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Research Article
Jojoba Irrigated with Diluted Seawater as Affected by Ascorbic Acid Application

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Abstract
Background: Application of antioxidant materials like ascorbic acid to alleviate salinity stress and promote jojoba growth is a high effectiveness target, whereas, jojoba is an attractive industrial and biofuel crop. **Materials and Methods:** A greenhouse experiment was conducted in the National Research Centre at Dokki, Giza, Egypt to evaluate the effect of different salt stress degrees on the growth, photosynthetic pigments (chlorophyll and carotenoids) and mineral status characters of jojoba plant which sprayed by ascorbic acid. The treatments were as follows: (1) Irrigation by two concentrations of diluted seawater (S1 = 2500 and S2 = 5000 ppm), the control treatment irrigated with tap water (TW = 250 ppm), (2) Spraying ascorbic acid with two concentrations (As1 = 100 and As2 = 200 ppm), the control plants received the same quantity of distilled water (C = distilled water). **Results:** Irrigation with seawater negatively affected growth parameters in spites of that the differences not great enough to reach the significant levels. The high salinity level used sharply decreased chlorophyll a, chlorophyll b, carotenoids and total chlorophyll concentrations in comparable with that irrigated regularly with fresh water. Ascorbic acid application increased stem, leaves, shoot and seed protein, compared to control (foliar with distilled water), whereas, As2 was super than As1. Carbohydrate and carbohydrate/protein ratio also were increased with ascorbic acid application but As1 was the superior. **Conclusion:** Generally, it can be used diluted seawater in irrigation of jojoba plant with spraying ascorbic acid to alleviate the harmful effect of salinity.

Key words: Jojoba, salinity, seawater, ascorbic acid, growth, carbohydrate, chlorophyll, protein, minerals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jojoba (*Simmondsia chinensis* (Link) Schneider) is an attractive alternative and industrial crop that is adapted to moisture and heat. It is grown commercially in arid and semi-arid regions because of the promising applications of its seed oil to cosmetics and biodiesel production[1].

Despite the essentiality of chloride as a micronutrient for all higher plants and of sodium as mineral nutrient for many halophytes and some species, salt accumulation may convert agricultural areas in unfavorable environments, reduce local biodiversity, limit growth and reproduction of plants and may lead to toxicity in non-salt-tolerant plants, known as glycophytes[2,3].

As well known, salinity is one of the most important limiting factors and environmental stresses that adversely affect the plant growth and metabolism particularly in the arid and semi-arid regions through the world[4]. Irrigation castor bean plants with 20% diluted seawater decreased plant height, green leaves number, leaves area and dry weight of root, stem, leaves and whole plant compared to plants that of irrigated regularly with tape water[5].

The suppression of shoot growth under salinity may either be refer to osmotic reduction in water availability or to excessive accumulation of ions, known as specific ion effect[6]. Inhibition of shoot growth has been considered a whole plant adaptation to salt stress or water stress[7]. Growth and physiological activities in different plants grown as affected by salinity conditions were reported by El Sayed[8], Hussein and Alva[9] and Hussein *et al.*[10].

Ascorbic acid application resulted in increases in growth and chemical properties of different plants as found by Hussein and Alva[9], Malik and Ashraf[11] and Ahmad *et al.*[12].

The objective of this study is to evaluate the response of jojoba growth, mineral status and photosynthetic pigments which irrigated with diluted seawater to ascorbic acid rates.

MATERIALS AND METHODS

A pot experiment was conducted in the greenhouse of the National Research Centre at Dokki, Giza, Egypt to evaluate the effect of different salt stress degrees on the growth (stem, leaves and top weight), carbohydrate, photosynthetic pigments (chlorophyll and carotenoids) and mineral status characters (N, P, K, Ca, Mg and its relations). The experiment was included that: (1) Irrigation by two concentrations of diluted seawater (2500 and 5000 ppm) more than the control treatment (irrigated by tap water 250 ppm). (2) Three concentrations of ascorbic acid (control = distilled water, As1 = 100 and As2 = 200 ppm).

All pots (40 cm in diameter and 50 cm in depth) were contained 30 kg of air dried clay loam soil. Soil was taken from Giza governorate and its particles size distribution was as follows: 7.1% coarse sand, 21.1% fine sand, 35.4% silt and 36.4% clay. The soil chemical analysis was as follows: pH 7.82, EC (dS m^{-1}) 1.3 and 0.5, 7.2, 3.1, 2.2, 0.4, 9.4 and 3.2 (mEq L^{-1}) for potassium, sodium, calcium, magnesium, bicarbonate, chloride and sulphate, respectively. The chemical analysis of used seawater was as follows: pH 8.10, EC (dS m^{-1}) 36.0 and 0.34, 10.5, 0.4, 1.3 and 18.9 (g L^{-1}) for potassium, sodium, calcium, magnesium and chloride, respectively. The physical and chemical characteristics of the investigated soil were determined according to Page *et al.*[13]. Jojoba seeds (*Simmondsia chinensis* (Link) Schneider) were sown in the first of April, plants were thinned twice. Ammonium sulfate (20.5% N), calcium super phosphate (15.5% P_2O_5) and potassium sulfate (48.5% K_2O) were added before sowing. One irrigation by diluted seawater and the next was by fresh water alternatively were started 30 days after sowing till before harvest.

Two plants from every pot were picked, cleaned, dried in electric oven at 70°C (until the weight was fixed) and grounded in stainless steel mill. Dry powder was digested using sulphuric and perchloric acids. Nitrogen, P, K, Ca, Mg and Na concentrations were determined as described by Cottenie *et al.*[14]. Carbohydrates were determined as described by Herbert *et al.*[15]. Cholorophylls a, b and carotenoids were measured in fresh leaves samples according to the method described by Von Wettstein[16].

Data of 9 treatments in 8 replicates were analyzed and Least Significant Difference (LSD) at 0.05 probability level was calculated using the methods described by Gomez and Gomez[17].

RESULTS AND DISCUSSION

Growth parameters: Data in Table 1 revealed that generally, high salinity level negatively affected growth parameters in spite of that the differences not great enough to reach the significant levels in weight of stem, leaves and carbohydrate content. Regardless the effect of As, salinity treatment especially the highest level decreased stem, leaves, whole plant dry weight (top) and protein, in contrast carbohydrate and carbohydrate/protein ratio in seeds were increased compared to the control.

Inhibition of shoot growth has been considered a whole plant adaptation to salt stress or water stress[7]. Salinity effects are generally summarized as water stress, salt stress and stress due to ionic imbalance[18]. Therefore, at least one part of salt stress is associated with water stress, which is a general

Table 1: Growth parameters, carbohydrate and protein of jojoba plants as affected by ascorbic acid spraying and salinity condition

Salinity treatments	As rates	Stem	Leaves	Top	Protein	Carbohydrate	Carbohydrate/protein ratio
TW	DW	75.81	14.85	90.66	10.94	9.28	0.85
	As1	113.05	37.93	150.98	13.88	24.87	1.79
	As2	170.32	46.13	216.45	14.88	23.85	1.60
Mean		119.73	32.97	152.70	13.23	19.33	1.41
S1	DW	76.52	14.77	91.29	9.94	12.99	1.31
	As1	83.78	37.90	121.68	8.94	21.71	2.43
	As2	165.90	31.17	197.07	11.94	31.25	2.62
Mean		108.73	27.95	136.68	10.27	21.98	2.12
S2	DW	54.59	10.50	65.09	8.94	8.54	0.96
	As1	72.86	13.76	86.62	8.94	16.32	1.83
	As2	157.72	12.95	170.67	9.94	23.36	2.35
Mean		95.06	12.40	107.46	9.27	16.07	1.71
General mean	DW	68.97	13.37	82.35	9.94	10.27	1.04
	As1	89.90	29.86	119.76	10.59	20.97	2.02
	As2	164.65	30.08	194.73	12.25	26.15	2.19
$LSD_{0.05}$		S = ns	S = ns	S = 23.3	S = 2.6	S = ns	S = 0.5
		As = 20.9	As = ns	As = 23.3	As = ns	As = 6.7	As = 0.5
		S×As = 36.2	S×As = ns	S×As = 40.3	S×As = ns	S×As = 11.7	S×As = 0.8

TW, S1 and S2: Irrigation by tap water, 2500 and 5000 ppm, DW, As1 and As2: Spraying by distilled water, 100 and 200 ppm ascorbic acid

condition and it can be expected that plant adaptation to salinity may show features similar to those characteristic of adaptation to water stress. The suppression of shoot growth under salinity may either be refer to osmotic reduction in water availability or to excessive accumulation of ions, known as specific ion effect[6]. The highest salinity level significantly decreased growth parameters, while carbohydrates were gradually increased with increasing the concentration of salinity[1].

Ascorbic acid application increased stem, leaves, shoot and seed protein, compared to control (foliar with distilled water), whereas, As2 was super than As1. Carbohydrate and carbohydrate/protein ratio also were increased with As application but As1 was the superior (Table 1).

Examination of data in Table 1 cleared a positive relationship between As application and the growth parameters. Continuous increases were detected with increase in As concentration in the sprayed solution except for No. of leaves, the high increase was by 100 ppm concentration compare to control or 200 ppm As treatment. Ascorbic acid play important role in plant metabolism through its effect on the physiological processes, enzymes activity, the polyamine synthesis and help modulate of the tocopherol synthesis which mitigate the environmental stresses[12,19]. Ascorbia acid application through roots or leaves significantly increased growth of wheat plants[11,12] indicated that applied As via leaves increased growth, enzymes activity and membrane stability of maize plants. Hussein and Alva[9] demonstrated that ascorbic acid improved growth of millet plants.

The highest increment in plant height was obtained by 200 ppm under severe salinity while for no of leaves it was by the same level of As and plants irrigated continuously by fresh water. Moreover, top increases was reached its higher values with 200 ppm As when plants irrigated by 5000 ppm salts followed by that under fresh water irrigation (Table 1).

El Sayed[8] on tomato revealed that salinity decreased all growth parameters but soaked seeds in ascorbic acid increased these parameters under different salinity used. Wang *et al.*[20] indicated that exogenous As differentially enhanced salinity tolerance and alleviated salinity-induced damage in the two rice cultivars. Protein concentration in leaves of jojoba plants was decreased parallel to the increase in salt stress (Table 1). However, carbohydrate concentration increased slightly with the moderate salinity and tended to decrease with the severe salinity to be less than that in the control plants. Sadok and Mostafa[21] pointed out that salinity induced reduction in protein of faba bean seeds. Dhanapackiam and Ilyas[22] found that soluble, non soluble and total carbohydrate of *Sesbania grandflora* L., seedlings were increased with salinity. Mahboobeh and Akbar[23] showed that significant increases were detected in carbohydrate and chlorophyll as a result of salinity and the reverse was true for protein content in *Nicoitana blumbaginifolia* L. High salinity decreased protein content of *Poulownia impirialis* L.[24].

Application of As on jojoba plants caused a remarkable increase in either protein and carbohydrate concentrations or its ratio in comparable with the untreated plants. Nassar[25] concluded that ascorbic acid has a promoting effect on carbohydrate and protein contents.

Table 2: Chlorophyll and carotenoids concentrations of jojoba plants as affected by ascorbic acid spraying and salinity condition

Salinity treatments	As rates	Chlorophyll a	Chlorophyll b	Carotenoids	Chl.a+Chl. b	Chl.a:Chl.b	(Chl.a+chl.b):carotenoids
TW	DW	9.24	6.09	5.14	15.33	1.52	2.98
	As1	13.09	3.52	5.46	16.61	3.72	3.04
	As2	8.98	3.52	4.86	12.50	2.55	2.57
Mean		10.44	4.38	5.15	14.81	2.60	2.86
S1	DW	10.18	13.53	4.15	23.71	0.75	5.71
	As1	10.83	11.14	3.03	21.97	0.97	7.25
	As2	6.58	5.22	2.71	11.80	1.26	4.35
Mean		9.20	9.96	3.30	19.16	0.99	5.77
S2	DW	4.10	3.04	3.21	7.14	1.35	2.22
	As1	5.85	3.64	4.75	9.49	1.61	2.00
	As2	2.73	3.95	3.28	6.68	0.69	2.04
Mean		4.23	3.54	3.75	7.77	1.22	2.09
General mean	DW	7.84	7.55	4.17	15.39	1.21	3.64
	As1	9.92	6.10	4.41	16.02	2.10	4.10
	As2	6.10	4.23	3.62	10.33	1.50	2.99
$LSD_{0.05}$		S = 2.3	S = ns	S = ns	S = 6.9	S = ns	S = 1.8
		As = 2.3	As = ns	As = ns	As = ns	As = ns	As = ns
		S×As = 3.9	S×As = ns	S×As = ns	S×As = 12.0	S×As = ns	S×As = 3.1

TW, S1 and S2 Irrigation by tap water, 2500 and 5000 ppm, DW, As1 and As2: Spraying by distilled water, 100 and 200 ppm ascorbic acid

The interactive effect of As and salinity were illustrated in Table 1. Plants received As showed improvement in carbohydrate and protein content. This was true under different salinity treatments. Also, carbohydrate to protein ratio showed similar response. Abdul Qados[26] reported the depression in sugars and total carbohydrate and added that water stress induced new proteins and disappear of the others than the control soy bean plants. Application of 100 or 200 ppm As led decrease the adverse effect on protein. This effect may be attributed to the increase in N content which condensate the decrease caused by stress. Amino acids in seeds showed approximately the same response. Ejaz *et al.*[27] noticed that exogenous application of As with different methods used significantly alleviated the adverse effect of salinity on biochemical parameters of sugarcane plants. El Sayed[8] indicated that the organic compounds, such as protein and carbohydrate and also the enzymes and antioxidant activity increased in tomato tissues by salinity and when seeds soaked in As the content of these compounds and activities were increased more.

Chlorophyll, caroteoids and their relations: The salinity could seriously change the photosynthetic carbon metabolize, leaf chlorophyll content as well as photosynthetic efficiency. Chlorophyll a content and carotenoids were decreased with increasing salinity concentration (Table 2). Chlorophyll b, total chlorophyll and the ratio between total chlorophyll and carotenoids values were increased with the first salinity rate and decreased with second one. In another mean, the high salinity level used sharply decreased chlorophyll a, carotenoids and chl.a:chl.b concentrations in comparable with that irrigated regularly with fresh water. Chlorophyll b, total chlorophyll and chl.a+chl.b:carotenoids ratio gave their higher values with moderate salinity, however for chlorophyll a, total carotenoids and chl.a:chl.b ratio it was by fresh water treatment. These may be due to seawater is a source of salinity not NaCl salt only. So, it contains Mg and Fe ions that enter in chlorophyll composition. While, under high salinity level, the harmful effect of salinity are appears clearly. Our results are in agreement with many researchers who revealed that, the photosynthetic pigments content (chl.a, chl.b, chl.a:chl.b and carotenoids) in jojoba leaves was reduced at all levels of salinity treatments as compared to the control[28,29]. The highest salinity level significantly decreased leaf chlorophyll content and protein[1].

Ascorbic acid spraying at rate of 100 ppm led to the high concentrations of chlorophylls a, b, total chlorophyll, carotenoids and the ratios between them compared to As2 treatment and control. Malik and Ashraf[11] observed that chlorophyll increased with As spraying on leaves or through soaking seeds. Ahmad *et al.*[12] showed that application of ascorbic acid increased the chlorophyll content of maize. Furthermore, El Sayed[8] noticed that photosynthetic pigments increased as salinity increased specially under 8000 ppm level whatever with ascorbic acid treatment or not.

Nutrient concentrations: The mineral nutrition of the leaves and seeds was assessed (Fig. 1-3). Each level of salinity stress affected the nutrient status of the explants distinctively. It can be observe that N in shoots decreased as salinity increased but reversely ascorbic acid increased it under different salinity treatments. The degree of increases was more under fresh water treatment and lesser under high salinity. Potassium

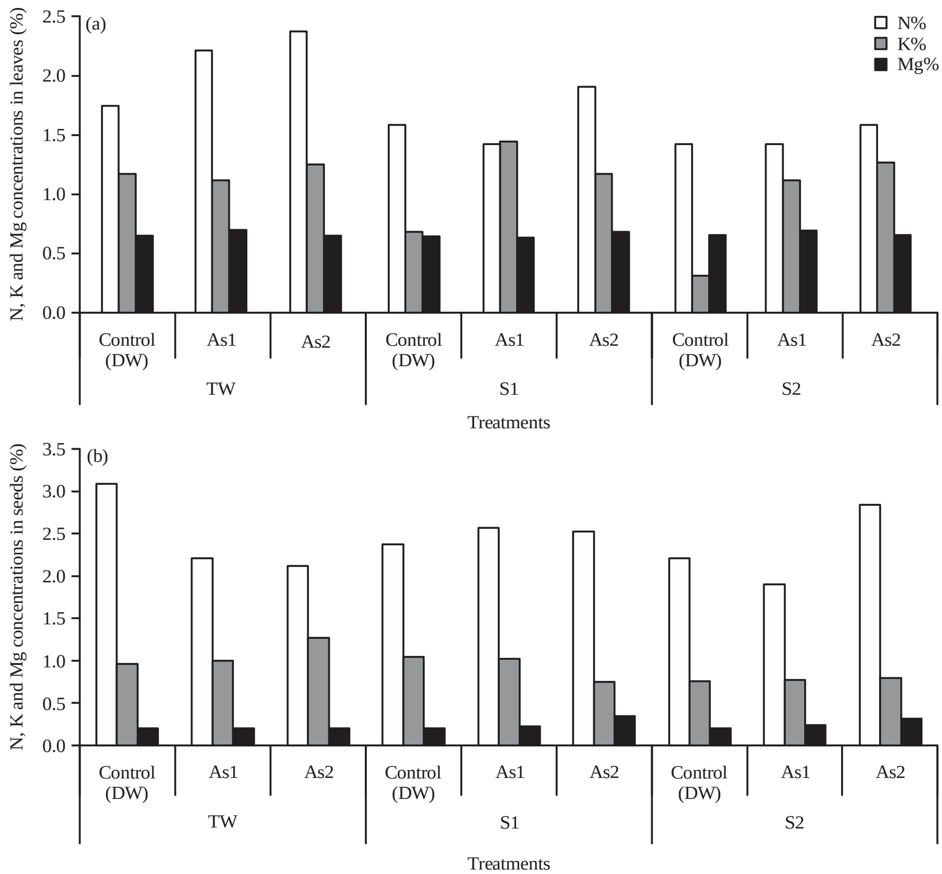

Fig. 1(a-b): N, K and Mg concentrations (%) in (a) Leaves and (b) Seeds as affected by ascorbic acid spraying and salinity condition, TW, S1 and S2: Irrigation by tap water, 2500 and 5000 ppm, DW, As1 and As2: Spraying by distilled water, 100 and 200 ppm ascorbic acid

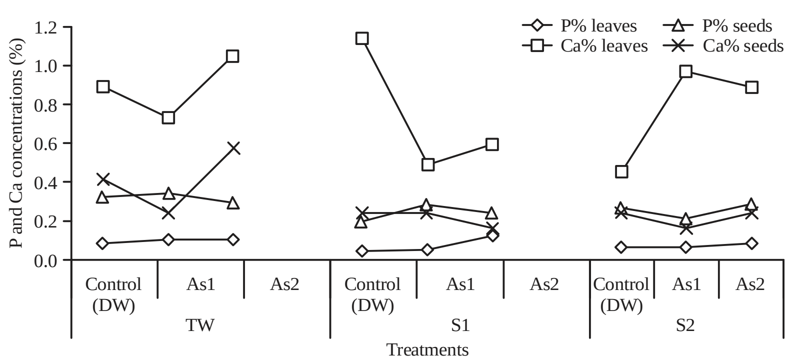

Fig. 2: P and Ca concentrations (%) in both leaves and seeds as affected by ascorbic acid concentration and salinity condition, TW, S1 and S2: Irrigation by tap water, 2500 and 5000 ppm, DW, As1 and As2: Spraying by distilled water, 100 and 200 ppm ascorbic acid

increased slightly by As under fresh water but under high salinity, the percentage of increase markedly increased parallel to the increase in As concentration (As2>As1>control). The high rate of As led to decrease salinity effect, especially under S1. Furthermore, Mg concentration increased under S1 more than control (TW) and differences as a result of As or salinity still low compare to the plants without receiving the vitamin.

Jojoba explants tolerate salinity up to a level of sodium chloride concentration (113 mM) without showing any stress symptoms. Above this level, the salinity stress impact was observed as succulence and chlorosis of leaves and shoots[30].

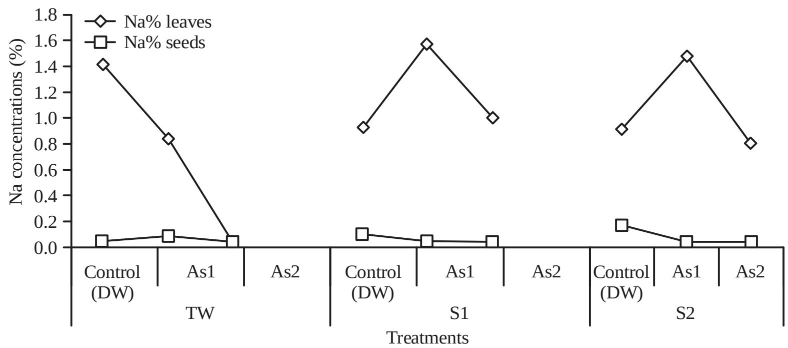

Fig. 3: Na concentrations (%) in leaves and seeds as affected by ascorbic acid concentration and salinity condition, TW, S1 and S2: Irrigation by tap water, 2500 and 5000 ppm, DW, As1 and As2: Spraying by distilled water, 100 and 200 ppm ascorbic acid

The results in Fig. 1b cleared that N concentration in seeds decreased with salinity but the decreases with 100 ppm. As under moderate salt stress more than that under high salinity. As in the rate of 200 ppm increased as salinity increased surrounding the jojoba plants roots. The K concentration increased by antioxidant spraying under fresh water irrigation but decreased with this material under moderate salinity, However no response to vitamin when high saline water used in irrigation (Fig. 1b).

Nitrogen concentration decreased with both salinity and As application. Nitrogen decreased with the As spraying under fresh water irrigation while slightly increased under moderate salinity but under high salinity it was decreased with 100 ppm As, however, increased markedly in seeds with 200 ppm As treatment. Also, K in shoots increased by the high As concentration in spite of the depression in this element with salinity in control treatment. Furthermore, K in seeds is slightly affected by salinity or ascorbic acid treatment.

Calcium in shoots decreased with the 1st As concentration (100 ppm) and tended to increase with the 2nd level of As but its value still less than the control. The reverse was true under high salinity stress (Fig. 2). The Ca% responses in seeds were less than in leaves under all salt stress treatments. In addition P either in shoots or in seeds slight increased by 100 ppm and decreased with 200 ppm vitamin under fresh water used in irrigation but in seeds and shoots responded reversely in between. The response of P in shoots and seeds showed the same trends but in seeds with small degree. Generally, Ca concentration in leaves was high than that in seeds but the reverse was true as for phosphorus.

Pronounced decreases were obtained in Na concentration in shoots of plants irrigated by fresh water however, it was increased with 100 ppm as and tended to decrease to be less than the control wit 200 ppm concentration of vitamin treatment. Meanwhile, in seeds this mineral slightly affected by the salt stress or vitamin spraying compare to that shown in shoots (Fig. 3).

Ali *et al.*[1] indicated that N, P, K and Ca were decreased with increasing salinity concentrations, while sodium and chloride were increased.

Generally, decreasing nutrient concentrations under the interaction between irrigation with tap water and spraying ascorbic acid may be attributed to the dilution effect resulted by increasing growth[31]. This claim confirmed by the growth data in Table 1. While decreasing nutrient concentration under salinity treatments is a logic trend and refer to (1) Osmotic pressures and chemical induced drought, (2) Nutrient imbalance, (3) Toxicity of Na and Cl and (4) Death of some useful microorganisms as concluded by Abou-Baker and El-Dardiry[32]. Salinity can directly affect nutrient uptake, such as Na^+ reducing K^+ uptake or by Cl^- reducing NO_3^- uptake. Salinity can also cause a combination of complex interactions that affect plant metabolism[33,34].

The Na/K, Na/Ca, Mg:(Na+K) and Ca:(Na+K) sharply depressed with As application under fresh water irrigation. In spite of the decreases in Na/Ca caused by salinity, pig increases when plants sprayed by 100 As (Fig. 4a). Raised the concentration of As to 200 ppm increased this ratio but still less than that induced by 100 ppm treatment (Fig. 4a). These ratios of plants irrigated by severe salinity showed similar trends of those under fresh water irrigation.

The Na/K and Na (Mg+K) ratio increased slightly and tended to decrease, while Na/Ca increased sharply with the increase in salinity. Addition of As decreased Na/Ca under salinity treatments however, under fresh water irrigation raised by 100 ppm but decreased by 200 ppm As (Fig. 4b).

From Fig. 5, it is clear that As K/Ca ratio more high than in leaves and at the rate of 100 ppm gave the highest ratios

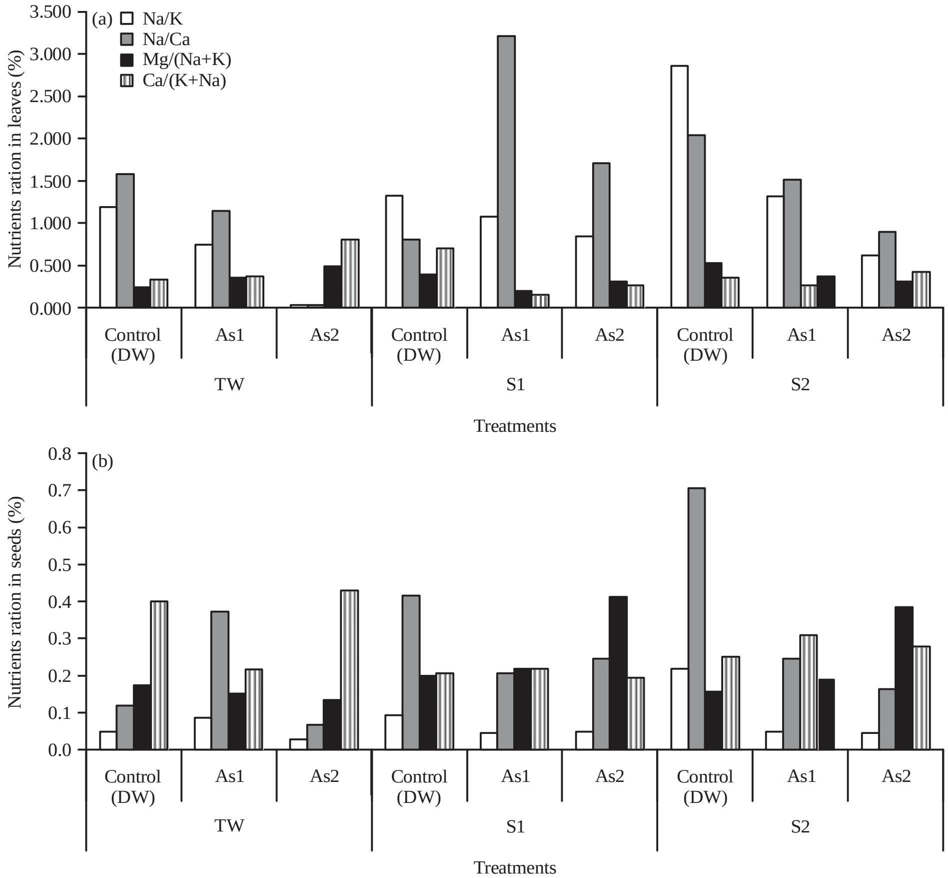

Fig. 4(a-b): Na ratios with the other nutrients (concentrations basis in shoots) in (a) Leaves and (b) Nutrient ratios in seeds (%) as affected by ascorbic acid spraying and salinity condition, TW, S1 and S2: Irrigation by tap water, 2500 and 5000 ppm, DW, As1 and As2: Spraying by distilled water, 100 and 200 ppm ascorbic acid

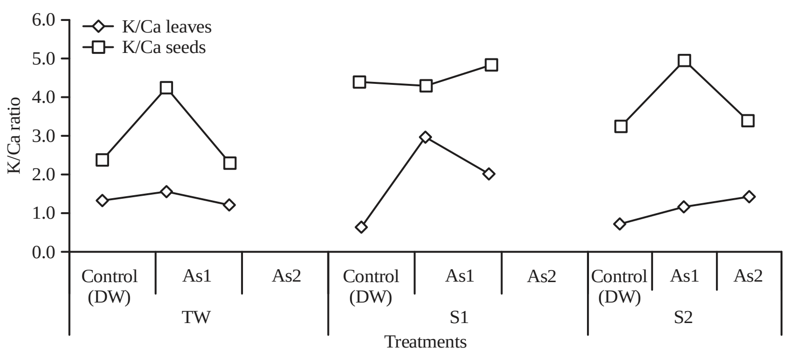

Fig. 5: K/Na ratio in shoot and seeds as affected by ascorbic acid concentration and salinity condition, TW, S1 and S2: Irrigation by tap water, 2500 and 5000 ppm, DW, As1 and As2: Spraying by distilled water, 100 and 200 ppm ascorbic acid

K/Ca in leaves and seeds K/Ca in seeds under different salinity treatments except for K/Ca under moderate salinity.

Numerous studies demonstrated that salinity induces an increase rather than a decrease in nutrients accumulation not only in glycophyte species, such as rice[35], tomato[36] and *Aeluropus* halophyte plant[37]. There were significant differences ($p \leq 0.05$) on N, P, K, Mg and Ca uptake by increasing NaCl level. Sodium and Cl accumulation by evaluate salinity level were increased in tissue of both root stocks. In mature leaves of 2 pomegranates cultivars, Fe, Zn and Mn contents were decreased but phosphors and boron were increased significantly as a result of increasing the salinity of nutrient solution from 500-1750 ppm[38]. Increasing salinity to 3 dSm^2 significantly increased the saffron leaf concentration of Na, Ca and Cl by about 4.0, 1.4 and 1.5 times, respectively but decreased K, N and P by about 30, 20 and 39%, respectively[39]. In another hand, salinity (200 mM NaCl) had no effect on *Purpletop vervain* leaves content of phosphorus, potassium, magnesium, copper, zinc and iron[40].

CONCLUSION

The combined efforts of governments and farmers in finding alternative irrigation water resources (such as diluted seawater) should be taken seriously, especially after increasing climate change hazards like water shortage and increasing salinity as most hazards abiotic stresses affected plant production. In addition to, studying how to increase plant alleviation to abiotic stress is a good approach. From this study, it can be concluded that (1) Although, irrigation with seawater negatively affected most growth parameters, there is no significant difference was observed between different salinity levels. (2) Ascorbic acid application led to increasing growth parameters, photosynthetic pigments and nutrient content compared to control (foliar with distilled water). (3) The high rate of ascorbic (200 ppm) was superior than low one (100 ppm). (4) Seawater is huge water reservoir, so using diluted concentrations of it in irrigation with ascorbic acid application is recommended.

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