16 Egypt. J. Agron. **Vol. 42**, No. 2, pp. 209- 224 (2020)

Egyptian Journal of Agronomy
http://agro.journals.ekb.eg/

Nutrient Content and Growth Responses of Sugar Beet Plants Grown under Salinity Condition to Citric Acid and Algal Extract

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THIS INVESTIGATION aimed to study the response of growth and mineral status of sugar beet plants which sprayed with antioxidant, citric acid (DW, 75 and 150ppm) and algal extract in the rateof 30cm/L, on sugar beet plants grown under salt stress condition (diluted sea water, TW, 5000 and 10000ppm). Increases in salt concentration in the irrigation water caused a continuous decrease in the dry weight of roots. Dry weight of leaves increased with 5000ppm salts treatment and tended to decrease to be approximately equal of the control. However, the whole plant dry weight did not affect with the first salinity treatment but decreased markedly with the second salinity level. Increased salinity decease the concentration of N, P, K and Fe in leaves of sugar beet. On the opposite side, Ca Na, Mn and Cu concentrations increased by raising salts concentration in the root media of plants. Meanwhile, Mg and Zn % were slightly affected. The concentration of P as well as Cu increased by both antioxidant treatment but the values by the combination of CA and algal extract were more than that of CA alone. Na and Mn increased by CA treatment and Citric acid + algal extract but the increase with CA more than that of the combined between them. The reverse was true of K concentration. Fe, Zn and Mg slightly affected with these treatments. Ca % decreased by CA and tended to increase by Citric acid + algal extract but still more than the control while N% increased by the application of Ca and tended to decrease by Citric acid + algal extract to be less than the control ones.

Keywords: Algal extract, Citric acid, Growth, Mineral status, Salinity, Sugar beet (*Beta vulgaris* L.).

Introduction

The availability of fresh water for use for growing the food production in agriculture to face the increasing demand of the high rate of population increases is increasingly becoming limited. In reverse, the brackish water availability and more saline water is practically unlimited and increases as a result of environmental changes. If sea water and brackish water could be used for crop cultivation or extension in saline soils, then vast quantities of fresh water would be saved (Rozema & Flowers, 2008; Hussein, et al., 2015a, 2017). Saline agriculture that exploits salinized soils and brackish water can deliver not only for the production of human food, human utilization, for example, vegetables and organic products, yet additionally feed for cattle, raw materials for use in the industry of biofuel biodiesel and/or bioethanol (Rozema et al., 2015). Saline agriculture is mainly need of salt-tolerant crops as well as tolerant varieties (Singh et al., 2012).

Citic acid (CA) is a six carbon natural corrosive (OA), having a significant job in citrus extract cycle inside mitochondria that makes

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Received 6/8/ 2020; Accepted 27/8/ 2020
DOI: 10.21608/agro.2020.38200.1223

vitality of cells by phosphorylative oxidation responses. It is made by expansion of acetyl-CoA to oxaloacetic corrosive that is convert to succinate and malate in next responses. It has been demonstrated that the exudation of citrate and malate from foundations of calcicole plants (plant utilized in soluble soils) empowers them to remove phosphorus and iron from various soils (Lopez-Bucio & Nieto-Jacobo, 2000). Citric acid via leaves alone or in combination with iron sources have been utilized to recuperate numerous in chlorosis of plants (Eidyan et al., 2014). Later studies uncovered that the citrus acid impact isn't only because of changes in pH and there are an assortment of physiological reactions to splash citrus extract. Utilization of CA exclusively expanded the fundamental oil creation of sweet basil and dill (Jaafari & Hadavi, 2012). Results on the citric acid application, on some physiological characters in different crops such as tuberose (Eidyan et al., 2014), and lilium (Darandeh & Hadavi, 2012) were promising.

Also, citrus acid (CA) is one of the TCA intermediates, which use as the wellspring of carbon skeleton and vitality of cells, which are used in the pattern of respiratory framework and other biochemical pathways (da Silva, 2003), tolerance of heavy metal stress (Gao et al., 2010) and salinity stress (Sun & Hong, 2011). Moreover, citrate is one of the compounds that help transport Fe through the tissues of indoor plants (Hell & Stephan, 2003). Moreover, the complex has been able to build vase life and chlorophyll and tubereuse (Darandeh & Hadavi, 2012). Zhao et al. (2015) underscored that the CA served significantly as a cell reinforcement respiratory digestion middle including the pathway of protection because of high temperature.

Use of plant extracts in treating plants grown under abiotic stresses considered one from the recent aspects in crop plants production (Taha et al., 2015; Ibrahim, 2016; Rinez et al., 2016).

Recently, algae and algal extracts produced commercially as a food source, animal feeding and bio-fertilizer characterized with high nutritional value (Selem, 2019). Ali & Mostafa (2009) tested the effect of spraying extracts or using soil application methods of potassium-humate and *S. platensis* extract, either individually or in combination and used both as a bio-organic fertilizer on sesame plant. They observed that the combined foliar spray showed the highest rates in plant height, number of capsules, number of branches, seed weight per plant and weight of one thousand seeds. While, combined soil application showed the highest yield and production of seeds and straw.

Several researches were done in the use of algal extracts on alleviation of abiotic stresses (Ibrahim, 2016; Rinez et al., 2016).

The goal of this research is to evaluate the response of growth and mineral status of sugar beet plants to application of ascorbic acid and algal extract grown under high salinity condition.

Materials and Methods

This investigation aimed to study the response of growth and mineral status of sugar beet plants which sprayed with antioxidant, citric acid (DW, 75 and 150ppm) and algal extract in the rate of 30cm/L, on sugar beet plants grown under salt stress condition (diluted sea water, TW (250ppm), 5000 and 10000ppm). Soil and sea water physical and chemical analysis were illustrated in Table 1.

The experiment included 9 treatments which included three salt stress in combination with three antioxidant treatments. The design of the experiment was split plot with six replicates. Seeds of sugar beet (*Beta vulgaris* L.).cv., Farida were sown November, 4, 2015 in a BVC pots 40cm in diameter and 60cm in height, filled with 30kg clay loam soil. The properties of water and soil used in the experiment were recorded in Table 1 a and 1 b. Plants thinned twice, the 1st was at 15 days after sowing and the second, 15 days later. Calcium super phosphate (15.5% P_2O_5) and potassium sulphate (48.5% K_2O) in the rate of 1.0 and 0.75g/pot broadcasted and mixed with 10cm depth of soil while ammonium sulphate in the rate of 2g/pot in two equal portions, the 1st added 21 days from sowing and the 2nd, 15 days later. Citric acid and algal extract were sprayed two times, the 1st at 35 and the 2nd, two weeks later. Two plants from every replicate were picked, cleaned and dried in electric oven at 70°C until the weight stable. Determination of nutrients were done using the methods described by Cottenie et al. (1982). Collected Data were subjected to the proper statistical analysis according to the methods described by Snedecor & Cochran (1980).

TABLE 1. Soil and water chemical properties.

- Soil analysis

a-Soil physical properties

Soil component	E. C 1: 2.5 (dS. m^{-1})	pH 1: 2.5	$CaCO_3$ %	Organic matter %	Sand %	Silt %	Clay %	Texture
	1.68	8.19	1.65	1.68	20.4	34.2	45.4	**Clay loam**

b- Soil chemical properties

Soil component	K	Ca	Mg	Na	SO_4	Cl	HCO_3	Fe	Mn	Zn	Cu
	mg. kg $Soil^{-1}$										
	194	462	282	168	132	127	69.0	5.63	8.23	2.65	1.2

- Sea water chemical properties

Soil component	E. C (dS. m^{-1})	pH	K (g/L)	Ca (g/L)	Mg (g/L)	Na (g/L)	Cl (g/L)	HCO_3 (g/L)	SO_4 (g/L)
	48.8	8.51	0.42	0.32	1.62	10.88	20.1	0.11	2.64

Results and Discussion

Growth parameters of sugar beet plants

Effect of salt stress on some growth parameters of sugar beet plants

It is evident from the results in Table 2 that increases in salt concentration in the irrigation water caused a continuous decrease in the dry weight of roots. Dry weight of leaves increased with 5000ppm salts treatment and tended to decrease to be approximately equal of the control. However, the whole plant dry weight did not affected with the first salinity treatment but decreased markedly with the second salinity treatment. Furthermore, R/L as well as R/Whole plant dry weight ratios decreased with the 1st salt concentration and tended to increase by the 2nd salt treatment, however, its values still more than the control. Ghoulam et al. (2002) stated that high sodium chloride (NaCl) fixations instigated an extraordinary decrease in growth parameters, for example, leaf zone, and new just as dry load of root and leaves, yet the leaf number was less influenced. It is clear that expanding salt concentration in water, led to a clear decrease in the growth and this was reflected in the yield and quality of the sugar beet plant. This may be due to the fact that salinity exposure during growth caused stunted growth and changes in structural at various levels of organization (Hussein et al., 2019; Naveed et al., 2020). Moreover, such reduction in sugar beet growth, yield and quality may be attributed to various stresses such as moisture stress (Khalil et al., 2012), salt stress and ion imbalance stress (Hussein & Abu-Bakr, 2018). Salinity stress appears to adversely affect growth, yield and yield quality through toxic effect of Na and/or Cl ions and/or the soil solution osmotic potential (Hussein & Orabi, 2008; Hussein et al., 2015b).

TABLE 2. Dry matter of sugar beet plants response to salinity stress.

Salinity	Root	Leaves	Whole	R/L	R/W
T.W.	11.41	9.18	20.59	1.24	0.55
5000ppm	5.23	14.87	2010	0.35	0.26
10000ppm	3.82	9.92	13.74	0.39	0.38
LSD at 5%	3.91	3.85	7.42	……	……..

Tw: Tap water.

Moreover, the increasing in salt concentration of water used in irrigation induced an imbalance in the plant chemical composition which greatly disturb the metabolic and activities of physiological processes (Abdel-Mawly & Zanouny, 2004; Hussein et al., 2007; Orabi et al., 2018). Sugar beets seemed osmotic modification in two different ways: With their phenological advancement and towards saltiness on thusly on carbohydrate assimilate. Because of the last change, sugar beets can keep up the capability of turgor potential similar incentive for lower estimations of water potential in leaves, to keep up stomatal conductance and photosynthesis process and toward the end their creation under serious water stress (Katerji et al., 1997). Rozema et al. (2015) noticed that the rate of growth decrease at higher saltiness was connected with the diminished in leaf area at the entire plant level (LAR, leaf area ratio) and at the individual leaf level (SLA, specific leaf area). The leaf weight portion was not affected by expanded saltiness. On the opposite side, succulence and leaf thickness and the net assimilation (NAR) per unit of leaf area expanded because of salt treatment, in this manner, mostly neutralizing diminished catch of light by the lowering in leaf area. Moreover, the increase in biomass due to increase the salinity per unit area of leaves (ULR) for sugar beet species stronger and continuously, so the sugar beet watered with seawater indicates that the absorption of carbon dioxide during photosynthesis (CO_2) through the stomata and stabilization is not adversely affected by salinity (Rozema et al., 2015). The plant and crop salt tolerance may be assessed through comparing both growth rate of plants under both saline and non-saline status (Shannon & Grieve, 1999). Meanwhile, by looking at the relative growth rate (RGR), instead of the supreme growth of plants, in response to the increase in salinity, the assessed salt resistance will be less dependent to the growth stages (Rozema & Schat, 2013). Rozema et al. (2015) mentioned that along with relative growth rater (RGR), leaf area ratio (LAR) and by increasing the salinity the specific leaf area (SLA) was decreased; LWF has not changed, but leaf succulence (LS) and leaf thickness (LT) were increase (50–90% increase at 30 dS m^{-1} in comparable with 0.4 dS m^{-1}). Thus, with increasing salinity stress, there is a close correlation between the decrease in growth rate (GR) of sugar beet varieties with decreased leaf area, at the whole plant level or single plant leaf level (SLA), with increased leaf thickness (LT) and freshness. (LS). It can be interpreted by decreased the expansion of cell and/or cell division due to lowered water uptake from a salt stress condition in environment surrounding the plant root.

Recent studies showed that the lipid peroxidation (MDA) changes and the possible involvement of the antioxidant system in relation to the salt stress tolerant (TSS) was studied in the cultivated beet *Beta vulgaris* L. *cv. ansa* and its wild salt-tolerant relative *Beta maritima* TR 51196. The 40 days in age beet seedlings were subjected to 0, 150 and 500mM sodium chloride for 12 days. In *B. maritima* constitutive level of lipid peroxidation (LP) was lower, but activities of peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APOX) and glutathione reductase (GR) were inherently higher than in *B. vulgaris cv. Ansa.* Skorupa et al. (2019) reported that there was a decrease of about three fold in transpiration and the rate of photosynthesis was detected in the leaves of plants adapted to salt stress, when compared with the control treatment. Whereas, there was an increase in the percentage of sodium (Na) and chlorine (Cl) (~ 2 and 10 times, respectively) in the leaves of plants affected by salinity, with the exclusion of genotype and excess stress.

Effect of citric acid and algal extract on some growth parameters of sugar beet plants

Results in Table 3 showed that the highest values of root, and whole plant dry weight were achieved by spraying a combined of citric acid and algal extract, but for top dry weight it was by spraying citric acid only. Furthermore, R\L or R\W ratios showed decreases in treatments of citric acid. Antioxidants play an important role in stress tolerance of crop plants which helps plants to ameliorate the bad effect of stress. Organic acids as antioxidants (such as citric acid and oxalic acid) play an important role in plant metabolism (Singh et al., 2010).

Different works were done for different responses of phenological and physiological response to the application of citric acid as antioxidants as da Silva (2003) stated that internal organic acids (OR) are the source of both carbon skeleton and cells energy and are used in the respiratory system and other biochemical pathways. The plant fresh and dry weight was significantly increased by citric acid (CA) in the rate of 100 and 300mg L^{-1}. Both the plant height and length of peduncle were significantly increased in all applied levels of CA (Talebi et al., 2014). Jafari & Hadavi (2012) noticed that plant vegetative parameters were improved by 0.3% citric acid. Hu et al. (2016) concluded that citric acid alleviate determined effect of stress on growth and physiological processes by decreasing the electrolyte leakage (EL) and malonaldehyde (MDA). The depression in chlorophyll content and the activity of SOD, POD, CAT and root activity of tall fescue plants were ameliorated by citric acid application. This treatment may be responsible for the maintaining the membrane stability. Sadak & Orabi (2015) revealed that citric acid treatment with different concentrations at the selected two dates showed marked increases in the studied growth parameters and improved grain nutritional values of the yielded grains. In addition, to increases in the amount of IAA, GA3 and cytokines, DNA and RNA with decreases in ABA contents, MDA and H_2O_2 contents compared with untreated plants. The activities of CAT and SOD enzymes increased significantly in response to different treatments of citric acid. Macro algae, such as *Ascophyllum nodosum* (rockweed), have been explored as a commercial source of different bio effectors and of bio stimulants for plant growth (Van Oosten et al., 2017). They added that there are many companies working to prepare commercial extracts of *A. nodosum* to support agricultural projects. The brown seaweed in general is characterized by its activity that is beneficial to plant growth and is widely used in agricultural projects. *Ascophyllum nodosum* extract (ANE) supports the growth of roots and shoots in Arabidopsis, which can be considered as a representative biotype for many crop plants, via the metabolism of phytohormones that regulate the treated plant (Rayorath et al., 2015). The data of Behairy et al. (2017) indicated that spraying of alga extract using 2.5g/L or 3.5g/L led to significantly high values of chlorophyll a, b and carotenoids (photosynthetic pigments), vegetative growth characters of sugar beet plants (root diameter, root and leaves fresh weight), root and top dry weight/plant, extractable sugar %, quality index %, nitrogen, phosphorus, potassium, boron contents in leaves and root, top and sugar yields/fed., as well as the lowest content of sodium in roots in both seasons.

Effect of interaction between salinity stress, citric acid and algal extract on some growth parameters of sugar beet plants

Data in Table 4 showed that root dry weight increased either with citric acid or citric acid + algal extraction but the increment with the combined of citric acid+algal extraction was more than that of citric acid alone. Weight of leaves/plant increased with the 1st salinity treatment and tended to decrease with the second one. The reverse was true for the R/L and R/Whole dry weight of plants.

Also, citrus acid (CA) is one of the TCA intermediates, which use as the wellspring of carbon skeleton and vitality of cells, which are used in the pattern of respiratory framework and other biochemical pathways (da Silva, 2003), tolerance of heavy metal stress (Gao et al., 2010) and salt stress (Sun & Hong, 2011). Moreover, citrate is one of the compounds that help transport Fe through the tissues of indoor plants (Hell & Stephan, 2003). Additionally, the complex has been confirmed to increase vase life and chlorophyll content of tuberose and lilium (Darandeh & Hadavi, 2012). Sun & Hong (2011) found that exogenous CA increase saline stress tolerance in the halophyte *Leymus chinensis* (Trin.) by improving photosynthesis and relative growth rate (RGR) and increasing enzymes activities of oxidative defense. El-Tohamy et al. (2013) found that spraying of CA increased the content of total chlorophyll in bean plants under stress. Behairy et al. (2017) showed that significant decreasing of shoot length, dry weight of seedlings, seedling vigor and chlorophyll content with the increasing sodium chloride %. The pretreatment of seeds with CA induced increase in seedling dry weight, shoot length, seedlings vigor and chlorophyll content compared to control. The activities of catalase, proline dehydrogenase and ascorbate oxidase enzymes dramatically increased owing to salinity stress. Furthermore, the highest values of catalase and proline dehydrogenase were observed with citric acid, respectively at NaCl rate (2000ppm). They concluded that pretreatment of seeds with 100ppm, CA as soaking significantly reduces the harmful effect of increased salinity and improves all the observed parameters (Hu et al., 2016).

TABLE 3. Dry matter of sugar beet response to spray with antioxidant and Algal extract.

Antioxidant	Root	Leaves	Whole	R/L	R/W
DW.	4.58	3.46	8.04	1.32	0.57
Citric A.	6.46	7.04	13.50	0.92	0.48
Cit+Alg.	8.46	6.94	15.40	1.219.	0.55
LSD at 5%	1.85	2.12	3.08	………	….

- Dw: Distilled water, Cit+Alg.: Citric acid + algal extract.
- LSD: Least significant different.

TABLE 4. Dry matter of sugar beet plants response to antioxidant spraying and salinity stress.

Salinity	Antioxi.	DM Root	DM Leaves	DM Who	R/L	R/Who
Tw	Dw	7.08	4.86	11.94	1.46	0.59
	Citric A	9.85	11.12	20.97	0.90	0.47
	Cit+Alg..	17.31	11.57	28.83	1.50	0.60
5000ppm	Dw	4.72	2.76	7.48	1.71	0.63
	Citric A	5.63	6.38	12.01	0.89	0.47
	Cit+Alg.	5.33	5.73	11.06	0.93	0.49
10000ppm	Dw	1.83	2.78	4.61	0.66	0.40
	Citric A	3.89	3.62	9.37	1.59	0.61
	Cit+Alg.	5.75	3.52	9.27	1.63	0.62
LSD		3.20	N.S	5.33	…..	…..

- Tw: Tap water, Dw: Distilled water, Cit+Alg.: Citric acid + algal extract.
- LSD: Least significant differences.

Concerning the salinity effect through water potential and osmotic pressure adjustment, El-Tohamy et al. (2013) confirmed that when adding citric acid at a rate of 1.5g/ L, it led to an improvement in the water condition of bean plants under drought conditions, based on the higher rate of leaf consumption compared to the control treatment. The total chloride content of the leaves was similar. Also, all the measured plant data were optimized by applying CA in comparison with control plants. And now there are several studies exploring the relationships between CA accumulation in plants and improved drought. Levi et al. (2011) found the enhancement in the ability of some cotton lines to cope with drought due to the accumulation of some organic acids (OA) as well as CA. Shukla et al. (2018) concluded that the accumulation of these metabolites could contribute to enhance tolerance of drought tolerance. Other investigators reported that, the use of citric acid is clearly important to produce high quality fruits (as tomato, pear and citrus crops) and there is an accumulation of those receptors under moderate water stress (Kang et al., 2009; Iwasaki et al., 2011). Sun & Hong (2011) observed that plant growth was significantly improved by CA spraying during saline stress conditions. They added that, there is the importance of using citric acid to cope with stress in *L. chinensis*. Darandeh & Hadavi (2012) noticed that the results observed that the application of 0.15% citric acid increased the life of the vase from 11.8 to 14 days compared to the control plants. This is because CA is considered a a vital organic acid, closely related with stresses (Sun & Hong, 2011).

Concerning the effects of Algal extracts, Ibrahim (2016) stated that marine macro algae originate a variety of highly bioactive, with structures that cannot be found in other organisms. Although, many products obtained from algae have economic importance and are broadly used, in a report, which demonstrates the application of five marine algal extract to overcome the deleterious effect of salt stress on wheat seedlings. The results confirmed the increases in growth and antioxidant content seedlings stressed by salinity of wheat as a result of that presoaking of wheat grains in algal extracts especially that of red alga *L. obtusa*. Abd El-Baky et al. (2008) mentioned that spraying wheat plants with extracts obtained from *Chlorella ellipoida* and *Spirulina maxima* (5g L^{-1}, dry weight in 0.1% Tween solution) cultivated under normal and saline conditions and found that application of algal extracts via leaves significantly increased the contents of total chlorophyll and antioxidant. Moreover, application of algal extracts exhibited strong positive correlation with increase in fresh weight, grain weight and yield traits. Shukla et al. (2018) concluded that the bioactive components of a commercial *A. nodosum* extract enhanced tolerance to salinity stress in *Arabidopsis thaliana* by modulating the expression of different miRNAs through the priming of an extensive network of pathways, some of them related to nutrient uptake and their in plant transfer.

Mineral status of sugar beet plants

Effect of salt stress on the mineral status of sugar beet plants

It is clear from Tables 5, 6 and Figs. 1, 2 that increased salinity deceases the concentration of N, P, K and Fe in leaves of sugar beet. In the opposite side, Ca Na, Mn and Cu concentrations were increased by raising salts concentration of salts in the root media of plants. Meanwhile, Mg and Zn % were slightly affected. The content of macro and micronutrients content were decreased parallel to the increase in salt concentration in irrigation solution (Table 5). Ghoulam et al. (2002) mentioned that change in growth was associated with decreased in the relative water content and K^+ concentrations, but Na^+ and Cl^- contents were highly increased in the leaves.

Several researches were done done to explain the effects of salinity on mineral status, where, El-Tayeb (2005) stated that Sodium, soluble sugars, soluble proteins, free amino acids and also proline content and lipid peroxidation (LP) level and peroxidase activity were increased in the two organs of plant with increasing of sodium chloride level. Electrolyte leakage (EL) from leaves of plants was found to increase with salinity level. Mahmoud (2017) noticed that salt stress significantly increased the concentration of α-amino-N and sodium ions in the storage root. In contrast, potassium content tended to decrease at high levels of salt treatment. Ghoulam et al. (2002) concluded that salinity stress increased the α-amino-N and sodium ions concentration in the sugar beet. The manageable outcomes found out the importance impact of saltiness levels of water on potassium, sodium or potassium/sodium proportion in sugar beet. Collection of high measures of dissolvable salts in soil arrangement is a trademark in dry and sub-dry locales, in spite of the fact that not so much restricted to such regions. The capacity of plants to endure abundance salts in the rhizosphere is of extensive significance in dry and semi-parched areas where salinization of soil generally wins. Likewise, significantly number of studies have been given to study the damage effects of salinity on balance of nutrients (Abdel-Mawly & Zanouny, 2004; Hussein & Abo Bakr, 2018; Hussein et al., 2019).

TABLE 5. Mineral concentrations of sugar beet plants response to salt stress.

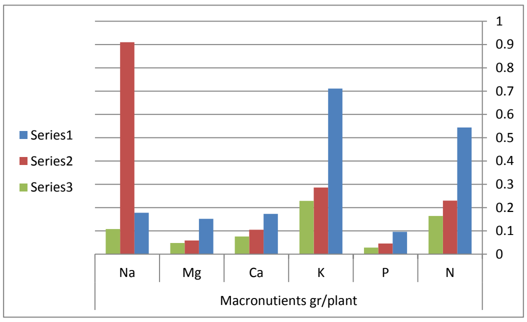
Salinity	Macronutrients%						Micronutrients (ppm)			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Tw	2.43	0.47	3.46	0.83	0.76	0.82	141	34.7	32.7	8.34
5000ppm	2.59	0.44	2.89	1.04	0.59	0.90	128	39.0	34.1	9.67
10000ppm	2.19	0.36	3.05	1.01	0.62	1.02	132	29.3	34.2	10.19

TW: Tap water

TABLE 6. Mineral content of sugar beet plants response to salt stress.

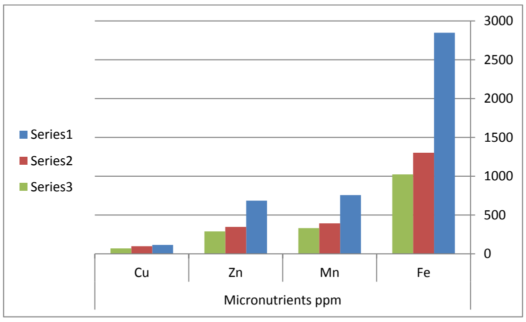
Salinity	Macronutrients gr/plant						Micronutrients ppm			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Tw	0.544	0.0960	0.711	0.173	0.152	0.178	2848	757	685	115
5000ppm	0.230	0.0459	0.286	0.105	0.059	0.108	1303	393	347	98
10000ppm	0.164	0.0284	0.229	0.076	0.048	0.91	1024	332	281	71
LSD	0.041	0.031	0.928	0.089	0.026	0.037	271	67.0	54.34	0.0393

Tw: Tap water.



Series 1: Tap water, Series 2: 5000ppm, Series 3:10000ppm.

Fig. 1. Response of macronutrients content in sugar beet plants as affected by salinity stress.



Series 1: Tap water, Series 2: 5000ppm, Series 3:10000ppm.

Fig. 2. Response of micronutrients content in sugar beet plants as affected by salinity stress.

Effect of citric acid and algal extract on the mineral status of sugar beet plants

As the results in Tables 6, 7, 8 and Fig. 3, 4 it could be mentioned that concentration of P as well as Cu increased by both antioxidant treatment but the values by the combination of CA and algal extract were more than that of CA alone. Na and Mn increased by CA treatment and CA+Alg. but the increase with CA more than that of the combined between them. The reverse was true of K concentration. Fe, Zn and Mg were slightly affected with these treatments. Ca % decreased by CA and tended to increase by CA+Alg. but still more than the control, while N % increased by the application of CA and tended to decrease by CA+Alg. to be less than the control ones. Data in Table 8 indicated that N, Na and Mn showed the higher content by citric acid application, while P, Mg and Cu gave its higher content by the combined application compared with that treatment sprayed by distilled water. The reverse was true for Ca and Zn content, meanwhile Fe decreased either by organic acid alone or by its combination with algal extract.

Many authors worked with the positive effect of citric acid and abiotic stresses such as, Sharma et al. (2013) and Hu et al. (2016). The increase in the different growth characters could be occurred through retarding the biosynthesis degradation hormone enzymes and/or repressing their activities or through preventing the transformation of these active substances into inactive forms. These obtained data was supported by those obtained by Sadak et al. (2013). The function of carboxylic acids in plant responses to environmental of CA, stress is complex and is just beginning to be understood. Citrate is considered to be the most powerful organic anion, followed by oxalate and malate, to mobilize phosphorous in the soil (Bolan et al., 1994). The beneficial effect of this physical-chemical reactions in the roots of wheat, buckwheat, triticale and legumes can be interpreted by the formation of stable molecular complexes between carboxylic acids and metallic cations favoring the availability and absorption with an increase in the plant vigor (Yang et al., 2000).

TABLE 7. Mineral concentrations of sugar beet plants response to antioxidant and algal extract.

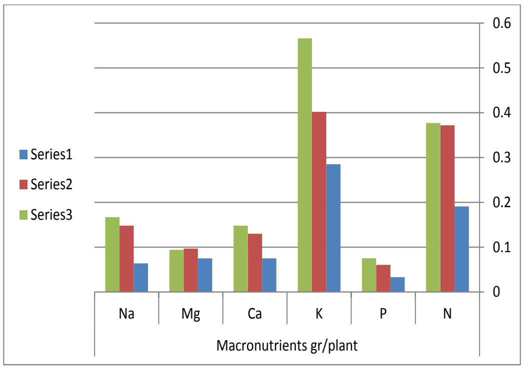
Antiox.	Macronutrients %						Micronutrients ppm			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
DW	2.37	0.39	3.50	1.00	0.72	0.84	132	33.0	34.1	8.56
Citric A	2.35	0.41	2.88	0.91	0.62	0.88	135	31.2	34.1	9.11
Cit+Alg	2.48	0.47	3.03	0.96	0.64	1.02	134	38.8	32.6	9.12

- Dw: Distilled water, Cit+Alg.: Citric acid + algal extract.
- LSD: Least significant different.

TABLE 8. Mineral content of sugar beet plants response to antioxidant and algal extract.

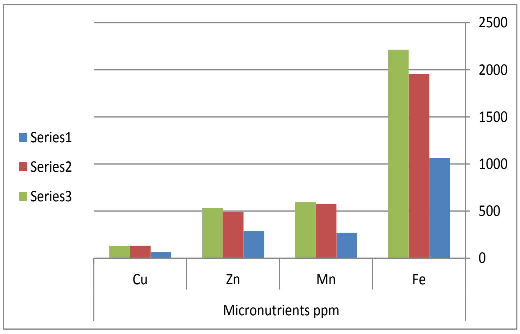
Antiox.	Macronutrients (g/plant)						Micronutrients (ppm)			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
DW	0.193	0.0331	0.285	0.075	0.075	0.064	1061	270	289	66
Citric A	0.369	0.0653	0.376	0.130	0.097	0.148	1926	578	489	125
Cit+Alg.	0.377	0.0753	0.566	0.149	0.094	0.167	2189	623	535	105
LSD	0.097	0.0210	0;146	N.S	0.029	0.55	745	16.67	70.30	0.0393

- Dw: Distilled water, Cit+Alg.: Citric acid + algal extract.
- LSD: Least significant different.



Series 1: Distilled water, Series 2: Citric acid, Series 3: Citric acid + algal extract.

Fig. 3. Macronutrients of sugar beet as affected by antioxidants.



Series 1: Distilled water, Series 2: Citric acid, Series 3: Citric acid + algal extract.

Fig. 4. Micronutrients of sugar beet as affected by antioxidants

The hypothesize that exogenous organic acids may protect larch against nutrient deficiency. Therefore, the possible beneficial role of oxalic acid (OA) and citric acid (CA) on *L. olgensis* seedlings grown in nutrient deficient soil is evaluated by applying concentrations of OA and CA found in *L.olgensis* forest litters (Song & Chui, 2003). Organic acids increase survival rate and biomass accumulation of root, leaf, and stem and lipid peroxidation, superoxide dismutase (SOD), proline, chlorophyll and total carotenoids measures of plant condition all improve. Hence, exogenous organic acids (OA) alleviate the damage of nutrient deficiency to *L. olgensis* seedlings, and improve their resistance to deficiency of nutrients. The relation between organic acids and nutrients were studied by several author i.e., Kpomblekou & Tabatabai (2003) and Palomo et al. (2006).

Algal extract (new biofertilizer) containing some macro and micro nutrients, i.e. (N, P, K, Ca, Mg and S) and (Zn, Fe, Mn, Cu, Mo and Co) as well as some growth regulators, vitamins and polyamines required to be applied for improving nutritional status and enhancing growth, yield and fruit quality in different fruits trees (Abd El-Migeed et al., 2004; Abd El-Moniem et al., 2008). El-Sharony et al. (2015) noticed that application of algal extract at 2% either solitary or combined with one of two plants extracts (10% roselle and 5% garlic), particularly both plant extracts if applied together surpassed in most cases other investigated spray treatments for improving most parameters of fruiting (fruit set, retention, yield and fruit quality), vegetative growth, and nutritional status, (leaf N, P and K contents). Adams (1999) reported that algal extract increased growth parameters and, nitrogenous compounds in wheat compared to the control. Lozano et al. (1999) showed that application of algal extract so through foliage or soil increased carbohydrate, ash and protein of potatoes plants. Abd El-Baky et al. (2009) suggested that the application of algal extracts on wheat plants irrigated by diluted seawater is beneficial for enhancement of salinity tolerance. This effect can be triggered by the stimulation of protein content and antioxidant components. In spite of the effect of organic acid concentration on leaf potassium % and iron ppm, also, although the effect of organic acid concentration on leaf potassium and iron was significant, the impact on leaf magnesium % was not a strongly decreasing trend as observed in roots (Song et al., 2016).

Effect of salinity stress, citric acid and algal extract on the mineral status of sugar beet plants

It is obvious from data in Table 9 that nitrogen increased with citric acid and tended to decrease with the mixture of citric acid and algal extract. Na and Mn showed the same response. However, P and Cu content were increased by citric acid or both antioxidant while Zn and K content seemed to be decreased by antioxidants or its combination.

The ratios of Na with Ca, Mg and Ca (Na+K) concentration showed similar trends either by salinity or by foliar spraying of antioxidants which increased by the both factors. The higher values of Na ratios with K and Ca by the spraying the mixture under control and 5000ppm salt treatments were obtanined, while under high salt treatment, the higher values were obtained with citric acid addition. Na/Mg ratio showed approximately similar values under 5000ppm with that treatment sprayed by distilled water, but under 10000ppm salt treatment, similar values were obtained by mixture as well as by citric acid solitary. In addition. The value of Ca (Na+K) was higher by citric acid than use distilled water as a control under tap water supply while under moderate or high salinity citric acid application slightly decreased and tended to slight increase by the combined treatment (Table 10).

The interaction effect of antioxidant application on sugar beet plants grown under salinity condition on macro and micronutrients content were illustrated in Table 11. These effects showed that P, K, Mg, Na and Fe contents increased by both antioxidant treatments whatever the salt treatment level. N, Zn and Mn only showed the higher response under fresh water irrigation by the combined antioxidant treatment. On the other hand, under the two salinity treatments the higher values with citric acid and lowered with the mixture treatments but still more than the control. Mg under moderate salinity and cu under fresh water irrigation showed the similar values by both antioxidant treatments.

TABLE 9. Effect of antioxidant spraying on macronutrients concentration of sugar beet plants grown under salinity stress.

Salinity	Antioxi.	Macronutrients g/100g						Micronutrients ppm			
		N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu
TW	DW	2.48	0.48	3.83	0.73	0.73	0.64	136	30	37	6.7
	Citric A	2.83	0.49	2.70	0.69	0.93	0.78	148	36	33	7.7
	Cit+Alg.	2.57	0.43	3.85	0.54	0.82	1.03	138	38	32	6.3
5000ppm	DW	2.33	0.36	3.13	0.62	1.13	0.93	126	37	33	10.7
	Citric A	2.58	0.36	3.23	0.55	0.99	0.81	131	41	35	9.7
	Cit+Alg.	1.85	0.61	2.87	0.68	0.99	0.97	126	39	34	8.7
10000ppm	DW	2.32	0.33	3.53	0.58	1.13	0.95	133	32	39	8.3
	Citric A	2.25	0.38	2.70	0.62	0.81	1.95	127	52	38	10.3
	Cit+Alg.	2.01	0.37	2.93	0.65	1.08	1.05	137	39	33	12.3

- Tw: Tap water, Dw: Distilled water, Cit+Alg.: Citric acid + algal extract.
- LSD: Least significant different.

TABLE 10. Effect of antioxidant spraying on ratios of Na with the other macronutrients concentration of sugar beet plants grown under salinity stress.

Salinity	Antioxi.	Na/K	Na/Mg	Na/Ca	Ca (Na+K)
Tw	Dw	0.167	0.877	o.877	0.163
	Citric A	0.289	1.131	0.839	0.267
	Cit+Alg.	0.269	1.907	0.278	0.168
5000ppm	Dw	0.297	1.500	0.823	0.278
	Citric A	0.252	1.473	0.828	0.225
	Cit+Alg.	0.338	1.427	0.980	0.236
10000ppm	Dw	0.269	1.638	0.841	0.252
	Citric A	0.723	3.145	0.733	0.175
	Cit+Alg.	0.353	1.615	0.972	0.271

- Tw: Tap water, Dw: Distilled water, Cit+Alg.: Citric acid + algal extract.
- LSD: Least significant different.

TABLE 11. Effect of antioxidant spraying on macronutrients content of sugar beet plants grown under salinity stress.

Salinity	Antioxi.	Macronutrients. gr\plant						Micronutients. ppm			
		N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
TW	DW	0.296	0.0573	0.457	0.087	0.153	0.077	1627	385	442	80
	Citric A	0.594	0.1028	0.566	0.195	0.145	0.164	3014	755	692	162
	Cit+Alg.	0.741	0.1241	1.110	0.236	0.156	0.297	3903	1095	922	102
5000ppm	DW	0.175	0.0269	0.234	0.085	0.046	0.070	943	277	246	80
	Citric A	0.311	0.0432	0.308	0.119	0.066	0.097	1573	492	420	117
	Cit+Alg.	0.205	0.0675	0.317	0.110	0.066	0.107	1393	411	376	98
10000ppm	DW	0.107	0.0152	0.163	0.052	0.027	0.044	613	148	180	38
	Citric A	0.201	0.0356	0.253	0.076	0.058	0.183	1190	487	356	97
	Cit+Alg.	0.186	0.0343	0.272	0.100	0.060	0.097	1270	362	306	114
LSD		0.368	N.S	N.S	N.S	N.S	0.094	N.S	38.87.	121.8	N.S

- Tw: Tap water, Dw: Distilled water, Cit+Alg.: Citric acid + algal extract.
- LSD: Least significant different.

Abdelmotlb et al. (2019) revealed that plants irrigated with lower saline water (1000ppm) had better antioxidant enzyme activity and accumulated higher K and lower Na and Cl than plants grown under high salinity irrigation water (4000ppm). The anti-salinity application positively enhanced the yield components under all salinity stress levels and the highest yield was significantly detected with glycine betaine application followed by molasses and lastly coming untreated plants. According to the triple applications of sulfur + compos microbial inoculation with *Azolla* and Cyanobacteria extract are recommended to ameliorate the saline-sodic soilon one hand and to attain better crop yield on the other one (Helmi et al., 2018). In addition, Selem (2019) emphasized that application of *Spirulina platensis* ameliorated adverse effects of salinity by enhancing total protein level, sodium, phosphorus and potassium and photosynthetic activity (CO_2 assimilation). This first attempt to evaluate the potential of *Spirulina platensisas* growth enhance under salt-stressed *Vicia faba* indicated that exogenously applied *Spirulina platensis* (100mg/L) provided more benefit against salinity stress.

Data illustrated in Table 12 and Fig. 5 showed that Na/Ka ratio (in content basis) increased as the salt concentration increased and also increased by antioxidant treatments. The highest value of this ratio was obtained with citric acid under high salinity and fresh water treatments but under moderate salinity the highest value was by citric acid+algal extract treatment. For the Na/Mg ratio, regardless the antioxidant treatments, this ratio decreased with the increasing salt concentration, while it was decreased slightly when plants sprayed with citric acid and tended to increase with citric acid+algal extract treatments under moderate salinity irrigation but the reverse was true under the high salinity treatment. It was clear from the same Table and Fig. that Na/Ca ratio increased parallel to the increase of salts in the irrigation solution up to the highest level used under either tap water or moderate salinity condition, however, under high salinity this ratio increased by citric acid and tended to decrease by the combined of this organic acid and algal extract mixture.

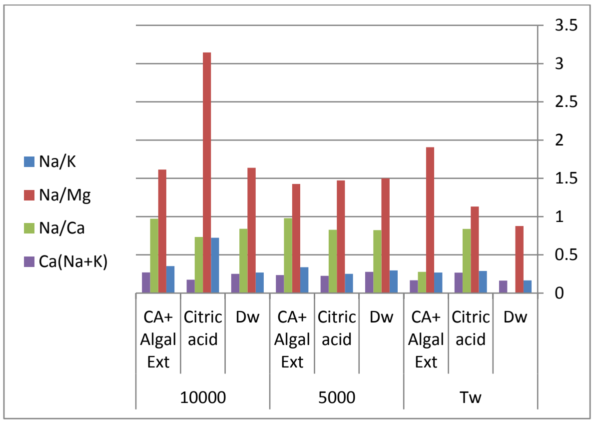
Concerning the Ca (Na+K) ratio,it was increased only by citric acid spraying under fresh water treatment but under 5000ppm salinity treatment is quaintly increased but clearly decreased with citric acid+algal extract spraying. Moreover, this ratio markedly decreased with citric acid and return to increase by the mixture of antioxidant application in comparable with that in plants received distilled water.

Mansour et al. (2019) mentioned that respecting to the 1st and 2nd order interacted of factors under this investigation effect on such parameters; the results showed that there were many characters that improved significantly, however, microalgae have shown their potential as bio-stimulants and bio-fertilizers (Khan et al., 2009; Garcia-Gonzalez & Sommerfeld, 2016). Recently, Microalgae production is attractive to the development of agricultural enterprises, especially MBS or MBF, and there is also interest from producers and agrochemical plants with the aim of improving the sustainability of crop production (Calvo et al., 2017). The occurrence of bioactive compounds in algal extracts such as flavonoids, , ascorbic acid (AA), proline, CA and plant hormones could potentially participate in the salinity stress alleviations (Ibrahim, 2016). They added that this results supported the presoaking technique of marine algal extract is an effective technology in solving one of the important economic problems in agriculture.

TABLE 12. Effect of antioxidant spraying on ratios of Na with the other macronutrients content of sugar beet plants grown under salinity stress.

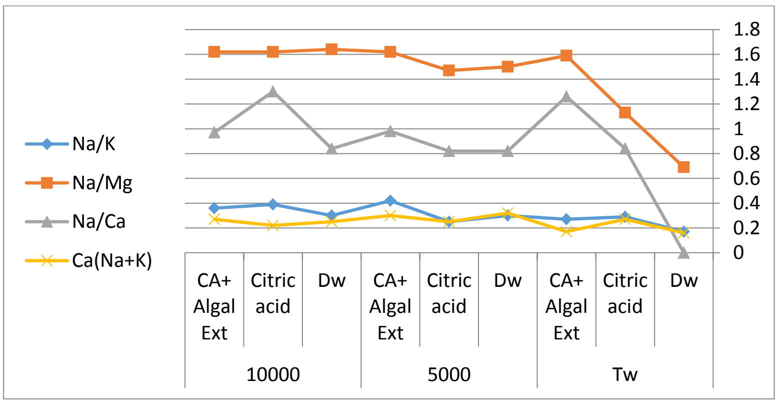
Salinity	Antioxi.	Na/K	Na/Ca	Na/Mg	Ca (Na+K)
	Dw	0.169	0.8^5	0.503	0.163
Tw	Citric A	0.290	0.841	1.131	0.267
	Cit+Alg.	0.268	1.259	1.904	0.168
	Dw	0.299	0.824	1.522	0.280
5000ppm	Citric A	0.315	0.815	1.470	0.294
	Cit+Alg.	0.338	0.973	1.621	0.260
	Dw	0.270	0.846	1.628	0.251
10000ppm	Citric A	0.723	2.408	3.155	0.174
	Cit+Alg.	0.357	0.970	1.616	0.271

- Tw: Tap water, Dw: Distilled water, Cit+Alg.: Citric acid + algal extract.
- LSD: Least significant different.



[Dw: Distilled water Tw: Tap water, CA+Algal Ext: Citric acid + algal extract].

Fig. 5. Na ratios with some other macronutrients as affected by antioxidants and salinity stress.



[Dw: Distilled water Tw: Tap water, CA+Algal Ext: Citric acid + algal extract].

Fig. 6. Na ratios with other macronutrients of sugar beet as affected by antioxidants and salinity stress.

Conclusion

Salinity stress is the most factor of adverse effect of environmental stresses and use natural products for ameliorate this reverse condition considered, nowaday, a retch field of research for its impotency in economic point of view and diminishing ecological pollution. In this study, the salinity stress affected badly growth and mineral content of plants. On reverse, the exogenous application of citric acid or its mixture with algal extract depressed the negative effect of salts through its role in improving growth and balancing the sodium concentration and content and the other nutrient elements in sugar beet plants.

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