



SALINITY STRESS AMELIORATION USING HUMIC ACID AND MYCORRHIZAE ON PEPPER PLANTS

Naser M. El-Sarkassy*, Seham A. Ibrahim and E.M. Desoky

Agric. Bot. Dept., Fac. Agric., Zagazig Univ., Egypt

Received: 10/9/2017; Accepted: 30/10/2017

ABSTRACT: Salinity is destructive factor and one of various abiotic stresses which strongly affects crop productivity and limits marketable yield of vegetable crops, for example pepper plants. This work studied the effects of sole application of each of Humic Acid (HA) and Arbuscular Mycorrhizal Fungi (AMF), *Glomus fasciculatum* inoculation and their combinations in order to reduce the harmful effects of salinity stress on growth, yield and endogenous bio-constituents in pepper cultivar top star. Growth parameters at 75 days after transplanting and yield components were decreased under levels of salt stress (2000 or 4000 ppm). Applied of humic acid and mycorrhizae, partially counteracted the harmful effects of salinity stress levels. By increasing salt stress levels, proline, peroxidase and catalase activities and Na content were increased, but photosynthetic pigments in leaves and N, P, K content in shoots of pepper plants were decreased. In addition, all treatments of humic acid and mycorrhizae either alone or in combination under different salt stress levels, slightly increased the content of proline, N, P, K and photosynthetic pigments, peroxidase and catalase activities while, Na content was decreased in pepper plants. It was observed that, the interaction between humic acid and mycorrhizae increased the positive effect of mycorrhizae inoculation. Results indicated also that, mycorrhizal inoculation help in ameliorating the damage caused by salt stress conditions in pepper plants. Application of humic acid combined with mycorrhizae, promote much more plant growth. These results provide support for the field application of humic acid and mycorrhizae to decline the harmful effects of salty soils.

Key words: Pepper, *Capsicum annuum*, salt stress, symbiosis agents, mycorrhizae, bio-stimulants, humic acid.

INTRODUCTION

Pepper (*Capsicum annuum* L.) is an important vegetable crop, because of its economic importance, and nutritional value. Pepper fruits are an excellent source of bioactive products. Salinity is among the major constrains restricting the growth and development of plants (Martínez *et al.*, 2014 and 2015).

Under protected glasshouse conditions, Kijne (2003) reported that, pepper is grown in temperate regions and in the open field under warm Mediterranean climates. It is often exposed to salinity conditions brought about by widespread use of irrigation containing trace

amounts of salts including sodium chloride. Fahramand *et al.* (2014) showed that, salinity stress is the major factor of abiotic stress which affect approximately every side of biochemistry and physiology of a plant, resulting in a reduction in its yield. Salts in soil or water had adverse effects on agriculture, because it causes a stress condition to crop plants. Salt-affected soil is one of the severe abiotic stresses that reason a reduction in development, plant growth and productivity worldwide (Sheng *et al.*, 2011). Most commonly, salt stress caused by high amount of Na⁺ and Cl⁻. Salt stress has three-fold effects which causes ion imbalance on disturbances in ion homeostasis and toxicity and reduces water potential. This altered water status

*Corresponding author: Tel.: +201004109568

E-mail address: desoky_s@yahoo.com

leads to limitation of plant productivity and initial growth reduction. Salt stress affects some major processes such as germination, growth and chlorophyll content (Paridam and Das, 2005). Under salinity stress, Al-Sobhi *et al.* (2006) noticed that, the decreased in chlorophyll content is a commonly reported phenomenon and in various studies, because of its harmful effects on membrane stability. With increasing salinity stress, the increase of proline content was significantly higher in leaves than in roots (Beltrano *et al.*, 2013).

In most cases, the adverse 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 interrupting different plant mechanisms. Although both Na⁺ and Cl⁻ are the major ions, Cl⁻ is the most dangerous, which produce many physiological disorders in plants (Tavakkoli *et al.*, 2010).

The conclusion of salinity effects may cause nutrients imbalance, membrane damage, enzymatic inhibition, altered levels of growth regulators and metabolic dysfunction, including photosynthesis which eventually leads to plant death (Hasanuzzaman *et al.*, 2012). Under salinity stress in pepper plants, Saker *et al.* (2012) reported that photosynthetic pigments, N, P and K contents were decreased, whilst increasing proline and Na contents.

The use of bio-stimulants to plants include humic substances as humic acid leads to positive metabolic changes, higher content of nutrients in plant tissue (Nardi *et al.*, 2016). The plants that grow in soils containing sufficient amounts of humic acid are less stressed because of the humic substances are an important component of soil organic matter and shows anti-stress effects. (Hanafy *et al.*, 2010).

Effects of arbuscular mycorrhizal (AM) symbiosis showed that, it could be improving plant tolerance to biotic and abiotic stresses, improving uptake of low mobile ions, improving quality of soil structure and soil nutrient cycling, improving rooting and plant establishment, (Smith and Read, 2008). Rouphael *et al.* (2015) showed that, arbuscular mycorrhizal (AM) fungi are obligate biotrophs that have a mutualistic association with the majority of the plants. For the plant partner, they play different useful roles

i.e. mitigation of drought and salinity stresses, bio-regulation, bio-fertilization, and bio-protection. Beltrano *et al.* (2013) indicated that, the mycorrhizal inoculation ameliorated the damage caused by salinity stress conditions on pepper plants. Highlight the significance of arbuscular mycorrhizal fungi mitigation of salt stress and their beneficial effects on plant growth and productivity (Porcel *et al.*, 2012)

Hashem *et al.*, (2015) reported that, on cowpea growth parameters, AMF ameliorated the negative influence of salinity stress which enhanced the activity of antioxidant enzymes such as catalase (CAT), peroxidase (POD) and also, AMF inoculation further improved their activity, thus strengthening the defense system of plants. In salt stressed plants as well as AMF-inoculated plants providing efficient protection against salt stress, proline content increased. Also AMF increased uptake of mineral elements which have direct effect on the osmoregulation of the plants. To some extent, AMF has been considered as bio-ameliorations of saline soils (Rao, 1998). The importance of AMF due to its great capability on the increase in yield of pepper and plant growth under certain conditions. Major reason for this increase in yield and the growth can be attributed to ability of plants in relations with AMF to uptake some nutrients efficiently, such as phosphorous. (Demir, 2004)

The soil application of either humic acid (HA) or arbuscular mycorrhizal fungi (AMF) could be relatively successful in attaining some salinity levels. Therefore, this work aimed to evaluate the impact of dual application of humic acid as bio stimulants and mycorrhiza as symbiosis agents on salinity stress and ameliorate the harmful effects of salinity stress on pepper growth parameters, yield and its endogenous bio-constituents.

MATERIALS AND METHODS

Two pot experiments were carried out during two successive growing seasons 2014 and 2015 under greenhouse conditions at the Experimental Station, Faculty of Agriculture, Zagazig University, Sharkia Governorate, Egypt, to study the response of certain phytochemical constituents, growth and yield of pepper plant to different levels of salinity stress, and how to

ameliorate its harmful effects through application of humic acid as bio stimulant and mycorrhizae as symbiosis agents.

Plant Materials and Experimental Conditions

In this study, seeds of pepper (*C. annuum* L.) cv top star provided by Sacata Co. Cairo, Egypt. Seeds were sown on 28th February in both seasons, seedlings were transplanted after 45 days (6-7 leaves) from sowing into plastic pots (50 cm inner diameter and 25cm in depth). Each pot filled with 15 kg of air dried loamy soil and contained two plants.

Mycorrhizal Inoculation

Two kg of arbuscular mycorrhizal inoculation (*Glomus fasciculatum*) were mixed with 100 kg sand soil. The inoculation obtained from plant pathology Research Institute, Agriculture Research Center, Giza, Egypt. Then the pots were inoculated with using 5 g of inoculum per pot placed at 5 cm depth. After inoculation, a thin layer of soil was added, then the pots were planted and covered with soil. Control plants received mycorrhiza free inoculum. After planting the pots were subjected to salinity stress. Irrigation solutions containing one of 3 levels of sodium chloride NaCl were used: 320 ppm as control; 2000 ppm as conc.1; 4000 ppm as conc.2; Irrigation solutions were supplied daily according to plant need and to maintain a slight reserve of water on the pot saucer. Before planting, the soil was treated with; humic acid at the rate of 10g per pot as bio-stimulant and mycorrhiza as symbiosis agents at 5 g of inoculum per pot.

According to the recommendations by Egyptian Ministry of Agriculture and Land reclamation, all agricultural practices were conducted as follow: fertilizers were applied to each pot of growing pepper plant, nitrogen (N) fertilizer in the form of ammonium sulphate (20.5% N) at the rate of 2.5 g per pot, phosphorous (P) fertilizer as calcium superphosphate (15.5% P₂O₅) at the rate of 1.5 g per pot and potassium (K) as potassium sulphate (K₂O) at the rate of 1 g per pot were mixed with the soil before planting. Also, N (ammonium sulphate 20.5% N) added at 30, 60, and 120 days after transplanting at the rate of 1.5 g per pot.

In a complete randomized design, each experiment included 3 salinity levels and 4 applications of humic acid and mycorrhiza (12 treatments) replicated 6 times.

In both growing seasons, at 75 days after transplanting, three pots/sample were taken from each treatment and the following vegetative growth characters of pepper plant were recorded: plant height (cm); number of leaves/plant; leaf area (cm²/plant); and shoot dry weight (g)/plant. Three pots were taken from each treatment and the yield components of pepper plant were recorded: number of fruits/plant (Total fruit yield); fresh weight of fruits/plant (g); dry weight of fruits/ plant (g). From each treatment at 180, and 210 days from transplanting, two fruit picking were taken and total fruit yield was calculated as summation of the two-fruit picking).

The following biochemical constituents of pepper plant namely: photosynthetic pigments; proline content; catalase and peroxidase activities were estimated in leaves and nutrient element contents: such as nitrogen, phosphorus, potassium and sodium contents were estimated in shoots of pepper plant.

Determination of Photosynthetic Pigments

Fresh leaf samples were used for estimation of Photosynthetic pigments as described by Wettstein (1957), and then calculated as mg/g fresh weight.

Determination of Proline

Proline was estimated due to the following method of Bates *et al.* (1973). Approx. 0.5 g of leaf fresh weight (FW) were homogenized in 10 ml of 3% (W/V) aqueous solution sulphosalicylic acid and filtered. Two ml of the filtrate was reacted with 2 ml of acid ninhydrin, followed by the addition of 2 ml of glacial acetic acid and boiled for 1 hour at 100°C. The mixture was extracted with toluene and free proline was quantified spectrophotometrically at 520 nm absorbance from the organic phase.

Proline cocentration was calculated per unit of FW according to: $\mu\text{mols proline g}^{-1} \text{FW} = [(\mu\text{g proline/ml} \times \text{ml toluene}) / 115.5 \mu\text{g}/\mu\text{mols}] / (\text{g FW}/5)$.

Determination of Catalase and Peroxidase Activity

Enzyme extraction

The extraction was carried out according to the method reported by Vitoria *et al.* (2001). Collected fresh leaf samples were transported in an ice-box and brought to the laboratory. The leaves were washed with distilled water and wiped out of surface moisture and 0.5 g of leaves was homogenized in ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA with pre-chilled mortar and pestle. Then transferred the homogenate to centrifuge tubes and was centrifuged at 4°C in Beckman refrigerated centrifuge at 15000×g for 15 min. The supernatant was transferred to 30 ml tubes and referred to enzyme extract.

Catalase (CAT) was assayed spectro-photo-chemically according to Chance and Maehly (1955). The enzyme extract (100 µl) was added to 100 µl of 100 mM H₂O₂ and the total volume was made up to 1 ml by 250 mM- phosphate buffer pH (6.8). The decreasing in optical density at 240 nm against blank was recorded every minute.

Peroxidase (POD) activity was estimated due to the method of Thomas *et al.* (1982). The enzyme was assayed using guaiacol as the substrate. The reaction mixture was consisted of 3 ml of phosphate buffer (0.1M, pH 7.0), 30 ml of H₂O₂ (20 mM), 50 ml of enzyme extract and 50 ml of guaiacol (20mM). The reaction mixture was incubated in a cuvette for 10 min at room temperature. The optical density was measured at 436 nm and the enzyme activity was expressed as number of absorbance unitsg⁻¹ fresh weight of leaves.

Determination of minerals contents

N, P, K and Na in shoot pepper plant: Total nitrogen (N) was determined colorimetrically using Nessler solution according to the method described by Naguib (1963). Total phosphorus (P) was determined colorimetrically by the hydroquinone method as described by Snell and Snell (1954). Total potassium (K) and sodium (Na) contents were estimated by flame photometry (Peterburgski, 1968).

Statistical Analysis

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

RESULTS AND DISCUSSION

Plant Growth Parameters

Results presented in Table 1 show that all growth parameters of pepper plants including plant height, leaf area, number of leaves and shoot dry weight were significantly decreased with exposure pepper plants to salinity stress levels (2000 ppm and 4000 ppm), the greatest reduction was noticed at 4000 ppm of salinity at 75 days after transplanting. On the other hand, application of humic acid and mycorrhiza alone or in combination gave positive effect and led to growth improvements at all levels of salt stress and therefore acting as growth stimulants. In this case, it's likely to mention that any of each of humic acid and mycorrhiza could be counteracted the harmful effect of salt stress levels (2000 ppm and 4000 ppm) which enhanced all growth parameters of pepper plant under high salinity levels.

Humic acid + mycorrhiza treatment achieved the best protection against salt stress at 4000ppm and improved plant height, shoot dry weight, leaf area and number of leaves/plant. From the results of the present work, it is obvious that salt stress reduced plant growth characters of pepper plants. However, humic acid and mycorrhiza alone and their combination ameliorated the harmful effect of salt stress on the growth parameters.

The effect of salt stress levels and soil application by humic acid and mycorrhiza and their combination on total fruit yield and fresh, dry weights of pepper plant fruits observed in Table 2. As for salt stress levels, it could be clearly indicated that total fruits yield and fresh, dry weights of pepper fruits were significantly reduced by increasing the level of salt concentration.

In the present work, pepper plants subjected to salinity stress levels (2000 ppm and 4000 ppm) showed a clear reduction in all morphological

Table 1. Effect of humic acid and arbuscular mycorrhizal fungi (AMF) and their combination on growth characters of pepper plant under salinity stress (average of two growing seasons 2014 and 2015)

	Salinity levels							
	Control	Conc.1	Conc.2	Mean	Control	Conc.1	Conc.2	Mean
	Plant height (cm)				No. of leaves/plant			
Control	49.50	46.90	41.23	45.877	60.83	40.50	32.50	44.612
HA	50.37	48.13	46.77	48.420	64.17	47.33	37.33	49.613
AMF	45.27	48.50	47.74	47.168	68.33	47.83	43.33	53.168
HA + AMF	50.89	49.37	48.55	49.602	71.17	55.33	50.67	59.058
Mean	48.378	47.843	45.243		64.447	45.223	37.723	
LSD at 0.05%	HAM: 0.575 Salt: 0.498 Interaction: 0.996				HAM: 2.034 Salt: 1.762 Interaction: 3.524			
	Leaf area (cm²)/plant				Shoot dry weight (g)/plant			
Control	3248.84	1189.92	567.07	1668.6	9.30	6.74	4.20	6.745
HA	3710.12	1873.78	975.89	2186.5	12.87	7.79	7.12	9.258
AMF	4299.84	2090.65	1353.07	2581.1	13.56	8.14	6.95	9.547
HA + AMF	4615.23	2593.28	1938.55	3049.0	14.10	8.87	7.43	10.130
Mean	3752.92	1718.11	965.34		11.907	7.557	6.087	
LSD at 0.05%	HAM: 214.96 Salt: 186.16 Interaction: 372.32				HAM: 0.359 Salt: 0.311 Interaction: 0.622			

HA: Humic acid. AMF: Arbuscular mycorrhizal fungi. HAM: Humic acid and /or Arbuscular mycorrhizal
Control: 320 ppm, Conc.1: 2000 ppm, Conc.2: 4000 ppm

Table 2. Effect of humic acid and arbuscular mycorrhizal fungi (AMF) and their combination on yield of pepper plant under salinity stress (average of two growing seasons 2014 and 2015)

	Salinity levels											
	Control	Conc.1	Conc.2	Mean	Control	Conc.1	Conc.2	Mean	Control	Conc.1	Conc.2	Mean
	Number of fruits/plant				Fresh weight of fruits /plant				Dry weight of fruits /plant			
Control	5.00	4.17	3.67	4.27	111.94	52.11	39.78	67.94	14.97	6.95	4.64	8.850
HA	6.00	6.00	5.00	5.66	142.40	92.46	65.01	99.95	19.03	12.32	8.66	13.335
AMF	6.50	6.17	6.17	6.27	150.80	101.15	83.81	111.92	20.09	13.48	11.18	14.913
HA + AMF	6.67	6.67	6.17	6.49	161.91	116.93	86.82	121.88	21.53	15.62	11.70	16.282
Mean	6.041	5.749	5.249		141.763	90.663	68.856		18.903	12.090	9.043	
LSD at 0.05%	HAM:0.555 Salt: :0.481 Interaction: 0.962				HAM: 12.58 Salt: 10.90 Interaction: 21.80				HAM: 1.62 Salt: 1.40 Interaction: 2.80			

HA: Humic acid. AMF: Arbuscular mycorrhizal fungi. HAM: Humic acid and /or Arbuscular mycorrhizal
Control: 320 ppm, Conc.1: 2000 ppm, Conc.2: 4000 ppm

parameters and yield of pepper plant including plant height; number of leaves; leaf area; shoot dry weight; number of fruits/ plant (Total fruit yield); fresh and dry weight of fruits/plant (g). These reductions may be due to the inhibition of cell division and cell elongation exerted by the high salinity levels (Aroca *et al.*, 2013; Ahanger *et al.*, 2014). Salinity in soil or water irrigation is of increasing importance to agriculture because it causes a stress condition to crop plants. Salt-affected soil is one of the dangerous abiotic stresses that cause a reduction plant growth, productivity worldwide and development (Sheng *et al.*, 2011).

Furthermore, Sakr *et al.* (2007) noticed that the reduction in leaf area index caused reduction in the supply of carbon assimilates which causes a decrease in the net photosynthetic rate and biomass accumulation. On the other hand, salt stress significantly decreased plant height, root and shoot weights, total biomass, and leaf number. However, leaf area was not affected by salt stress. Dolatabadian *et al.* (2011) concluded that salinity stress decreased photosynthetic pigments and potassium uptake, all of which will ultimately decrease pepper yield. Sakr *et al.* (2004) reported that a reduction in fruit yield are largely attributable to decreases in the viability of pollen or the receptivity of the stigmatic surface and substantially increased flowers or young fruit abscission due to ethylene induction by salinity.

On the other hand, soil application of humic acid and mycorrhiza and their combination increased total fruit yield, fresh and dry weights of pepper fruit averaged during the two growing seasons. HA + mycorrhiza treatment was the most effective where it increased fruit yield more than the other two-treatments compared to the untreated plants (control). All of the bio-stimulant and symbiosis agents counteracted the negative effects of 2000 ppm of salt stress and partially offset the effects of 4000 ppm of salt stress. Also, it could be noticed that the combination between HA + mycorrhiza was the most effective in this respect.

The improving effect of humic acid on amelioration of drought or salinity stress may be through a stimulation of germination and plant growth and vigour of seed by accelerated cell division, increasing the rate of development in

root systems, (Clapp *et al.*, 2002). Porcel *et al.* (2012) emphasized the significance of arbuscular mycorrhizal fungi amelioration of salinity stress and their useful effects on plant growth and productivity. Mycorrhizal inoculated plants grown under salinity conditions experienced increase in root length, weights of fresh and dry shoots and content of photosynthetic pigments observed by Shekoofeh and Sepideh (2011). Selvakumar and Thamizhiniyan (2011) suggest the general results that mycorrhizal colonization improves host plant mineral concentration and thereby alters fruit production and quality of fruits under salinity stress in chilli plants.

Biochemical Constituents

The results in Table 3 clearly show that chlorophyll a, b and carotenoids in the fresh leaves of pepper plants were significantly reduced by salinity stress treatments (2000 ppm and 4000 ppm).

From the mentioned results, it could be concluded that photosynthetic pigments were increased under low salinity level of NaCl, thereafter decreased with increasing salinity level, these reductions supported by the results reported by Arafa *et al.* (2014) who reported that photosynthetic pigments were significantly increased thereafter decreased by increasing of salinity levels. The obtained results concerning the effect of salinity stress on photosynthetic pigments in pepper leaves, it was significantly decreased 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). The decrease in Chl contents under salinity stress is a generally reported phenomenon in various studies and the Chl concentrations were used as a sensitive indicator of the cellular metabolic state (Chutipaijit *et al.*, 2011). In the present study, salinity decreased carotenoid content due to degradation of α -carotein and formation of zeaxanthin, which protect the plant against photo inhibition (Sharma and Hall, 1991).

However, soil application with humic acid and mycorrhiza alone or in combination caused considerable increase in the photosynthetic pigments in the leaves of pepper plants compared with control treatment. Furthermore, the results showed that HA as bio stimulants and mycorrhiza as symbiosis agents

Table 3. Effect of humic acid and arbuscular mycorrhizal fungi (AMF) and their combination on Photosynthetic pigments of pepper plant under salinity stress (average of two growing seasons 2014 and 2015)

	Salinity levels							
	Control	Conc.1	Conc.2	Mean	Control	Conc.1	Conc.2	Mean
	Chl. A (mg/g)				Chl. B (mg/g)			
Control	1.017	0.772	0.499	0.763	0.769	0.619	0.348	0.579
HA	1.052	0.879	0.552	0.828	0.812	0.652	0.440	0.634
AMF	1.074	0.954	0.717	0.915	0.846	0.704	0.539	0.696
HA + AMF	1.181	0.960	0.725	0.955	0.898	0.742	0.589	0.743
Mean	1.081	0.891	0.623		0.831	0.679	0.479	
LSD at 0.05%	HAM: 0.099			Salt: 0.086	HAM: 0.059			Salt: 0.051
	Interaction: 0.172				Interaction: 0.103			
	Chl. A+B (mg/g)				Carotenoids (mg/g)			
Control	1.313	1.391	0.847	1.183	0.232	0.199	0.141	0.191
HA	1.397	1.531	0.992	1.306	0.244	0.204	0.156	0.201
AMF	1.577	1.657	1.255	1.496	0.249	0.212	0.175	0.212
HA + AMF	1.686	1.702	1.313	1.567	0.262	0.230	0.192	0.228
Mean	1.493	1.570	1.102		0.247	0.211	0.166	
LSD at 0.05%	HAM: 0.126			Salt: 0.109	HAM: 0.027			Salt: 0.023
	Interaction: 0.219				Interaction: 0.046			

HA: Humic acid. AMF: Arbuscular mycorrhizal fungi. HAM: Humic acid and /or Arbuscular mycorrhizal

Control: 320 ppm, Conc.1: 2000 ppm, Conc.2: 4000 ppm

ameliorated the adverse effect of salinity stress on photosynthetic pigments in the leaves of pepper plant. HA + mycorrhiza treatment was most effective in this respect. Hither, the mycorrhizal colonization significantly improved chlorophyll concentration. This is because NaCl has an antagonistic effect on N absorption which is an essential component of the structure of chlorophyll molecule.

Concerning the effect of salt stress levels and soil application with HA as bio stimulants and mycorrhiza as symbiosis agents on proline accumulation and catalase and peroxidase activities in leaves of pepper plants as shown in Table 4, it could be noticed that all salinity stress levels (2000 ppm and 4000 ppm) slightly increased proline accumulation, catalase and peroxidase activities in leaves of pepper plants.

Changes were incrementally related to the increase in salt stress. On the other hand, the treatment with humic acid and Mycorrhiza alone or in combination, increased proline content, catalase and peroxidase activities in leaves of pepper plant. It could be shown from the results that HA and mycorrhiza mitigated the harmful effect of salinity stress and also promoted proline accumulation, catalase and peroxidase activities in leaves of pepper plants. Moreover, HA + mycorrhiza treatment was most effective in this concern.

It could be seen the importance to ameliorate the negative impact of salt stress on pepper plants by using soil treated with humic acid and mycorrhiza. Humic acid which had protective enzymes activity, as one of the protection mechanisms by inhibiting or quenching free

Table 4. Effect of humic acid and arbuscular mycorrhizal fungi (AMF) and their combination on Proline, Catalase and Peroxidase activities of pepper plant under salinity stress (average of two growing seasons 2014 and 2015)

	Salinity levels											
	Control	Conc.1	Conc.2	Mean	Control	Conc.1	Conc.2	Mean	Control	Conc.1	Conc.2	Mean
	Proline (mg/g Dw)				Catalase				Peroxidase			
Control	5.335	14.320	16.730	12.128	58.875	208.945	305.45	191.090	15.045	31.965	47.615	31.542
HA	9.345	15.070	17.285	13.900	76.075	211.210	377.84	221.710	18.205	34.010	44.025	32.080
AMF	12.160	15.945	17.715	15.273	110.475	242.405	464.38	272.420	20.250	37.195	47.420	34.955
HA + AMF	13.595	16.245	17.710	15.850	153.450	294.280	503.56	317.097	22.510	39.485	60.700	40.898
Mean	10.109	15.395	17.360		99.719	239.210	412.80		19.003	35.664	49.940	
LSD at 0.05%	HM: 0.211 HAM: 0.183 Salt: 0.183				HM HAM: 32.61 Salt : 28.24				HAM: 3.98 Salt: 3.455			
	Interaction: 0.367				Interaction: 56.49				Interaction: 6.91			

HA: Humic acid. AMF: Arbuscular mycorrhizal fungi. HAM: Humic acid and /or Arbuscular mycorrhizal
Control: 320 ppm, Conc.1: 2000 ppm, Conc.2: 4000 ppm

radicals. It is concluded that humic acid may play a significant role in the response to salinity and protection is largely through enzymatic processing of activated forms of oxygen.

It's evident that salinity stress levels increased proline content in leaves of pepper plant averaged during the two growing seasons. Several functions are proposed for the proline accumulation in tissues submitted to stress including osmotic adjustment, stabilization of proteins and cell membranes, being a scavenger of free radicals, improvement of the stability of some cytoplasmic and mitochondrial enzymes, and increased protection of proteins and enzymes. (Ozdemir *et al.*, 2004 ; Sakr *et al.*, 2007). On the growth parameters of cowpea, Hashem *et al.* (2015) reported that, AMF ameliorated the negative influence of salinity stress which enhanced the activity of catalase (CAT), peroxidase (POD), thus strengthening the defense system of plants. Proline accumulation is thought to be an adaptive feature under salinity stress in AM (Jindal *et al.*, 1993). Arbuscular mycorrhizal plants enhance the activity of antioxidant enzymes in order to cope with the reactive oxygen species generated by salinity (Porcel *et al.*, 2012).

The results in Table 5 clearly show the effect of salt stress and soil application with HA and mycorrhiza on N P K and Na in shoot pepper

plants. All salinity stress (2000 and 4000 ppm) caused a significant reduction in nutrients percentage N P K while Na percentage increased. On the other hand, applied humic acid and mycorrhiza alone or in combination increased N P K while Na was decreased in shoot of pepper plant.

The results also showed that, salinity stress levels decreased potassium and increased sodium contents in the shoots of pepper plants which is a typical response of plants in saline conditions arising from the inability of plants to distinguish between potassium and sodium ions (Storey *et al.*, 1983). In the vacuole, increasing Na⁺ content mainly 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.

Bio-stimulants can alleviate the adverse effect of drought or salinity stress through improving leaf water status and possibly by reduced uptake of ions Na and Cl (Nabati, 1994) and as a consequence increase Ca and K content in the leaves stimulating chloroplast development and enhancing phloem loading and delaying senescence (Demir *et al.*, 2004).

Also, humic acid has been shown to increase the permeability of plant membranes, promoting the uptake of nutrients N, P, K, Ca, and Mg

Table 5. Effect of humic acid and arbuscular mycorrhizal fungi (AMF) and their combination on N, P, K and Na percentage of pepper plant under salinity stress (average of two growing seasons 2014 and 2015)

	Salinity levels							
	Control	Conc.1	Conc.2	Mean	Control	Conc.1	Conc.2	Mean
	Nitrogen (N%)				Phosphorus (P%)			
Control	2.310	1.905	1.239	1.818	0.410	0.299	0.189	0.299
HA	2.454	2.020	1.350	1.941	0.442	0.327	0.198	0.322
AMF	2.410	2.100	1.594	2.035	0.479	0.362	0.229	0.357
HA + AMF	2.502	2.314	1.735	2.183	0.487	0.385	0.237	0.369
Mean	2.419	2.085	1.479		0.455	0.343	0.213	
LSD at 0.05%	HAM: 0.075			Salt: 0.065	HAM: 0.002			Salt: 0.002
	Interaction: 0.131				Interaction: 0.004			
	Potassium (K%)				Sodium (Na%)			
Control	2.920	2.295	2.020	2.412	1.145	1.630	1.995	1.590
HA	3.305	2.705	2.115	2.708	1.130	1.420	1.945	1.498
AMF	3.420	2.785	2.225	2.810	1.090	1.255	1.825	1.390
HA + AMF	3.470	2.915	2.265	2.883	0.970	1.200	1.715	1.295
Mean	3.279	2.675	2.156		1.084	1.376	1.870	
LSD at 0.05%	HAM: 0.040			Salt: 0.035	HAM: 0.038			Salt: 0.033
	Interaction: 0.070				Interaction: 0.066			

HA: Humic acid. AMF: Arbuscular mycorrhizal fungi. HAM: Humic acid and /or Arbuscular mycorrhizal
Control: 320 ppm, Conc.1: 2000 ppm, Conc.2: 4000 ppm

(Mackowiak *et al.*, 2001) and enhancing root development (Vaughan and Macdonald, 2005). Humic acids also are claimed to chelate sodium in the soil which helps plants tolerate higher soil sodium concentrations avoiding toxicity and osmotically related problems (Super, 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 provide support for the field application of exogenous protectants as HA under salt stress condition has been found to be very much effective to alleviate salt-induced damages in pepper plants, according to Saker *et al.* (2015). Major reason for the increase in the growth and yield can be attributed to ability of plants in associations with AMF to uptake some

nutrients efficiently, such as phosphorous. (Demir, 2004). These positive impacts are explained by improved host plant nutrition, higher K⁺/Na⁺ ratios in plant tissues and a better osmotic adjustment by accumulation of compatible solutes such as soluble sugars, glycine betaine, or proline. (Porcel *et al.*, 2012)

Conclusion

In the present study, salinity resulted in altered growth of pepper plants (*C. annuum* L.) cv top star due to its effects on the various physiological and biochemical parameters studied. It is possible to reduce the harmful effects of salinity stress by the application of humic acid as bio-stimulants and mycorrhiza as symbiosis agents to ameliorate the negative impact of salinity.

AMF allayed the salt stress by preventing the excess uptake of Na⁺ and at the same time causing further enhancement in activities of catalase and peroxidase enzymes, thus ensuring better scavenging of ROS. Besides this AMF and humic acid also increased proline accumulation, activities of enzymes as well as the uptake of mineral elements which have direct effect on the osmoregulation of the plants. Thus, the harmful effects of salinity on growth, antioxidant enzymes and mineral nutrients in pepper plants can be alleviated by AMF and Humic acid and their combination application.

Therefore, it is advisable that the dual application of HA and mycorrhiza could be an adequate strategy to alleviate the deleterious effects of salinity stress and promote much more growth and yield in pepper plants (*Capsicum annuum* L.) cv top star grown in salty condition.

REFERENCES

- Ahanger, M.A., A. Hashem, E.F. Abd Allah and P. Ahmad (2014). Arbuscular mycorrhiza in crop improvement under environmental stress, In: Ahmad, P., Rasool, S. (Eds.), Emerging Technologies and Management of Crop Stress Tolerance, 2 : 69-95.
- Al-Sobhi, O.A., H.S. Al-Zahrani and S.B. Al-Ahmadi (2006). Effect of salinity on chlorophyll and carbohydrate contents of *Calotropis procera* seedlings. Sci. J. King Faisal Univ., Basic and Appl. Sci., 7: 105-115.
- Arafa, A.A., M.A. Khafagy, A.M. Abo-El Kheer and M.F. El-Banna (2014). Response of certain phytochemical constituents in sweet pepper leaves to some Bio-stimulants under two types of salinity. J. Adv. in Nat. Sci., 2 (2): 83-99.
- Aroca, R., J.M. Ruiz-Lozano, A. Zamarreno, J.A. Paz, J.M. Garcia-Mina, M.J. Pozo and J.A. Lopez-Raez (2013). Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. J. Plant Physiol., 170 : 47-55.
- Bates, L., R.P. Waldren and I.D. Teare (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205-207
- Beltrano, J., M. Ruscitti, M.C. Arango and M. Ronco (2013). Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and p levels. J. Soil Sci. and Plant Nut., 13 (1): 123-141.
- Chance, B. and A.C. Maehly (1955) Assay of Catalase and Peroxidase. Methods in Enzymol., 2: 764-775.
- Chutipaijit, S., S. Chaum and K. Sompornpailin (2011). High contents of proline and anthocyanin increase protective response to salinity in *Oryza sativa* L. spp. *indica*. Aust. J Crop Sci., 5:1191-1198
- Clapp, C.E., Y. Chen, M. Hayes, A.J. Palazzo and V.W. Cline (2002). Stimulation of plant growth by humic substances. World Cong. Soil Sci., 192.
- Demir, D., A. Günes, A. Inal and M. Alpaslan (2014). Effects of humic acids on the yield and mineral nutrition of cucumber (*Cucumis sativus* L.) grown with different salinity levels. Acta Hort., 492:95-104
- Demir, S. (2004). Influence of arbuscular mycorrhizae (AM) on some physiological growth parameters of pepper. Turkish J. Biol., 28 : 85-90.
- Dolatabadian, A., S.A.M. Modarressanavy and F. Ghanati (2011). Effect of salinity on growth, xylem structure and anatomical characteristics of soybean. Not. Sci. Biol., 3: 41- 45.
- Fahramand (2014). Influence of abiotic stress on proline, Photosynthetic enzymes and growth. Intl. Res. J. Appl. Basic. Sci., 8 (3) : 257-265
- Gomez, K.A. and A.A. Gomez (1984). Statistical Procedures for Agricultural Research. 2nd Ed., Wiley and Sons, New York. USA.
- Hanafy, A.A.H., M.R. Nesiem, A.M. Hewedy and H.E.I-S. Sallam (2010). Effect of some simulative compounds on growth, yield and chemical composition of snap bean plants grown under calcareous soil conditions. J. Ame. Sci., 6 (10): 552-569.

- Hasanuzzaman, M., M.A. Hossain, J.A.T. da Silva and M. Fujita (2012). Plant responses and tolerance to abiotic oxidative stress: antioxidant defenses is a key factors. In: Bandi V, Shanker AK, Shanker C, Mandapaka M (eds) Crop stress and its management: perspectives and strategies. Springer, Berlin, 261–316.
- Hashem, A., E.F. Abd-Allah, A.A. Alqarawi and D. Egamberdieva (2015). Induction of salt stress tolerance in cowpea [*Vigna unguiculata* (L.) Walp.] by arbuscular mycorrhizal fungi. Legume Res., 38 (5) : 579-588.
- Jindal, V., A. Atwal, B.S. Sekhon and R. Singh (1993). Effect of vesicular-arbuscular mycorrhizae on metabolism of moong plants under NaCl salinity. Plant Physiol. and Biochem., 3: 475-481.
- Kijne, J.W. (2003). Unlocking the Water Potential of Agriculture. FAO Corporate Document Repository, 70, ISBN 9251049114.
- Mackowiak, L., P.R. Gross and B.G. Bugbee (2001). Beneficial Effects of humic acid on micronutrient availability to wheat. Soil Sci. Soc. Ame. J., 65: 1744-1750.
- Martinez, A.S., F.D. Amor, M.I. Fortea, C.L. Abellan, S.L. Miranda and E.N. Delicado (2014). Effect of plant age and saline water on antioxidants and peroxidase activity in sweet pepper Fruit. J. Agric. Sci., 6 (12): 139-151.
- Martínez, A.S., M.I. Fortea, F.M. Del Amor, C. Lucas-Abellán, M.T. Mercader-Ros, J.A. Gabaldón, A. Martínez-Cachá and E.N. Delicado (2015). Parameters of pepper fruit quality. Trends in Food Technol., 75-98.
- Nabati, D.A. (1994). Responses of two grass species to plant regulators, fertilizer N, chelated Fe, salinity and water stress. Ph.D. Crop and Soil Environ. Sci. Dept., Virginia Tech, Blacksburg.
- Naguib, M.I. (1963). Effect of colochicine of the nitrogen metabolism and fate of phosphorus observed during the formation of fungal of *cunninghamella* spp. Arch. Microbiol., 47: 154 – 160.
- Nardi, S., D. Pizzeghello, M. Schiavon A. Ertani (2016). Plant bio stimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. Sci. Agric., 73 (1): 18-23.
- 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. Ecotoxicol. and Environ. Safety, 60: 324-349.
- Peterburgski, A.V. (1968). Handbook of Agronomic Chemistry. Kolop Publishing House, Moscow, Russia.
- Porcel, R., R. Aroca and J.M. Ruiz-Lozano (2012). Salinity stress alleviation using arbuscular mycorrhizal fungi. A Rev. Agron. Sustain. Dev., 32:181–200.
- Rao, D.L.N. (1998). Biological amelioration of Salt affected Soils. In: Microbial Interactions in Agricultural and Forestry, Sci., Publ., Enfield, USA., 1 : 221-238.
- Rouphael, Y., P. Franken, C. Schneider, D. Schwarz, M. Giovannetti, M. Agnolucci, S. De Pascale, P. Bonini and G. Colla (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Scientia Hort., 196: 91-108.
- Saha, P., P. Chatterjee and A. Biswas (2010). NaCl pretreatment alleviated salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek). Ind. J. Exp. Bio., 48: 593-600.
- Saker, M.T., N.M. El-Sarkassy and M.P. Fuller (2012). Exogenously applied antioxidants and biostimulants alleviate salt stress in sweet pepper. Zagazig J. Agric. Res., 39 (4): 605 - 617
- 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, 295-321.
- Sakr, M.T., M.E. El-Emery, R.A. Fouda and M.A. Mowafy (2007). Role of some antioxidants in alleviating soil salinity stress. *J. of Agric. Sci., Mansoura Univ.*, 32: 9751-9763.
- Sakr, M.T., N.M. El-Sarkassy and M.P. Fuller (2015). Minimization the effects of salt stress on sweet pepper plants by exogenous protectants application. *Zagazig J. Agric. Res.*, 42 (6)-1397-1410
- Selvakumar, G. and P. Thamizhiniyan (2011). The effect of the arbuscular mycorrhizal (AM) fungus (*Glomus intraradices*) on the growth and yield of Chilli (*Capsicum annuum* L.) under salinity stress. *World Appl. Sci. J.*, 14 (8): 1209-1214.
- Sharma, P.K. and D.O. Hall (1991). Interaction of salt stress and photo -inhibition on photosynthesis in barley and sorghum. *J. Plant Physiol.*, 138: 614-619.
- Shekoofeh, E. and H. Sepideh (2011). Effect of mycorrhizal fungi on some physiological characteristics of salt stressed *Ocimum basilicum* L. *Iraninan. J. Plant Physiol.*, 1(4): 215-222.
- Sheng, M., M. Tang, F. Zhang and Y. Huang (2011). Influence of arbuscular mycorrhiza on organic solutes in maize leaves under salt stress. *Mycorrhiza.*, 21 : 423–430
- Smith, S.E. and D.J. Read (2008). *Mycorrhizal Symbiosis*. Academic Press, Inc., San Diego, USA
- Snell, F.D. and C.T. Snell (1954). In: *Colorimetric methods of analysis* 3rd Ed. D.Van Nostrand Company. New York, 512 (513): 516-518.
- Storey, R., M.G. Pitan and R. Stelzer (1983). X-Ray Microanalysis of cells and cell compartments of *Atriplex spongiosa* root. *J. Exp. Bot.*, 34: 1196-1206.
- Super, G.R. (2006). How Does Fulvic Acid Affect Plant Permeability?
- Tavakkoli, E., P. Rengasamy and G.K. McDonald (2010). High concentrations of Na⁺ and Cl⁻ ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *J. Exp. Bot.*, 61:4449–4459
- Thomas, R.L., J.J. Jen and C.V. Morr (1982). Changes in soluble and bound peroxidase-IAA oxidase during tomato fruit development. *J. Food Sci.*, 47: 158-161.
- 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.
- Vitoria, A.P., P.J. Lea and R.A. Azevedo (2001). Antioxidant enzymes responses to cadmium in radish tissues. *Phytochem.*, 57: 701-710.
- Wettstein, D. (1957). Chlorophyll–Lethal undder submink roskopische formivechoel der plastiden. *Exp. Cell. Res.*, 12: 427 – 433.

تحسين الإجهاد الملحي باستخدام حمض الهيوميك والميكورهيذا في نباتات الفلفل

ناصر محمد السرکسي - سهام عبد العال ابراهيم - السيد محمد دسوقي حسن

قسم النبات الزراعي - كلية الزراعة - جامعة الزقازيق - مصر

الملوحة هي عامل مدمر ومن بين عوامل الإجهادات اللاحيائية المختلفة، والتي تؤثر بقوة علي إنتاجية المحصول وقابليته للتسويق في محاصيل الخضر مثل نباتات الفلفل، في هذا البحث تم دراسة الآثار المترتبة على التطبيق المزدوج لحمض الهيوميك والتلقيح بالميكورهيذا منفردا أو خليط منهما والتي خفضت وحدثت من آثار الإجهاد الملحي الضارة على النمو والمحصول والمكونات الحيوية الداخلية في نبات الفلفل صنف توب ستار، ويتضح من هذا البحث إن الإجهاد الملحي يتركز (٢٠٠٠، ٤٠٠٠ جزء في المليون) عند عمر ٧٥ يوم من الشتل يتسبب في نقص قياسات النمو وأيضا مكونات وجودة محصول الفلفل، ولكن عند استعمال الميكورهيذا وحمض الهيوميك كل منهما منفردا أو خليط منهما قد تغلبت علي التأثير الضار لمستويات الملوحة، وأدت مستويات الاجهاد الملحي الي زيادة في تراكم البرولين وعنصر الصوديوم ونشاط انزيم الكتاليز والبيروكسيداز بينما أدت إلى نقص لمحتوي النيتروجين والفوسفور والبوتاسيوم في المجموع الخضري وكذلك صبغات التمثيل الضوئي في أوراق نباتات الفلفل، بينما أدت إضافة الميكورهيذا وحمض الهيوميك المستخدمة سواء بصورة منفردة أو متداخلة مع المستويات المختلفة للملوحة إلى زيادة محتوى النيتروجين والفوسفور والبوتاسيوم في المجموع الخضري وتراكم البرولين وصبغات التمثيل الضوئي ونشاط انزيم الكتاليز والبيروكسيداز في الأوراق وتقليل عنصر الصوديوم في المجموع الخضري وكان لمعاملة الميكورهيذا وحمض الهيوميك معا تأثيراً متزايداً في هذا الإتجاه، تبين النتائج أيضا أن التلقيح بحمض الهيوميك أو الميكورهيذا منفردا أو خليط منهما له القدرة على تخفيف الآثار الناجمة عن الإجهاد الملحي على نبات الفلفل مما انعكس على محصول النبات، هذه النتائج تقدم الدعم للتطبيق الحقل لحامض الهيوميك والميكورهيذا لتقليل الآثار الضارة لملوحة التربة.

المحكمون:

١- أستاذ النبات الزراعي - كلية الزراعة - جامعة المنصورة.
 ٢- أستاذ النبات الزراعي - كلية الزراعة - جامعة الزقازيق.

١- أ.د. محب طه صقر
 ٢- أ.د. نادية حسين كامل