Ameliorative Effect of α-tocopherol on *Capsicum Annuum* (L.) Plants Exposed to Short-Term Sea Water Stress

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ABSTRACT

Alpha-tocopherol (α-TOC); one of the important vitamins in plants, is considered as a non-enzymatic antioxidant .It plays an important role in ameliorating a number of abiotic stresses, including salinity. A pot experiment was conducted in 2017 at Albaha region, Saudi Arabiato study the ameliorative effect of α-TOC (2 mM)on sweet pepper (*Capsicum annuum* L., cv. 'California Wonder') plants under irrigation with diluted sea water (DSW). The DSW was obtained by mixing sea water (54.7 dS m^{-1}) with fresh water to decline the electrical conductivity to12.5 dS m^{-1}. Results show that short term irrigation with DSW significantly reduced growth and yield characteristics, photosynthetic pigment contents, relative water content (RWC), membrane stability index (MSI), contents of K^+ and Ca^{2+} and ratios of K^+/Na^+ and Ca^{2+}/Na^+, while increased the contents of proline, soluble sugars, α-TOC, and Na^+. However, exogenous α-TOC ameliorated the salt stress effects and significantly increased growth and yield characteristics, photosynthetic pigment contents, RWC, MSI, contents of K^+ and Ca^{2+}, ratios of K^+/Na^+ and Ca^{2+}/Na^+. It further increased the contents of proline, soluble sugars, and α-TOC, while significantly reduced Na^+ content compared to the corresponding controls. These results recommend the use of 2.0 m Mα-TOC as a commercial formulation to improve growth and productivity of sweet pepper plants exposed to short term saline (EC = 12.5 dS m^{-1}) water irrigation.

Keywords: Sweet pepper; salt stress; α-tocopherol; growth and yield; tissue health

1. INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is an important crop due to its economic importance and its nutritional value, and its fruits are an excellent source of bioactive products that are important for human health (Howard ***et al.***, 2000). Sweet pepper is classified as a moderately sensitive crop to salt stress (Lee, 2006).

Salinity is one of the major constrains, particularly in dry (arid and semi-arid) regions. It restricts plant growth and development because of its adverse effects on physiological, biochemical and molecular levels (Tester and Davenport, 2003). Salt stress can also disturb the photosynthetic processes by increase in the endogenous accumulation of Na^+, Cl^-, and other undesired ions, causing disturbance in water and osmotic potential. Where, high salinity reduces the soil water potential and causes physiological drought in plant growth medium (Yusuf ***et al.***, 2007). Plants grown under saline conditions change their metabolism to overcome this adverse environmental condition. They can adapt to salt stress through different mechanisms. Changes in morphological and developmental patterns, as well as biochemical responses are of these mechanisms (Bohnert ***et al.***, 1995).One mechanism adopted by plants to overcome salt stress is the accumulation of compatible osmolytes such as

freeproline and soluble sugars (Semida and Rady, 2014; Bargaz ***et al.***, 2016). The synthesis and accumulation of proline and soluble sugars by plant tissues during salt and/or water stress is an adaptive response (Rady ***et al.***, 2015). In addition, low molecular weight antioxidants like ascorbic acid, glutathione, α-tocopherol, etc., besides enzymatic antioxidants (i.e., superoxide dismutase, catalase, as corbate peroxidase, glutathione reductase, etc.) represent a major part of antioxidative defense system that act toalleviate salt stress damages and increase resistance to salt stress (Semida and Rady, 2014; Bargaz ***et al.***, 2016; Semida ***et al.***, 2016). Such mechanisms/systems may be induced or activated by spraying plants with antioxidants, including vitamins (Semida ***et al.***, 2014, 2016).

Vitamins are organic compounds used in trace amounts to maintain normal growth and correct development in all organisms. These compounds are considered as an essential part of the regulation of plant metabolism by acting as enzyme co-factors (Rady ***et al.***, 2015). One of them is α-tocopherol (α-TOC) that is a lipophilic membrane-located vitamin compound in chloroplasts (Semida ***et al.***, 2014; Rady ***et al.***, 2015). Theα-TOC is a lipid-soluble antioxidant, localizing the chloroplast envelope and thylakoid membranes of green plants (Matringe ***et al.***, 2008). It is synthesized exclusively in photosynthetic organisms (Della Penna, 2005). It has been postulated that α-TOC increases plant tolerance to the adverse effects of salt stress on crop performance (Rady ***et al.***, 2015; Semida ***et al.***, 2014, 2016).

Therefore, the objective of this study was to assess the response of sweet pepper plants exposed to a short term irrigation with diluted sea water (DSW) with EC = 12.5dS m^{-1} to foliar application of 2.0 m Mα-TOC. In addition, this study aimed to investigate the integrative effects of α-TOC and DSW on plant performance (growth and yield), nutrient status, cell and tissue health and activity of the non-enzymatic antoxidative defense system, including α-TOC in sweet pepper plants.

2. MATERIALS AND METHODS

2.1 Growth conditions and treatments

A three-replicated pot experiment was conducted in a wire-house at Albaha University (latitude 20° 17' 41"N, longitude 41° 38' 35"E), elevation 1651.88m above sea level, Albaha, Saudi Arabia. The climate of the study area is semiarid (Zabin and Howladar, 2015) and is characterized as follows: the mean annual temperature varies from a minimum of 17.8˚C and a maximum of 29.9˚C. The average annual rainfall is about 62.45 mm. The relative humidity min. 15% and max 87%, the mean wind speed around 6 Kts/Deg (PMEP, 2017).

Sweet pepper seedlings (45-day-old, had 6-7 leaves for each) were obtained and two healthy seedlings were transplanted in each 50 cm-diameter plastic pot, containing air-dried 12 kg of a sandy loam soil. According to the recommended doses of agricultural practices, nitrogen (N) as ammonium sulphate (20.5% N) at 2.5 g per pot, phosphorous (P) as calcium superphosphate (15.5% P_2O_5) at 1.5 g per pot, and potassium (K) as potassium sulphate (48% K_2O) at 1 g per pot were added to each pot before planting. In addition, further N doses (ammonium sulphate 20.5% N) were added at 30, 60, and 120 days after transplanting at 1.5 g per pot.

The experimental treatments were arranged in a completely randomized design as one level (12.5 dS m^{-1}) of diluted sea water(DSW)and one level (2 mM) of α-TOC(Hangzhou Toyond Biotech Co. Ltd., Zhejiang, P. R. China) and their combination (DSW × α-TOC), besides the control (Tap water), each with 20 replicates (pots) per treatment. Starting at 16 days after transplanting, all plants per treatment (n = 40) were sprayed two-times, at 6-day intervals with tap water or 2mMα-TOC. Theα-TOC at 2mM was chosen as the best level to apply based on a preliminary study in which we tested 1.0, 1.5, 2.0, 2.5 or 3.0 mMα-TOC (data not shown). In the control

treatment, plants (n = 40) were irrigated with tap water having an electrical conductivity (EC) of 0.34dS m^{-1} and sprayed with tap water in place of 2mMα-TOC. To induce salinity stress, sea water (EC = 54.7dS m^{-1}) was mixed with tap water (EC = 0.34dS m^{-1}) to obtain the used level of DSW (12.5dS m^{-1}). Batches of plants (n = 40) were then watered four consecutive times with an equal volume of DSW. The first DSW irrigation was applied with the first foliar application with α-TOC and the third DSW irrigation was applied with the second foliar application with α-TOC. The EC and pH values and the contents of cations and anions in the soil used for the experiments are shown in Table 1.The soil water-holding capacity was measured by saturating the soil in each pot with water and weighing it after it had drained for 48 h. The water-holding capacity of the soil in each pot was 36% (w/v) soil:water. Soil water contents were maintained at approx. 90% (w/v) of the soil water-holding capacity. The level of soil moisture was controlled by weighing each pot and any water loss was supplemented daily.

Table 1. Physico-chemical characteristics of a sandy loam soil prior to the experiments in two seasons.

Bulk density (g cm^{-3})	CEC ($cmol^{+}/kg$)	pH	EC (dS m^{-1})	OC*	N	P	K	Ca	Fe	Mn	Zn
				(g kg^{-1})				(mg kg^{-1})			
1.29	7.9	7.7	2.3	8.9	0.82	15	70	85	6.0	4.0	2.1

*OC, organic content.

2.2 Measurements

To measure growth and yield characteristics of sweet pepper plants, five sample pots were taken randomly from each treatment at 75 days after transplanting. Number of leaves per plant was counted and leaf area per plant (m^2) was assessed using a digital Planimeter. Plant dry weight (g) was measured after oven-drying at 70 °C for 48 h. At harvest, five plants from each treatment were taken and yield of pepper plants in terms of fruit number and fruit weight per plant were recorded.

Total chlorophylls and total carotenoids were extracted and determined according to the methods described by Arnon (1949) using 80% (v/v) acetone to homogenize samples, and then centrifugation at 10,000 × *g* for 10 min was performed. Absorbances of extracts were measured at 663, 645, and 470 nm using a UV-160A UV-visible recording spectrometer (Shimadzu, Kyoto, Japan).

Relative water content (RWC) of tissue was measured according to Hayat *et al.* (2007) 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 saturate discs with water. Any adhering water was gently dried and the turgid mass (TM) was recorded. Dry mass (DM) was taken after drying the discs at 70°C until the constant weight. RWC was then calculated using the following formula:

$$\mathrm{RWC}(\%) = \left[\frac{(\mathrm{FM} - \mathrm{DM})}{(\mathrm{TM} - \mathrm{DM})}\right] \times 100$$

Membrane stability index of plant tissues (MSI) was determined according to the method of Rady (2011) using two equivalent samples of fully-expanded leaf tissues. First sample was placed in test-tube containing double-distilled water. The content of the test-tube was heated at 40°C in a water bath for 30 min, and the electrical conductivity (EC1) of the solution was recorded using a conductivity bridge. Second sample was boiled at 100°C for 10 min, and the conductivity was measured (EC2), and MSI was calculated using the formula:

$$\mathrm{MSI}(\%) = \left[1 - \left(\frac{\mathrm{EC1}}{\mathrm{EC2}}\right)\right] \times 100$$

Free proline was determined following the method of Bates ***et al***. (1973). Samples were grinded in 3% (v/v) sulphosalicylic acid, and centrifugation at 10,000 × *g* for 10 min was then done. 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 90ºC 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 Bauschand Lomb-2000 Spectronic Spectrophotometer.

Total soluble sugars were determined according to Irigoyen ***et al***. (1992). Samples were homogenized in 5 ml of 96% (v/v) ethanol, and then washed with 5 ml 70% (v/v) ethanol. The extracts were centrifuged at 3500 × g for 10 min, and the supernatants were stored at 4°C 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 was then cooled. The absorbance was recorded at 625 nm using a Bauschand Lomb-2000 Spectronic Spectrophotometer.

Alpha-tocopherol (α-TOC) 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 hydroxytoluene (BHT) was dissolved in this solvent mixture. Using R-TOC, standard solutions (20 – 200 µg/ml) were prepared from stock solution (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 °C 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 °C, 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 in a separatory funnel. Organic phases were combined and washed with 100-ml portions of water until free of alkali that was 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 °C). The residues were dissolved each in 20 ml of *n*-hexane (HPLC grade) and stored in a freezer at –20 °C. The αTOC was determined on a HPLC system using a 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).

Contents of Na^+ and K^+ were determined as follows: 0.2 g of dried leaf was digested with sulphuric acid in the presence of H_2O_2 (Wolf, 1982). The mixture was then diluted with distilled water. The total leaf concentrations of Na^+ and K^+ were measured directly using Flame Spectrophotometry (Lachica ***et al.***, 1973). The content of Ca^{2+} was determined using a Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer (Chapman and Pratt, 1961). Results were calculated and expressed as mg g^{-1} dry weight.

2.3 Statistical analysis

The completely randomized design was the layout for the current work. Data were statistically analyzed using ANOVA followed by Tukey's HSD test (SPSS 14.0; SPSS Chicago, IL, USA). Significant differences were analyzed based on $P \leq 0.05$ between four means of four treatments.

3. RESULTS

Data presented in Table 2 show that growth and yield characteristics (i.e., number of leaves per plant, leaf are per plant, plant dry weight, number of fruits per plant and fruit yield per plant) of sweet pepper plants were significantly increased with foliar spray of α-tocopherol (α-TOC) when plants irrigated with tap water compared to the normal control (irrigated with tap water). Under short term irrigation with diluted sea water (DSW), the pepper growth and yield characteristics were significantly reduced compared to the normal control. However, application of α-TOC as foliar spray significantly increased these characteristics compared to the salt-stressed control (irrigated with DSW). These increases were 64% for number of leaves per plant, 71% for leaf area per plant, 67% for plant dry weight, 100% for number of fruits per plant, and 124% for fruits weight per plant, respectively.

Table 2. Effect of α-tocopherol (TOC; 2 mM) application on some growth and yield characteristics of *Capsicum annuum* plants irrigated with diluted sea water

Treatments	Leaves No. plant^{-1}	Leaf area plant^{-1} (m^2)	Plant dry weight (g)	Fruit No. plant^{-1}	Fruit yield plant^{-1} (g)
Tap water (control)	49 ± 5 b	0.14 ± 0.02 b	69 ± 7 b	12 ± 2 b	360 ± 32 b
Tap water + $TOC_{2.0}$	54 ± 5 a	0.16 ± 0.02 a	74 ± 7 a	14 ± 2 a	400 ± 38 a
Sea water ($Salin_{12.5}$)	25 ± 3 d	0.07 ± 0.00 d	36 ± 4 d	5 ± 1 d	98 ± 10 d
$Salin_{12.5}$ + $TOC_{2.0}$	41 ± 4 c	0.12 ± 0.01 c	60 ± 6 c	10 ± 2 c	220 ± 19 c

Values are means (n = 5) and differences between means were compared by least-significant difference test (LSD; $P \leq 0.05$). Means followed by different letters are significantly different.

Data shown in Table 3 show that the contents of leaf photosynthetic pigments (i.e., total chlorophylls and total carotenoids) of sweet pepper plants were significantly increased, while relative water content (RWC) and membrane stability index (MSI)were not affected by foliar spray of α-TOC when plants irrigated with tap water compared to the normal control. Under short term irrigation with DSW, the contents of total chlorophylls and total carotenoids, RWC and MSI were significantly decreased compared to the normal control. However, application of α-TOC as foliar spray significantly increased the contents of total chlorophylls and total carotenoids, RWC and MSI compared to the salinized controls. These increases were 80% for total chlorophylls, 56% for total carotenoids, 52% for RWC, and39% for MSI.

Table 3. Effect of α-tocopherol (TOC; 2 mM) application on leaf contents of photosynthetic pigments (mg g^{-1} FW), relative water content (RWC), and membrane stability index (MSI) of *Capsicum annuum* plants irrigated with diluted sea water.

Treatments	Total chlorophylls	Total carotenoids	RWC (%)	MSI (%)
Tap water (control)	1.92 ± 0.05 b	0.55 ± 0.01 b	74 ± 2 a	68 ± 2 a
Tap water + $TOC_{2.0}$	2.08 ± 0.07 a	0.65 ± 0.01 a	78 ± 2 a	71 ± 2 a
Sea water ($Salin_{12.5}$)	0.89 ± 0.03 d	0.32 ± 0.00 d	42 ± 1 c	44 ± 1 c
$Salin_{12.5}$ + $TOC_{2.0}$	1.60 ± 0.04 c	0.50 ± 0.01 c	64 ± 2 b	61 ± 1 b

Values are means (n = 5) and differences between means were compared by least-significant difference test (LSD; $P \leq 0.05$). Means followed by different letters are significantly different.

Data shown in Table 4 show that the contents of antioxidants/osmoprotectants (i.e., free proline and soluble sugars) were not affected, while the content of the antioxidant α-TOC was significantly increased by foliar spray of α-TOC when plants irrigated with tap water compared to the normal control. Under short term irrigation with DSW, the contents of free proline, total soluble sugars and α-TOC were significantly increased compared to the normal control. Application of α-TOC as foliar spray significantly further increased the contents of these osmoprotectants/antioxidants compared to the salinized controls. These increases were 58 and 20% for free proline, 130 and 44% for total soluble sugars, and 158 and 63% for α-TOC compared to the normal and salinized controls, respectively.

Table 4. Effect of α-tocopherol (TOC; 2 mM) application on leaf contents of free proline(μg g^{-1} DW), total soluble sugars (mg g^{-1} DW), and α-tocopherol (α-TOC; μg g^{-1} DW) of *Capsicum annuum* plants irrigated with diluted sea water.

Treatments	Free proline	Soluble sugars	α-TOC
Tap water (control)	62 ± 1.1 c	20 ± 0.4 c	24 ± 0.3 d
Tap water + $TOC_{2.0}$	64 ± 1.3 c	21 ± 0.4 c	30 ± 0.3 c
Sea water ($Salin_{12.5}$)	82 ± 1.6 b	32 ± 0.7 b	38 ± 0.4 b
$Salin_{12.5}$ + $TOC_{2.0}$	98 ± 1.8 a	46 ± 0.9 a	62 ± 0.5 a

Values are means (n = 5) and differences between means were compared by least-significant difference test (LSD; $P \leq 0.05$). Means followed by different letters are significantly different.

Data shown in Table 5 exhibit that the contents of K^+, Ca^{2+} and Na^+, and the ratios of K^+/Na^+ and Ca^{2+}/Na^+ of sweet pepper plants were not affected by foliar spray of α-TOC when plants irrigated with tap water compared to the normal control. Under short term irrigation with DSW, the contents of K^+ and Ca^{2+}, and the ratios of K^+/Na^+ and Ca^{2+}/Na^+ were significantly decreased, while the content of Na^+ was significantly increased compared to the normal control. However, application of α-TOC as foliar spray significantly increased the contents of K^+ and Ca^{2+}, and the ratios of K^+/Na^+ and Ca^{2+}/Na^+, while significantly decreased the content of Na^+ compared to the salt-stressed controls. The increases in the contents of K^+ and Ca^{2+} were 91 and 55%, respectively, and the ratios of K^+/Na^+ and Ca^{2+}/Na^+ were 332 and 247%, respectively. The reduction in the content of Na^+ was 55%.

Table 5. Effect of α-tocopherol (TOC; 2 mM) application on leaf contents of K^+ and Ca^{2+}(mg g^{-1} DW), and the ratios of K^+/Na^+ and Ca^{2+}/Na^+in *Capsicum annuum* plants irrigated with diluted sea water

Treatments	K^+	Ca^{2+}	Na^+	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio
Tap water (control)	23 ± 0.6 a	9.6 ± 0.2 a	4.2 ± 0.1 c	5.48 ± 0.1 a	2.29 ± 0.0 a
Tap water + $TOC_{2.0}$	23 ± 0.6 a	9.8 ± 0.2 a	4.1 ± 0.1 c	5.61 ± 0.1 a	2.39 ± 0.0 a
Sea water ($Salin_{12.5}$)	11 ± 0.3 c	5.8 ± 0.1 c	18.2 ± 0.5 a	0.60 ± 0.0 c	0.32 ± 0.0 c
$Salin_{12.5}$ + $TOC_{2.0}$	21 ± 0.5 b	9.0 ± 0.2 b	8.1 ± 0.2 b	2.59 ± 0.0 b	1.11 ± 0.0 b

Values are means (n = 5) and differences between means were compared by least-significant difference test (LSD; $P \leq 0.05$). Means followed by different letters are significantly different.

4. DISCUSSION

In the arid and semiarid regions that are characterized by water scarcity, salt stress adversely affects different processes during seed germination, growth and flowering that reflect in plant productivity (Semida ***et al.***, 2016). These saline effects occur by stimulating the overproduction of reactive oxygen species (ROS) through various organelles and enzymes and to avoid these effects, plants adopt several strategies, including ion homeostasis, osmotic adjustment, and enhancing the antioxidant defense system (Xiong and Zhu, 2002). In the current study, the reduced plant growth and yield (Table 2) under the adverse conditions of the short term irrigation with diluted sea water (DSW; EC = 12.5dS m^{-1}) could be attributed to the osmotic effect of salt stress, causing a disturbance in the water balance of the stressed plants that are clearly shown in Table 3 (strongly reduced RWC). This imbalance tissue water content leads to stomatal closure, ionic imbalance (Table 5), reduction in photosynthetic pigments (Table 3) and consequently photosynthesis process, accumulation of toxic ions and consequently inhibition of growth and productivity (Table 2) (Semida and Rady, 2014; Semida ***et al.***, 2014, 2016).

Salt stress negatively affects plant growth by causing disruptions in various physiological and biochemical processes, including photosynthesis, antioxidant capacity and ion homeostasis (Semida ***et al.***, 2016), resulting in damages of growing cells which, therefore, cannot perform their functions (Chen and Murata, 2000). Spraying the sweet pepper seedlings with 2.0mMα-TOC two times significantly improved all plant growth and yield characteristics, alleviating the harmful effects of salt stress on growth and yield of pepper plants and increased plant dry matter accumulation (Table 2). The α-TOC as an antioxidant, deactivates photosynthesis-derived ROS, and prevents the increase in lipid peroxidation by scavenging lipid peroxyl radicals in thylakoid membranes (Liu ***et al.***, 2008; Semida ***et al.***, 2016). Levels of α-TOC have been found to change differentially in response to environmental restrictions, depending on the magnitude of the stress and species-sensitivity to stress. The α-TOC considers an important part of the plant defense machinery, maintaining the integrity and normal function of the photosynthetic apparatus (Liu ***et al.***, 2008). Foyer and Noctor (2005) concluded that α-TOC acts directly to neutralize superoxide radicals ($O_2^{\bullet-}$) or singlet oxygen ($^1O_2$) in plant cells. It also affects many physiological processes positively under saline conditions such as the regulation of growth, differentiation and metabolism of plants and the increase in the physiological availability of water and nutrients (Semida ***et al.***, 2016). It has been reported that applied α-TOC protects metabolic processes against H_2O_2 and other toxic derivatives of oxygen, affects many enzyme activities, minimizes the damage caused by oxidative processes through synergic function with other antioxidants and stabilizes tissue membranes, and consequently obtaining healthy plant growth and satisfactory yield under salt stress conditions (Semida ***et al.***, 2016). These positive effects of α-TOC were performed by the increase in its endogenous content in the current study (Table 4).

Salt stress, in the current study, adversely affected photosynthesis by the reduction in leaf photosynthetic pigments (chlorophylls and carotenoids; Table 3), while α-TOC application repaired the photosynthetic machinery from salt-induced ROS, and increased chlorophyll and carotenoid contents. The reduced content of chlorophylls under salt stress generated by irrigation of pepper plants with DSW might have been due to that salt induced increase in the activity of chlorophyll degrading enzyme chlorophyllase (Rao and Rao, 1981).

Reduced performances (growth and yield) of sweet pepper plants grown under salt stress have been associated with the reduction in water potential that decreased the relative water content (RWC) and membrane stability index (MSI), while the application of α-TOC weakened these adverse effects and increased RWC and MSI (Table 3). Application of α-TOC enabled plant tissues to maintain high levels of RWC by regulating the leaf osmolality (free proline and soluble sugars; Table 4), alleviating the negative effects of salt stress and reflecting in

the increase in MSI. The increase in water potential and osmotic potential might help the stabilization of protein and increases photosynthesis (Ashfaque ***et al***., 2014). The exogenous application of α-TOC exhibited alleviation in the deleterious salt effects and increased RWC and MSI, maintaining turgidity of tissue cells for healthy metabolic processes and membranes integrity. In addition, free proline and soluble sugar contents were increased with foliar application of α-TOC acting as solutes for intercellular osmotic adjustment and further important factors of adaptation to salinity (Semida ***et al***., 2016). This result is found to be in a parallel line with the results of the present study (Table 4). These increased contents of free proline and soluble sugars that act as osmoprotectants supported the crucial role of α-TOC as an antioxidant in alleviating the deleterious salt effects. In the present study, the increased proline content was observed in pepper leaves under salt stress, and was further increased after the application of α-TOC. The increase in leaf proline content under saline stress might be caused by increased proline synthesis from glutamate, decreased use for protein synthesis, or enhanced protein turnover. Thus, proline may be the major source of energy and N during immediate post-stress metabolism. The accumulated proline supplies plants with energy for growth and survival, thereby increasing the salt tolerance (Gad, 2005). Application ofα-TOC alleviated the salt stress injury and the increased content of proline accompanied with the increase in total soluble sugar content. Since proline biosynthesis is a highly energy-demanding process, reduced proline synthesis could benefit plants by saving energy to cope with stress (Gad, 2005). Total soluble sugars are considered as key osmolytes for osmotic adjustment. Accumulation of total soluble sugars is a common phenomenon under stress conditions (Murakeozy ***et al***., 2003). Irrigation of pepper plants with DSW significantly increased total soluble sugars content compared to normal control plants. Haq ***et al***. (2011) reported an increase in total soluble sugars under salt stress, which plays an important role in osmoregulation and reduced the osmotic potential.

Salinity caused both hyper-ionic and hyper-osmotic stress, leading to plant death (Hasegawa ***et al***., 2000). It has been reported that plants grown under saline conditions are affected in three ways: reduced water potential in the root zone, causing water deficit stress; phytotoxicity of Na^+ and Cl^- ions; and nutrient imbalances due to lowered uptake and transport of nutrients such as K^+ and Ca^{2+} studied in the current study (Table 5). Na^+ 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 DSW caused significant increases in Na^+ ions content in leaves, with significant decreases in K^+ and Ca^{2+}ions contents, and in the $K^+/Na^+$and Ca^{2+}/Na^+ratios. The K^+ ions are the main cation and are an important component of the osmotic potential of cells (Reggiani ***et al***., 1995).Exogenous spray application of 2.0 mMα-TOC alleviated the harmful effects of salinity on ions (Ca^{2+} and K^+) contents due to the reduction in Na^+ ion accumulation (Table 5), as well as the increase in the contents of Ca^{2+} and K^+ led to the increase in the $K^+/Na^+$and Ca^{2+}/Na^+ ratios when compared with the salinized controls without α-TOC treatment. The positive effects of α-TOC arose through its role in increasing osmotic tolerance and/or through regulating processes such as the absorption of nutrients from the soil solution. In addition, the beneficial effects of α-TOC may be due to its roles in improving membrane permeability and increasing soluble protein contents, which protected membranes and membrane-bound enzymes. The α-TOC thus protected the 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).

Alpha-TOC acts as membrane stabilizers and multifaceted antioxidant that scavenge the ROS. It reacts with peroxy radicals formed in the bilayer as they diffuse to the aqueous phase, scavenging cytotoxic H_2O_2, reacts non-enzymatically with other ROS such as singlet oxygen, superoxide radical and hydroxyl radical, and stabilizes membrane structures (Blokhina ***et al***., 2003). In addition, α-TOC has appeared to play a major role in chloroplastic antioxidant network of plants, contributing to preserve an adequate redox station in chloroplasts, and to maintain thylakoid membrane structure and function during plant development and in plant responses to stress (Munne´-Bosch, 2005). Salt stress tolerance in pepper plants, in this study, was improved by foliar application of

α-TOC that was effective in alleviating the water salinity stress by better chlorophyll, nutrients and osmoprotectants contents, and plant growth and productivity. This might be attributed to cytokinin mediated stay green effect in leaves. Findings of the present study suggested that the exogenous application of α-TOC, particularly at the level of 2.0mM, improves the expression of stress–response genes and increases salt stress tolerance in pepper plants. In addition, inducing the expression of ROS-related stress–response genes by α-TOC application is an effective means of enhancing resistance to subsequent stress (Rady ***et al.***, 2015; Semida ***et al.***, 2016).

Results of the current study recommend the use of 2.0 mM α-TOC as a commercial formulation to improve the growth and productivity of sweet pepper plants when exposed to short term saline water irrigation (EC = 12.5 dS m^{-1}).

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