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Ameliorating the deleterious effects of saline water on the antioxidants defense system and yield of eggplant using foliar application of zinc sulphate

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ABSTRACT

To alleviate the harmful effects of NaCl salinity on the development, antioxidants defense system and yield of eggplant (*Solanum melongena* L.) using foliar application of zinc sulphate ($ZnSO_4$), a pot experiment was conducted during two summer seasons 2017 and 2018 at the Experimental Farm, Faculty of Agriculture, Ain Shams University. Eight treatments were the combination of two sodium chloride levels of irrigation water 0 and 100 mM, and four levels of $ZnSO_4$ 0, 0.7, 1.5 and 3 mM. Results indicated that, under 100 mM NaCl concentration of the macronutrients (N, K, Mg and Ca) in leaves significantly decreased, while sodium concentration increased in both leaves and fruits which led to an increase in the concentration of malondialdehyde (MDA) which in turn reflecting on the reduction of vegetative growth, photosynthetic pigments, flowers number, fruits number, fruit setting and total yield of eggplant in both seasons. The foliar application of $ZnSO_4$ alleviated the adverse effects of salinity by stimulating the growth, antioxidants defense system in the form of superoxide dismutase (SOD), total soluble sugars, proline, free amino acids and total soluble protein, which enhanced the flowers number, fruits number, fruit setting and total fruits yield. The most effective application that antagonized the adverse effects of salinity stress was 1.5 mM $ZnSO_4$.

1. Introduction

Eggplant (*Solanum melongena*, L.) is a popular vegetable cash crop grown in many countries throughout the subtropics, tropics and Mediterranean area like Egypt. Also, it has been largely consumed in world due to nutritive and medical advantages which consider as higher antioxidant and nutritional value i.e. a very good source of vitamins, dietary fibres, minerals, phenolic compounds such as caffeic and chlorogenic acid and flavonoids, such as nasunin or delphinidin-3-(coumaroylrutinoside)-5-glucoside (Dias, 2012). However, eggplant is moderately sensitive to salinity stress with a threshold of 1.5 dS/m, and the relative fruit yield reduced to 56% when the electrical conductivity of irrigation water increased to 10 dS/m (Ünlükara et al., 2010).

Furthermore, plant growth, development and yield of plants are limited under salinity stress by different ways i.e. osmotic effect (water uptake), specific-ion toxicity and/or nutritional imbalances due to nutrients availability, uptake and translocate within plant cells (Gupta and Huang, 2014; Maathuis, 2007; Munns, 2002; Yasin Ashraf et al., 2014).

Salinity affected morphological, biochemical and physiological processes of plants (Nawaz et al., 2010; Osman and Salim, 2016a; Parida and Das, 2005). Therefore, using saline water (sodium chloride) caused reductions in vegetative growth, yield and quality of vegetable crops such as eggplant (Hegazi et al., 2015).

Zinc (Zn) required in growth, development, flowering, pollination, biosynthesis of tryptophan and hormones mainly indole acetic acid (IAA) and component of several metallo-enzymes which necessary for several functions in plant metabolism (Broadley et al., 2012; Marschner, 1995). Foliar application of Zn improved the vegetative growth, productivity and minimized the adverse effects of salinity on plant biomass, water relation, gas exchange characteristics and yield of rice. Zinc was also useful under non stressed conditions (Yasin Ashraf et al., 2014). Likewise, Pandey et al. (2010) stated that, the harmful effects of salinity stress on growth and yield of mungbean were minimized after $ZnSO_4$ foliar spray.

The objectives of present research were to study the impact of zinc sulphate in mitigating the adverse effects of saline water stress on eggplant and its role in improving the photosynthetic pigments, osmolytes, antioxidants defense system and mineral nutrients status.

2. Materials and methods

To investigate how could the foliar spray with zinc in the form of

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$ZnSO_4$ be effective in ameliorating the negative impact at eggplant plants subjected to salt stress, a two years pot experiments were conducted at the Experimental Farm, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, during two successive summer seasons at 21 March 2017 and 2018.

2.1. Soil preparation and transplantation

Seedlings of eggplant "long white" (45 day old) were selected and transplanted into plastic pots (40 cm diameter) containing 15 kg of acid washed sandy soil with one plant/pot. The seedlings were irrigated with Hoagland solution (Hoagland and Arnon, 1950).

2.2. Experimental design and treatments

Two sets of pots were used to test the effect NaCl salinity on eggplant growing plants. The first set of pots served as control of salinity (0 mM NaCl) where the full-strength Hoagland nutrient solution was used without the addition of NaCl. The second set of pots was the same full-strength Hoagland nutrient solution plus 100 mM NaCl. The irrigation with saline solution started at two weeks after transplanting at rate 3 L per pot every 3 days. After every two times of irrigation with saline water and before applying the next one, pots were irrigated with tap water to avoid salt accumulation in the soil. Zinc sulphate ($ZnSO_4$) was used as a source of Zn which applied as a foliar treatment with the following concentrations; 0, 0.7, 1.5 and 3 mM. The application of the foliar spray with $ZnSO_4$ was three times with 15-day intervals started at two weeks after transplanting.

The pots of experiment were arranged to be in a complete randomized block design with three replicates, where the eight treatments as following:

1) NaCl at 0 mM + $ZnSO_4$ at 0.0 mM
2) NaCl at 0 mM + $ZnSO_4$ at 0.7 mM
3) NaCl at 0 mM + $ZnSO_4$ at 1.5 mM
4) NaCl at 0 mM + $ZnSO_4$ at 3.0 mM
5) NaCl at 100 mM + $ZnSO_4$ at 0.0 mM
6) NaCl at 100 mM + $ZnSO_4$ at 0.7 mM
7) NaCl at 100 mM + $ZnSO_4$ at 1.5 mM
8) NaCl at 100 mM + $ZnSO_4$ at 3.0 mM

2.3. Vegetative growth characteristics

Plant height, leaves number/plant, average leaf area, average leaf fresh and dry weights, the percentage between leaf dry weight/fresh weight and leaf area index (LAI) were recorded at 60 days after transplanting (DAT) to explore the status of vegetative growth under the studied treatments. Average leaf area was calculated by analyzing the images of total plant leaves by Image-pro plus software (version 6.2, Media Cybernetics Inc., USA). Leaf area index was calculated according to the equation of Hunt (1990).

$$LAI = \frac{\text{Total leaf area per plant}}{\text{Total ground area per plant}}$$

2.4. Flowering, fruit setting and yield

At flowering and ripening stages, the flowers number/plant were accounted at 40, 55, 70, 85 and 100 DAT, whereas the full-size fruits were harvested five times (55, 70, 85, 100 and 115 DAT) to calculate the fruit setting ratio and the fruit set ratio over control (FS. T/C %) by the equation described by Osman and Salim (2016b):

$$FS.\ T/C\% = \left(\frac{FS.\ T}{FS.\ C} - 1\right) \times 100$$

where, FS. T = the percentage of fruit setting of the treatment, FS. C = the percentage of fruit setting of the control under non-stressed conditions.

Additionally, fruits number/plant, average fruit fresh weight, fruit length and total fruits yield/plant were recorded.

2.5. Determination of biochemical active compounds

2.5.1. Photosynthetic pigments

The methods as described by Arnon (1949) were used to extract the photosynthetic pigments of eggplant fresh leaves. Leaves sample were homogenized and centrifuged in the presence of 80% acetone. Concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophylls and carotenoids were calculated using formulae described by Lichtenthaler and Wellburn (1983), after measuring the absorption of the extracted pigments using a spectrophotometer (Mapada UV 1200) at 470, 647 and 664 nm.

2.5.2. Osmolytes and soluble proteins

The major plants' osmolytes (total free amino acids, total soluble sugars and proline) were determined to study its role in plant response to changes in environmental conditions from non-stressed to stress condition (salinity). Free amino acids concentrations in eggplant leaves were analyzed using ninhydrin method described by Swamy (2008). The developed color with ninhydrin reagent was measured using a spectrophotometer (Mapada UV 1200) at 570 nm. Whereas, the concentrations of proline in the same leaves were extracted in 3% (*w/v*) aqueous sulfosalicylic acid, then determined using the acid ninhydrin reagent and reading at 520 nm as described by Bates et al. (1973). Total soluble sugars were extracted from leaves sample using 80% hot ethanol (A.O.A.C., 2005), then spectrophotometrically determined using anthrone reagent (100 mg anthrone +50 ml 95% H_2SO_4) as described by Sadasivam and Manickam (2010) at 620 nm using glucose as standard. Total soluble protein was determined in the leaves extracts using the colorimetric method described by Bradford (1976), based on binding of proteins to Coomassie Brilliant Blue G-250 reagent then reading at 595 nm using bovine serum albumin as the standard.

2.5.3. Assessing the antioxidant status of SOD and oxidative damage

2.5.3.1. Activity of superoxide dismutase. One gram of eggplant leaves samples were freezing in liquid nitrogen to prevent proteolytic activity followed by grinding with 5 ml of cold extraction buffer (0.1 M phosphate buffer, pH 7, containing 0.5 mM EDTA and 2% (*w/v*) polyvinylpyrrolidone (PVP)), then centrifuged for 20 min at 10,000 $\times g$ to use the supernatant as enzyme extract. The specific activity of SOD was determined using the nitro-blue tetrazolium (NBT) photochemical assay at 560 nm as described by Beauchamp and Fridovich (1971).

2.5.3.2. Lipid peroxidation. The production of malondialdehyde (MDA) in plant cells considered a good indicator for the oxidative damage to membrane lipids which determined by reacts with thiobarbituric acid (TBA) to form a pink solution. Lipid peroxidation rates were estimated by measuring the malondialdehyde equivalents according to improving method by Hodges et al. (1999).

2.5.4. Determination some macro, micronutrients and sodium

At 70 days from planting, eggplant leaves and fruits samples were cut gently at random from each treatment, plant samples were dried at 60 °C in a forced air oven for 72 h to determine the concentration of N, P, K, Mg, Ca, Fe, Mn, Zn, Cu and Na. Samples (0.5 g) were wet digested using H_2SO_4 and H_2O_2 mixture. Total nitrogen concentration was determined by using the micro-Kjeldahl method as described by Horneck and Miller (1998). The molybdenum blue method described by Bernhart and Wreath (1955) was used to determine total P. The flame photometer were used as described by Horneck and Hanson (1998) to determine the concentration of K and Na. The inductively coupled

Table 1
Influence of foliar spray with $ZnSO_4$ on the vegetative characteristics of eggplant plants growing under different levels of NaCl salinity (0 and 100 mM) during 2017 and 2018 growing seasons (main of two seasons).

Treatments (mM)		Plant height (cm)	Leaves no./plant	Average leaf FW (g)	Average leaf DW (g)	DW/FW %	Average leaf area (cm^2)	LAI
NaCl	$ZnSO_4$							
0	0	42.5 ± 3.1^{bc}	16.3 ± 2.5^{c}	3.26 ± 0.2^{b}	0.47 ± 0.0^{d}	14.5 ± 0.5^{cd}	42.4 ± 7.6^{ab}	1.0 ± 0.3^{bc}
	0.7	45.6 ± 1.6^{abc}	19.8 ± 2.0^{bc}	3.42 ± 0.1^{ab}	0.63 ± 0.1^{ab}	18.4 ± 0.9^{ab}	68.8 ± 11.8^{ab}	1.9 ± 0.2^{ab}
	1.5	48.3 ± 3.5^{abc}	23.9 ± 3.2^{ab}	3.61 ± 0.1^{a}	0.67 ± 0.1^{a}	18.7 ± 1.9^{ab}	74.1 ± 17.1^{a}	2.5 ± 0.5^{a}
	3	54.0 ± 1.0^{a}	23.6 ± 2.3^{ab}	3.39 ± 0.1^{ab}	0.6 ± 0.0^{abc}	17.8 ± 0.6^{ab}	77.8 ± 19.7^{a}	2.6 ± 0.7^{a}
100	0	39.5 ± 3.8^{c}	13.7 ± 3.8^{c}	3.38 ± 0.1^{ab}	0.45 ± 0.0^{d}	13.2 ± 0.8^{d}	34.7 ± 5.3^{b}	0.7 ± 0.2^{c}
	0.7	44.2 ± 1.7^{bc}	15.0 ± 2.6^{c}	3.36 ± 0.1^{ab}	0.58 ± 0.0^{bc}	17.3 ± 0.5^{b}	37.7 ± 3.5^{b}	0.8 ± 0.2^{c}
	1.5	47.8 ± 1.6^{abc}	24.0 ± 3.6^{ab}	3.27 ± 0.1^{b}	0.66 ± 0.0^{ab}	20.1 ± 0.3^{a}	61.6 ± 11.5^{ab}	2.1 ± 0.3^{ab}
	3	51.3 ± 5.5^{ab}	28.3 ± 3.2^{a}	3.24 ± 0.1^{b}	0.54 ± 0.0^{cd}	16.6 ± 1.3^{bc}	52.0 ± 7.1^{ab}	2.1 ± 0.5^{a}
MSD 0.05		9.61	6.88	0.32	0.09	2.34	35.48	1.09
Mains of $ZnSO_4$	0	41.0^{c}	15.0^{b}	3.32^{a}	0.46^{c}	13.9^{c}	38.6^{b}	0.8^{b}
	0.7	44.9^{bc}	17.4^{b}	3.39^{a}	0.61^{b}	17.8^{b}	53.2^{ab}	1.4^{b}
	1.5	48.1^{ab}	23.9^{a}	3.44^{a}	0.67^{a}	19.4^{a}	67.8^{a}	2.3^{a}
	3	52.6^{a}	25.9^{a}	3.32^{a}	0.57^{b}	17.2^{b}	64.9^{a}	2.3a
Mains of NaCl	0	47.6^{a}	20.9^{a}	3.42^{a}	0.60^{a}	17.3^{a}	65.7^{a}	2.0^{a}
	100	45.7^{a}	20.2^{a}	3.31^{a}	0.56^{b}	17.1^{a}	46.5^{b}	1.4^{b}
MSD $ZnSO_4$		5.6	4.0	0.19	0.05	1.4	20.7	0.6
MSD NaCl		2.9	2.1	0.10	0.03	0.7	10.8	0.3

Means (±SD) followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test. Where MSD = minimum significant difference, DW = dry weight, FW = fresh weight, LAI = leaf area index.

plasma atomic emission spectroscopy (ICP-AES) were used as described by Stefánsson et al. (2007) to determine Mg, Ca, Zn, Fe, Mn and Cu.

2.6. Statistical analysis

Data of the two seasons were arranged and statistically analyzed using CoStat software (version 6.4, CoHort Software, USA) according to the method described by Gomez and Gomez (1984). Two-way analysis of variance (ANOVA) was used to test for significant differences among salinity levels and foliar spray with $ZnSO_4$ at $P < 0.05$, followed by Tukey's HSD test.

3. Results

3.1. Vegetative growth

Irrigation with saline water (NaCl) had a negative impact on all vegetative growth parameters of eggplant as presented at Table 1. Although, the significance test between means showed that the individual treatment of NaCl at 100 mM didn't significantly different from the individual treatment of 0 mM NaCl for all the studied vegetative growth parameters (plant height, leaves no./plant, leaves FW and DW, average leaf area and LAI), salinity at 100 mM NaCl decreased the growth. These insignificant decreases in all vegetative growth parameters may be refer to the presence of $ZnSO_4$ (the second studied factor), which recorded a high positive increase in the values of vegetative growth parameters, which in turn had indirect effect on the significance test. The main effect of salinity supports this hypothesis, where application of 100 mM NaCl didn't significantly affect plant height, leaves no./plant, average leaf FW and leaves DW/FW %. In contrast, the most influenced parameters under NaCl-salinity were leaf DW, leaf area and LAI (Table 1). The foliar applications of $ZnSO_4$ (0.7, 1.5, 3 mM) significantly increased the values of the most studied parameters comparing with the control level (0 mM $ZnSO_4$) either under 0 mM NaCl or 100 mM NaCl. Although the application of $ZnSO_4$ at 3 mM recorded the highest significant values for plant height either under stressed or non-stressed conditions, the most effective treatment under the same conditions was $ZnSO_4$ at 1.5 mM which highly increased and promoted the vegetative growth (Table 1). Between all the vegetative growth parameters, the most influenced one by $ZnSO_4$ treatments were leaf area and LAI which recorded an increase ranged from 1.5:1.8 and 2:2.5 times respectively over control under both salinity levels.

3.2. Flowering and fruit yield

Flowering is a very sensitive stage to the changes of environmental factors. Responses of the different flowering and yield parameters indicated to the strength of flowering stage under salinity stress (100 mM NaCl) comparing with control conditions (0 mM NaCl) were recorded and illustrated in Fig. 1. Generally, irrigation with saline water (100 mM NaCl) led to a significant reduction in flowers no. and fruits no. which consequently affected the percentage of fruit setting (Fig. 1A–C).

Not always values of fruit set % consider a better indicator for the power of treatment on the characteristics of flowering and yield especially when the growth conditions are instable which contributes substantially to affect on the total number of flowers. In view of the fact that value of the fruits no./plant will never exceed the value of the flowers no./plant, so the most important parameters would indicate to the changes in flowering under saline conditions are both of flowers no. per control and fruits no. per control of non-stressed plants. Regarding the values (8 and 12.7) of the flowers no./plant under saline and non-saline conditions respectively, the individual treatment of NaCl at 100 mM reduced the flowers no./plant to 40% less than control (0 mM NaCl) (Fig. 1A). Respecting the production and development of fruits under the same conditions, 75% of flowers (8) would be able to produce fruits under 100 mM NaCl comparing with 87% (12.7 flower) under 0 mM NaCl, which indicate that the reduction in fruit set % over control was −12%, while focusing on the values of the fruits no. under saline and non-saline conditions (6 and 11 fruit/plant) revealed that the fruits no. per control had been reduced to reach 55% under saline water treatment (Fig. 1A–C).

Foliar application with $ZnSO_4$ at the lowest level (0.7 mM) boosted the development and stability of flowers and fruits of eggplant under either stressed or non-stressed conditions (Fig. 1A–C). Flowers no. under 100 mM NaCl was increased to record 12.3 flower/plant with application of 0.7 mM $ZnSO_4$ which become near enough from control (12.7 flower/plant) of non-stressed plant with a proportion reach 97% based on control. The same observation was obvious for fruits no./plant, where the percentage per control reach 91% after treated with 0.7 mM $ZnSO_4$ (10 compared to 11 fruit/plant). The same level of $ZnSO_4$ encouraged the plants irrigated with non-saline water to

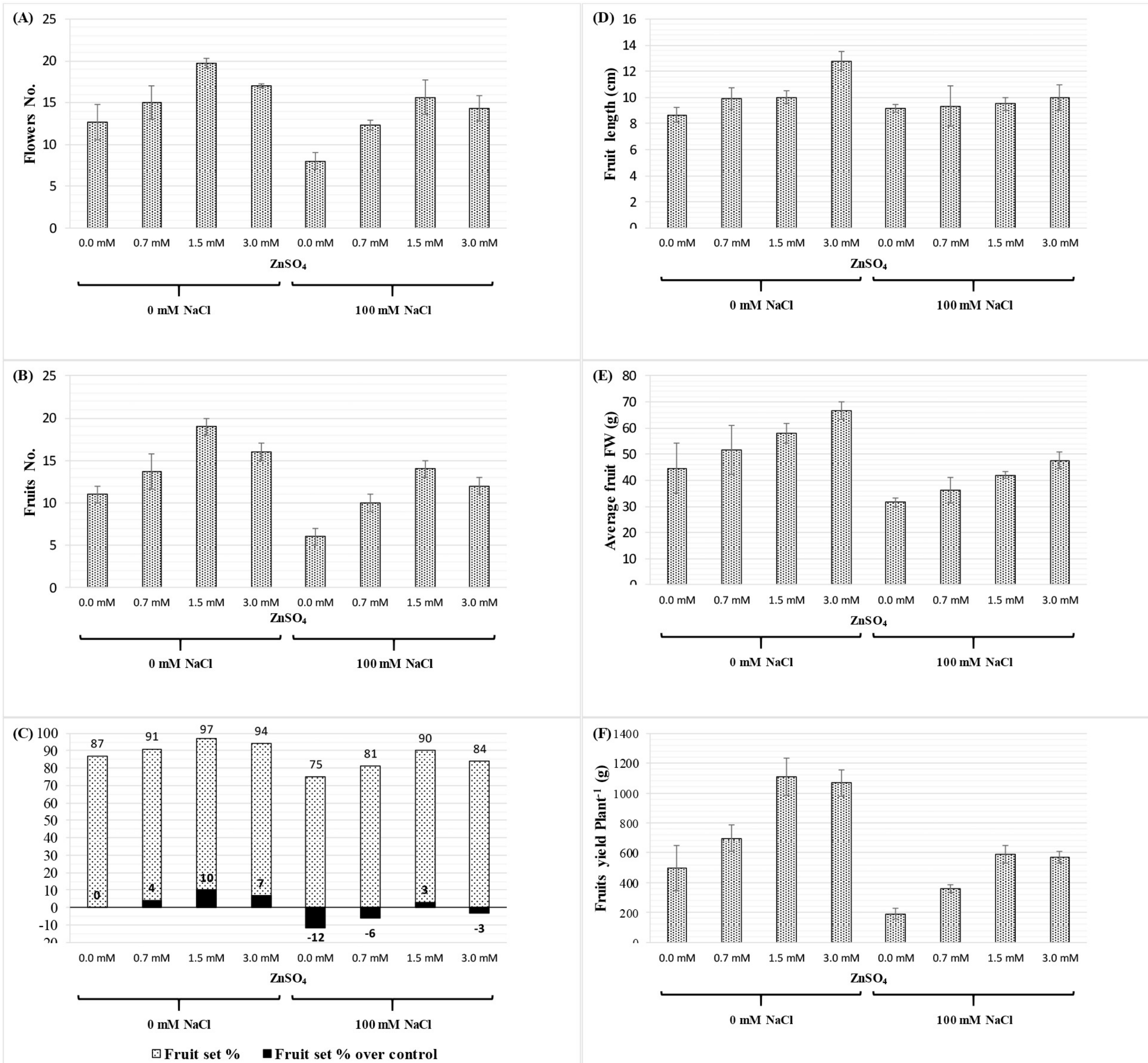

Fig. 1. Influence of foliar spray with $ZnSO_4$ on the flowering and fruit yield characteristics of eggplant plants growing under different levels of NaCl salinity (0 and 100 mM) during 2017 and 2018 seasons (main of two seasons). Means (±SD as error bar).

improve the development of flowering stage by increasing both flowers and fruits numbers to record an increase by 18% and 24% over control of flowers and fruits numbers parameters under the same condition. Furthermore, increasing the level of applied $ZnSO_4$ to 1.5 mM boosted the values of flowers and fruits numbers to its highest significant levels either under normal conditions or stressed conditions, where recorded an increase under non-stressed conditions by 55% and 73% respectively over control (19.7 compared to 12.7 flower/plant, and 19 compared to 11 fruit/plant), whereas the same treatment (1.5 mM $ZnSO_4$) under 100 mM NaCl recorded an increase over the control of both stressed condition and normal condition by 23% and 27% for flowers and fruits numbers respectively regarding its means (15.7 compared to 12.7 flower/plant, and 14 compared to 11 fruit/plant). Although increasing the level of $ZnSO_4$ to 3 mM led to an increase in the values of all flowering parameters either under stressed or non-stressed conditions over what recorded with $ZnSO_4$ at 0.7 mM or control, unexpectedly the means values for these parameters were in the second order after the treatment of $ZnSO_4$ at 1.5 mM (Fig. 1A–C). This observation directed the thinking to looking for where that much of wasted energy go or it might be had a negative effect on some physiological functions which led to a retreat in the growth acceleration of flowering parameters. The possible answer for this hypothesis comes from the results of plant height (Table 1), where $ZnSO_4$ at 3 mM recorded the highest values between all treatments, which indicate that type of wasted energy was directed to support the vegetative growth instead of the reproductive growth.

The yield of fruits/plant is the outcome of fruits number and average fruit FW. Irrigation with Saline water reduced the fruits yield by 62% comparing with control. The treatment of $ZnSO_4$ at 1.5 mM recorded the highest significant values between all $ZnSO_4$ treatments either under non-stressed or stressed conditions with an increase 122% and 18% over control of 0 mM NaCl respectively (Fig. 1F). Since the highest values for average fruit FW recorded with the treatment of $ZnSO_4$ at 3 mM under both salinity levels (Fig. 1E), so the significant in fruits yield readings refer mainly to fruits no./plant (Fig. 1B).

3.3. Biochemical changes

Salinity stress had a negative impact on the amount of chlorophylls. Applications of $ZnSO_4$ slightly improved the chlorophyll concentrations

Table 2
Influence of foliar spray with $ZnSO_4$ on the photosynthetic pigments concentration of eggplant plants growing under different levels of NaCl salinity (0 and 100 mM) during 2017 and 2018 growing seasons (main of two seasons).

Treatments (mM)		Chl *a* (mg/g FW)	Chl *b* (mg/g FW)	Chl *a*+*b* (mg/g FW)	Carotenoids (mg/g FW)	Chl *a*:*b* (Ratio)
NaCl	$ZnSO_4$					
0	0	1.13[b]	0.39[c]	1.53[b]	0.10	2.87
	0.7	1.37[a]	0.50[a]	1.86[a]	0.10	2.76
	1.5	1.28[ab]	0.47[ab]	1.75[a]	0.12	2.74
	3	1.29[ab]	0.42[bc]	1.71[a]	0.14	3.05
100	0	0.66[c]	0.27[d]	0.94[c]	0.08	2.42
	0.7	0.70[c]	0.27[d]	0.97[c]	0.09	2.61
	1.5	0.79[c]	0.28[d]	1.07[c]	0.12	2.92
	3	0.77[c]	0.28[d]	1.05[c]	0.13	2.83
MSD 0.05		0.18	0.07	0.17	NS	
Mains of $ZnSO_4$	0	0.90[b]	0.33[b]	1.23[b]	0.09	
	0.7	1.04[a]	0.38[a]	1.42[a]	0.10	
	1.5	1.04[a]	0.38[ab]	1.41[a]	0.12	
	3	1.03[a]	0.35[ab]	1.38[a]	0.13	
Mains of NaCl	0	1.27[a]	0.44[a]	1.71[a]	0.12	
	100	0.73[b]	0.28[b]	1.01[b]	0.10	
MSD $ZnSO_4$		0.11	0.04	0.10	NS	
MSD NaCl		0.06	0.02	0.05	NS	

Means followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test.
NS = Not significant at $p \leq 0.05$.

but didn't reach the significant level under 100 mM NaCl. Foliar application of $ZnSO_4$ at all studied levels enhanced the values of photosynthetic pigments under irrigation with non-saline water to record the highest significant level by $ZnSO_4$ at 0.7 mM for Chlorophyll *a*, Chlorophyll *b* and total chlorophylls, whereas the highest values of carotenoids were recorded by $ZnSO_4$ at 3 and 1.5 mM respectively (Table 2).

Concentrations of free amino acids, proline, total soluble proteins and total soluble sugars in leaves were presented in Fig. 2. Salinity insignificantly decreased the values of free amino acids and total soluble proteins while recorded insignificantly increase for proline and total soluble sugars over control. Foliar applications of $ZnSO_4$ increased the levels of osmolytes and soluble proteins especially under salinity stress. The most effective treatment boosted the osmolytes concentration under non-stressed conditions was $ZnSO_4$ at 3 mM, whereas $ZnSO_4$ at 1.5 mM was the most effective treatment for soluble protein concertation either under stress or non-stress conditions (Fig. 2).

Oxidation of membrane lipids recorded the highest significant values in the form of MDA in the plants irrigated with saline water at 100 mM NaCl (Fig. 3A). Application of $ZnSO_4$ reduced the values of MDA under both salinity levels. The most effective treatment in reducing the levels of MDA was 1.5 mM $ZnSO_4$. Superoxide dismutase enzyme considers the first line of defense against oxidative damage caused by many environmental stresses. Application of NaCl at 100 mM duplicated the activity of SOD comparing with control (Fig. 3B), which indicated that eggplant has some resistance to salinity stress by upholding the activity of SOD enzyme. Application of $ZnSO_4$ boosted the activity of SOD especially for the level of 1.5 mM which recorded an increase in activity reach to 2.5-fold the activity in control.

Salinity led to an increase in the concentration of micronutrients and a decrease in the concentration of macronutrients at leaves except for P concentration which was increased under saline condition in leaves (Figure 4). Foliar application of $ZnSO_4$ reduced the concentration of Ca, Mg, Fe and Na in eggplant leaves either under non-stressed or stressed conditions comparing with control at 0 and 100 mM respectively. Also, the concentration of N in leaves were decreased under non-stressed condition and increased under stressed condition influenced by of $ZnSO_4$ application, whereas the opposite occurred with fruits since $ZnSO_4$ application increased the N concentration (Fig. 4). The promoting effect of $ZnSO_4$ on the concentration of K either in leaves or fruits of eggplant, was achieved under salinity stress, whereas under non-stress condition, the concentrations of K were increased in eggplant leaves only after applications of $ZnSO_4$. Leaves of eggplant are more

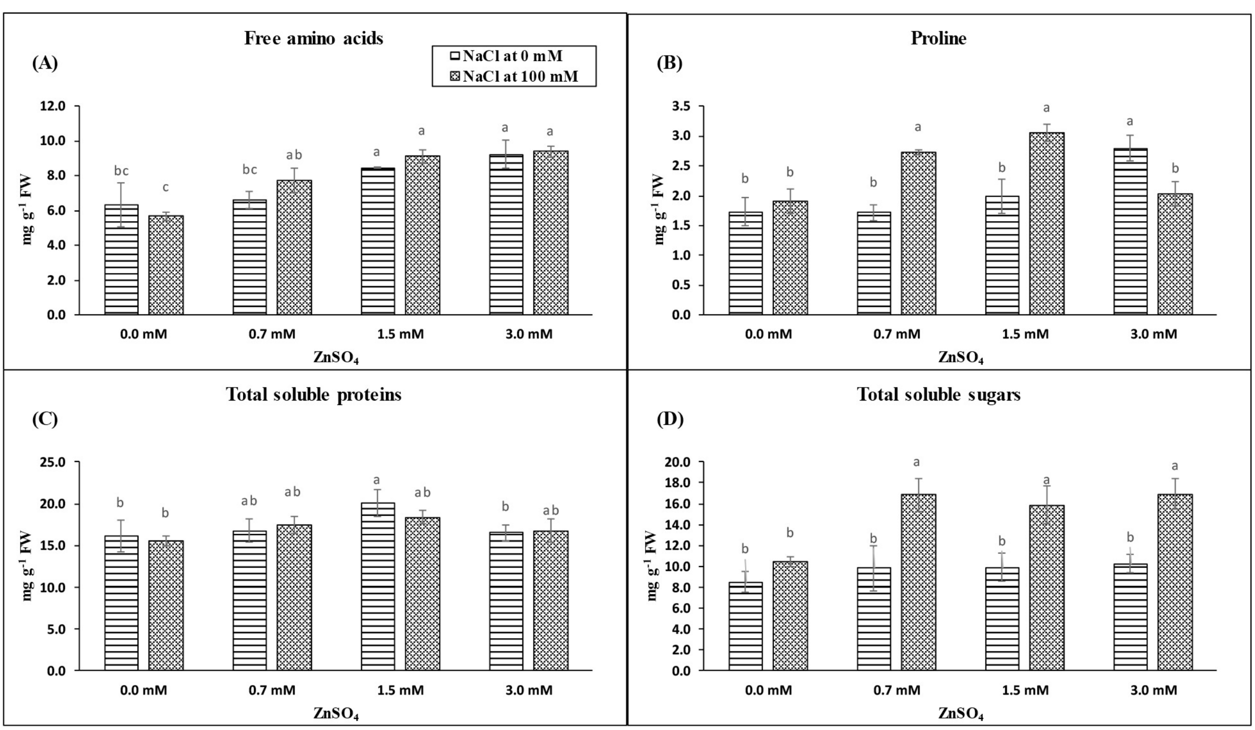

Fig. 2. Influence of foliar spray with $ZnSO_4$ on A) the concentration of total free amino acids, B) proline, C) total soluble protein and D) total soluble sugar in the leaves of eggplant plants growing under different levels of NaCl salinity (0 and 100 mM) during 2017 and 2018 seasons (main of two seasons). Means (±SD as error bar) followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test.

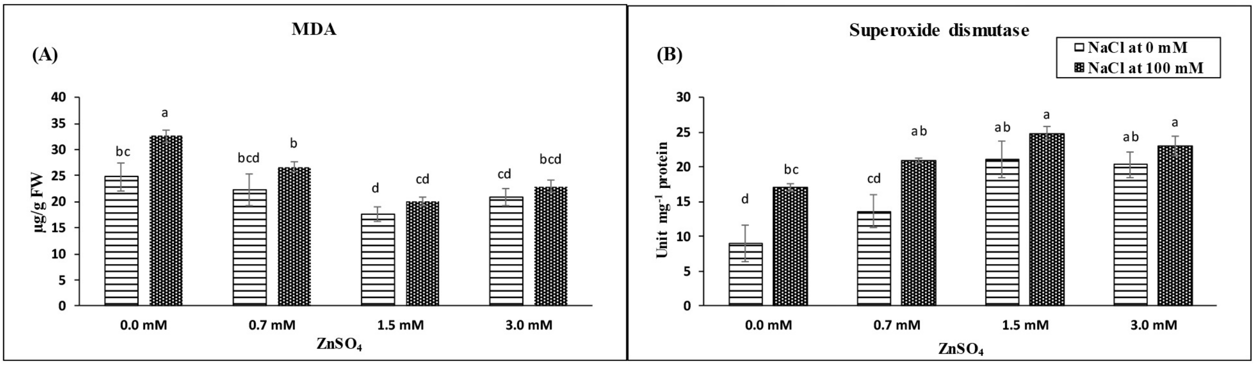

Fig. 3. Influence of foliar spray with $ZnSO_4$ on A) the concentration of malondialdehyde (MDA) and B) the activity of superoxide dismutase (SOD) in leaves of eggplant plants growing under different levels of NaCl salinity (0 and 100 mM) during 2017 and 2018 seasons (main of two seasons). Means (±SD as error bar) followed by different letters are significantly different at $P<$ 0.05 level; Tukey's HSD test.

sensitive than fruits in accumulation of Na^+ ions. The most effective treatment that reduced the levels of Na in leaves or fruits is $ZnSO_4$ at 1.5 mM which also recorded the highest K concentration under salinity comparing with its control.

4. Discussion

All types of abiotic stresses have a an intense effect on the yield of vegetable crops, due to most of them are sensitive or moderate tolerant to the abiotic stresses which resulting to a pronounced reduction in the produced yield (leaves, roots, fruits, etc.). Salinity stress considers the most harmful stress between all abiotic stresses which contributes substantially in limit the growth and productivity of several crops. Salinity stress has a dual deleterious effect on plant growth, one as osmotic stress and the other as ions toxicity, which induces a secondary oxidative stress. Several studies reported that eggplant is moderately sensitive to salinity stress (Chartzoulakis and Loupassaki, 1997; Ünlükara et al., 2010; Assaha et al., 2013), which indicated that it has some abilities to tolerate the moderate abiotic stress. The current study aimed to motivate this ability by foliar spray of zinc in the form of $ZnSO_4$.

In the present study, the individual application of 100 mM NaCl led to a reduction in leaves no./plant, average leaf area and LAI, while the fresh and dry weights of the leaves didn't affect significantly (Table 1), which in turn indicate that between all vegetative parameters, the most influenced parameter under salinity is leaf area. Additionally, individual treatment of 100 mM NaCl had the lowest value in leaf DW/FW % which reveal that salinity stress had a positive effect on leaves water content, which could be a result of accumulating Na^+ ions to its highest level in cell vacuole which in turn decrease its osmotic potential which led to pull up more water to the leaf cells, or could be refer to that accumulation of Na^+ ions at leaves have a negative effect on the leaves dry matter. These results are in good agreement with Chartzoulakis and Loupassaki (1997), who reported that the most significant reduction in eggplant seedlings under salinity stress were for plant height and leaf area, while the leaf area was the most sensitive parameter due to its highest Na^+ ion concentration comparing with other organs. Furthermore, the reduction in leaves no./plant (Table 1) under NaCl salinity directly reduced the flowers number per plant (Fig. 1A), which consequently, lessened the number of formed fruits per plant and total yield (Fig. 1B, F). Salinity not only affected the total yield by reducing the total fruits no./plant (Fig. 1B), but also by reducing the average fruit fresh weight (Fig. 1E). In this regard, Mahjoor et al. (2016) referred the reduction in fruit FW to the considerable effects of salinity on decreasing the osmotic potential in fruit cells which consequently causes less water translocation.

Between all recorded nutrients in leaves and fruits of eggplant, the only element reported a markedly decrease in both leaves and fruits parallel with increasing the concentration of Na^+ ions under salinity stress, was K. Additionally, K concentration in fruits is about 2-fold its concentration in leaves (Fig. 4), which indicate to the importance of K on fruit development which motivating the plant to support its translocation to fruits. In this regard, the eggplant food data report by USDA (2019) illustrated that between all elements located at eggplant fruit, K is the highest one which for example recorded 229 mg/100 g FW comparing with Ca 9 mg/100 g FW, while Michałojć and Buczkowska (2009) mentioned that eggplant fruits are abundant in K, where its amount ranges from 200 to 600 mg K/100 g FW, depending on a variety. This accumulation could be responsible for modulating the osmotic regulation during fruit cells expansion and controlling the transport of photo-assimilates from the source to sink, which reflected on fruit quality by maintaining the desirable sugar to acid ratio (Kumar et al., 2006; Mäser et al., 2002). Also, Akinci et al. (2004) found a revers relationship between concentration of Na^+ and K^+ ions in eggplant leaves subjected to salinity stress and decreasing the ratio between K^+/Na^+ which reflected on the delay and reduction of fruit development.

The most effective osmolyte in eggplant leaves subjected to 100 mM NaCl are proline and total soluble sugars which increased comparing with control. On the contrary, the concentration of free amino acids reduced under same comparison, whereas total soluble protein nearly not affected by salinity (Fig. 2). Furthermore, the activity of SOD increased with increasing the amount of lipid peroxidation (Fig. 3). These observations indicated that eggplant irrigated with saline water depending mainly in osmoregulation process on total soluble sugars and proline, while the share of free amino acids in this osmoregulation was reduced, to serve in maintaining the amount of functional proteins (total soluble proteins) near normal values of non-stressed conditions. Additionally, these increases in proline, total soluble sugars and the activity of SOD under salinity stress, affirm that is moderately sensitive to salinity stress (Ünlükara et al., 2010).

Maximizing the performance of antioxidants machinery under salinity stress which reflecting on growth and development of eggplant, were studied by foliar spray of $ZnSO_4$. The nutrients uptake under NaCl salinity inhibited as a result of competitive interactions between Na^+ and other nutrients, resulting in deficiencies of these elements, which reflecting on reducing plant growth (Assaha et al., 2013). Zinc deficiency is recognized as the most critical one under saline conditions (Novo et al., 2014). Foliar application of $ZnSO_4$ under salinity stress increased the concentration of N, P, K, Cu and Zn in the leaves and fruits which accompanying with a reduction in Na concentration in the leaves and fruits of eggplant (Fig. 4). Zinc has the ability to inhibit the uptake, translocation and repressed the transport of Na^+ ions in plant which improve plant growth (Shahriaripour et al., 2010; Weisany et al., 2014). This concomitant improvement in eggplant growth with Zn application not only due to its role in enhancing the nutrients balance, but also to its direct or indirect role on enhancing the physiological

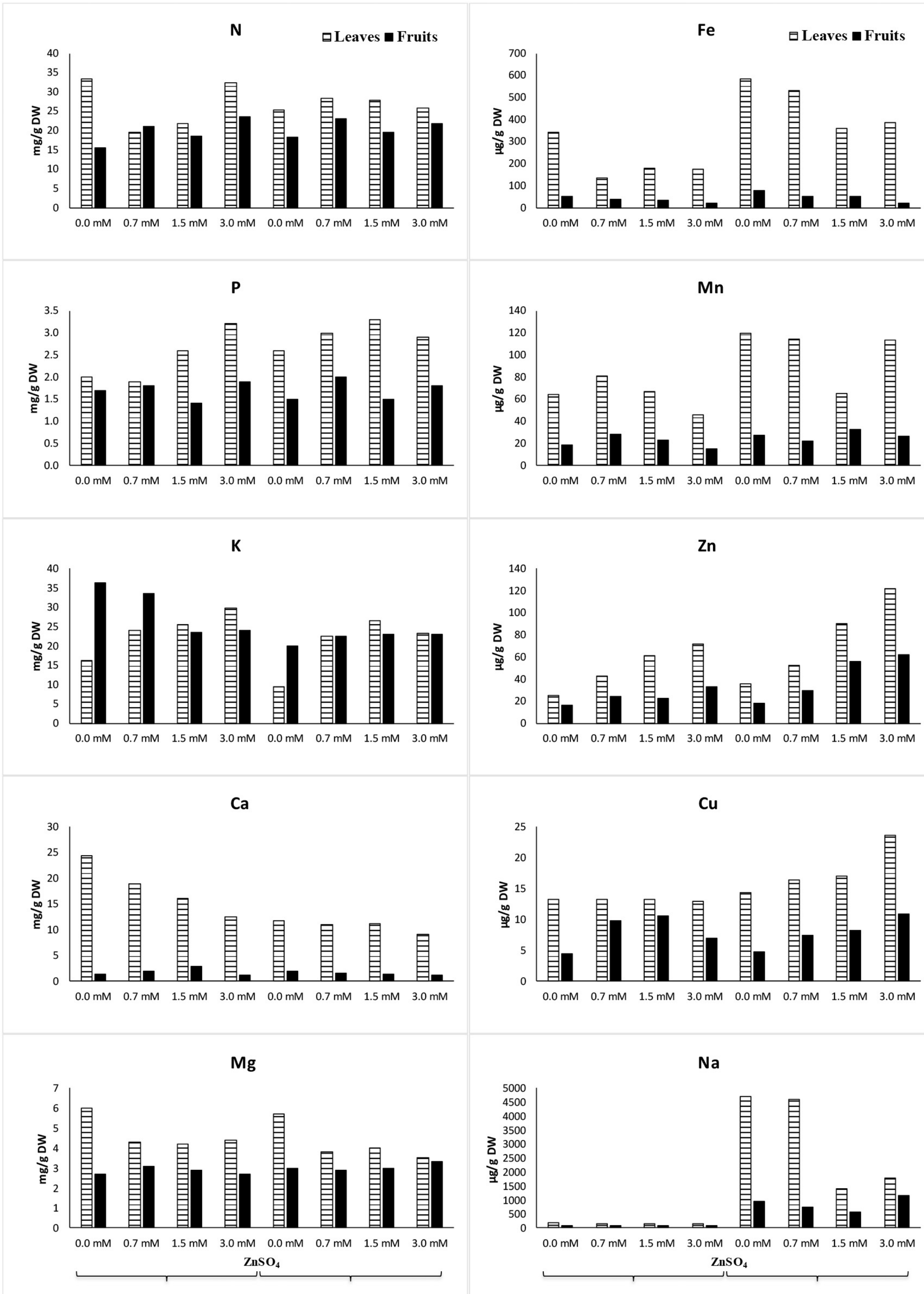

Fig. 4. Influence of foliar spray with $ZnSO_4$ on the concentration of macro-, micro-elements and sodium in leaves and fruits of eggplant plants growing under different levels of NaCl salinity (0 and 100 mM) during 2017 and 2018 seasons (main of two seasons).

functions and metabolism which increased the levels of assimilates and its derivatives macromolecules (Fig. 2), boosted the activity of antioxidants (SOD), leading to a marked reduction in the lipid peroxidation (Fig. 3), which in turn maintained the membrane integrity and restore its complete functions. These enhancements have a positive effect on water and nutrients uptake resulting in improving eggplant vegetative growth (Table 1) and fruits yield (Fig. 1). All the studied $ZnSO_4$ levels (0.7, 1.5 and 3 mM) comparing with 0 level, increased all the mentioned parameters, but the most effective level is $ZnSO_4$ at 1.5 mM either under saline or non-saline conditions. Regarding Zn importance, Zn has a fundamental role in the biosynthesis of chlorophyll and conversion of starch to sugars (Tavallali et al., 2009). Zinc is the only metal represented in the six classes of enzymes which making Zn has a very important role in plant metabolism including nitrogen assimilation, cell division, pollen formation, photosynthesis and synthesis of carbohydrates, maintaining integrity of membranes and reduce lipids peroxidation (Galal, 2019; Shahriaripour et al., 2010).

5. Conclusion

Results of this study concluded that eggplant not only consider as a moderate sensitive plant to NaCl salinity, but also foliar application with $ZnSO_4$ increased its level of tolerance to a higher one, which enhancing the growth and productivity of eggplant under 100 mM NaCl as a consequence of reducing the accumulation of Na in eggplant leaves and fruits tissues to a non-toxic level, which reflecting on increasing the tolerance to salinity by enhancing the activity of the antioxidant enzyme (SOD) and nutrients concentration. The best Zn application eliminated the negative effects of salinity on qualitative and quantitative traits of eggplant was the foliar treatment with $ZnSO_4$ at 1.5 mM.

References

A.O.A.C, 2005. Official Methods of Analysis of AOAC International, Ed 18th. Association of Official Analytical Chemists, Washington, D.C., USA.

Akinci, I.E., Akinci, S., Yilmaz, K., Dikici, H., 2004. Response of eggplant varieties (*Solanum melongena*) to salinity in germination and seedling stages. New Zealand J. Crop Hort. Sci. 32, 193–200.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1–15.

Assaha, D.V.M., Ueda, A., Saneoka, H., 2013. Comparison of growth and mineral accumulation of two solanaceous species, *Solanum scabrum* Mill. (huckleberry) and *S. melongena* L. (eggplant), under salinity stress. Soil Sci. Plant Nut. 59, 912–920.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207.

Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44, 276–287.

Bernhart, D.N., Wreath, A.R., 1955. Colorimetric determination of phosphorus by modified phosphomolybdate method. Anal. Chem. 27, 440–441.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Broadley, M., Brown, P., Cakmak, I., Rengel, Z., Zhao, F., 2012. Chapter 7 - function of nutrients: micronutrients. In: Marschner, P. (Ed.), Marschner's Mineral Nutrition of Higher Plants, Third edition. Academic Press, San Diego, pp. 191–248.

Chartzoulakis, K.S., Loupassaki, M.H., 1997. Effects of NaCl salinity on germination, growth, gas exchange and yield of greenhouse eggplant. Agric. Water Manag. 32, 215–225.

Dias, J.S., 2012. Nutritional quality and health benefits of vegetables: a review. Food Nut. Sci. 3, 1354–1374.

Galal, A., 2019. Exogenous application of zinc mitigates the deleterious effects in eggplant grown under salinity stress. J. Plant Nut. 42, 915–927.

Gomez, K.A., Gomez, A.A., 1984. Statistical Procedures for Agricultural Research, Ed 2nd. 329–387. John Wiley and Sons, New York, pp. 20–29.

Gupta, B., Huang, B., 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Inter. J. Genom. 2014, 701596 (DOI: 701510.701155/702014/701596).

Hegazi, A.M., El-Shraiy, A.M., Ghoname, A.A., 2015. Alleviation of salt stress adverse effect and enhancing phenolic anti-oxidant content of eggplant by seaweed extract. Gesunde Pflanzen. 67, 21–31.

Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. California Agric. Exper. Stat.Circ. 347, 1–32.

Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta. 207, 604–611.

Horneck, D.A., Hanson, D., 1998. Determination of potassium and sodium by flame emission spectrophotometry. In: Kalra, Y.P. (Ed.), Handbook of Reference Methods for Plant Analysis, pp. 153–155.

Horneck, D.A., Miller, R.O., 1998. Determination of total nitrogen in plant tissue. In: Kalra, Y.P. (Ed.), Handbook of Reference Methods for Plant Analysis, pp. 75–83.

Hunt, R., 1990. Simple ratios. *In* Basic Growth Analysis. Springer Netherlands, pp: 35–54.

Kumar, A.R., Kumar, N., Kavino, M., 2006. Role of potassium in fruit crops-a review. Agric. Rev. 27, 284–291.

Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soci. Transact. 11, 591–592.

Maathuis, F.J., 2007. Transport across plant membranes. In: Yeo, A., Flowers, T. (Eds.), Plant Solute Transport. Blackwell Publishing Ltd, pp. 75–98.

Mahjoor, F., Ghaemi, A.A., Golabi, M.H., 2016. Interaction effects of water salinity and hydroponic growth medium on eggplant yield, water-use efficiency, and evapotranspiration. Inter. Soil Water Conserv. Res. 4, 99–107.

Marschner, H., 1995. Functions of mineral nutrients: micronutrients. In: Marschner, H. (Ed.), Mineral Nutrition of Higher Plants, Ed 2nd. Academic Press, London, pp. 313–404.

Mäser, P., Gierth, M., Schroeder, J.I., 2002. Molecular mechanisms of potassium and sodium uptake in plants. In: Horst, W.J., Bürkert, A., Claassen, N., Flessa, H., Frommer, W.B., Goldbach, H., Merbach, W., Olfs, H.W., Römheld, V., Sattelmacher, B., Schmidhalter, U., Schenk, M.K., Wirén, N. (Eds.), Progress in Plant Nutrition: Plenary Lectures of the XIV International Plant Nutrition Colloquium: Food Security and Sustainability of Agro-ecosystems Through Basic and Applied Research. Springer, Netherlands, Dordrecht, pp. 43–54.

Michałojć, Z., Buczkowska, H., 2009. Content of macroelements in eggplant fruits depending on varied potassium fertilization. J. Elem. 14, 111–118.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25, 239–250.

Nawaz, K., Hussain, K., Majeed, A., Khan, F., Afghan, S., Ali, K., 2010. Fatality of salt stress to plants: morphological, physiological and biochemical aspects. African J. Biotec. 9, 5475–5480.

Novo, L.A.B., Covelo, E.F., González, L., 2014. Effect of salinity on zinc uptake by *Brassica juncea*. Inter. J. Phytoremed. 16, 704–718.

Osman, H.S., Salim, B.B., 2016a. Improving yield and quality of kohlrabi stems growing under NaCl salinity using foliar application of urea and seaweed extract. Journal of Horticultural Science & Ornamental Plants. 8, 149–160.

Osman, H.S., Salim, B.B.M., 2016b. Influence of exogenous application of some phytoprotectants on growth, yield and pod quality of snap bean under NaCl salinity. Ann. Agric. Sci. 61, 1–13.

Pandey, S.K., Bahuguna, R., Madan, P., Trivedi, A., Hemantaranjan, A., Srivastava, J., 2010. Effects of pre-treatment and foliar application of zinc on growth and yield components of mungbean (*Vigna radiata* L.) under induced salinity. Indian. J. Plant Physiol. 15, 164–167.

Parida, A.K., Das, A.B., 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxic. Environ.l Safety. 60, 324–349.

Sadasivam, S., Manickam, A., 2010. Biochemical Methods, Ed 3rd. New Age International Publishers, pp. 1–19.

Shahriaripour, R., Pour, A.T., Mozaffari, V., Dashti, H., Adhami, E., 2010. Effects of salinity and soil zinc application on growth and chemical composition of pistachio seedlings. J. Plant Nut. 33, 1166–1179.

Stefánsson, A., Gunnarsson, I., Giroud, N., 2007. New methods for the direct determination of dissolved inorganic, organic and total carbon in natural waters by Reagent-Free™ Ion Chromatography and inductively coupled plasma atomic emission spectrometry. Anal. Chim. Acta 582, 69–74.

Swamy, P.M., 2008. Laboratory Manual on Biotechnology. Rastogi Publications, India, pp. 80–82.

Tavallali, V., Rahemi, M., Maftoun, M., Panahi, B., Karimi, S., Ramezanian, A., Vaezpour, M., 2009. Zinc influence and salt stress on photosynthesis, water relations, and carbonic anhydrase activity in pistachio. Sci. Hortic. 123, 272–279.

Ünlükara, A., Kurunç, A., Kesmez, G.D., Yurtseven, E., Suarez, D.L., 2010. Effects of salinity on eggplant (*Solanum melongena* L.) growth and evapotranspiration. Irrig. Drain. 59, 203–214.

USDA, 2019. FoodData Central, Eggplant, Raw. Ed FDC ID: 169228 Vol 4/1/2019. USDA. https://fdc.nal.usda.gov/fdc-app.html#/food-details/169228/nutrients.

Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A., Badakhshan, H., 2014. Effects of zinc application on growth, absorption and distribution of mineral nutrients under salinity stress in soybean (*Glycine max* L.). J. Plant Nut. 37, 2255–2269.

Yasin Ashraf, M., Iqbal, N., Ashraf, M., Akhter, J., 2014. Modulation of physiological and biochemical metabolites in salt stressed rice by foliar application of zinc. J. Plant Nut. 37, 447–457.