Zagazig Journal of Agricultural Research
www.zu.edu.eg/agr/journals

EXOGENOUSLY APPLIED ANTIOXIDANTS AND BIOSTIMULANTS ALLEVIATE SALT STRESS IN SWEET PEPPER

Moheb T. Sakr[1]*, N.M. El-Sarkassy[2] and M.P. Fuller[3]

1. Agricultural Botany Department, Agriculture Faculty, Mansoura University, Egypt
2. Agricultural Botany and Plant Pathology Department, Agriculture Faculty, Zagazig University, Egypt
3. School of Biomedical and Biological Sciences, Faculty of Science and Technology, Plymouth University, UK

ABSTRACT

Saline soils are responsible for the restriction in yield of many crop plants and/or the limitation of marketable yield of several vegetable fruit crops such as sweet pepper. Whilst genetic solutions to these problems are being sought, exogenously applied ameliorants are needed to reduce the effects of salt. Experiments were carried out during the two growing seasons 2007 and 2008 to investigate the effect of salinity stress on growth, yield and endogenous bio-constituents and to examine whether salinity stress can be offset by the exogenous application of some antioxidant materials on sweet pepper (*Capsicum annuum* L. cv. Orlando). Salinity stress (2, 4 or 6 g L^{-1}) decreased growth at 75 days after transplanting and marketable yield. Applied antioxidants and bio-stimulants counteracted the harmful effects of low and moderate salinity stress levels (2 and 4 g L^{-1}) and partially couteracted the harmful effects under the highest salinity stress level (6 g L^{-1}). Ascorbic acid and sea weed extract (SWE) were the most effective agents in this respect Salinity stress levels increased super oxide dismutase (SOD), ascorbic peroxidase (APX) activity, proline content, and Na content but decreased photosynthetic pigment in the leaves and K in shoot of pepper plants. In addition, all of the applied antioxidants alone or combined with different salinity stress levels slightly increased the content of ascorbic acid and glutathione and the activity of SOD, APX. These results provide support for the field application of bio-stimulants and antioxidant compounds to alleviate the symptoms and effects of salty soils.

Keywords: Antioxidants, sweet pepper, *Capsicum annuum,* salt stress, seed presoaking.

INTRODUCTION

Pepper is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent source of natural colors and antioxidant compounds important for human health (Howard *et al.,* 2000). Pepper is grown under protected glasshouse conditions in temperate regions and in the open field under warm Mediterranean climates. Where it is grown in the soil, it is frequently exposed to saline conditions brought about by extensive use of irrigation containing trace amounts of salts including sodium chloride (Kijne, 2003). Soil salinity imposes stress conditions on crop plants (Hajer *et al.*, 2006) and affect germination, growth and chlorophyll content (Paridam and Das, 2005) and has been shown to limit pepper yield (Navarro *et al.,* 2002).

Plants subject to salinity stress conditions produce cytotoxic activated oxygen that can seriously disrupt normal metabolism, through oxidative damage of lipids, proteins, and nucleic acids (Abbaspour, 2012). To defend against such oxidants, plants have evolved specific protective mechanisms, involving antioxidant

***Corresponding author:** Tel.: +201112582556
E-mail address: sakrmohb@yahoo.com

molecules and enzymes that protect against the potentially-cytotoxic species of activated oxygen. Several studies have shown that the effects of cytotoxicity induced by salt stress can be ameliorated by the exogenous application of antioxidants (Sakhabutdinova *et al.*, 2003) or by compounds that enhance the natural defense systems of the plant (Demir *et al.*, 2004, Schmidt 2005). If such amelioration can be sustained then such treatments offer the opportunity for in-field protection against this stress.

The objectives of this work were to study the effect of salinity stress on growth, yield and endogenous bio-constituents in sweet pepper (*Capsicum annuum* L.) and to examine whether the harmful effects of salinity stress can be offset by the exogenous application of some antioxidants and biostimulant materials.

MATERIALS AND METHODS

Two pot experiments were performed at the Experimental Station Farm, Faculty of Agriculture, Mansoura University during two successive summer seasons (2007 and 2008). Sweet pepper cv Orlando seeds provided by Gohara Co. Cairo, Egypt were sown on 15 th February in both seasons, and seedlings were transplanted at 45 days (6-7 leaves) on the 1st of April into pots (50cm inner diameter) containing 8 kg of air-dried loamy soil at of two plants/pot. Each pot before planting was supplied with 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 (K_2O) at 1 g per pot. Also further N (ammonium sulphate 20.5% N) was added at 30, 60, and 120 days after transplanting at 1.5 g per pot.

Irrigation solutions containing one of four levels sodium chloride were used : 0.32 g L^{-1}; 2 g L^{-1}; 4 g L^{-1}; 6 g L^{-1}. Irrigation solutions were supplied daily according to plant need and to maintain a slight reserve of water in the pot saucer.

The exogenously applied treatments were:

1- Tap water

2- Humic acid (1000 mg L^{-1})

3- Salicylic acid (250 mg L^{-1})

4- Ascorbic acid (250 mg L^{-1})

5- Seaweed extract (1000 mg L^{-1}).

The humic acid (a.i. 80% leonardite-based) was provided by Plant Wise Biostimulants (Louisville, KY) and the seaweed extract (extract of *Ascophyllum nodosum*) was supplied by Acadian Sea plants Ltd. (Dartmouth, Nova Scotia, Canada), both products are used commonly in Turfgrass management programmes.

Seeds were presoaked in the antioxidants for 8 hours before sowing, and then plants were foliar sprayed to run-off with the same antioxidant at 30, 60, 90, 120, and 150 days after transplanting.

Each experiment therefore included 4 salinity levels and 5 exogenous spray treatments, (20 treatments) replicated 6 times in a completely randomized design.

In both growing seasons, 6 sample pots were taken at 75 days after transplanting and the following vegetative growth characters recorded: plant height (cm); number of branches/ plant; number of leaves/plant; shoot fresh weight (g); shoot dry weight (g); leaf area (cm^2/plant).

Fruit setting percentage was also determined as the number of fruits set per number of flowers produced. Two fruit picking were taken from each treatment at 180, and 210 days from transplanting, total yield was calculated (summation of the two pickings). Six plants from each treatment were taken and the following were recorded: number of fruits/plant; fresh weight of fruits/plant (g); dry weight of fruits/ plant (g).

The following chemical determinations were investigated in shoots at 75 days after transplanting: photosynthetic pigments; total soluble solids (T.S.S.); total soluble sugar content; proline content; total free amino acids; total ascorbic acid; total glutathione; super oxide dismutase (SOD) activity; ascorbate peroxidase activity; potassium content and sodium content.

Photosynthetic pigments were measured in fresh leaf samples (0.5 g from the 3rd terminal leaf) extracted by methanol for 24h at laboratory temperature after adding a trace of sodium carbonate. Chlorophylls and carotenoids were determined spectrophotometrically (Spekol Π at

wave-lengths 452, 650 and 665 nm) and calculated according to the methodology of Mackinney (1941).

Total soluble solids were determined by hand-held refractometer A.O.A.C. (1975). Reducing and non–reducing sugars were extracted from 5 g crude dried material of the 3rd terminal leaf using 70% ethanol and kept overnight at room temperature according to Kayani, *et al.* (1990) and then was filtered and recorded as total soluble sugar content (TSS).

Total free amino acids were measured in fresh leaf samples, which were extracted with 80% hot ethanol three times and evaporated to dryness. The dried film was then dissolved in 10% aqueous isopropanol. A ninhydrin solution was prepared by dissolving 2.0 g ninhydrin in 25 mL of acetone following by the addition of 250 ml 0.2 M acetate buffer (pH 5.5) and stored in a brown bottle to be protected from light degradation (Jayaraman,1985). Sample extracts were pipetted into a series of test tubes, and the total volume was made up to 4.0 mL with distilled water. One mL of the ninhydrin reagent was added to each tube and mixed well, and the tubes were kept in a boiling water bath for 15 min. The tubes were then cooled and the volume made up to 10 mL in a measuring flask with 50 % ethanol. The developed pink color was measured using a spectrophotometer at 570 nm. The concentration of free acids were calculated from a standard curve of lysine. Proline content was determined in leaves by the modified ninhydrin method of Troll and Lindsley, (1955).

Total ascorbic acid content was determined using the dye 2,6 dichlorophenol indophenol as described by Ranganna (1979) and recorded as mg/100 g fresh weight.

Total glutathione (GSH) was determined with Ellman's reagent according to De Vos *et al.* (1992). 300 µL of sample buffer was mixed with 630 µL of 0.5 M K_2HPO_4 and 25 µL of 5 mM 5, 5-dithiobis (2-nitrobenzoic acid) (final pH 7.0). The absorbance at 412 nm was read after 2 min and compared to a GSH standard.

Super oxide dismutase (SOD) activity was measured in fresh leaf samples collected in an ice bucket and brought to the laboratory. Leaves were washed with distilled water and then blotted dry. Leaf samples (0.5 g) were homogenized in an ice cold previously prepared 0.1M pH 7.5 phosphate buffer containing 0.5 mM EDTA in a pre-chilled pestle and mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 4^0C in a refrigerated centrifuge for 15 min at 15000 X g. The supernatant was transferred to 30 mL tubes, and SOD activity was estimated by recording the decrease in absorbance of superoxide-nitro blue tetrazolium complex by the enzyme. About 3 mL of reaction mixture containing 0.1 mL of 1.5M sodium carbonate, 0.2 mL of 200mM methionine, 0.1 mL of 2.25mM Nitro-blue tetrazolium, 0.1 mL of 3mM EDTA, 1.5 mL of 100mM potassium phosphate buffer, 1 mL distilled water and 0.05 mL of enzyme were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 mL riboflavin (60 mM) and placing the tubes below a light source of two 15 w florescent lamps for 15 min. The reaction was stopped by switching off the light and covering the tube with black cloth. Tubes without enzyme developed maximal color. A non-irradiated complete reaction mixture which did not develop color served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples 50% of comparison with types lacking enzymes (Dhindsa *et al.*, 1981).

Ascorbate peroxidase (APX) was assayed spectrophotochemically according to Fielding (1978). The assay was carried out at 25°C in a 1.0 cm light path cuvette and the reaction mixture consisted of 1500 µL phosphate buffer, 20 µL EDTA, 1000 µL sodium ascorbate and enzyme extract (20 µL). After mixing the reaction was initiated by adding the 480 µL H_2O_2 and measuring the decrease in optical density at 290 nm against a blank (without extract) was recorded at one and two minute .

Potassium (K) and sodium (Na) contents were estimated by flame photometry (Peterburgski, 1968).

The determination of enzymatic and non-enzymatic antioxidants as well as nutrient elements were only determined in the first experimental season.

The data of all experiments were analyzed statistically using analysis of variance according to Gomez and Gomez, (1984). The treatment means were compared using the least significant differences (LSD).

RESULTS

All growth characters of sweet pepper plants were decreased with increasing salinity stress levels with the greatest reduction observed at the highest salinity (Table 1). Applied antioxidant materials led to growth improvements at all levels of salt stress including the lowest level (0.32 gL^{-1}) and were therefore acting as growth stimulants (Table 1). It was shown that the applied antioxidants could counteract the effects of low salt stress (2 gL^{-1}) and partially counteract the harmful effect of medium and high salt stress (4 and 6 g L^{-1}). Ascorbic acid and seaweed extract gave the best protection against salt stress.

Fruit setting, total fruit yield and fresh and dry weights of pepper fruit decreased with increasing level of salinity stress with the high salt stress reducing fruit yield by 67% (Table 2). Applied antioxidants increased fruit setting, fruit yield, and fresh and dry weights of pepper fruit during both growing seasons and ascorbic acid was the most effective of the antioxidants, increasing fruit set and fruit size more than two-fold compared to the untreated plants at the lowest salt treatment. All of the antioxidants counteracted the negative effects of low and medium salt stress and partially offset the effects of high salt stress. Ascorbic acid and seaweed extract were the most effective of the antioxidant applications.

Data in Tables 3, 4, 5 and 6 show that all salinity stress levels (2, 4 and 6 g L^{-1}) slightly decreased total chlorophyll and carotenoids in the leaves whereas T.S.S. content, total amino acids and K while Na content increased. Changes were incrementally related to the increase in salt stress.

The applied antioxidants increased chlorophyll, carotenoids in leaves, and T.S.S. contents, total amino acids and contents of ascorbic acid and glutathione and increased the activity of SOD and APX, and the potassium to sodium ratio. Furthermore the data show that the applied antioxidants completely counteracted the harmful effects of salinity stress levels (2 and 4 g L^{-1}) on photosynthetic pigments in the leaves. The ascorbic acid and seaweed extract treatments were most effective in increasing photosynthetic pigments in most cases. Humic acid was most effective in alleviating the harmful effect of salinity stress in terms of enhancing TSS content.

Applied antioxidants also promoted soluble sugar accumulation, proline accumulation, total amino acid content and K, content, and seaweed extract was the most effective in this respect.

DISCUSSION

The inhibitory effects of salinity on growth of pepper plant reported here were typical of the effects of high soil salt availability and are probably due to decreased water absorption and perturbed metabolic processes leading to decreased meristematic activity and/or cell enlargement (Khadr *et al.*, 1994) coupled with an increase in respiration rate resulting from higher energy requirements. Yang *et al.* (1990) reported that there are two ways that salinity could retard growth, by damaging growth cells so that they cannot perform their functions or by limiting their supply of essential metabolites. Salinity stress is known to retard plant growth through its influence on several vital factors of plant metabolism, including osmotic adjustment (Sakr and El-Metwally, 2009) nutrient uptake (Saied, *et al.*, 2005), protein and nucleic acid synthesis, photosynthesis (Zaibunnisa, *et al.*, 2002), organic solute accumulation, enzyme activity, hormonal balance and reduced water availability at the cell level all of which result in reduced plant growth and ultimately reduced yield. Khan and Panda (2002) attributed the depressing effects of salt stress on plant growth to an increase in reactive oxygen species which play an important role in damaging all classes of biologically important macromolecules including DNA and the generation of H_2O_2 and lipid hydro-peroxides which cause membrane changes. Reductions in fruit yield are largely attributable to decreases in the viability of pollen or the receptivity of the stigmatic surface (Sakr *et al.*, 2004) and substantially increased abscission of

Table 1. Effect of salt stress and exogenously applied antioxidants on growth characters of pepper plant at 75 days after transplanting averaged across two growing seasons (2007 & 2008); HA: Humic acid, SA: Salicylic acid, ASA: Ascorbic acid, SWE: Seaweed extract

	Plant height					No. of leaves/plant				
	Salinity levels					Salinity levels				
	Control	Low	Med.	High	Mean	Control	Low	Med	High	Mean
	0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}		0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}	
Water	39.0	29.0	24.8	19.4	28.00	46.0	37.5	29.7	26.0	34.75
HA (1000 mgL-1)	53.8	44.3	35.3	27.2	40.10	61.4	50.5	38.3	28.5	44.65
SA (250 mgL-1)	51.6	43.5	33.2	29.4	39.40	61.2	50.5	41.6	28.3	45.30
ASA (250 mgL-1)	58.2	46.0	37.2	31.8	43.25	66.5	56.9	42.1	30.3	50.00
SWE (1000 mgL-1)	60.4	48.1	36.5	30.9	43.90	65.9	53.6	40.5	26.4	48.05
Mean	52.57	42.18	33.38	27.72		60.18	49.78	38.43	27.88	
LSD at 5%	Antioxidant 2.22		Salinity 1.56		Interaction 4.35	Antioxidant 2.9		Salinity 1.9		Interaction 5.8

	Leaf area (cm2)					Shoot dry weight (g)				
	Salinity Levels					Salinity Levels				
	Control	Low	Med	High	Mean	Control	Low	Med	High	Mean
	0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}		0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}	
Water	1433.0	1192.0	826.5	495.0	986.00	10.9	7.6	6.1	3.1	6.85
HA (1000 mgL^{-1})	1878.0	1474.5	1126.5	688.0	1291.50	17.5	11.6	8.3	5.2	10.55
SA (250 mgL^{-1})	1942.5	1331.0	990.0	644.5	1227.00	18.1	11.9	7.4	5.8	10.75
ASA (250 mgL^{-1})	2070.0	1582.5	1315.0	806.5	1443.50	20.2	13.7	10.8	6.9	12.85
SWE (1000 mgL^{-1})	1959.5	1541.5	1027.0	495.5	1256.00	20.0	14.0	10.4	7.0	12.80
Mean	1856.60	1424.30	1057.00	625.90		17.31	11.73	8.56	5.57	
LSD at 5%	Antioxidant 52.2		Salinity 36.1		Interaction 103.2	Antioxidant 1.02		Salinity 0.71		Interaction 2.01

Table 2. Effect of salt stress levels and exogenously applied antioxidants on yield of pepper plant at 75 days after transplanting averaged over two growing seasons (2007 & 2008 HA: Humic acid, SA: Salicylic acid, ASA: Ascorbic acid, SWE: Seaweed extract

	Fruit set (%)					No. of fruits/plant (Total fruit yield)				
	Salinity Levels					Salinity Levels				
	Control	Low	Med.	High	Mean	Control	Low	Med	High	Mean
	0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}		0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}	
Water	13.0	11.6	8.7	3.7	7.98	7.6	5.1	3.9	2.7	3.88
HA (1000 mgL^{-1})	23.6	18.3	13.8	10.5	14.18	9.5	8.0	7.0	4.0	6.32
SA (250 mgL^{-1})	23.1	17.9	15.1	12.3	15.07	9.7	8.9	6.4	3.0	6.07
ASA (250 mgL^{-1})	27.0	20.2	17.8	13.5	17.12	9.3	8.2	7.5	4.7	6.77
SWE (1000 mgL^{-1})	26.0	21.5	15.3	12.5	16.38	9.7	9.5	7.0	4.0	6.83
Mean	22.51	17.87	14.10	10.47		9.15	7.91	6.35	3.66	
LSD at 5%	Antioxidant 1.35	Salinity 0.92	Interaction 2.81			Antioxidant 1.01	Salinity 0.67	Interaction 1.92		

	Fresh weight of fruits/plant (g)					Dry weight of fruits/plant (g)				
	Salinity Levels					Salinity Levels				
	Control	Low	Med	High	Mean	Control	Low	Med	High	Mean
	0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}		0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}	
Water	299.5	211.5	145.0	78.0	183.50	20.0	11.5	9.9	6.2	9.20
HA (1000 mgL^{-1})	551.0	368.0	238.0	118.0	241.33	37.7	20.7	16.3	8.2	15.02
SA (250 mgL^{-1})	589.5	428.5	276.0	108.0	270.83	35.6	25.3	19.7	8.7	17.87
ASA (250 mgL^{-1})	649.5	473.0	294.0	138.0	301.67	47.2	24.8	22.9	9.5	19.05
SWE (1000 mgL^{-1})	597.0	469.0	294.5	135.5	299.67	41.6	30.7	21.9	8.5	20.35
Mean	537.30	390.00	249.50	115.50		36.39	22.59	18.10	8.20	
LSD at 5%	Antioxidant 29.2	Salinity 19.4	Interaction 57.8			Antioxidant 6.9	Salinity 5.7	Interaction 8.7		

Table 3. Effect of salt stress and exogenously applied antioxidants on photosynthetic pigments in the leaves of pepper plant at 75 days after transplanting averaged over two growing seasons (2007 & 2008), HA: Humic acid, SA: Salicylic acid, ASA: Ascorbic acid, SWE: Seaweed extract

	Total Chlorophyll content (mg/g)					Carotenoids content (carotene mg/g)				
	Salinity Levels					Salinity Levels				
	Control	Low	Med.	High	Mean	Control	Low	Med	High	Mean
	0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}		0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}	
Water	1.6	1.2	1.0	0.7	0.99	0.4	0.4	0.5	0.6	0.48
HA (1000 mgL^{-1})	2.8	2.3	1.6	1.3	1.73	0.4	0.5	0.6	0.6	0.57
SA (250 mgL^{-1})	2.6	2.1	1.7	1.1	1.64	0.4	0.5	0.5	0.6	0.53
ASA (250 mgL^{-1})	3.0	2.6	2.1	1.3	1.97	0.5	0.5	0.6	0.6	0.57
SWE (1000 mgL^{-1})	3.0	2.7	1.9	1.5	2.04	0.5	0.5	0.5	0.6	0.57
Mean	2.60	2.18	1.66	1.17		0.45	0.48	0.53	0.62	
LSD at 5%	Antioxidant 0.22	Salinity 0.14	Interaction 0.36			Antioxidant 0.027	Salinity 0.017	Interaction 0.06		

Table 4. Effect of salt stress and exogenously applied antioxidants on biochemical constituents in pepper plant shoots at 75 days after transplanting averaged over two growing seasons (2007 & 2008), HA: Humic acid, SA: Salicylic acid, ASA: Ascorbic acid, SWE: Seaweed extract

	Total soluble solids (TSS %)					Total soluble sugars (mg/g d.w.)				
	Salinity Levels					Salinity Levels				
	Control	Low	Med.	High	Mean	Control	Low	Med	High	Mean
	0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}		0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}	
Water	6.8	6.1	5.4	4.5	5.30	73.5	82.0	98.5	133.5	104.67
HA (1000 mgL^{-1})	10.4	9.9	8.0	6.8	8.22	161.5	175.0	211.0	233.5	206.50
SA (250 mgL^{-1})	10.0	9.0	7.2	6.2	7.43	149.0	165.0	188.0	194.0	182.33
ASA (250 mgL^{-1})	10.3	10.0	8.0	6.3	8.07	165.5	189.5	222.0	249.5	220.33
SWE (1000 mgL^{-1})	9.9	8.5	7.8	6.7	7.67	166.5	188.0	199.5	226.0	204.50
Mean	9.47	8.68	7.26	6.07		143.20	159.90	183.80	207.30	
LSD at 5%	Antioxidant 0.91	Salinity 0.65	Interaction 1.87			Antioxidant 0.71	Salinity 0.73	Interaction 1.68		
	Proline concentration (mg/g d.w.)					**Total free amino acids (mg/100g d.w.)**				
	Salinity Levels					Salinity Levels				
	Control	Low	Med	High	Mean	Control	Low	Med	High	Mean
	0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}		0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}	
Water	2.2	3.85	5.05	6.5	4.40	72.5	63.0	54.0	42.5	53.17
HA (1000 mgL^{-1})	1	1.15	3.1	3.85	2.70	134.0	99.5	81.0	59.0	79.83
SA (250 mgL^{-1})	1.35	1.8	2.5	4.05	2.78	110.0	92.0	83.5	79.5	85.00
ASA (250 mgL^{-1})	1.25	1.55	2.55	3.15	2.42	132.0	124.0	88.0	82.5	98.17
SWE (1000 mgL^{-1})	1.3	1.55	2.95	3.5	2.67	147.5	109.0	90.5	80.5	93.33
Mean	1.42	1.98	3.23	4.21		119.20	97.50	79.40	68.80	
LSD at 5%	Antioxidant 0.45	Salinity 0.30	Interaction 1.00			Antioxidant 4.91	Salinity 3.33	Interaction 9.59		

Table 5. Effect of salt stress and exogenously applied antioxidants on enzymatic and non-enzymatic antioxidant content in pepper plant shoots at 75 days after transplanting during the season 2007, HA: Humic acid, SA: Salicylic acid, ASA: Ascorbic acid, SWE: Seaweed extract

	SOD (units/g f.w.)					APX (units/g protein/min)				
	Salinity levels					Salinity levels				
	Control	Low	Med.	High	Mean	Control	Low	Med	High	Mean
	0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}		0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}	
Water	307.0	350.0	355.0	362.0	355.67	125.0	133.0	137.0	141.0	137.00
HA (1000 mgL^{-1})	310.0	335.0	347.0	358.0	346.67	130.0	135.0	140.0	146.0	140.33
SA (250 mgL^{-1})	312.0	320.0	325.0	328.0	324.33	130.0	145.0	150.0	154.0	149.67
ASA (250 mgL^{-1})	314.0	350.0	360.0	390.0	366.67	135.0	155.0	160.0	170.0	161.67
SWE (1000 mgL^{-1})	315.0	360.0	380.0	390.0	376.67	140.0	148.0	155.0	160.0	154.33
Mean	311.60	343.00	353.40	365.60		132.00	143.20	148.40	154.20	
LSD at 5%	Antioxidant 0.71 Salinity 0.73 Interaction 1.65					Antioxidant x0.71 Salinity 0.73 Interaction 1.65				

	Total GSH (umol/g f.w.)					AsA (mg/100 g f.w.)				
	Salinity levels					Salinity levels				
	Control	Low	Med	High	Mean	Control	Low	Med	High	Mean
	0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}		0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}	
Water	220.0	225.0	238.0	246.0	232.25	129.0	153.0	184.0	215.0	184.00
HA (1000 mgL^{-1})	222.0	227.0	230.0	240.0	232.33	154.0	177.0	275.0	294.0	248.67
SA (250 mgL^{-1})	227.0	232.0	235.0	242.0	236.33	164.0	203.0	280.0	290.0	257.67
ASA (250 mgL^{-1})	245.0	260.0	280.0	240.0	260.00	175.0	245.0	300.0	364.0	303.00
SWE (1000 mgL^{-1})	259.0	270.0	280.0	295.0	281.67	174.0	196.0	299.0	322.0	272.33
Mean	234.60	242.80	252.60	252.60		159.20	194.80	267.60	297.00	
LSD at 5%	Antioxidant 3.1 Salinity 2.8 Interaction 4.5					Antioxidant 10.9 Salinity 7.3 Interaction 21.8				

Table 6. Effect of salt stress and exogenously applied antioxidants on nutrient contents (K & Na) in pepper plant shoots at 75 days after transplanting averaged over two growing seasons (2007 & 2008), HA: Humic acid, SA: Salicylic acid, ASA: Ascorbic acid, SWE: Seaweed extract

	Na (%)					K (%)				
	Salinity levels					Salinity levels				
	Control	Low	Med.	High	Mean	Control	Low	Med	High	Mean
	0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}		0.32 gL^{-1}	2 gL^{-1}	4 gL^{-1}	6 gL^{-1}	
Water	4.7	2.8	2.2	2.0	2.32	0.75	1	1.4	1.85	1.42
HA (1000 mgL^{-1})	5.5	4.8	4.1	2.5	3.78	0.55	0.7	1.1	1.6	1.13
SA (250 mgL^{-1})	5.7	5.0	3.8	2.8	3.85	0.65	0.75	1.05	1.6	1.13
ASA (250 mgL^{-1})	6.1	5.2	4.3	3.1	4.17	0.45	0.6	0.9	1.35	0.95
SWE (1000 mgL^{-1})	6.7	5.4	4.3	3.3	4.30	0.55	0.7	0.85	1.55	1.03
Mean	5.73	4.62	3.72	2.71		0.59	0.75	1.06	1.59	
LSD at 5%	Antioxidant 0.71 Salinity 0.73 Interaction 1.67					Antioxidant 0.71 Salinity 0.74 Interaction 1.68				

flowers or young fruit due to ethylene induction by salinity. Other factors affecting cell division and cell expansion, such as tissue water status and the concentration of certain plant hormones e.g. ABA are also involved in the regulation fruit set under stress. Also increasing salinity decreased economic of fruit yield due to the decreased number of perfect flowers fruit set and imperfect fruit production and this has been reported elsewhere (Grattan *et al.,* 2002). Furthermore a reduction in leaf area index results in reduction in the supply of carbon assimilate due to a decrease in the net photosynthetic rate and biomass accumulation (Sakr *et al.,* 2007). According to the data recorded in this investigation, it was shown that salinity stress decreased many parameters including leaf number, leaf area, accumulation of dry matter, photosynthetic pigments, potassium uptake, and sugar content all of which will ultimately decrease pepper yield.

Photosynthetic pigments in pepper leaves were significantly decreased with increasing salinity levels and this reduction may be related to enhanced activity of the chlorophyll-degrading enzyme, chlorophyllase, as suggested by Mishra and Sharma (1994) who indicated that increasing saline increased oxidation of chlorophyll leading to its decreased concentration (Pell and Dann, 1991). Carotenoids are synthesized and accumulate in chloroplasts where they play a critical role in the light harvesting coupling assembly and function. Carotenoids protect chlorophyll against photo-oxidation and their decreased content leads to severe chlorophyll degradation under stress (Munne-Bosch *et al.,* 1999).

Salinity stress levels and applied antioxidants both increased proline content in pepper shoots during the two growing seasons. Several functions are proposed for the accumulation of proline in tissues submitted to stress including osmotic adjustment, stabilization of proteins and membranes, being a scavenger of free radicals, improvement of the stability of some cytoplasmic and mitochondrial enzymes, and increased protection of proteins and enzymes or membranes (Ozdemir *et al.,* 2004 and Sakr *et al.,* 2007).

The data show that salinity stress levels increased sodium and decreased potassium contents in the shoot of pepper plants which is a typical response of plants in saline environments arising from the inability of plants to distinguish between sodium and potassium ions (Storey *et al.,* 1983). The increase in Na^+ content mainly in the vacuole provides an osmotic adjustment of salt affected plants (Sakr *et al.,* 2007). This accumulation might be due to the important role of sodium in increasing osmotic pressure. The most abundant inorganic cation in vacuoles is K^+, which plays a large part in maintaining cell turgor pressure and K^+ homeostasis between cytoplasm and vacuole (Heelebust *et al.,* 1976). Excess of Na^+ and Cl^-, the predominant ions in saline soils, creates high ionic imbalance that may impair the selectivity of root cell membranes (Bohra and Doerffling, 1993).

To mitigate and repair damage initiated by reactive oxygen, plants have developed a complex antioxidant system. The primary components of this system include carotenoids, ascorbate and enzymes such as superoxidase dismutase (SOD). Many components of these antioxidant defence system can be found in different sub-cellular compartments (Vaidyanathan *et al.,* 2003). These antioxidant enzymes and metabolites are reported to increase under various environmental stresses (Yu and Rengel, 1999) and this increase was also evident in the current work. Salinity stress levels clearly affected ascorbic acid (ASA) in pepper shoot.

The increased water potential values in SA pre-treated pepper plants under osmotic stress suggest that accumulation of inorganic or organic osmolytes increases the relative water contents of tissues (Szepesi *et al.,* 2005). Salicylic acid decreased the Na+/K+ ratio in the roots and increased it significantly in the leaves. Na+, accumulated in the leaf tissues where it functions as an inorganic osmolyte, and results in an increased water potential and water content and SA has been reported to improve the photosynthetic performance of plants under stress conditions (Ananieva *et al.,* 2004). Our results suggest that SA-pre-treatment may improve the gross rate of carbon assimilation during osmotic stress. The application of SA led to an accumulation of different compatible osmolytes including sugars, sugar alcohol and

proline. Superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) have all been reported to exhibit different changes in plants at 10^{-7}M SA or 10^{-4}M SA (Sakhabutdinova *et al.*, 2003). Senaratna *et al.* (2000), reported that SA and APX confer tolerance to pepper plants and the tolerance was associated with changes in antioxidants such as glutathione reductase, dehydroascorbate reductase and monode-hydroascorbate reductase. SA treatment also increased the level of reduced glutathione (GSH) with an increase in the ratio of reduced to oxidised glutathione (GSSG) indicating higher antioxidant potential (Srivastava and Dwivedi, 1998). Proline is one of the important components of the adaptation of plants to salinity (Kuznetsov and Shevyakova, 1999) and pretreatment with SA also contributed to accumulation of this amino acid under stress possibly through maintaining an enhanced level of ABA in the plants (Ervin, 2005).

Biostimulants such as sea-weed extracts (SWE) can alleviate the harmful effect of salinity or drought stress through enhancing leaf water status and possibly by reduced uptake of Na and Cl ions (Nabati, 1994) and as a consequence increase K and Ca content in the leaves stimulating chloroplast development and enhancing phloem loading and delaying senescence (Demir *et al.*, 2004). Also, stimulation of the uptake of N, P, K, Mg, Ca, Zn, Fe, and Cu by the plants also can alleviate the inhibitory effect of Na toxicity and restore growth (Nelson and Van Staden, 1984). In addition, enhancement of antioxidant enzymes (SOD, GR, ASP) for protection against adverse environmental conditions (Schmidt, 2005) and stimulation the biosynthesis of tocopherol, ascorbic acid and carotenoids in chloroplasts which can protect the PSII photosynthetic apparatus (Zhang and Schmidt, 2000). The enhancing effect of humic acid on alleviation of salinity or drought stress may be through a stimulation of germination and vigour of seed and plant growth by accelerated cell division, increasing the rate of development in root systems, (Clapp *et al.*, 2002). Also, humic acid has been shown to increase the permeability of plant membranes, promoting the uptake of nutrients N, P, K, Ca, and Mg (Mackowiak *et al.*, 2001) and enhancing root development (Vaughan and Macdonald, 2005). In addition, the manufacturers claim that it aids photosynthesis, stimulates plant enzymes and acts as an organic catalyst. Humic acids also are claimed to chelate sodium ions in the soil which helps plants tolerate higher soil sodium concentrations avoiding toxicity and osmotically related problems (Super-Grow, 2006). It is also possible that these biostimulants are capable of stimulating the genetic pathways leading to improve plant defense mechanisms evidenced by the improved end product enhancement of antioxidants.

The results presented here clearly indicate that it is possible to ameliorate the effects of salinity by the exogenous application either of anti-oxidants or compounds known to upregulate the plants natural defences against salt stress. No attempt was made to model the cost-effectiveness of these treatments from an agricultural point of view, but it was interesting to note that the relatively inexpensive and commercially available compounds, SWE and humic acid, were both effective as protectors against salt. The implications of this work are that it may be possible to develop field applied protection against salt stress.

REFERENCES

Abbaspour, H. (2012). Effects of salt stress on lipid peroxidation, antioxidative enzymes and proline accumulation in pistachio plants. Journal of Medicinal Plants Research, 6: 526-529.

A.O.A.C. (1975)."Official Methods of Analysis" Association of Official Agricultural Chemists, 13th Ed. Washington, D.C., USA.

Ananieva, K.I., J. Malbeck, M. Kamínek and J. Van Staden (2004). Methyl jasmonate down-regulates endogenous cytokinin levels in cotyledons of *Cucurbita pepo* (zucchini) seedlings. Physiologia Plantarum, 122: 496 – 503.

Bohra, J.S. and K. Doerffling (1993). Potassium nutrition of rice (*Oryza sativa* L.) varieties under NaCl salinity. Plant and Soil, 152: 299-303.

Clapp, C.E., Y. Chen, M. Hayes, A.J. Palazzo and V.W. Cline (2002). Stimulation of plant growth by humic substances. World Congress of Soil Science, p: 192.

De Vos, C.H., M.J. Vonk, R. Vooijs and S. Hek (1992). Glutathione depletion due to copper-induced phytochelation synthesis causes oxidative stress in *Silene cucblus*. Plant Physiology, 98: 859-868.

Demir, D., A. Günes, A. Inal and M. Alpaslan (2004). Effects of humic acids on the yield and mineral nutrition of cucumber (*Cucumis sativus* L.) grown with different salinity levels. Acta Horticulturae, 492.

Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Throne (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. Journal of Experimental Botany, 32: 93-101.

Ervin, D. "Key Issues (2005). Evaluation of Payments – Mid-term Evaluation of Rural Development Plans,' in Evaluating Agri-Environmental Policies: Design, Practice and Results, OECD, 101-102.

Fielding, J. (1978). A biochemical and cytochemical study of peroxidase activity in root of *Pisum sativum*. Journal of Experimental Botany, 29: 969-981.

Gomez, K.A. and A.A. Gomez (1984). Statistical procedures for agricultural research. 2nd Ed., Wiley and Sons, New York. U.S.A.

Grattan, S.R., L.H. Zeng, M.C. Shannon and S.R. Roberts (2002). Rice is more sensitive to salinity than previously thought. California-Agriculture., 56 (6): 189-195.

Hajer, A.S., A.A. Malibari, H.S. Al-Zahrani and O.A. Almaghrabi (2006). Responses of three tomato cultivars to sea water salinity 1. Effect of salinity on the seedling growth. African Journal of Biotechnology, 5 (10): 855-861.

Heelebust, J.A. (1976). Osmoregulation. Annual Review of Plant Physiology, 27 : 485-505.

Howard, L.R., S.T. Talcott, C.H. Brenes and B. Villalon (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum spp.*) as influenced by maturity. Journal of Agricultural and Food Chemistry, 48: 1713–1720.

Jayaraman, J. (1985). Post harvest biological control. Wiley: Eastern, New Delhi (India) Ltd.

Kayani, S.A., H.H. Naqvi and I.P. Ting (1990). Salinity effects on germination and mobilization of reserves in Jojoba seed. Crop Science, 30 (3): 704-708.

Khadr, I., F. Nyireda, F. Shanahan, C. Nielsen and R. Andria (1994). Ethephon alters corn growth under drought stress. Agronomy Journal, 86: 283-288.

Khan, M.H. and S.K. Panda (2002). Induction of oxidative stress in roots of *Oryza sativa* L. in response to salt stress. Biological Plantarum, 45(4): 625-627.

Kijne, J.W. (2003). Unlocking the Water Potential of Agriculture. FAO Corporate Document Repository, 70 pp, ISBN 9251049114.

Kuznetsova, V. and N.I. Shevyakova, (1999). Proline under stress conditions: biological role, metabolism, and regulation. Russian Journal of Plant Physiol, 46: 321–336.

Mackinney, G. (1941). Absorption of light by chlorophyll solution. Journal of Biological Chemistry, 140: 315- 322.

Mackowiak, L., P.R. Gross and B.G. Bugbee (2001). Beneficial Effects of Humic Acid on Micronutrient Availability to Wheat. Soil Science Society of America Journal, 65: 1744-1750.

Mishra, S.N. and I. Sharma (1994). Putrescine as a growth inducer and as a source nitrogen for mustard seedling under sodium chloride salinity. Indian Journal of Experimental Physiology, 32: 916-918.

Munne-Bosch, S., K. Schwarz and L. Alegre (1999). Enhanced formation of tocopherol and highly oxidized abietane diterpense in water-stressed rosemary plants. Plant Physiology, 121: 1047-1052.

Nabati, D.A. (1994). Responses of two grass species to plant regulators, fertilizer N, chelated Fe, salinity and water stress. Ph.D. diss. Department of Crop and Soil Environmental Science, Virginia Tech, Blacksburg.

Navarro, J.M., C, Garrido, M. Carvajal and V. Martinez (2002). Yield and fruit quality of pepper plants under sulphate and chloride salinity. Journal of Horticultural Science and Biotechnology, 77: 52–57.

Nelson, W.R. and J. Van Staden (1984). The effect of seaweed concentrate on the growth of nutrient-stressed, greenhouse cucumbers. Horticultural Science, 19: 81–82.

Ozdemir, O., B. Melike, D. Tije and T. Ismail (2004). Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. Plant Growth Regulation, 42: 203-211.

Paridam, A.K. and A.B. Das (2005). Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety, 60: 324-349.

Pell, E.J. and M.S. Dann (1991). Multiple stress-induced foliar senescence and implication for whole plant longevity. In H.A. Mooney, V.E. Vinner, E.J.Pell, E. Chu, eds, Response of plants to multiple stresses. Academic Press Inc, pp: 189-204.

Peterburgski, A.V. (1968). Handbook of Agronomic chemistry, Klop publishing House Moscad, pp:29-86.

Ranganna, C. (1979). Manual of analysis of fruit vegetable products. Tatame. McGraw Hill publishing company limited New Delhi (2nd ed).

Sakhabutdinova, A.R., D.R. Fatkhutdinova, M.V. Bezrukova and F.M. Shakirova (2003). Salicylic acid prevents the damaging action of stress factors on wheat plants. Bulgarian Journal of Plant Physiology, Special Issue, 314–319.

Sakr, M.T. and M.A. El-Metwally (2009). Alleviation of the harmful effects of soil salt stress on growth, wield and endogenous antioxidant content of wheat plant by application of antioxidants. Pakistan Journal of Biological Science, 12 (8): 624-630.

Sakr, M.T., M.E. El-Emery, R.A. Fouda and M.A. Mowafy (2007). Role of some antioxidants in alleviating soil salinity stress. Journal of Agricultural Science, Mansoura University, 32: 9751- 9763.

Sakr, M.T., M. El-Hadidy, A.M. Abo El-Kheer, and S. Farouk, (2004). Physiological studies of some osmo-regulator on kanulla. International conversation microbiology and biotechnology in Africa and Arab Regions, 27th to 29th. pp: 295-321.

Saied, A.S., A.J. Keutgen and G. Noga (2005). The influence of NaCl salinity on growth, yield and fruit quality of strawberry Cvs. 'Elsanta' and 'Korona'. Scientia Horticulturae, 103, 289-303.

Schmidt, R.E. (2005). Biostimulants function in turfgrass nutrition. Ph.D. Emeritus Virginia Tech.

Senaratna, T., D. Touchell, E. Bunn and Dixon (2000). Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regulation, 30: 157–161.

Srivastava, M.K. and U.N. Dwivedi (1998). Salicylic acid modulates glutathione metabolism in pea seedlings. Journal of Plant Physiology and Molecular Biology, 43: 439–463.

Storey, R., M.G. Pitan and R. Stelzer (1983). X-Ray Microanalysis of cells and cell compartments of *Atriplex spongiosa* root. Journal of Experimental Botany, 34: 1196-1206.

Super-Grow, (2006). How Does Fulvic Acid Affect Plant Permeability. www.hydro-gardens.com/organic.htm.

Szepesi, A., J. Csiszár, S. Bajkán, K. Gémes, F. Horváth, L. Erdei, A.K. Deér, M.L. Simon and I. Tari (2005). Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt- and osmotic stress. Acta Biologica Szegediensis, 49 (1-2):123-125.

Troll, W. and J. Lindsley (1955). A photometric method for the determination of proline. Journal of Biological Chemistry, 215: 655-660.

Vaidyanathan, H., P. Sivakumar, R. Chakrabarty, G. Thomas, P. Sivakumar and R. Chakrabarty (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) differential response in salt-tolerant and sensitive varieties. Plant Science, 165 (6): 1411-1418.

Vaughan, D. and I.R. Macdonald (2005). Effects of Humic Acid on Protein Synthesis and Ion Uptake in Beet. J. Exp. Bot., 22 (2): 400-410.

Yang, Y., R. Newton and F. Miler (1990). Salinity tolerance in sorghum. Crop Science, 30: 781-85.

Yu, Q. and Z. Rengel (1999). Drought and salinity differentially influence activities of superoxide dismutase in narrow leafed lupines. Plant Science, 141: 1–11.

Zaibunnisa, A., M.A. Khan, T.J. Flowers, R. Ahmad and K.A. Malik (2002). Causes of sterility in rice under salinity stress. In: Ahmad, R. (Ed.) Prospects for saline-agriculture. Kluwer. The Netherlands, 177-187.

Zhang, X. and R.E. Schmidt (2000). Application of Trinexapac-ethyl and Proiconazole enhances photochemical activity in creeping bentgrass (*Agrostis stoloniferous* var. *palustris*). Journal of the American Society of Horticulture, 125: 47-51.

مضادات الأكسدة والمنشطات الحيوية تقلل الإجهاد الملحي في نبات الفلفل الحلو

محب طه صقر[١] – ناصر محمد السركسي[٢] – ميك فوللر[٣]

١- قسم النبات الزراعي - كلية الزراعة - جامعة المنصورة- مصر

٢- قسم النبات الزراعي وأمراض النبات - كلية الزراعة - جامعة الزقازيق- مصر

٣- كلية العلوم والتكنولوجيا – مدرسة العلوم البيولوجية والطبية- جامعة بلايموث- انجلترا

تعتبر ملوحة التربة أحد العوامل الرئيسية في انخفاض المحصول في معظم المحاصيل الحقلية وانخفاض الجودة التسويقية لمختلف ثمار الخضر منها الفلفل الحلو. يعتبر دراسة التحسين الوراثي واستخدام بعض المحسنات الحيوية ومضادات الاكسدة ضرورية لتقليل التأثير الضار للملوحة . قد أجريت تجارب خلال موسمي ٢٠٠٧ و ٢٠٠٨ لدراسة تأثير الاجهاد الملحي علي النمو و المحصول والمركبات الحيوية بالنبات واختبار امكانية تقليل تأثير الملوحة بالاستخدام الخارجي لبعض المحسنات الحيوية و مضادات الأكسدة على نبات الفلفل الحلو أورلاندو . الاجهاد الملحي بتركيز(٢،٤،٦جم/لتر) تسببت في نقص النمو عند عمر ٧٥ يوم من الشتل. وكذلك تسببت في نقص جودة المحصول التسويقي. استخدام المحسنات الحيوية و مضادات الأكسدة تغلبت على التأثير الضار للملوحة عند المستوي المنخفض وكذلك المتوسط لمستوي الملوحة (٢،٤جم/لتر) وقد تم ابطال الاثر الضار للملوحة جزئيا عند التركيز العالي منها (٦جم/لتر). حمض الاسكوربيك ومستخلص أعشاب البحر(SWE) كانت الافضل في هذا الاتجاه. مستويات الملوحة أدت إلى زيادة في نشاط انزيم سوبر اكسيد ديسميوتيز (SOD) وانزيم الأسكوربيك بيروكسيديز (APX) ومحتوى البرولين وتراكم واضح في عنصر الصوديوم بينما أدت إلى نقص صبغات البناء الضوئي في الاوراق وكذلك البوتاسيوم في المجموع الخضري لنبات الفلفل الحلو. علاوة علي ذلك فان كل مضادات الأكسدة المستخدمة سواء منفردة أو متداخلة مع مستويات الملوحة المختلفة أدت إلى زيادة في محتوي حمض الاسكوربيك والجلوتاثيون وكذلك نشاط انزيم (SOD) و(APX). هذه النتائج تدعم استخدام المحسنات الحيوية ومضادات الاكسدة لتقليل الاثر الضار لملوحة التربة.