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MINIMIZATION THE EFFECTS OF SALT STRESS ON SWEET PEPPER PLANTS BY EXOGENOUS PROTECTANTS APPLICATION

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ABSTRACT

Among the abiotic stresses, salinity is the most destructive factor which limits yield productivity of many crop plants and/or limitation of marketable yield of several vegetable fruit crops such as sweet pepper. Exogenously applied protectants are needed to alleviate the effects of salt stress. Two experiments were carried out to study the effect of salt stress on growth, yield and endogenous bio-constituents on sweet pepper (*Capsicum annuum* L. cv. Orlando) and to examine whether salinity stress can be offset by the application of exogenous protectants of some antioxidant and bio-stimulant compounds. Salinity stress (2, 4 or 6 g l^{-1}) decreased growth parameters at 75 days after transplanting and yield components. Exogenously applied protectants counteracted the harmful effects of low and moderate salinity stress levels (2 and 4 g l^{-1}) and partially counteracted the harmful effects under the highest salinity stress level (6 g l^{-1}). Salinity stress levels increased proline and Na contents but decreased sugar content, K in shoots and fruits, and photosynthetic pigments in the leaves of pepper plants. In addition, all of the applied antioxidants alone or combined with different salinity stress levels slightly increased the content of sugar, K and decreased Na and proline content. Citric, humic acid, Putrescine, and seaweeds extract (SWE) were the most effective agents in this respect and ascorbic acid is the best. These results provide support for the field application of antioxidant and bio-stimulant compounds to alleviate the effects of salty soils.

Key words: Sweet pepper, *Capsicum annuum*, antioxidants, bio-stimulants, exogenous protectants, salt stress, foliar spray.

INTRODUCTION

Pepper is an important agricultural crop, not only because of its economic importance, but also by its nutritional value (Martinez *et al.*, 2015). Sweet pepper (*Capsicum annuum* L.) fruits are an excellent source of bioactive products but the content of the same is related with the plant response to stressful conditions. Salinity is among the major constrains restricting plant growth and development, and optimizing irrigation strategies could improve fruit quality while saving good quality water (Martínez *et al.*, 2014).

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 resulting from extensive use of irrigation water containing trace amounts of salts including sodium chloride (Kijne, 2003). Salinity is one of the major stresses in arid and semi-arid regions causing adverse effects at physiological, biochemical, and molecular levels, (Munns, 2002) limiting crop productivity (Tester and Davenport 2003). Salt stress can disturb growth and photosynthetic processes by causing changes in the accumulation of Na^+, Cl^-, and nutrients, and disturbance in water and osmotic potential.

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In most cases, the negative effects of salinity have been attributed to increase in Na^+ and Cl^- ions in different plants hence these ions produce the critical conditions for plant survival by intercepting different plant mechanisms. Although both Na^+ and Cl^- are the major ions which produce many physiological disorders in plants, Cl^- is the most dangerous (Tavakkoli *et al.*, 2010). The outcome of these effects may cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction, including photosynthesis which ultimately leads to plant death (Mahajan and Tuteja, 2005; Hasanuzzaman *et al.*, 2012). Moreover, salinity stress decreased photosynthetic pigments, K and P contents, whilst increasing proline, soluble sugars, ascorbic acid, Na and Cl contents in Canola plants (Saker *et al*., 2012b).

Several studies have shown that the effects of cytotoxicity induced by salt stress can be alleviated 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.

Exploring suitable ameliorants or stress alleviant is one of the tasks of plant biologists. In recent decades, exogenous protectants such as osmoprotectants (proline, glycinebetaine, trehalose, *etc.*), plant hormone (gibberellic acids, jasmonic acids, brassinosterioids, salicylic acid, *etc.*), antioxidants (ascorbic acid, glutathione, tocopherol, etc.), signaling molecules (nitric oxide, hydrogen peroxide, *etc.*), polyamines (spermidine, spermine, putrescine), trace elements (selenium, silicon, *etc.*) have been found effective in mitigating the salt induced damage in plant (Azzedine *et al.*, 2011; Hasanuzzaman *et al.*, 2011a, b; Poor *et al.*, 2011; Rawia *et al.*, 2011; Ahmad *et al.*, 2012; Ioannidis *et al.*, 2012; Nounjan *et al.*, 2012; Tahir *et al.*, 2012; Yusuf *et al.*, 2012). These protectants showed the capacity to enhance the plant's growth, yield as well as stress tolerance under salinity.

Therefore, the aims of this work were undertaken to study the effect of exogenous application of some protectant materials to alleviate the harmful effects of salt stress on growth, yield and endogenous bio-constituents in sweet pepper (*Capsicum annuum* L.)

MATERIALS AND METHODS

Two pot experiments during two successive summer seasons 2012 and 2013 were carried out on the Experimental Station Farm, Faculty of Agriculture, Mansoura University, Egypt.

Plant Material and Stress Application

In this study, Sweet pepper cv Orlando seeds provided by Gohara Co. Cairo, Egypt were sown on 17th February in both seasons, seedlings were transplanted at 45 days (6-7 leaves) on the 3rd of April into plastic pots (50cm inner diameter) containing 8 kg of air-dried loamy soil, with two plants/pot. 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. Also, further N doses (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 the 4 levels of sodium chloride NaCl were used: 0.32 g l^{-1} as control; 2 g l^{-1} as Low; 4 g l^{-1} as Med.; 6 g l^{-1} as High. Irrigation solutions were supplied daily according to plants need and to maintain a slight reserve of water in the pot saucer. The plants were treated with tap water or the exogenous protectants application; Humic acid at 1000 mg l^{-1}) , Salicylic acid at 250 mg l^{-1}, Ascorbic acid at 250 mg l^{-1}, Seaweeds extract at 1000 mg l^{-1} , Tocopherol at 250 mg l^{-1}, Reduced glutathione at 250 mg l^{-1}, Citric acid at 250 mg l^{-1} and Putrescince at 1 mg l^{-1}. The plants of each salinity stress level were foliar sprayed until run-off with the same applied antioxidants and biostimulants as exogenous protectants at 30, 60, 90, 120 and 150 days after transplanting.

In a completely randomized design, each experiment included 4 salinity levels and 9 exogenous foliar spray treatments, (36 treatments) replicated 6 times.

In both growing seasons, six sample pots were taken randomly from each treatment at 75

days after transplanting, the growth characters of pepper plant were recorded: plant height (cm); number of leaves/ plant; leaf area (cm^2/plant); shoot dry weight (g).Six plants from each treatment were taken and the yield of pepper plant were recorded: number of fruits/plant (Total fruit yield); fresh weight of fruits/plant (g); dry weight of fruits/plant (g). Fruit setting percentage was also determined. Total fruit yield was calculated as summation of the two fruits picking which were taken from each treatment at 180, and 210 days from transplanting.

The following biochemical constituents in pepper plant: photosynthetic pigments, total soluble sugar content, proline content; Nutrient element contents: potassium and sodium contents were estimated in shoots and fruits of pepper plant as the follows:

Photosynthetic pigments were measured in fresh leaf samples (0.5 g from the 3rd terminal leaf) extracted by methanol for 24hr., 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 Mackinney (1941).

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.

Proline content was determined in leaves by the modified ninhydrin method of Troll and Lindsley, (1955). Potassium (K) and sodium (Na) contents were estimated by flame photometry (Peterburgski, 1968).

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

Data presented in Table 1 show that all growth characters of sweet pepper plants including plant height, number of leaves/plant, leaf area (cm^2/plant), shoot dry weight (g/plant) were significantly decreased with increasing the salinity stress levels (2 gl^{-1}, 4 gl^{-1} and 6 g l^{-1}) with the greatest reduction observed at the highest salinity stress level, at 75 days after transplanting. On the other hand, exogenous application of antioxidant materials and bio stimulants as protectants such as humic acid and seaweeds extract at (1000 mg l^{-1}), salicylic acid, ascorbic acid, tochopherol, glutathione and citric acid at (250 mg l^{-1}), and putrescince at (1mgl^{-1}) gave positive effects and led to growth improvements at all levels of salt stress including the lowest level and were therefore acting as growth stimulants. In this case, the applied antioxidants completely mitigated the harmful effect of 2 gl^{-1} salinity level on growth of pepper plant. It's likely to mention that any of each antioxidant materials and bio stimulants could be counteracted the effects of low salt stress (2 g l^{-1}) and partially counteract the harmful effects of medium and high salt stress (4 and 6 g l^{-1}) which enhanced all growth parameters under high salinity level. Ascorbic acid (ASA) gave the best protection against salt stress, and citric acid putr, and SWE were the most effective in this respect. From the results of the present study, it is obvious that salt stress reduced plant growth parameters of sweet pepper plants. However, exogenous applied protectants alleviated the adverse effects of salt stress on the growth parameters.

Data in Table 2 show the effect of salinity stress levels and foliar application of antioxidant materials and bio stimulants as protectants on fruit setting, total fruit yield and fresh and dry weights of pepper fruits. As for salinity levels, it could clearly indicate that fruit setting, total fruit yield and fresh and dry weights of pepper fruit were decreased with increasing the level of salinity stress, with the high salt stress reducing fruit yield by 65%. On the other hand, foliar application of antioxidant materials and bio stimulants increased fruit setting, fruit yield, and fresh and dry weights of pepper fruit averaged across two growing seasons. Ascorbic acid was the most effective over all the antioxidants, increasing fruit set and fruits number more than two- folds compared to the untreated plants at the lowest salt treatment. All of the antioxidant materials and bio stimulants counteracted the negative effects of low and medium salt stress and partially offset the effects of high salt stress.

Table 1. Effect of some exogenous protectants on growth parameters of pepper plant, 75 days after transplanting, grown under salinity stress condition (averaged across two growing seasons 2012 and 2013)

	Plant height (cm)					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	33.0	25.0	21.0	17.5	24.1	39.5	32.5	26.9	18.5	29.3
SA (250 mgl^{-1})	47.6	37.8	28.0	24.5	34.4	57.5	46.5	36.3	25.2	41.3
ASA (250 mgl^{-1})	52.8	39.3	31.3	26.0	37.3	60.2	50.5	35.5	29.1	43.8
Toco (250 mgl^{-1})	47.3	36.2	27.7	19.4	32.6	59.5	45.5	30.8	21.6	38.9
GSH (250 mgl^{-1})	48.8	37.6	28.9	24.4	34.9	59.5	46.4	35.5	28.5	42.5
Citric (250 mgl^{-1})	48.5	39.4	30.8	26.2	36.2	62.2	50.0	35.5	24.9	43.1
Put.z (1 mgl^{-1})	48.2	40.7	30.7	27.2	36.7	65.5	48.7	36.1	25.3	43.9
SWE (1000 mgl^{-1})	49.2	40.3	29.4	24.0	35.7	65.0	51.4	32.2	25.0	43.3
HA (1000 mgl^{-1})	49.0	37.7	29.5	23.0	34.8	60.0	45.5	34.2	25.1	41.2
Mean	47.1	37.1	28.6	23.6		58.8	46.3	33.6	24.8	
LSD at 5%	Protectants: 2.12	Salinity:1.42	Interaction: 4.22			Protectants: 2.4	Salinity:1.45	Interaction: 5.45		
	Leaf area (cm^2)/plant					**Shoot dry weight (g)/plant**				
Water	1276.5	936.5	769.0	360.0	835.0	9.5	7.6	7.0	3.0	6.4
SA (250 mgl^{-1})	1657.5	1370.0	1056.0	562.0	1161.5	15.4	11.0	10.3	6.2	10.7
ASA (250 mgl^{-1})	1927.0	1462.5	1055.5	695.0	1285.0	17.7	12.3	13.7	7.5	12.8
Toco (250 mgl^{-1})	1680.0	1347.0	1045.5	480.0	1138.5	15.8	11.3	10.9	5.2	10.8
GSH (250 mgl^{-1})	1710.5	1373.5	1005.0	499.0	1147.5	15.7	10.9	11.4	5.6	10.9
Citric (250 mgl^{-1})	1738.5	1482.5	1076.5	539.5	1209.5	17.9	13.6	12.3	7.8	12.9
Put. (1 mgl^{-1})	1822.0	1456.0	915.0	461.5	1163.5	17.8	13.0	13.4	5.5	12.4
SWE (1000 mgl^{-1})	1690.5	1432.0	979.5	527.0	1157.5	16.3	13.3	14.1	6.0	12.4
HA (1000 mgl^{-1})	1634.5	1296.0	1007.0	579.0	1129.5	15.6	10.7	10.8	5.5	9.1
Mean	1681.9	1350.7	989.9	522.6		15.7	11.5	11.5	5.8	
LSD at 5%	Protectants: 52.2	Salinity 36.1	Interaction 103.2			Protectants: 1.02	Salinity 0.71	Interaction 2.01		

HA: Humic acid, SA : Salicylic acid, ASA: Ascorbic acid, Toco : Tocopherol, GSH : Glutathione, Putr : Putrescine, SWE : Seaweeds extract.

Table 2. Effect of some exogenous protectants on yield of pepper plant, grown under salinity stress condition (averaged across two growing seasons 2012 and2013)

	Fruit setting (%)					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	13.7	10.9	9.9	5.0	9.8	7.5	5.8	4.9	2.5	5.3
SA (250 mgl^{-1})	21.2	17.6	14.3	6.3	14.8	10.7	9.8	7.5	4.1	8.0
ASA (250 mgl^{-1})	23.5	19.9	15.5	9.7	17.1	10.3	10.6	8.8	4.5	8.5
Toco (250 mgl^{-1})	23.5	16.2	14.2	9.2	15.7	10.7	7.0	7.2	4.8	7.4
GSH (250 mgl^{-1})	21.5	17.3	12.9	6.7	14.5	10.7	6.7	6.0	4.2	6.9
Citric (250 mgl^{-1})	22.5	17.6	15.5	7.5	15.8	11.5	8.3	7.3	5.4	8.1
Put. (1 mgl^{-1})	21.5	22.0	15.3	10.0	17.1	10.9	10.2	7.2	4.3	8.1
SWE (1000 mgl^{-1})	20.3	19.4	12.3	9.5	15.3	10.5	9.0	7.4	3.9	7.7
HA (1000 mgl^{-1})	20.3	15.7	13.9	7.8	14.4	9.7	9.1	8.0	4.3	7.7
Mean	20.9	17.4	13.7	7.9		10.3	8.5	7.1	4.2	
LSD at 5%	protectants : 1. 36 Salinity: 0.96 Interaction: 2.87					protectants : 1.01 Salinity: 0.66 Interaction: 1.91				
	Fresh weight of fruits/(g plant) (Total yield)					**Dry weight of fruits/ (g plant) (Total yield)**				
Water	262.5	176.5	135.0	56.9	157.8	18.2	12.6	9.7	3.9	11.0
SA (250 mgl^{-1})	530.5	357.0	253.0	99.0	309.9	29.4	23.5	16.4	8.5	19.4
ASA (250 mgl^{-1})	489.5	397.0	254.0	107.9	312.1	38.2	26.2	18.3	10.1	23.1
Toco (250 mgl^{-1})	439.5	305.0	215.5	74.2	258.6	30.6	18.2	15.9	7.7	18.1
GSH (250 mgl^{-1})	447.5	286.6	195.9	82.7	253.2	33.0	21.8	13.6	8.7	19.2
Citric (250 mgl^{-1})	452.5	335.5	211.4	98.2	274.4	33.2	22.4	17.9	9.5	20.7
Put. (1 mgl^{-1})	445.0	363.8	243.9	101.8	288.6	32.1	26.4	20.6	9.4	22.1
SWE (1000 mgl^{-1})	507.5	367.9	270.5	111.1	314.3	34.0	25.6	19.6	10.2	22.3
HA (1000 mgl^{-1})	505.5	343.3	221.5	86.7	289.2	29.1	15.0	15.9	9.1	17.3
Mean	453.3	325.8	222.3	90.9		30.8	21.3	16.4	8.5	
LSD at 5%	protectants : 29.3 Salinity: 19.5 Interaction: 58.6					protectants : 6.8 Salinity: 5. 5 Interaction: 8.4				

HA: Humic acid, SA: Salicylic acid, ASA: Ascorbic acid, Toco : Tocopherol, GSH : Glutathione, Putr : Putrescine, SWE : Seaweeds extract.

Also, it could be noticed that ascorbic acid, putrescince, citric acid and seaweeds extract were the most effective of the antioxidant applications.

The obtained results in Table 3 indicate that all salinity stress levels (2, 4 and 6 g l^{-1}) slightly decreased chlorophyll a, b and increased carotenoids in the leaves of pepper plants. However, applied different protectants increased photosynthetic pigments in the leaves of pepper plants. Furthermore, the data show that the exogenous applied protectants completely counteracted the adverse effects of salinity stress levels (2 and 4 g l^{1}) on photosynthetic pigments in the leaves of pepper plant. ASA, citric acid and SWE treatments were the most effective in increasing photosynthetic pigments in most cases.

Data in Tables 4 and 5 show the effect of salinity stress levels and foliar application of antioxidant materials and bio stimulants on total soluble sugars content, proline content, K and Na content in both shoots and fruits of pepper plants. All salinity stress levels (2, 4 and 6 g l^{-1}) slightly increased proline content, total soluble sugars and Na% but decreased K content either in shoots or fruits of pepper plants. These changes were incrementally related to the increase in salt stress. On the other hand, the applied protectants (HA, SA, ASA, GSH, tochopherol, citric, putrescince and SWE) increased, total soluble sugars content, and K but decreased proline content and Na in both shoots and fruits of pepper plant. It could be show from the data that each applied antioxidant completely counteracted the harmful effect of low and moderate salinity stress levels (2 and 4 g l^{-1}) on proline content and total soluble sugars in both shoots and fruits of pepper plants. Moreover, HA, ASA and SWE were the most effective in ameliorating the adverse effect of salinity stress level on total soluble sugar, and proline content in both shoot and fruits of pepper plant.

DISCUSSION

According to the data recorded in this investigation, it was shown that all salinity stress levels (2, 4 and 6 g l^{-1}) slightly decreased all growth parameters of sweet pepper plant including plant height, number of leaves, leaf area, shoot dry weight. 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). Furthermore, a reduction in leaf area index, resulted in reduction supply of carbon assimilates due to a decrease in the net photosynthetic rate and biomass accumulation (Sakr *et al.*, 2007). In addition**,** Dolatabadian *et al.* (2011) observed that salinity stress, significantly decreased shoot and root weight, total biomass, plant height and leaf number of soybean. However, leaf area was not affected by salinity stress. It was shown that salinity stress decreased photosynthetic pigments and potassium uptake, all of which will ultimately decrease pepper yield. 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 flowers or young fruit due to ethylene induction by salinity. 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).

The obtained results concerning the effect of salinity stress on photosynthetic pigments in pepper leaves, it were significantly decreased, Chl a, Chl b but increased Carotenoids content with increasing salinity levels and this reduction may be related to enhanced activity of the chlorophyll-degrading enzyme, chlorophyllase, as suggested by Saha *et al.* (2010) who observed a linear decrease in the levels of total Chl, Chl a, and Chl b bas well as the intensity of Chl fluorescence in *Vigna radiata* under increasing concentrations of NaCl treatments. Compared to control, the pigment contents decreased on an average, by 31% for total Chl, 22% for Chl *a*, and 45% for Chl b. The decrease in Chl content under salt stress is a commonly reported phenomenon and in various studies and the Chl concentrations were used as a sensitive indicator of the cellular metabolic state (Chutipaijit *et al.* 2011).

It's evident that salinity stress levels increased proline content and decreased by applied antioxidants in both shoots and fruits of pepper plant averaged across two growing seasons. Several functions are proposed for the

Table 3. Effect of some exogenous protectants on photosynthetic pigments in the fresh leaves of pepper plant 75 days after transplanting and grown under salinity stress condition (averaged across two growing seasons 2012 and 2013)

	Chlorophyll a content (mg/g)					Chlorophyll b content (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 g^{-1}	
Water	1.540	0.870	0.750	0.505	0.916	0.629	0.445	0.290	0.210	0.393
SA (250 mgl^{-1})	2.135	1.825	1.310	0.775	1.511	0.932	0.763	0.597	0.339	0.657
ASA (250 mgl^{-1})	2.450	1.995	1.495	0.910	1.713	0.994	0.932	0.738	0.465	0.782
Toco (250 mgl^{-1})	1.700	1.875	1.340	0.990	1.476	0.904	0.763	0.547	0.386	0.650
GSH (250 mgl^{-1})	2.415	1.900	1.215	0.915	1.611	0.913	0.832	0.629	0.423	0.699
Citric (250 mgl^{-1})	2.445	1.980	1.710	1.290	1.856	1.255	1.087	0.657	0.527	0.881
Put. (1 mgl^{-1})	2.335	1.725	1.605	1.045	1.678	1.090	0.867	0.641	0.396	0.748
SWE (1000 mgl^{-1})	2.600	2.255	1.665	1.115	1.909	1.150	1.005	0.648	0.448	0.813
HA (1000 mgl^{-1})	2.220	1.930	1.350	0.885	1.596	0.949	0.786	0.499	0.303	0.634
Mean	2.204	1.817	1.382	0.937		0.979	0.831	0.583	0.388	
LSD at 5%	Protectants: 0.16 Salinity: 0.10 Interaction: 0.33					Protectants: 0.28 Salinity: 0.18 Interaction: N.S.				
	Chlorophyll a+ b content (mg/g)					Carotenoids content (mg/g)				
Water	2.169	1.315	1.040	0.715	1.310	0.375	0.438	0.475	0.568	0.464
SA (250 mgl^{-1})	3.067	2.588	1.907	1.114	2.169	0.463	0.479	0.516	0.593	0.513
ASA (250 mgl^{-1})	3.444	2.927	2.233	1.375	2.495	0.417	0.469	0.528	0.629	0.511
Toco (250 mgl^{-1})	2.604	2.638	1.887	1.376	2.126	0.465	0.475	0.537	0.636	0.528
GSH (250 mgl^{-1})	3.328	2.732	1.844	1.338	2.310	0.446	0.479	0.531	0.679	0.534
Citric (250 mgl^{-1})	3.700	3.067	2.367	1.817	2.738	0.403	0.461	0.514	0.635	0.503
Put. (1 mgl^{-1})	3.425	2.592	2.246	1.441	2.426	0.412	0.475	0.539	0.590	0.504
SWE (1000 mgl^{-1})	3.750	3.260	2.313	1.563	2.721	0.416	0.467	0.538	0.636	0.514
HA (1000 mgl^{-1})	3.169	2.716	1.849	1.188	2.230	0.427	0.522	0.527	0.629	0.526
Mean	3.184	2.648	1.965	1.325		0.425	0.474	0.523	0.621	
LSD at 5%	Protectants: 0.24 Salinity: 0. 14 Interaction: 0.20					Protectants: 0.023 Salinity: 0.013 Interaction:0.04				

HA : Humic acid, SA : Salicylic acid, SA: Ascorbic acid, Toco : Tocopherol ,
GSH : Glutathione, Putr : Putrescine, SWE : Seaweeds extract.

Table 4. Effect of some exogenous protectants on total soluble sugars and proline concentration in pepper shoots and fruits, grown under salinity stress condition (averaged across two growing seasons 2012 and 2013)

	Total soluble sugars (mg/g. D.w.)					Proline concentration (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}	
Shoots										
Water	62.0	71.5	139.0	141.5	103.50	2.90	3.80	5.30	7.30	4.81
SA (250 mgl^{-1})	99.5	143.5	169.0	176.0	147.00	1.50	2.20	3.50	5.10	3.05
ASA (250 mgl^{-1})	138.0	159.5	181.0	198.5	169.25	1.70	2.00	3.00	3.80	2.59
Toco (250 mgl^{-1})	100.0	141.5	164.5	178.0	146.00	2.00	2.40	3.30	4.40	3.00
GSH (250 mgl^{-1})	98.5	131.5	169.5	190.0	147.38	1.90	2.50	3.80	5.90	3.49
Citric (250 mgl^{-1})	112.5	145.5	166.5	192.0	154.13	1.90	2.30	4.20	5.10	3.35
Put. (1 mgl^{-1})	112.5	159.5	183.0	191.5	161.63	1.90	2.50	4.20	4.90	3.34
SWE (1000 mgl^{-1})	117.0	157.5	187.0	195.5	164.25	1.80	2.40	3.60	4.50	3.06
HA (1000 mgl^{-1})	112.0	150.0	177.0	181.5	155.13	1.30	1.50	3.30	4.70	2.66
Mean	105.8	140.0	170.7	182.7		1.90	2.40	3.80	5.10	
LSD at 5%	Protectants : 0.71 Salinity: 0.73 Interaction:1.67					Protectants : 0.48 Salinity: 0.32 Interaction: 1.04				
Fruits										
Water	39.5	52.5	62.0	71.5	56.37	0.837	0.900	1.400	2.850	1.497
SA (250 mgl^{-1})	49.0	67.0	79.0	85.0	70.00	0.585	0.703	0.945	1.700	0.983
ASA (250 mgl^{-1})	59.5	75.5	92.0	95.0	80.50	0.485	0.500	0.919	1.400	0.826
Toco (250 mgl^{-1})	54.0	72.5	81.5	85.5	73.37	0.693	0.805	1.050	1.950	1.124
GSH (250 mgl^{-1})	53.0	68.0	75.5	87.5	71.00	0.590	0.740	0.966	1.550	0.962
Citric (250 mgl^{-1})	57.0	71.0	79.5	89.5	74.25	0.565	0.645	0.958	1.550	0.929
Put. (1 mgl^{-1})	52.0	67.5	75.5	90.5	71.37	0.660	0.703	0.953	1.250	0.891
SWE (1000 mgl^{-1})	55.5	73.0	84.5	88.5	75.37	0.670	0.738	0.950	1.200	0.889
HA (1000 mgl^{-1})	47.5	71.5	90.0	92.5	75.37	0.535	0.544	0.960	1.400	0.860
Mean	51.88	68.72	79.94	87.27		0.624	0.697	1.011	1.650	
LSD at 5%	Protectants : 3.3 Salinity: 2.1 Interaction: 5.2					Protectants : 0.18 Salinity: 0.12 Interaction: 0.37				

HA : Humic acid, SA : Salicylic acid, ASA: Ascorbic acid, Toco : Tocopherol , GSH : Glutathione, Putr : Putrescine, SWE : Seaweeds extract.

Table 5. Effect of some exogenous protectants on K (%) and Na (%) in pepper shoots and fruits, grown under salinity stress condition (averaged across two growing seasons 2012 and 2013)

	K (%)					Na (%)				
	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}	
Shoots										
Water	4.150	2.250	1.950	1.500	2.463	0.850	1.050	1.600	1.950	1.363
SA (250 mgl^{-1})	4.950	4.150	3.300	2.050	3.613	0.650	0.800	1.250	1.750	1.113
ASA (250 mgl^{-1})	5.300	4.600	3.700	2.500	4.025	0.450	0.650	0.950	1.500	0.888
Toco (250 mgl^{-1})	5.050	4.400	3.400	2.000	3.713	0.700	0.850	1.100	1.600	1.063
GSH (250 mgl^{-1})	5.200	4.450	3.700	2.350	3.925	0.600	0.750	1.300	1.700	1.088
Citric (250 mgl^{-1})	5.150	4.550	3.950	2.450	4.025	0.500	0.600	0.900	1.450	0.863
Put. (1 mgl^{-1})	5.600	4.850	3.650	2.450	4.138	0.650	0.800	1.200	1.450	1.025
SWE (1000 mgl^{-1})	5.550	4.850	3.650	2.700	4.188	0.650	0.800	1.350	1.450	1.063
HA (1000 mgl^{-1})	5.200	4.250	3.450	2.150	3.763	0.650	0.900	1.100	1.850	1.125
Mean	5.128	4.261	3.417	2.239		0.633	0.800	1.194	1.633	
LSD at 5%	Protectants : 0.71 Salinity: 0.73 Interaction:1.65					Protectants : 0.71 Salinity: 0.73 Interaction: 1.65				
Fruits										
Water	2.25	1.60	1.45	1.00	1.58	0.65	0.75	0.95	1.80	1.04
SA (250 mgl^{-1})	3.10	2.50	1.80	1.60	2.25	0.55	0.70	0.80	1.40	0.86
ASA (250 mgl^{-1})	3.45	2.70	2.00	1.90	2.51	0.35	0.55	0.75	1.00	0.66
Toco (250 mgl^{-1})	2.95	2.55	1.80	1.45	2.19	0.45	0.65	0.85	1.30	0.81
GSH (250 mgl^{-1})	2.85	2.50	1.95	1.45	2.19	0.50	0.65	0.75	1.30	0.80
Citric (250 mgl^{-1})	3.00	2.80	2.30	1.80	2.48	0.30	0.45	0.65	1.15	0.64
Put. (1 mgl^{-1})	3.20	2.80	2.15	1.60	2.44	0.40	0.60	0.75	1.10	0.71
SWE (1000 mgl^{-1})	3.20	2.65	2.15	1.60	2.40	0.40	0.50	0.65	1.10	0.66
HA (1000 mgl^{-1})	3.20	2.30	1.80	1.50	2.20	0.45	0.60	0.80	1.55	0.85
Mean	3.02	2.49	1.93	1.54		0.45	0.61	0.77	1.30	
LSD at 5%	Protectants : 0.71 Salinity: 0.73 Interaction:1.65					Protectants : 0.71 Salinity: 0.73 Interaction: 1.65				

HA : Humic acid, SA : Salicylic acid, ASA: Ascorbic acid, Toco : Tocopherol , GSH : Glutathione, Putr : Putrescine, SWE : Seaweeds extract.

accumulation of proline in tissues submitted to stress including osmotic adjustment, stabilization of proteins and cellular 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 ; Sakr *et al*., 2007).

The data show that salinity stress levels increased sodium and decreased potassium contents in the shoots and fruits 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.

Several methods of application (soaking the seeds prior to sowing, adding to the hydroponic solution, irrigating, or spraying with SA solution) have been shown to protect various plant species against abiotic stress by inducing a wide range of processes involved in stress tolerance mechanisms (Horvath *et al*., 2007). In mungbean plants SA alleviates salt-induced decrease in photosynthesis and minimizes the leaf Na^+, Cl^-, and H_2O_2 content (Nazar *et al*., 2011).

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).

The application of SA led to an accumulation of different compatible osmolytes including sugars, sugar alcohol and proline. Proline is one of the important components of the adaptation of plants to salinity (Kuznetsov and Shevyakova, 1999).

Exogenously applied ascorbic acid (ASA) were generally effective partially or completely countering the inhibitory effects of salt stress on net photosynthetic rate, pigments biosynthesis and membrane integrity by exerting a stimulatory action on these parameters, especially in plants subjected to moderate and low salinity levels (Hamada and Al-Hakimi, 2009). The application of vitamin C was effective to mitigate the adverse effects of salt stress on plant growth due to increased leaf area, improved Chl and Carotenoids contents, enhanced Proline accumulation and decreased H_2O_2 content, as reported by Azzedine *et al.* (2011).

However, the effect of Exogenous GSH could partially alleviate the harmful effects of salinity stress which reflected on growth and yield of *T. aestivum* plant. In *Tagetes erecta*, application of GSH (100 or 200 ppm) was found to be effective in increasing plant height, No. of branches, fresh and dry weight of herb and flowers, No. of flowers, total carbohydrates (%), total phenols, xanthophyll pigment contents and mineral ion percentage under saline (1,500 ppm NaCl) conditions (Rawia *et al*. 2011). Salt stressed wheat plants supplemented with a-tocopherol decreased the Na^+ and Cl^- contents but increased the K+, Ca2+ and Mg2+ contents (Farouk, 2011).

Zhang *et al.* (2011) observed that exogenous putr concentrations, significantly increased growth, photosynthesis and decreased lipid peroxidation under salt stress, and Quinet *et al.* (2010) found that exogenous putr reduced Na^+ accumulation in shoots and roots of salt-treated plants of susceptible cultivar while no change was obtained in tolerant one. Application of putr reduced photosynthetic rate, and pigments content of *Citrus karna* under saline conditions compared to plants exposed to NaCl in the absence of putr (Sharma *et al.* 2011). Biostimulants such as seaweeds extract (SWE) can alleviate the harmful effects of salinity or drought stress through enhancing leaf water status and possibly by reducing uptake of Na and Cl ions (Nabati, 1994) and as a consequence increase K and Ca contents in the leaves stimulating chloroplast development and enhancing phloem loading and delaying senescence (Demir *et al.,* 2004).

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). 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 provide support for the field application of exogenous protectants under salt stress conditions has been found to be very much effective to alleviate salt- induced damages, according to Saker *et al*. (2012a,b). The results indicate that it is possible to alleviate the effects of salinity stress by use of exogenous protectants either of antioxidants or compounds known to up regulate the plants natural defences against salt stress. putr., citric, humic acid and SWE , were the most effective as protectors against salt stress and ASA, is the best. The implications of this work are that it may be possible to develop field applied protection against salt stress.

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تقليل تأثيرات الإجهاد الملحي في نباتات الفلفل الحلو بالإضافات الوقائية الخارجية

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إن الملوحة أحد وأهم عوامل الإجهادات اللاحيوية التي تسبب انخفاضا لإنتاجية العديد من المحاصيل الحقلية وانخفاض الجودة التسويقية لمحاصيل ثمار الخضر مثل الفلفل الحلو. ومن هنا نحتاج إلى إضافة الواقيات الخارجية كالمنشطات الحيوية ومضادات الأكسدة لتخفيف تأثيرات الإجهاد الملحي، أجريت تجارب لدراسة تأثير إجهاد الملوحة علي النمو والمحصول والمركبات الداخلية بالنبات وإمكانية تخفيف التأثيرات الضارة للإجهاد الملحي بالإضافات الخارجية للواقيات كبعض المحسنات الحيوية ومضادات الأكسدة علي نباتات الفلفل الحلو صنف أورلاندو، عند عمر ٧٥ يوم من الشتل، تسبب الإجهاد الملحي بتركيز (٢،٤،٦ جم/ لتر) في نقص قياسات النمو وأيضا مكونات محصول الفلفل الحلو، وعند استعمال المنشطات الحيوية ومضادات الأكسدة كواقيات خارجية عند المستوى المنخفض والمتوسط للملوحة (٢، ٤جم/ لتر) فقد تغلبت على التأثير الضار للملوحة أما عند التركيز العالي منها (٦جم/ لتر) فلقد تم تخفيف الأثر الضار للملوحة جزئيا، أدت مستويات الإجهاد الملحي إلى زيادة في تراكم البرولين وعنصر الصوديوم بينما أدت إلى نقص لمحتوي السكر الذائب والبوتاسيوم في كلا من المجموع الخضري والثمار وكذلك صبغات التمثيل الضوئي في أوراق نباتات الفلفل الحلو، بالاضافة إلي ذلك فان اضافة الواقيات الخارجية رشا سواء منفردة أو متداخلة مع المستويات المختلفة للملوحة فقد أدت إلى زيادة محتوي السكر والبوتاسيوم وتقليل تراكم البرولين وعنصر الصوديوم في كلا من المجموع الخضري والثمار وكان لكلا من حمض الستريك والهيوميك والبتروسين ومستخلص الأعشاب البحرية تاثيراً في هذا الاتجاة وقد كان حمض الاسكوربيك الافضل في هذا الصدد، وتكون النتائج داعمة للتطبيق الحقلي لاستخدام المنشطات الحيوية ومضادات الاكسدة لتخفيف تأثيرات الأراضي الملحية.

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