlings each. Treatments were a combination of three levels of salinity (electrical conductivity (EC) of 2.5, 6.0 and 9.5 dS/m), adjusted with sodium chloride (NaCl) and three levels of

Si (0, 50 and 100 mg/L), applied by means of potassium silicate (K_2SiO_3). All plants were irrigated with a nutrient solution (N: 105, P: 21, K: 236, Ca: 100, Mg: 50, S: 75, Fe: 1, Mn: 0.5, B: 0.2, Zn: 0.2, Cu: 0.05 and Mo: 0.05 mg/L, pH 5.5-6). Irrigation was applied in a daily frequency regime, with a volume of 100 ml per tray, during the first 10 days after sowing; from day 11 to 20 the volume was increased to 200 ml per tray and from day 21 to 30 volume was increased to 300 ml per tray.

Variables: Variables were classified into four aspects:

1) Emergence: a) Emergence percentage (number of emerged plants / number of planted seeds), b) Days to beginning emergence (number of days from planting to first emerged plant), c) Days to full emergence (number of days from planting to day of last plant emerged), d) Days to maximum emergence (number of days from planting to day in which more plants emerged), e) Uniformity (average of plants emerged per day), and f) Survival rate (number of plants at day 30 / number of emerged plants) (data were collected every day using the ISTA (2012) methodology during the first 15 days, except for survival).

2) Growth: g) Seedling height, h) Stem diameter, i) Root length, j) Number of leaves and k) Leaf area, using ImageJ software (http://rsb.info.nih.gov/ij/) (average of 15 plants).

3) Biomass: l) Root, m) Stem and n) Leaf dry weight.

4) Nutritional concentration (root, stem and leaf) of: o) Potassium (K), p) Calcium (Ca), q) Magnesium (Mg), r) Iron (Fe), s) Manganese (Mn), t) Zinc (Zn), u) Copper (Cu) and v) Sodium (Na). Seedlings were washed with deionized water, and separated into roots, stem and leaves; organs were stored in paper bags and placed in a forced convection oven at 60 °C for 48 h; subsequently, samples were ground and sieved with a 0.5 mm mesh. Samples were digested in a muffle at 450 to 500 °C for 6 h. Analysis of the nutrient concentration were conducted by means of atomic absorption spectroscopy (UNICAM Solar®, model 9626).

Data analyses: Variables with percentage values were transformed by arcsine, while the values obtained by counting were transformed by square root. Data were subject to analysis of variance using the Statistical Package for Social Science (SPSS) V22.0 (IBM Corp, 2013). In the variables of emergence, growth, biomass and ion concentration, means were compared using the Tukey test ($p \leq 0.05$) when significant differences were observed in the analysis of variance.

Results

Effects of salinity and Si on seed emergence: Salinity had a negative effect on most of the emergence variables. Percentage of emergence decreased 2.9% and 81% when EC changed from 2.5 to 6.0 and 9.5 dS/m, respectively. Uniformity and survival also showed similar trends to percent emergence, decrease of these variables was less at lower level of salinity than at higher levels, with 0.0 survival at EC of 9.5 dS/m. Days to beginning emergence and days to maximum emergence increased as salinity increased; days of full emergence were similar at different salinity levels. The levels of Si application showed a positive effect on survival, particularly the solution with 50 mg/L, which increased plant survival by 23% (Table 1).

Effects of salinity and Si on growth and biomass: Salinity reduced growth and biomass. EC of 6.0 dS/m reduced 49% plant height, 7% stem diameter, 47% leaves number, 81% leaves area, 66% root dry weight, 55% stem dry weight, and 77% leaves dry weight (Table 2). On the other hand, application of Si had positive effect on leaves growth; Si had positive effects on leaf number, increasing by 23% when the concentration of Si in the nutrient solution was 50 mg/L and for leaves area, the concentration of 100 mg/L of Si increased this variable by 73%. The biomass produced was benefited by the presence of Si. Root dry weight increased 46% and stem dry weight increased 23% when Si concentrations was of 100 mg/L. Leaves dry weight increased 35% when Si concentration was 50 mg/L (Table 2).

Table 1. Effect of EC and Si in the emergence of *M. oleifera* Lam.

	E[1] %	DBE	DFE	MED	U	S%
EC (dS/m)						
2.5	90.79 a	4.56 c	6.56	6.33 b	5.44 a	85.48 a
6.0	87.94 a	5.89 b	6.22	7.22 ab	5.09 a	49.92 b
9.5	17.46 b	7.11 a	6.56	8.33 a	0.97 b	00.00 c
± SE	2.37	0.22	0.54	0.32	0.41	2.47
p	0.000	0.000	ns	0.001	0.000	0.000
Si (mg/L)						
0	64.76	5.89	7.11	7.44	3.30	39.17 b
50	66.98	5.67	6.78	7.11	3.89	48.35 a
100	64.44	6.000	5.44	7.33	0.31	47.88 ab
± SE	2.37	0.22	0.54	0.32	0.41	2.47
p	ns	ns	ns	ns	ns	0.028

[1)] E: Percentage emergence; DBE: Day of beginning emergence; DFE: Day of full emergence; MED: Maximum emergence day; U: Uniformity; S: Survival. Data are shown as mean ± SE (Standard error). Different letters mean significant difference.

Table 2. Effect of EC and Si on the growth and biomass of seedlings *M. oleifera* Lam.

	PH[1] cm	SD mm	RL cm	NL	LA cm^2	RDW	SDW	LDW
EC (dS/m)								
2.5	17.89 a	3.13 a	7.00	6.38 a	36.30 a	79.50 a	91.43 a	105.41 a
6.0	9.06 b	2.94 b	6.53	3.44 b	6.80 b	27.10 b	41.33 b	23.89 b
± SE	0.42	0.04	0.18	0.17	1.03	2.08	1.59	1.91
p	0.000	0.009	ns	0.000	0.000	0.000	0.000	0.000
Si (mg/L)								
0	12.71	2.97	6.56	4.20 b	15.52 b	45.00 b	60.39 b	53.17 b
50	13.84	3.01	6.90	5.30 a	22.25 a	49.10 b	64.52 b	71.57 a
100	13.87	3.13	6.84	5.23 a	26.90 a	65.80 a	74.23 a	69.20 a
± SE	0.51	0.05	0.22	0.21	1.26	2.55	1.95	2.34
p	ns	ns	ns	0.005	0.000	0.000	0.001	0.000

[1)] PH: Plant height; SD: Stem diameter; RL: Root length; NL: Number of leaves; LA: Leaf area; RDW: Root dry weight SDW: Stem dry weight; LDW: Leaf dry weight. Data are shown as mean ± SE (Standard error). Different letters mean significant difference.

Table 3. Effects of EC and Si in ion concentration in root of seedlings *M. oleifera* Lam.

	K	Ca	Mg	Zn	Cu	Mn
	mg/kg DW[1]					
EC (dS/m)						
2.5	15548.30 a	3966.79	4356.08 a	281.02 b	12.95 b	137.97 b
6.0	11077.66 b	3507.23	3304.06 b	545.26 a	17.55 a	178.51 a
± SE	525.80	203.68	129.93	16.19	0.86	8.47
p	0.000	ns	0.000	0.000	0.003	0.005
Si (mg/L)						
0	12826.42	3833.95 ab	3957.37	395.51	14.59	143.34
50	14602.04	4353.93 a	4044.45	442.55	15.80	179.07
100	12510.41	3023.16 b	3488.39	401.36	15.56	152.36
± SE	643.96	249.46	159.14	19.83	1.05	10.37
p	ns	0.009	ns	ns	ns	ns

[1)] DW: Dry weight. Data are shown as mean ± SE (Standard error). Different letters mean significant difference.

Effects of salinity and Si on plant ion concentration

Root ion concentration: Salinity had effect on root ion concentration. K and Mg decreased 29% and 24%, respectively, as salinity increased from 2.5 to 6.0 dS/m. Zn, Cu and Mn increased 94%, 35%, and 30%, respectively, as salinity increased from 2.5 to 6.0 dS/m. Ca was not affected by salinity. Si did not have effect on ion concentration, except for Ca. Statistical analyses showed that Si concentrations impacted root calcium concentration differently but were not significantly different from the control (Table 3). Analysis of variance showed interaction between CE and Si for Na and Fe. For Na, Si had not effect on Na concentrations at low salinity, however at high salinity (6.0 dS/m) the presence of Si in the nutrient solution reduced Na concentrations in the root by 12 and 10%, when the Si concentration was 50 and 100 mg/L, respectively. For root Fe concentration, at low EC, the concentration of 50 mg/L Si increased Fe concentration by 6%. At high EC the presence of Si in solution, increased the Fe concentration by 24% (Fig. 1).

Stem ion concentration: Salinity had effect on Ca, Mg and Na stem concentrations. Ca and Mg were reduced in 12% and 15%, respectively. While Na increased due to salinity in 108%. Si increased concentration of Mg in the stem by 23% when the nutrient solution contained 50 mg/L (Table 4).

Leaves ion concentration: The concentration of Mg, Na, Zn and Mn was affected by salinity and Si, but no interactions between these factors were found. Mg concentration decreased due to salinity in 48% and increased due to Si application in 33%. Na concentration increased by 189% when the nutrient solution had an EC of 6 dS/m, compared to an EC of 2.5 dS/m. Contrary to the foregoing, Si decreased Na concentration at all levels of salinity, average in 11%. Salinity had effect on Zn leaves concentration, with higher concentrations observed at high salinity. Zn concentration was not affected by Si. Mn concentration was not affected by salinity, but Si had effect on Mn leaves concentration, with higher concentration at 50 mg/L (Table 5). Statistical analysis showed an interaction of CE x Si for K, Ca, Cu and Fe leaves concentrations. At 2.5 dS/m salinity, K and Ca concentrations did not have statistical differences among Si levels, however, at 6.0 dS/m, 50 mg/L of Si increased its concentration in 51 and 84%, respectively. Cu concentration increased as Si concentration increased, however for 2.5 dS/m the maximum concentration was obtained at 50 mg/L, while for 6.0 dS/m the maximum Cu concentration was obtained at 100 mg/L. Fe concentration increased with the application of 50 mg/L of Si, at both EC levels. The greatest increase was observed at EC 6 dS/m, increasing the Fe concentration in the leaves by 126% (Fig. 2).

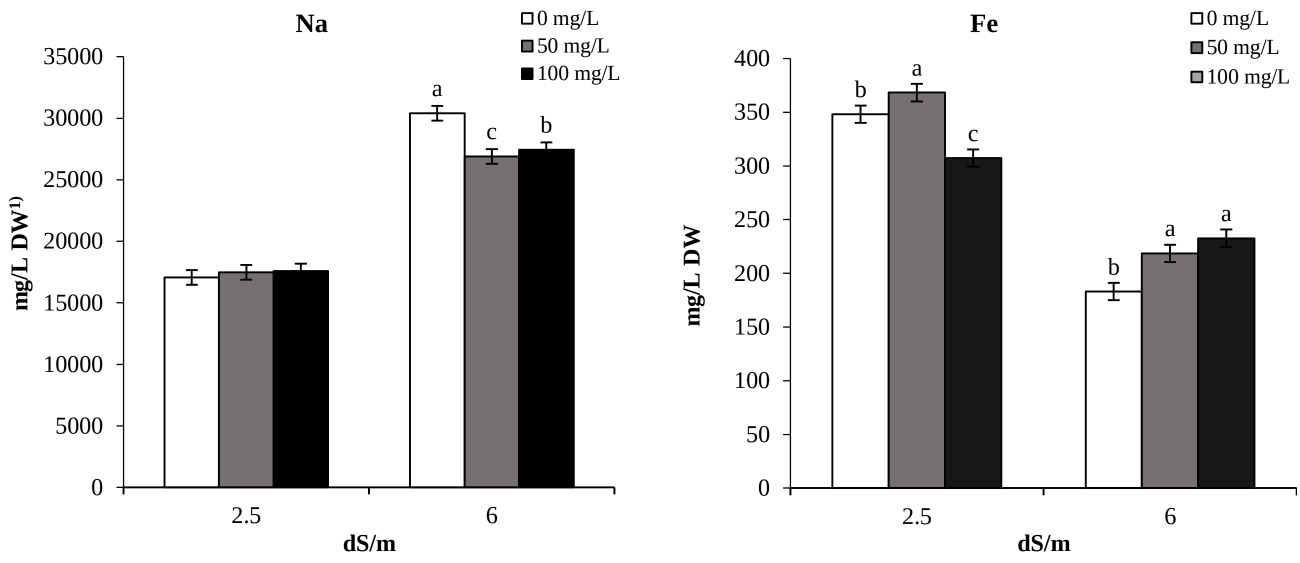

Fig. 1. Effects of interaction between EC and Si in ion concentration in root. [1)] DW: Dry weight. Data are shown as mean ± SE (Standard error). Different letters mean significant difference at p<0.05.

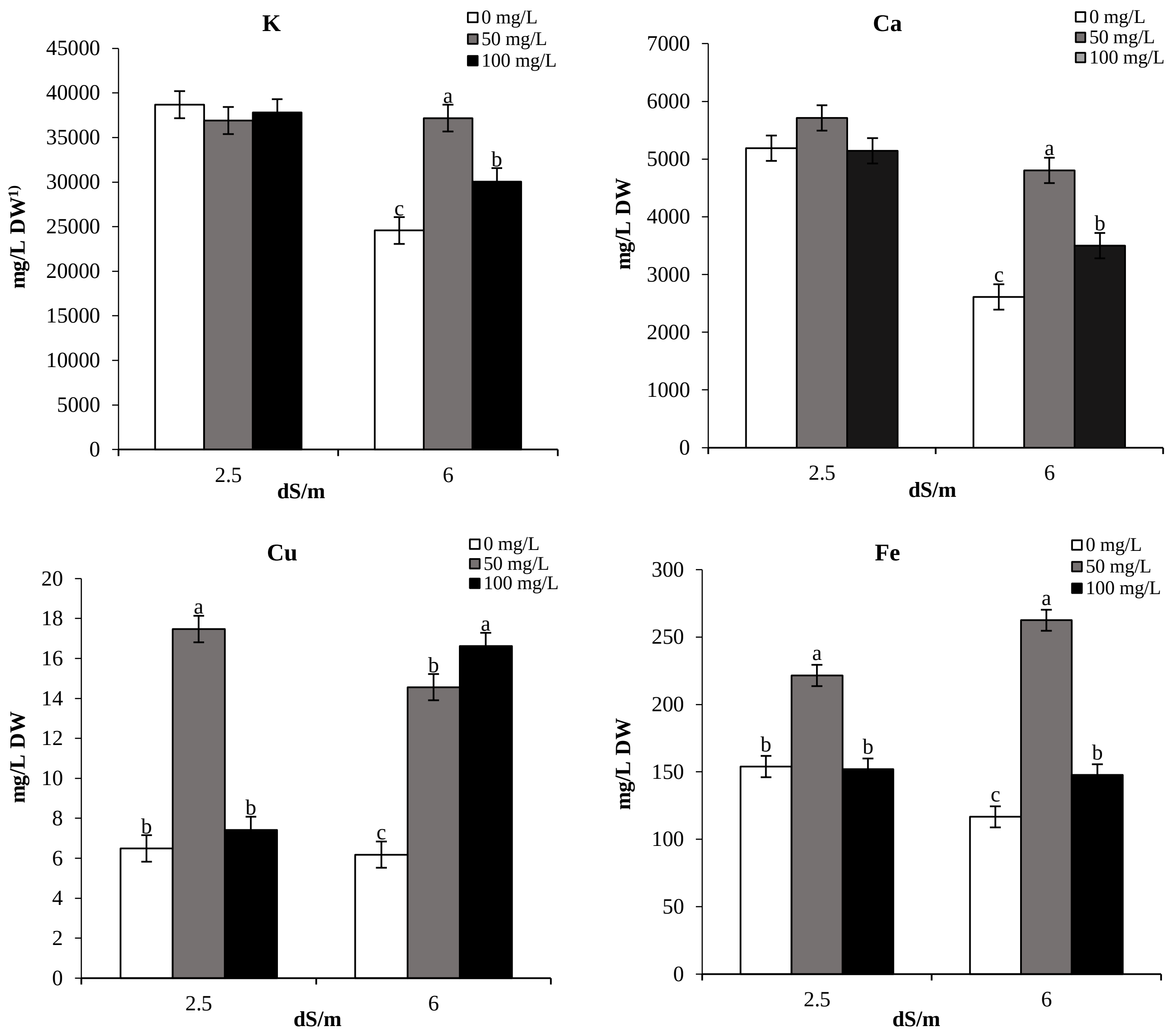

Fig. 2. Effects of interaction between EC and Si in ion concentration in leaves. [1)] DW: dry weight. Data are shown as mean ± SE (Standard error). Different letters mean significant difference at p<0.05.

Table 4. Effects of EC and Si or interaction between EC and Si in ion concentration in stem of seedlings *M. oleifera* Lam.

	K	Ca	Mg	Na	Zn	Cu	Mn	Fe
	mg/kg DW[1)]							
EC (dS/m)								
2.5	25592.85	4757.93 a	7313.38 a	13991.88 b	140.72	12.66	23.95	125.27
6.0	22832.56	4164.41 b	6229.61 b	29161.12 a	178.30	14.94	26.95	107.65
± SE	1230.13	161.67	293.41	1292.95	35.85	1.15	1.62	8.44
p	ns	0.023	0.023	0.000	ns	ns	ns	ns
Si (mg/L)								
0	25526.79	4566.83	6322.14 b	22334.47	115.72	14.00	24.81	108.69
50	25610.27	4774.42	7776.66 a	211693.96	211.78	16.31	25.70	124.51
100	24641.51	4042.26	6215.69 b	21225.07	151.05	11.09	25.83	116.17
± SE	2130.65	198.00	359.36	1583.53	43.91	1.41	1.99	10.34
p	ns	ns	0.017	ns	ns	ns	ns	ns

[1)] DW: dry weight. Data are shown as mean ±SE (Standard error). Different letters mean significant difference.

Table 5. Effects of EC and Si in ion concentration in leaves of seedlings *M. oleifera* Lam.

	Mg	Na	Zn	Mn
	mg/kg DW[1)]			
EC (dS/m)				
2.5	3121.56 a	10253.17 b	60.20 b	54.59
6.0	1610.18 b	29621.57 a	88.50 a	54.88
± SE	89.95	241.46	3.36	1.47
p	0.000	0.000	0.000	ns
Si (mg/L)				
0	1901.35 b	21530.88 a	70.95	44.23 c
50	2718.47 a	19165.12 b	76.46	63.48 a
100	2327.79 a	19116.12 b	475.65	56.49 b
± SE	110.17	418.21	4.11	1.81
p	0.001	0.000	ns	0.000

[1)] DW: Dry weight. Data are shown as mean ± SE (Standard error). Different letters mean significant difference.

Discussion

Effects of salinity and Si in emergence: Salinity reduced seed emergence of *Moringa oleifera* Lam. Our results agree with Alatar (2011) who found that the germination percentage of *Moringa peregrina* Forssk. decreased significantly when treated with a nutrient solution with an EC of 6 dS/m. Elhag & Hussein (2012) also found that emergence uniformity of *Moringa oleifera* Lam. decreased when a solution with 4 dS/m was used, while speed of emergence and emergence were reduced by 50% using solutions with 8 and 16 dS/m, respectively. Unlike other authors like Biju *et al.*, (2017) who reported that 2 mM of Si increased germination and seedlings emergence of *Lens culinaris* Medik.

Effects of salinity and Si on growth and biomass: The earliest response in salinity stress is a reduction in the rate of leaf expansion, followed by stomatal closure and depression of photosynthesis and transpiration (Meng & Fricke, 2017); therefore, production of biomass is compromised. This fact is in agreement with results obtained in this research, as those reported by Elhag & Hussein (2012) whom reported that a significant reduction in dry weight of *Moringa oleifera* Lam. subject to an EC of 4 dS/m; and those obtained for Nouman *et al.*, (2012), whom reported that at 8 dS/m salinity reduced root length and leaves number, and 12 dS/m reduced plant height in 30 day old seedlings. According with results obtained in this research, Si is a beneficial element for moringa plants, since increased the growth of the aerial part of the plant and biomass. The beneficial effect of Si on growth and plant biomass was found in another species, such as *Gossypium hirsutum* L., *Brassica napus* L., *Triticum aestivum* L. and *Oryza sativa* L. (Mehrabanjoubani *et al.*, 2015; Pati *et al.*, 2016). Li *et al.*, (2015) found that application of Si, in plants subjected to salt stress, regulates the expression of aquaporins, which contributes to the increase of root hydraulic conductivity and improves water absorption. Si also increases the stem hydraulic conductivity, which benefits the water supply to leaves, increasing the water status of the aerial part and growth of leaves and shoots.

Effects of salinity and Si on ion concentration: Results of this research agree with Nouman *et al.*, (2012) who reported that, at 8 dS/m salinity, the concentrations of K, Ca and Mg were reduced, and at 4 dS/m, the Na was increased in leaves and roots of moringa. The increase in Na concentration and decrease of K, Ca and Mg concentrations in plants with a nutrient solution high in Na salts was explained by Coskun *et al.*, (2013) and Kronzucker *et al.*, (2013); they mentioned that, Na can directly inhibit the functioning of K transporters, in addition to causing a depolarization of the plasma membrane, and added that disintegration of the plasma membrane occurs due to osmotic and ionic shock, causing a displacement of Ca, K and water. Results showed that an increment in EC caused increments of root Zn, Mn and Cu concentration and leaf Zn and Cu concentrations. These results also have been found in another species such as *Lactuca sativa* L. (Neocleous *et al.*, 2014), and Andrographis paniculata Nees (Talei *et al.*, 2012); however, Pérez-López *et al.*, (2014) reported that, in *Hordeum vulgare* L., Fe, Zn and Cu concentrations decreased, when the EC increased. These results show that salinity has different effects in Fe, Mn, Zn and Cu concentrations in different species. Results of this research showed that Si application to moringa increased leaf Ca, K, Mg, Cu, Mn and Fe concentrations and decreased leaf Na concentrations. These results have also been found with another species. Shahzad *et al.*, (2013) and Xu *et al.*,

(2015) found, in *Vicia faba* L. and *Aloe vera* L., respectively, higher K, Ca and Mg, and lower Na leaf concentration when Si was added to the nutrient solution. The lower Na concentrations have been explained due to effect of Si on the cell apoplast as a physical barrier that contributes to the reduction of Na absorption (Luyckx *et al.,* 2017; Rios *et al.,* 2017). This effect has also been explained by increasing the content of root organic solutes, caused by Si, which limits the absorption of Na and increases the absorption of K, Ca and Mg improving the stability of the cell membrane (Li *et al.,* 2015; Wu *et al.,* 2017; Xu *et al.,* 2015). The above data agree with Gottardi *et al*., (2012) who reported an increase in Cu and Zn plant concentrations of *Valerianella locusta* L. when Si was added to the nutrient solution; Mehrabanjoubani *et al.,* (2015) found similar results for Zn in *Brassica napus* L. and *Triticum aestivum* L., and Fe for *Brassica napus* L., *Gossypium hirsutum* L. and *Triticum aestivum* L. Results presented by Pavlovic *et al*., (2013) indicated that Si alleviated Fe deficiency in *Cucumis sativus* L. grown in calcareous soils, which have little Si available; the presence of Si increased Fe concentration in the apoplast of the root and improved its mobilization, due to the mediation in biosynthesis of Fe chelating compounds.

Conclusion

An EC of 6.0 dS/m caused negative effects on emergence, growth and biomass, as well as changes in ion concentrations; while at EC of 9.5 dS/m, moringa plants did not show any growth. Si increased the growth of the aerial part of the plant and biomass regardless of salinity. For ion concentration, the negative effect of salinity was lessened by application of Si at a dose of 50 mg/L. Si increased concentrations of K, Ca, Cu and Fe in leaves and Fe in roots, and reduced concentrations of Na in roots. Therefore, Si should be considered as an alternative to reduce the effects of salinity in plants.

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