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Effect of Exogenous α-Tocopherol on Sweet Pepper Plants Irrigated by Diluted Sea Water

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Abstract

Capsicum annum is one of the most cultivated summer crops in Egypt which is consider the most susceptible crop to harsh a biotic stresses as Salinity condition. Pots experiment was carried out at Faculty of Agriculture, Fayoum University, Egypt during two successive summer seasons of 2014 and 2015 to study the responsive of Sweet pepper (cv. California wonder) plants irrigated by diluted sea water (EC= 8.0 dSm^{-1}) to foliar applications of 1 mM alpha-Tocopherol (α TOC). Four treatments were arranged in a randomized block design:1) plants irrigated by sea water (SW) and sprayed by tap water (TW), 2) TW for irrigation and α TOC for foliar spray, 3) SW for irrigation and α TOC for foliar spray and 4) the control (TW for irrigation and foliar spray). Pepper plants irrigated by sea water recognized significant reductions in growth parameters (leave number, leaf area, plant dry weight, Fruit number and Fruit yield). Results also showed that, foliar application of pepper plant with αTOC caused a notable upgrading in growth and yield under saline conditions. The maximum increased growth was obtained when plants irrigated by TW and sprayed by 1 mM αTOC. The foliar

application of αTOC considerably boosted the activities of Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX) and Glutathione reductase (GR) of pepper plants contrasted to control treatment. The outcome of present experiment could be recommended for both new reclaimed lands suffering from salt water and regions exposure to salinity hazard in irrigated water.

Keywords: Alpha-Tocopherol, *Capsicum annum*, growth parameters, Enzymes activity, Saline water, Yield.

1. Introduction

Capsicum annuum, bell, sweet, or chili pepper with cultivated varieties containing bell, sweet, chili, and paprika peppers is a perennial herbaceous plant belong to Solanaceae , which originated in Central and South America and was cultivated over 5,000 years ago (Kraig *et al.*, 2006). Peppers from *C. annuum* have been elaborated into numerous varieties that are presently cultivated over a wide area for sweet and hot cultivars of green and red bell pepper and chili pepper, that are one of the most common used spices in the world, with dry forms containing paprika, chili powder and cayenne (Castanon-Najera *et al.*, 2008; Perez-Castañeda *et al*., 2008 and Prado, 2008). In Egypt, *Capsicum annum* is consider one of the mainly cultivated vegetable crop in summer season in new reclaimed land, which suffer from salinity, low productivity, and reduce soil building. Therefore, these adverse environments affect plants development and resulted in reduce yield. *Capsicum annum* is particularly susceptible to drought damages and is moderately susceptible to salinity (Rhoades *et al*., 1992). (Semiz *et al*., 2014) mentioned that yield decreased once irrigation water salinity become more than EC 2 dS m^{-1}. Approximately one-third of the 260 million hectares of irrigated soil worldwide, soil that provide 40% of the total food productivity is affected by salinization (United Nations, 2011). For instance, e.g., Egypt, Pakistan, India, the United States and Australia have saline land and drainage between 15 and 36% of their irrigated areas and are thereafter devoting real resources to clear this matter (Schwabe *et al.*, 2006).

Salinity caused a decrease in yield that is caused by adverse stimuli in water relations, thus initiating an ion unbalance in plants, which could cause ion toxicity (Munns *et al*., 2006), osmotic stress and exchanges to biochemical response in plants (Khan *et al.*, 2013), as well as it could effect in alimentation unbalance (Liu and Zhu 1998). Salinity can cause membrane instability of the photosynthetic apparatus (Munns and Termaat 1986), in addition to irreversible damage to cells and tissues (Meyer and Boyer, 1981).Salt stress decreases photosynthetic apparatus, plant development, and stimulates the activity of an antioxidant system (Rao,2006; Rady, 2011; Rady *et al*., 2013; Semida *et al.*, 2014; Semida and Rady, 2014).This problem-induced salinization is due to the triggering of oxidative stress in plant cells through an over reproduction of reactive oxygen species (ROS), successively damage proteins, DNA and lipids (Yasar *et al*., 2006). Chloroplasts are the organelles delivering the ROS such as the superoxide radical (O_2) hydrogen peroxide (H_2O_2) and singlet oxygen (O_1) through photosynthesis (Asada, 1992). ROS cause photo-degradation and membrane lipid peroxidation (Yildrimin *et al*., 2008). To relieve these salt toxicity, plants develop numerous mechanisms to make their tolerance, thus protecting their cells and sub-cellular from the toxic

effects of ROS with together, enzymatic and non-enzymatic antioxidant biological systems (Sairam and Srivastava, 2001; Mishra *et al*., 2009), containing ion homeostasis, osmotic modification, stress damage control and repair, and growth regulation (Zhu, 2002), salt stressed plants can osmotically modify to reduce theouter water potential by store toxic ions in the vacuole and by synthesizing compatible solutes in the cytoplasm (Hasgawa *et al.*, 2000). In addition the activation of the reactive oxygen scavenging system and regulation of cell growth rate can occur (Maggio *et al*., 2002).Such metabolic reaction allows acclimation to osmotically unfavorable environments but can decrease the final yield.

In addition to the physiological mechanisms of plants, various cultural methods are being used to reduce the harm effects of salinity on crop yield, for example, reproduction salinity resistant cultivars (Yang *et al.,* 2005), utilizing grafting methods on vegetable plants (Estan *et al*., 2005 and Edelstein *et al.,* 2005), spraying growth substance (Hamdia *et al.,* 2004), soil elaboration (Bacilio *et al.,* 2004),using organic fertilizers (Rady, 2012), humic substances application (Arancon *et al.,* 2006), applying of antioxidants such as ascorbic acid (Ejaz *et al.* 2012) and alpha-tocopherol (Abdallah *et al.,* 2013,Semida *et al.,* 2014 and Semida *et al*., 2016). Alpha-tocopherol (αTOC) is a low molecular weight lipophilic membrane-located antioxidant. It keeps cell membranes from oxidation (Asada, 1999) and polyunsaturated fatty acids from lipid peroxidation (Krieger-Liszkay and Trebst, 2006), it also has the potential to improve membrane stability, integrity and permeability. furthermore, αTOC has been shown to give an optimum conditions for the photosynthetic apparatus (Wise and Naylor, 1987), applying of αTOC was demonstrated to significantly improve salt tolerance in onion plants by reducing the endogenous lipid peroxidation and H_2O_2, at the same time increasing enzymatic for example superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase and non-enzymatic for example ascorbic acid and glutathione beside antioxidant activity (Semida *et al.,* 2016). Applying of αTOC at different concentrations led to decreases in Na and Cl and increase in Ca, K, Mg and P relative to controls. Exogenous applications of αTOC caused marked encouragement in yield attributes on three cultivars of flax plant (*Linum usitatissimum* L.) (Sadak and Dawood 2014), moreover αTOC treated bean plants grown under varying conditions, had enhanced all growth parameters and yield and its components compared to control plants. Other factors such as, performance index, relative water content, membrane stability index, nutrients status and their relations, stem and leaf parameters were enriched in αTOC treated plants, in comparison to untreated plants (Semida *et al*., 2014). The supplementation of 0.1g l^{-1} αTOC on soybean plants was shown to increase the rate of photosynthesis, growth rate, and N contents, (Rady *et al.,* 2015).When the influence of αTOC was carried out on sunflowers in saline conditions, the results showed an enhancement in both plant development and its yield (Sadak *et al.,* 2010).

This study was carried out with the primary objective of assessing the response of pepper plants irrigated with diluted sea water (EC= 8.0 dSm^{-1}) to foliar applications of 1 mM αTOC. It was a major aim to investigate the effects of αTOC and diluted sea water on the growth, yield, concentrations of photosynthetic pigments in leaves ,non-enzymatic antioxidants, and osmoprotectants (α-tocopherol, ascorbic acid, glutathione), K , Ca ,Na , as well as their relation with Na. The activities of superoxide dismutase, catalase, ascorbate peroxidase,

glutathione reductase and tissue health (relative water content and membrane stability index) were also overviewed.

2. Materials and Methods

Plant Experiments

In a greenhouse, two pot experiments were conducted during the summer seasons of 2014 and 2015 at the Faculty of Agriculture, Fayoum University, Southeast Fayoum, Egypt. The climate conditions were as follows: daytime temperatures ranging from 29.8 to 39.6 °C, with an average of 34.7 ± 2.3 °C, night temperatures ranging from 18.4 to 24.8 °C, with an average of 21.6 ± 1.4 °C and the daily relative humidity ranged from 38 to 76% with an average value of 57 ±5.8%.

Sweet pepper (cv. California wonder) seedlings (45 day old hold 6-7 leaves/ plant) were obtained from a privet outlet. Two healthy seedlings were transplanted in 50 cm-diameter plastic pot that contains 12 kg sandy loam soil. According to the recommended doses, 2.5 g nitrogen (N) as ammonium sulphate (20.5% N), 1.5 g phosphorous (P) as calcium superphosphate (15.5% P_2O_5), and 1.0 g potassium (K) as potassium sulphate (48% K_2O) were added to each pot before planting. Ammonium sulphate of 1.50 g as supplementary doses was added at 30, 60, and 120 days after transplantation for each pot.

In every season, four treatments were arranged in a randomized block design with 30 replicate, as 1) diluted sea water (EC 8.0 dS m^{-1}) for irrigation and tap water (EC 0.22 dS m^{-1}) for foliar spray, 2) tap water for irrigation and α-tocopherol (α-TOC; 1 mM, Hangzhou Toyond Biotech Co. Ltd., Zhejiang, P. R. China) for foliar spray, 3) sea water for irrigation and $\alpha TOC_{1.0}$ for foliar spray, and 4) control (tap water for irrigation and foliar spray). Fifteen days after transplantation, all pots were sprayed three-times at 15 days intervals with tap water or 1 mM α-TOC. The 1 mM α-TOC was chosen as the best spraying level based on a preliminary study where 0.5, 1.0, 1.5 or 2.0 mM α-TOC was tested (data not shown). The physio-chemical properties of tested sandy loam soil are shown in Table (1).

Table 1. Some physio-chemical properties of tested soil as an initial state

Property	pH	EC	OC	N	P	K	Ca	Fe	Mn	Zn	CEC
	(1: 2.5)	(dS m^{-1})	(g kg^{-1})		(mg kg^{-1})						(cmol $^{+}$/ k)
Value	7.75	2.35	8.75	0.78	15.40	72.20	82.60	6.00	3.60	2.00	8.00
pH = soil reaction EC = soil salinity OC = organic carbon CEC = cation exchange capacity											
all values are average of both seasons											

The soil water-holding capacity was measured by saturating each pot with water and left 48 h to be drained then its weight was recorded. The water-holding capacity in each pot was 35.8% (soil: water w/w). Soil moisture content was maintained at approximately 90% of water-holding capacity by daily weighing of each pot and compensates the evapo-transpiration water.

Yield Traits and Quality

To measure the sweet pepper plants growth and their yield characteristics of both seasons, plant samples were taken randomly from each treatment 75 days after transplanting. The leaves number/ plant were manually counted and its leaf area (m^2) was measured using a digital Planimeter. Plant dry weight (g) was assessed after oven-drying at 70 ℃ until constant weight was reached. At harvesting, the pepper plants in each treatment were taken and their yield in terms of fruit number and fruit weight/ plant was recorded.

Total chlorophyll and carotenoids were extracted and determined according to the methods described by (Moran, 1982).using 80% (v/v) acetone to homogenize samples centrifuged at 10,000 g for 10 min. Absorbance readings of the extracts was measured at 663, 645 and 470 nm using a UV60A UV-visible recording spectrometer (Shimadzu, Kyoto, Japan).

Relative water content (RWC) of the tissue was measured according to (Weatherly, 1950) using fully-expanded leaf discs. Discs were weighed (fresh mass; FM) and immediately floated on double-distilled water in Petri dishes for 24 h, in the dark, to be saturated. Any adhering water was gently dried and the turgid mass (TM) was recorded. Dry mass (DM) was taken after drying the discs at 70 ℃ until reaching a constant weight. RWC was then calculated using the following formula:

$$RWC\ (\%) = [(FM - DM) / (TM - DM)] \times 100$$

Membrane stability index of plant tissues (MSI) was determined according to the methods of (Gnanasiri *et al*. 1990), using two equivalent samples of fully-expanded leaf tissues. One sample was placed in test-tubes containing double-distilled water. The content of the test-tube was then heated at 40 ℃ in a water bath for 30 min, and the electrical conductivity (EC_1) of the solution was recorded using a conductivity bridge. The other one was boiled at 100 ℃ for 10 min, and the conductivity was measured (EC_2), and MSI was calculated using the following formula:

$$MSI = [1- (EC1 /EC2)] x100$$

Alpha-tocopherol (αTOC) concentration was measured using 900 ml of extraction solvent (*n*-hexane-ethyl acetate, *n*-hexane), that was mixed with 100 ml of ethyl acetate, and then 20 mg of butylated hydroxyl toluene (BHT) was dissolved in this solvent mixture. Using R-TOC, standard solutions (20–200 μg/ml) were prepared from stock solutions (50 mg/100 ml *n*-hexane). According to the method of (Konings *et al*. 1996), samples were prepared and saponified. Samples were sliced and dried in an oven at 40 ℃ and homogenized, and then 5 g from each sample was suspended in 30 ml of water in a 500-ml conical flask. To the flask, 21 g of KOH dissolved in 100 ml of ethanol was added and then 0.25 g of ascorbic acid g^{-1} test portion was added for protecting from oxidation and mixed. At 80 ℃, saponification was done for 40 min and the samples were immediately cooled to room temperature. Water (300 ml) was added to bring the ethanol/water ratio to 0.3 and then *n*-hexane/ethyl acetate [9:1 (3 × 100 ml)] was added, and the mixtures were extracted 3 times using a separator funnel. Organic phases were combined and washed with 100-ml portions of water until free of alkali, determined by where the reaction of washes to phenolphthalein was neutral (no visible pink

color). Organic phases were then filtered through anhydrous sodium sulphate into a beaker. The filtrates were evaporated to dryness under reduced pressure (at 40 ℃). The residues were dissolved in 20 ml of *n*-hexane (HPLC grade) and stored in a freezer at –20 °C. The αTOC was determined using an HPLC system of Waters Bondapak C_{18} reverse-phase column. The mobile phase (methanol/water 94:6) was used at a flow rate of 1.5 ml min^{-1} and the UV detector was set at 292 nm (Ching and Mohamed, 2001).

Ascorbic acid concentration (AsA) was resolved in fully-expanded leaves following the method of (Mukherjee and Choudhuri 1983). A sample of 500 mg was extracted with 10 ml of 6% (w/v) trichloro acetic acid (TCA). The extract was then mixed with 2 ml of 2% (w/v) dinitrophenylhydrazine, followed by the addition of 1 drop of 10% (w/v) thiourea in 70% (v/v) ethanol. The mixture was then boiled for 15 min in a water bath. After cooling to room temperature, 5 ml of 80% (v/v) H_2SO_4 was added at 0 ℃. The absorbance was recorded at 530 nm.

Glutathione (GSH) concentration was also measured using a fully-expanded leaf tissue by the method of (Griffth, 1980). Fresh 50 mg sample was homogenized in 2 ml of 2% (v/v) metaphosphoric acid and centrifuged at 17,000 × *g* for 10 min. The supernatant was neutralized by 10% (w/v) sodium citrate and three replicate determinations were made. Each 1.0 ml assay was consisted of 700 µl of 0.3 ml M NADPH, 100 µl of 6 ml M 5,5´-dithio-*bis*-2-nitrobenzoic acid, 100 µl distilled water and 100 µl of extract. The assay was stabilized at 25 ℃ for 3 – 4 min. Ten µl of 50 Units ml^{-1} GSH reductase was added and the absorbance was recorded at 412 nm.

Free proline was acquired based on the method of (Bates *et al.* 1973). Samples were grinded in 3% (v/v) sulphosalicylic acid, followed by centrifugation at 10,000 ×*g* for 10 min. In a test-tube, a 2-ml of freshly prepared acid-ninhydrin solution was added to 2 ml of the supernatant. The tubes were incubated in a water bath at 90 ℃ for 30 min, and the reactions were terminated in an ice-bath. Reaction mixtures were then extracted with 5 ml of toluene and vortex-mixed for 15 s. At room temperature, the tube was allowed to stand for at least 20 min in the dark to separate the toluene and aqueous phases. The toluene phase was then collected carefully into a test tube and the absorbance of the toluene phase was read at 520 nm using a Bausch and Lomb-2000 Spectronic Spectrophotometer.

Total soluble sugars assessments were carried out according to (Irigoyen *et al.* 1992). Samples were homogenized in 5 ml of 96% (v/v) ethanol, washed with 5 ml 70% (v/v) ethanol. The extracts were then centrifuged at 3500 × g for 10 min, and the supernatants were stored at 4 ℃ prior to determination. Reaction mixture of 0.1 ml of the ethanolic extract and 3 ml of freshly-prepared anthrone reagent [150 mg anthrone plus 100 ml of 72% (v/v) sulphuric acid] were placed in a boiling water bath for 10 min, and then cooled. The absorbance was recorded at 625 nm using a Bausch and Lomb-2000 Spectronic Spectrophotometer.

Concentrations of Na^+ and K^+ were calculated using 0.2 g dried leaves that were digested with sulphuric acid in the presence of H_2O_2 (Wolf, 1982). The mixture was then diluted with distilled water. The concentrations of Na^+ and K^+ were measured directly using Flame Spectrophotometry (Lachica *et al.*, 1973). Concentration of Ca^{+2} was determined using a

Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer (Chapman and Pratt, 1961).

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assessed by assessing the inhibition of the photochemical reduction of nitro bluete trazolium (NBT) (Giannopilitis and Ries, 1977; Beyer and Fridovicht, 1987). One unit of SOD activity was defined as the amount of enzyme required for the reduction of 50%NBT. Activity of SOD was expressed as $\Delta A_{564}min^{-1}g^{-1}$protein. Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the H_2O_2 consumption (Nakano and Asada, 1981). The reaction mixture consisted of 25 ml M Tris-acetate buffer, pH 7.0, 0.8 ml M Na-EDTA and 20 mM H_2O_2. The enzyme assay was performed at 25 ℃. Activity of CAT was expressed as $\Delta A_{290}min^{-1}g^{-1}$protein.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was assessed by the method of (Rao *et al.* 1996) by recording the optical density at 290 nm and the APX activity was expressed as $\Delta A_{290}min^{-1}g^{-1}$protein. Glutathione reductase (GR; EC 1.6.4.1) activity was measured after monitoring the oxidation of NADPH for 3 absorbances taken at 340 nm. Activity of GR was expressed as $\Delta A_{340}min^{-1}mg^{-1}$protein.

The obtained data was analyzed by analysis of variance analysis (ANOVA) and differences between the means were compared by Fisher least-significant difference (LSD) test at a probability level of 95%. Significance levels were expressed as $P\leq 0.05$, data was deemed significant at $P\leq 0.05$.

3. Results

The effect of α-TOC on growth parameters and yield of pepper plants irrigated by diluted sea water are appeared in Table 2. The data revealed that pepper plants irrigated by sea water (EC= 8.0 dS m^{-1}) resulted in a noteworthy reduction in the assessed growth variables (leaves number & area, plant dry weight, Fruit number and yield). The results also illustrated that foliar application of α-TOC on pepper plants improved growth and yield under salinity stress. The maximum increase was recognized in the treatment irrigated by tap water and sprayed by 1 mM α-TOC.

Table2. Effect of α-tocopherol application on some growth and yield characteristics of Capsicum annuum plants irrigated by diluted sea water

Growth season	Treatments	Leaves No. plant $^{-1}$	Leave area (m2)	Plant dry weight (g)	Fruit number plant^{-1}	Fruit yield plant $^{-1}$ (g)
2014	TW_I +TW_F (control)	47.3 b	0.15 b	65.4 b	11.7 b	352 b
	TW_I + TOC_F.	52.2 a	0.17 a	72.1 a	12.9 a	388 a
	SW_I + TW_F	26.7 d	0.08 d	34.7 d	6.1 d	102 d
	SW_I + TOC_F	38.3 c	0.11 c	53.5 c	8.9 c	190 c
2015	TW_I +TW_F (control)	48.5 b	0.16 b	73.2 b	12.2 b	364 b
	TW_I + TOC_F.	53.1 a	0.19 a	81.6 a	14.0 a	396 a
	SW_I + TW_F	26.9 d	0.08 d	39.1 d	5.8d	111 d
	SW_I + TOC_F	39.2 c	0.12c	59.8 c	9.4 c	203c
	TW_I = tap water for irrigation		TW_F = tap water for spray		SW_I = sea water for irrigation	
	TOC_F = α-tocopherol for spray		values are mean of 5 numbers			
	value followed by different latters are significantly different					

In terms of photosynthetic pigment, relative water content and membrane stability index of pepper plants that sprayed by α TOC and irrigated by tap water subsequently caused an increase in total chlorophyll, carotenoids, relative water content and membrane stability index compared to other treatments (Table 3). Spraying of α TOC to the salt-stressed pepper plants induced significant increase in total chlorophyll, carotenoids, relative water content and membrane stability index compared to unsprayed plants.

Table 3. Effect of α-tocopherol application on photosynthetic pigments and tissue health (relative water content and membrane stability index) of Capsicum annuum plants irrigated by diluted sea water

Growth season	Treatments	Total chlorophylls	Total carotenoids	RWC (%)	MSI (%)
2014	TW_I +TW_F (control)	2.11 b	0.51 b	74.2 a	67.4 a
	TW_I + TOC_F.	2.44 a	0.58 a	76.1 a	70.3 a
	SW_I + TW_F	0.84 d	0.34 d	45.3 c	49.8 c
	SW_I + TOC_F	1.73 c	0.43 c	61.5 b	59.5 b
2015	TW_I +TW_F (control)	2.26 b	0.53 b	75.2 a	68.5 a
	TW_I + TOC_F.	2.58 a	0.60 a	77.0 a	71.0 a
	SW_I + TW_F	0.86 d	0.31 d	46.1 c	48.2 c
	SW_I + TOC_F	1.69 c	0.42 c	63.2 b	58.6 b
TW_I = tap water for irrigation		TW_F = tap water for spray		SW_I = sea water for irrigation	
TOC_F = α-tocopherol for spray		RWC = relative water content		MSI =membrane stability index	
values are mean of 5 numbers		value followed by different latters are significantly different			

The results in Table 4 show that sprayed the pepper plants by α TOC under water stress led to an increase ascorbic acid, glutathione and free proline and total soluble sugars compared to the unsprayed plants. Also, the results revealed that, there was a significant increase in all leaf concentrations of non-enzymatic antioxidants and osmoprotectants contents when treat the plants by α TOC under normal conditions.

Table 4. Effect of α-tocopherol application on leaf concentrations of non-enzymatic antioxidants & osmoprotectants, ascorbic acid, glutathione, free proline and total soluble sugars in *Capsicum annuum* plants irrigated by diluted sea water.

Growth season	Treatments	TOC	ASA	GSH	Free proline	Soluble sugars
2014	TW_I +TW_F (control)	25.4 d	0.67c	5.21c	44.3 c	21.3 c
	TW_I + TOC_F	56.6 b	0.68 c	5.26 c	44.5 c	21.9 c
	SW_I + TW_F	34.1c	1.01 b	7.82 b	71.7 b	34.5 b
	SW_I + TOC_F	77.5 a	1.26 a	8.98 a	94.2 a	42.8 a
2015	TW_I +TW_F (control)	28.4 d	0.75 c	5.85 c	42.1 c	25.1 c
	TW_I + TOC_F	58.4 b	0.72 c	5.90 c	43.0 c	24.9 c
	SW_I + TW_F	36.2 c	1.18 b	7.92 b	67.9 b	42.1 b
	SW_I + TOC_F	76.3 a	1.47 a	9.48 a	83.2 a	62.2 a

TW_I = tap water for irrigation	TW_F = tap water for spray		SW_I = sea water for irrigation	
TOC_F = α-tocopherol for spray	ASA =ascorbic acid		GSH =glutathione	
values are mean of 5 numbers		value followed by different latters are significantly different		

Table (5) shows that irrigated pepper plants by diluted sea water led to a significant reduce in shoot concentrations of K, Ca, K/Na and Ca/Na, while shoot concentrations of Na significantly increased in contrast to the plants grown under other treatments. Cultivation of pepper in the presence of α TOC led to significant increase in shoot concentrations of K, Ca, K/Na and Ca/Na ratio and a decrease concentration of Na compared to other plants grown without spraying α TOC and irrigated by tap water or diluted sea water.

Table 5. Effect of α-tocopherol application on shoot concentrations of K, Ca and Na in *Capsicum annuum* plants irrigated by diluted sea water

Growth season	Treatments	K	Ca	Na	K/Na ratio	Ca/Na ratio
2014	TW_I +TW_F (control)	23.4 a	9.38 a	4.52 c	5.18 a	2.08 a
	TW_I + TOC_F	23.5 a	9.42 a	4.44 c	5.29 a	2.12 a
	SW_I + TW_F	12.5 c	6.44 c	16.12 a	0.78 c	0.40 c
	SW_I + TOC_F	18.2 b	8.12 b	7.66 b	2.38 b	1.06 b
2015	TW_I +TW_F (control)	22.6 a	9.82 a	4.74 c	4.77 a	2.07 a
	TW_I + TOC_F	22.5 a	9.80 a	4.66 c	4.83 a	2.10 a
	SW_I + TW_F	13.1 c	6.60 c	15.46 a	0.85 c	0.43 c
	SW_I + TOC_F	17.9 b	8.44 b	8.04 b	2.23 b	1.05 b

TW_I = tap water for irrigation	TW_F = tap water for spray		SW_I = sea water for irrigation	
TOC_F = α-tocopherol for spray	K potassium		Ca = calcium	Na = sodium
values are mean of 5 numbers		value followed by different latters are significantly different		

The activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) are shown in Table 6. Under saline conditions (ECe = 8.0 dS m^{-1}), the foliar application of α-TOC considerably improved the activities of SOD, CAT, APX as well as GR (9.31, 50.2, 62.5 and 32.6 min^{-1} g^{-1} protein, respectively) of pepper plants compared to those irrigated and sprayed by tap water (control treatment) since the corresponding values were 3.8, 34.5, 41.8 and 20.3 min^{-1} g^{-1} protein.

Table 6. Effect of α-tocopherol application on the activities of superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase in *Capsicum annuum* plants irrigated by diluted sea water

Growth season	Treatments	SOD	CAT	APX	GR
2014	TW_I +TW_F (control)	3.85 d	34.5 d	41.8 d	20.3 d
	TW_I + TOC_F	5.24 c	39.8 c	48.6 c	23.4 c
	SW_I + TW_F	8.22 b	46.2 b	56.2 b	28.4 b
	SW_I + TOC_F	9.31a	50.2 a	62.5a	32.6a
2015	TW_I +TW_F (control)	3.55 d	33.2 d	39.2 d	22.6 d
	TW_I + TOC_F	4.86 c	38.4 c	46.2 c	25.4 c
	SW_I + TW_F	7.53 b	44.8 b	55.6 b	31.2 b
	SW_I + TOC_F	8.82 a	49.4 a	64.2 a	35.6 a

TW_I = tap water for irrigation	TW_F = tap water for spray	SW_I = sea water for irrigation	
TOC_F = α-tocopherol for spray	SOD = superoxide dismutase	CAT = catalase	
APX = ascorbate peroxidase	GR = glutathione reductase		
values are mean of 5 numbers	value followed by different latters are significantly different		

4. Discussion

Concerning pepper plants growth and their yield, salinity effects occur by stimulating the over production of reactive oxygen species (ROS) through various organelles and enzymes (Rao,2006).In order to avoid these effects alongside other previously stated damages, the plants attempt to adopt several resistance strategies; include ion homeostasis, osmotic adjustment, and enhancing the antioxidant defense system (Xiong and Zhu, 2002). In the current study, the reduce in leaves number or their area, plant dry weight, fruit number and yield per plant (Table 2) caused by saline irrigation water (EC = 8.0 dS m^{-1}) could be ascribed to the osmotic effect of salt stress that elevated Na concentration in mean time reduced Ca, K, K/Na and Ca/Na (Table 5) created imbalance state of water content in the stressed plants. This phenomena includes, ionic imbalance, stomatal closure, reduction in photosynthesis, reserve of toxic ions and accordingly growth inhibition (Rady *et al.*, 2013; Semida and Rady, 2014 and Semida *et al.*, 2014).

Salinity limits plant development by its harmful effects on numerous physiological and biochemical actions, antioxidant magnitude, including photosynthesis (Orabi and Abdelhamid, 2014), resulting in damaging growth cells which, therefore, cannot perform their necessary roles (Chen and Murata, 2002). Spraying the pepper plants with 1 mM **α**-TOC significantly improved all plant growth characteristics (fruit number and its weight) resulting in an enhancement in the final yield (Table 2). (Sakr and El-Metwally, 2009) illustrated that **α**-TOC relieved the harmful effects of high salinity stress on the growth of wheat plants and

increased plant dry matter accumulation in salt soil. **α**-TOC is a main vitamin E compound located in the leaf chloroplasts. **α**-TOC as an antioxidant, that deactivates photosynthesis-derived reactive oxygen species (ROS), and prevents the enhance in lipid peroxidation by scavenging lipid peroxyl radicals in thylakoid membranes. **α**-TOC is considered as an important part of the plant defense machinery, which maintains the integrity and normal function of the photosynthetic apparatus (Liu *et al*., 2008). In addition, K+ ions are known activators of numerous enzymes that are essential for photosynthesis and respiration; thus, the reduction in K+ ion concentrations (Table 5) could result in an inhibition of photosynthesis and, eventually, a reduction of growth (Salisbury and Ross, 1992).

Regarding photosynthetic pigmentation, acquired data showed that salinity induced a decrease in total chlorophyll, carotenoids, while **α**-TOC application positively influenced these parameters (Table 3). These results are in approval with those postulated by (Rady *et al*., 2013). Carotenoids and chlorophyll contents reduced under salinity through the reduction of chlorophyll biosynthesis (Abd El-Mageed and Semida, 2015), leading to a lower absorption of daylight by the chloroplast and led to impairing photosynthesis (Semida *et al*., 2015). The effect of salt soil stress on the photosynthetic pigments may be due to the effect of salinity on the actions of photosynthetic enzymes, and reduced CO_2 partial pressure in the leaves caused by stomatal closure (DeRidder, and Salvucci, 2007). Also, (Desingh and Kanagaraj 2007) indicated that salinity stress might affect the photosynthesis by causing, a disorientation of the lamellar system of chloroplasts and a loss of chloroplast integrity leading to a diminution in the activities of photosystems. Application of **α**-TOC could relieve the inhibitory effect of salinity (Table 3). **α**-TOC may interfere with the protection of the chloroplasts and their membrane against salt toxicity and aid in maintaining their integrity (Hassanein, 2009) or vitamins protect chloroplasts from oxidative damage (Munne and Bosch, *et al*., 2001). Also **α**-TOC as an antioxidant protected photosynthetic machinery from salt-induced ROS. Under salinity stress, **α**-TOC significantly increased the concentrations of carotenoids, which are considered free radical scavenger (Sakr and El-Metwally, 2009), controlling the intensity of free radicals as well as peroxides (Apel and Hirt, 2004) and augmenting plant capacity to reduce the damage caused by ROS, which in turn increment chlorophyll contents in the leaves (Orabi and Abdelhamid, 2014).

The reduced growth and yields of pepper plants grown under diluted sea water have been associated with the reduction in membrane stability index (MSI) and relative water content (RWC) (Tables 2 and 3), while the application of **α**-TOC solved these adverse effects and increased RWC and MSI. This decrease in leaf RWC could be related to the lessened availability of water under stress conditions (Shalhevet, 1993), or to a root system that was not able to compensate the water lost via transpiration through a reduction in its water-absorbing surface (Gadallah, 2000). Stress-induced reductions in the RWC of leaves indicated a loss in cell turgidity that resulted in limited availability of water for cell extension (Katerji *et al*., 1997). The **α**-TOC spraying enabled the plant leaves to maintain a high level of RWC by regulating the leaf osmolality, assuaging the negative effects of salt stress. The effect of TOC may be due to it serving as an osmo-protectant to prevent cell damage from dehydration (Krieger-Liszkay and Trebst, 2006). The increase in water potential and osmotic

potential might help in stabilization of the associated proteins and increase photosynthesis processes (Ashfaque *et al*., 2014). Under salt stress, soil salts trigger the osmotic stress, and the over increase of ionic in the cells causes salt stress. In addition, (Abd El-Mageed and Semida 2015) reported a decrease in relative water content (RWC) and membrane stability index (MSI) under the effect of salt stress. This result confirms the findings in Table 3.In the present study, increased proline concentrations and total soluble were observed in pepper plants growing under salt stress (Table 4). The increase in leaf proline concentrations under saline stress might be caused by increased proline synthesis from glutamate, decreased use for protein synthesis, or enhanced protein turnover.

Some researchers have reported a higher proline content with foliar application of **α**-TOC acting as a solute for intercellular osmotic adjustment (Orabi and Abdelhamid, 2014 and Taie *et al*., 2013). Total soluble sugars are considered key osmolytes for osmotic adjustment. The accumulation of total soluble sugars is a common phenomenon under stress conditions (Haq *et al*. 2011 and Wu *et al*. 2013) conveyed an increase in total soluble sugars with a progressive escalation in salinity, which played an important role in osmo-regulation and abridged the osmotic potential (Martino *et al*., 2003). High salinity caused both hyper-ionic and hyperosmotic stress leading to plant death (Hasegawa *et al*., 2000). It has been stated that plants grown under saline conditions are affected in three ways: reduced water potential in the root zone causing water deficit stress; phyto-toxicity of Na^+ and Cl^- ions; and nutrient imbalances due to lowered uptake and transport of nutrients. Sodium ions compete with K^+ ions for the binding sites essential for cellular functions (Munns, 2002). However, data in Table 5 showed that irrigation of pepper plants with diluted sea water caused significant increases in Na concentrations in leaves, with significant decreases in N and K+ ion concentrations, and in the N/Na and K/Na ratio. Reductions in K^+ ion concentrations and K/Na ratios under saline conditions were confirmed by (Osman and Rady 2012 and Abdelhamid *et al*. 2013). It is known that a high concentration of Na ions in plant tissues under saline conditions decreases the K/Na ratio, which in turn impairs the selectivity of root cell membranes and results in the passive accumulation of Na ions in plant organs (Farouk, 2011). The increase in Na uptake under salinity was accompanied by corresponding declines in N and K concentrations, showing an ostensible antagonism of N and K ions against Na ions (Alam, 1994). Potassium ions are the main cation and are an essential component of the osmotic potential of cells (Reggiani *et al*., 1995). Exogenous foliar applications of TOC lightened the detrimental effects of salinity to ion concentrations due to a reduction in Na ion accumulation (Table 5), as well as an increase in the concentrations of K^+ and Ca which led to an increase in K/Na and Ca/Na ratios compared to control plants and plants grown under the salinity level without TOC treatment. The positive effects of TOC arose through its role in increasing osmotic tolerance and/or through regulating processes such as the absorption of nutrients from the soil solution. Furthermore, the beneficial effects of TOC may be due to its roles in improving membrane permeability and increasing soluble protein concentrations which protected membranes and membrane-bound enzymes. TOC thus protected plants against salt toxicity through its roles in maintaining the structural integrity of the plasma membrane and controlling the uptake of Na^+ and other toxic ions (Buschmann and Lichtenthaler, 1979).

Data of the present study shows that **α**-TOC spraying increased the activities of SOD, CAT, APX, ASA and GR compared to the salt stressed-control plants (Table 4 and 6). These increased activities of antioxidant enzymes may contribute compensations to pepper plants and enable a better performance in the various aspects of growth and metabolism as they defend against the harmful effect of salinity stress, mainly through the proliferation in these enzyme activities together with the increase of some antioxidant substances. The ASA and GSH (the substrates of the Halliwell–Asada cycle) also act as antioxidants in an isolated manner, meaning that they are directly involved in the reduction of ROS during different levels of stress (Del R ó *et al*., 2006). This situation is reflected within the evaluation of the total contents of **α**-TOC, ASA and GSH in the current study (Table 4). The specified compounds are increased by the application of **α**-TOC under salt stress, lessening the accumulation of O_2. The ASA can directly remove O_2 and H_2O_2 in a non-enzymatic manner (Foyer *et al*., 1991). **α**-TOC has suggested to play a major role in chloroplastic antioxidant of plants, contributing to preserve an adequate redox station in chloroplasts, and to maintaining thylakoid membrane structure and function during plant development and in the plants' response to stress (Munne ´-Bosch, 2005, Orabi & Abdehamid 2014 and Semida *et al*., 2016) recorded increases in the antioxidant enzymes in response to **α**-TOC application on bean and plants against oxidative stress.

5. Conclusion

Salt stress tolerance in pepper plants, was improved by the exogenous of **α**-TOC that was effective in boosting adaptability to soil salinity stresses by better chlorophyll, enzymatic and non-enzymatic antioxidants, plant growth and productivity. This might be attributed to cytokinins mediated staying green effect. Findings of this study suggested that the exogenous spraying of **α**-TOC, at the level of 1 mM, promotes the expression of stress–response genes and increases salt stress tolerance in pepper plants. While concurrently concluding that **α**-TOC spraying enhanced the expression of ROS-related stress–response genes, and that it is an effective means of enhancing resistance to subsequent stress.

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