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Effect of Putrescine and Humic Acid on Growth, Yield and Chemical Composition of Cotton Plants Grown under Saline Soil Conditions

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Abstract: This study was carried out to determine the effects of putrescine(Put) and humic acid (HA) foliar applications on growth, yield and chemical composition of Egyptian cotton (*Gossypium barbadense* L. cv. Giza 90)plants grown under saline soil condition. A soil mixture of, clay: sand (1:1), was used as a plant growing media. Three different doses of putrescine (0, 1 and 2 ppm) and humic acid (0, 1 and 2%) were sprayed eight times started at 45 days after planting. Before sowing cotton, different concentrations (0, 3000, 6000 and 9000ppm) of salt mixture (2 NaCl: 2 $CaCl_2$: 1 $MgSO_4$) were added into soil for each pot. Application of Put and HA positively affected cotton plants grown under salt stress. The results indicated that there is an increase in morphological characters e.g. plant height, number of leaves per plant, leaf area per plant, fruiting branches per plant, shoot fresh and dry weight. Also, Put and HA increased chemical constitutes related to salt tolerance either inorganic, N, P and K, while Na, Cl, Ca and Mg were decreased, or organic constitutes e.g. proline, total free amino acids, total sugars, total soluble phenols, chlorophyll a, b, total chlorophyll and total carotenoids. As a result of promoting growth induced by previous foliar applications, yield components e.g.; number of total, open and closed bolls, seed cotton yield/plant, lint percentage and seed index were increased. Application of 2 ppm Put and 1% HA recorded the highest values of growth and yield characters.

Key words: Cotton · Salinity · Putrescine · Humic Acid · Growth characters · Lint

INTRODUCTION

Egyptian cotton is a strategic crop with glorious history in Egypt. In past it was accounted as main source of Egyptian economy. Its impact on the economic development of the country is well established and it is rightly known as the white gold in Egypt. It is a strong competitor in the worldwide market against the other types of cotton. Recently, Egyptian cotton has faced serious problems that dramatically affected its production. Nowadays a lot of efforts have been made to get its position back among different cotton types. One of these strategies to improve cotton yield in Egypt is to extend cultivation area in new lands; yet, this new land may have a high level of salt in soil or ground water. Although cotton is considered as being fairly tolerant to salinity [1] yet its yield is drastically reduced due to poor germination and subsequent abnormal plant development under saline conditions [2]. Salinity inhibits plant growth and productivity by a range of mechanisms; include osmotic effects, direct ion toxicity and interference with the uptake of nutrients [3]. Plants growing in saline environments can cope with imposed salt stress by using various strategies at the whole plant as well as at the tissue and cellular levels to allow them to grow under these conditions. To cope with low water potential, plants accumulate organic metabolites and/or inorganic ions, which decrease the water potential of the plant. Exogenous application of different osmoprotectant has not only been proposed as a convenient approach for unveiling their implication in salinity response, but it is also considered as an effective approach for enhancing salt tolerance of crops and eventually improving crop productivity under high salinity [4]. Since polyamines (PAs) were reported to be involved in stabilization of membrane and scavenging of free radicals [5], osmotic adjustment [6], mineral nutrition

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[7-9] an implication of these compounds in salt resistance may be considered. Foliar applied PAs due to their ability to act as growth egulator is able to modulate the plant metabolism and the production of metabolites involved in stress tolerance [10]. Also, humic acid might show anti-stress effects under abiotic stress conditions such as unfavorable temperature, salinity, pH, etc. The major functional groups of humic substance include carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone and quinoid [11]. Humic substances are well known as stimulators of plant germination and growth [12]. It was also reported that HA application positively affected the plant parameters of plant grown in salinity condition [13].

The objective of this study was to investigate the mitigation effect of putrescine and humic acid on growth, yield and organic and inorganic constitutes of cotton plants under different salinity stress conditions.

MATERIALS AND METHODS

This experiment was carried out in the house wire of the Plant Physiology Section, Faculty of Agriculture, Cairo University, Giza, Egypt, during the two successive summer seasons (2011 and 2012), the planting date for both seasons was 29[th]March. Plastic pots of 40 cm in diameter and 40 cm in depth were used in this experiment. Each pot was filled with 11 kg soil obtained from the Experimental Farm, Faculty of Agriculture, Cairo Univ. Giza, Egypt. Mixture of soil was1:1 (clay: sand w/w) was used. In the two successive seasons, the experiment was divided into 4 main-groups of salinity levels (0, 3000, 6000 and 9000ppm), each group soil salinity was obtained by adding mixture of sodium chloride, calcium chloride and magnesium sulphate at the ratio of 2:2:1 by weight. Each group was divided into five sub-groups. The first sub-group was sprayed with tap water (control A), the second sub-group was sprayed with 1 ppm putrescine (Put) the third with 2 ppm putrescine-(tetramethyenediamine $C_4H_{12}N_2$, F.W. 88.15) was obtained from local market (Techno Gen). The fourth treatment with 1% humic acid (HA) and the fifth with 2% humic acid. Humic acid as a liquid was obtained from Seed Outlet in Agricultural Research Center, Giza, Egypt, "Super Canada" produced by the Egyptian Canadian for Humate Trade and Agricultural Consultancies in Egypt, its content from humic acid active 8%-folic acid active 1%, other organic materials 72.3% and neutral pH). Foliar application of different concentration from Put and HA were sprayed eight times started at the day 45 after planting (DAP) and repeated every 15 days.

Plant Sampling and Growth Measurements: In the two successive seasons plant sample was taken at 100 DAP (post-flowering stage). Plants were divided into roots and shoots and the following measurements were recorded: plant height (cm), number of leaves/plant, leaf area/plant (cm^2), number of fruiting branches/plant, shoot fresh and dry weight (g). In the both successive seasons, the first pick of cotton yield was performed by hand, on October 29, while the second pick was on November 18. Yield and yield components were estimated as follow: number of total bolls/plant, number of open bolls/plant, number of closed bolls/plant, boll weight (g), seed cotton yield/plant(g), seed index (%) estimated as an average weight of 100 seeds in gram and lint percentage (L%) calculated as the relative amount of lint in a seed cotton sample, expressed in percentage: L% = (weight of lint cotton in sample/weight of seed cotton in the same sample) x 100. Some of the technological fibre properties (fibre length, strength and fineness) were measured by using HVI according to A.S.T.M. [14] in the laboratories of the Cotton Research Institute, Agricultural Research Center, Giza, Egypt.

Chemical Analysis: Determinations of N, P, K, Ca, Mg, Cl and Na were carried out on the dry material. The wet digestion of 0.2g plant material with sulphuric and perchloric acids was carried out on shoots as reported by Piper [15]. Nitrogen concentration was determined by Nessler method according to A.O.A.C. [16]. Phosphorus was estimated calorimetrically by using the chlorostannous reduced molybdophosphoric blue color method according to Jackson [17]. Potassium and sodium concentrations were determined by using the flame photometer apparatus (GENWAY PFP-7). Calcium and magnesium concentrations were determined by using atomic absorption spectrophotometer (UNICO UV-2000). For organic components; photosynthetic pigments of fresh leaves were extracted with dimethylformamide and placed overnight at cool temperature (5°C). Chlorophyll a, b, total chlorophyll and total carotenoids were measured by spectrophotometer at wavelengths 663, 647 and 470 nm, respectively. Chlorophylls and carotenoids were calculated according to the equation described by Nornai [18]. Total sugars were determined by using the phenol sulphuric acid reagent according to Dubois *et al.* [19]. Total free amino acids were determined by using ninhydrin reagent according to Moore and Stein [20].Total soluble phenols were determined by using the Folin-Denis colorimetric method according to Swain and Hillis [21]. Free proline concentration was determined in the extraction of dry materials by u sing ninhydrin reagent according to Bates *et al.* [22].

Statistical Analysis: Data obtained were statistically analyzed by using factorial experiment in completely randomized design with six replicates according to Snedecor and Cochran[23], then the combined analysis of two seasons was done according to Steel and Torrie [24] an the treatment means were compared using the least significant difference test (L.S.D.) at 5% level of probability

RESULTS AND DISCUSSION

Growth Characters: Results presented in Table 1 showed the effect of putrescine (Put) and humic acid(HA)foliar applications on growth characters; plant height, number of leaves, leaf area, number of fruiting branches and shoot fresh and dry weights of cotton plants grown under different levels of salinity. Generally, increasing salinity stress gradually decreased all of the studied characters of whole treatments. The decline in dry weight in response to increased salinity may be attributed to a combination of osmotic and specific ion effects of Cl^- and Na^+ [25]. Also, Moradi and Zavareh [26] on chickpea stated that seedling dry weight was decreased with increasing salinity. The reduction in seedling growth under saline conditions may either be due to decrease in the availability for water or increase in sodium chloride toxicity, associated with increasing salinity. Growth inhibition by salt stress also occurs due to the diversion of energy from growth to the maintenance. Munns [27] reported that the reduction in dry weight of cotton tissues reach to 60% under salt stress conditions. High salinity levels led to reducing leaf area due to turgor pressure reduction resulting from salt stress which can cause inhibition of cell division and expansion [28]. Gale and Zeroni [29] concluded that under salt stress, turgor pressure decreased and closure of stomata takes place causing decreased photosynthesis. Ionic toxicity of Na^+ and Cl^- is considered to be the other reason for decreasing shoot fresh weight with increasing salinity [30, 31]. Salt-induced osmotic stress [32], altered metabolism, inability of apoplastic acidification and lack of turgor seemed to be the possible reasons of salinity-induced decrease in rice growth [33]; increase in Na^+ uptake also contributed towards this [34].

Both concentrations of Put and HA have a promoting effect on plant height under all salinity levels in comparison to control plants. This increment in plant height was significant at 1 ppm Put, 2 ppm Put and 1% HA under non-saline or saline soil conditions. On the other hand, although Put and HA showed promoting effect on leaves number/plant values and number of fruiting branches, this increase was non-significant for the interaction between sprayed Put or HA and all salinity levels compared to control plants. It was noticed that leaves number/plant and fruiting branches were decreased under all salinity levels with both control plants, or with Put and HA treated plants. Leaf area was decreased gradually with increasing salinity levels upto 9000 ppm. Put and HA enhanced leaf area, the highest values of leaf area was recorded at 2 ppm Put, 1% HA and 1 ppm Put, respectively. In this regard, shoot fresh weight, was decreased under all salinity levels. It worth mentioning that under salt stress, plants treated with 2 ppm Put, 1% HA and 1 ppm Put gave a significant increment in fresh weight, respectively. The highest values of shoot dry mass was obtained by foliar application of 2ppm Put, 1% HA and 1ppm Put, respectively. It worth noticing that plants sprayed with 2% HA recorded the lowest values in regard to the previously mentioned growth characters. The decrease in shoot dry weight with increasing salinity level from 0, 3000, 6000 to 9000 ppm was estimated by 29%, 53% and 73%, respectively as compared with control plants. On the other hand, foliar application of Put and HA increased shoot dry weight under all salinity levels or with control plants. The positive effect of these substances for cotton plants is in agreement with Boris *et al*. [35] who concluded that humic substances provided a bio-stimulating effect on growth of cucumber. In this respect, physiological mechanisms through which humic substances exert their effects may depend on hormones and, in particular, on the presence of auxin or auxin like components in their structure and, consequently its effect on plant growth and development [36]. Accordingly, Chen *et al*. [37] pointed that the direct effects of humic substances depends on biochemical actions on cell wall, membrane or cytoplasm, mainly hormonal acting, in manner similar to plant growth substances [38] and agricultural humic substances are reputed to drought tolerance, enhance nutrient uptake and overall plant performance resulting in increasing leaf area and biomass production, so this was in agreement with the findings of the present work. Moreover,

Table 1: Effect of spraying with putrescine (Put) and humic acid (HA), salinity treatments and their interaction on plant height, number of leaves/plant, leaf area/plant, number of fruiting branches/plant and shoot fresh and dry weight of cotton Giza 90 at 100 days DAP during 2011 and 2012 seasons.

Spray Salinity ppm	C	Put 1ppm	Put 2ppm	HA 1%	HA 2%	Mean A
			Plant height (cm)			
C	83.33	89.00	94.33	87.83	81.00	87.10
3000	72.83	81.33	85.50	81.67	73.17	78.90
6000	56.66	65.50	74.50	70.83	60.00	65.50
9000	42.17	45.83	55.67	53.33	44.67	48.33
Mean B	63.75	70.42	77.50	73.42	64.71	--
LSD 5%	A=2.035		B=2.275		A*B=4.550	
			Number of leaves/plant			
C	14.67	15.33	16.00	16.67	14.83	15.50
3000	11.83	13.83	14.83	14.17	12.33	13.40
6000	9.50	10.16	12.00	11.67	10.00	10.67
9000	6.50	7.50	9.33	9.00	7.00	7.87
Mean B	10.63	11.71	13.04	12.88	11.04	--
LSD 5%	A= 0.684		B= 0.765		A*B=N.S	
			Leaf area/plant (cm^2)			
C	14.60	15.04	17.52	17.42	15.05	15.93
3000	11.20	13.47	14.12	13.55	11.46	12.76
6000	8.93	10.25	12.19	11.44	8.82	10.32
9000	3.84	4.86	6.80	5.98	4.10	5.12
Mean B	9.64	10.91	12.66	12.10	9.86	--
LSD 5%	A= 0.644		B= 0.720		A*B=1.439	
			Number of fruiting branches/plant			
C	4.66	5.00	5.50	5.50	4.83	5.10
3000	3.50	3.83	4.66	4.33	4.00	4.07
6000	1.67	2.00	2.83	2.50	2.00	2.20
9000	0.00	0.00	0.17	0.33	0.33	0.17
Mean B	2.46	2.71	3.29	3.17	2.79	--
LSD 5%	A= 0.459		B=N.S		A*B=N.S	
			Shoot fresh weight (g)			
C	49.17	52.22	55.80	57.71	49.39	52.86
3000	35.15	42.54	46.37	46.24	36.01	41.26
6000	21.37	24.58	32.77	29.71	25.91	26.87
9000	13.82	15.69	22.68	18.62	12.02	16.57
Mean B	29.88	33.76	39.41	38.07	30.83	--
LSD 5%	A= 2.158		B= 2.412		A*B=4.825	
			Shoot dry weight (g)			
C	14.95	14.80	17.59	20.14	16.15	16.72
3000	9.98	12.55	13.33	12.46	11.08	11.88
6000	5.92	6.77	9.28	10.15	7.42	7.91
9000	3.53	4.22	6.73	4.97	3.19	4.53
Mean B	8.60	9.58	11.73	11.93	9.46	--
LSD 5%	A= 0.820	`	B= 0.916		A*B=1.832	

C: Control , A= Salinity, B= Spray, A*B= Interaction

Mora [39] mentioned that, the ability of humic substances to increase shoot growth in different plant species cultivated under diverse growth conditions might be attributed to H^+-ATPase activity and nitrate root-shoot distribution that, in turn, causes changes in the root-shoot distribution of certain cytokinins, polyamines and abscisic acid, thus affecting shoot growth. In contrast, Defline *et al*. [40]and Pavlikova *et al*. [41]indicated that application of potassium humate or humic acids foliar spray during the growth season of cultivated crops were not significant on yields these cultivated crops. Also, Cooper *et al*. [42] applied humic substances for creeping bentgrass, they found that the rate of application did not have any effect on the plant growth. This finding is in agreement with our present work showing that 2% of HA showed the lowest increments in all studied morphological traits and these increments were non-significant.

Classical approaches, using exogenous polyamine application and/or inhibitors of enzymes involved in polyamine biosynthesis, pointed to a possible role of these compounds in plant adaptation/defense to several environmental stresses [43, 44]. It has been reported that Put, spermidine (Spd) and spermine (Spm) also regulate stomatal responses by reducing their aperture and inducing closure [45, 46]. In addition, Put modulates ABA biosynthesis in response to abiotic stress. In other crops, Put also stimulated growth by increasing the amount of endogenous promoters (auxin, gibberellins and cytokinins) accompanied by a decrease in the content and activity of inhibitors (ABA) [47, 48]. Put improved the photosynthetic rate and stomatal conductance in salt-susceptible Karnakhatta under saline conditions. The ability Put to ameliorate the negative effects of salt stress on photosynthesis may be due to the reduced uptake of Na^+ and Cl^- and improvement in the concentrations of K^+ and Ca^{2+} in leaf tissues. Put induced stomatal closure in wheat which exhibited high water content [45].The salinity caused reduction in biomass was reversed by Put up to an extent. Put induced growth in control seedlings prove its nature of plant growth regulator in general. However, Put alleviation of growth in stressed seedlings might be through activating antioxidative defense system. PAs general nature to reduce membrane leakage and lipid peroxidation has been mentioned; in addition to similar nature of Put effect on stabilization of membrane damage under stress could be also due to its polycationic nature as suggested by Tiburcio *et al*. [49].

Chemical Constitutes: Data presented in Table 2 revealed that salt stress affects the accumulation of nutrients in shoot of the plants. It was noticed that the percentage of nitrogen, phosphorus and potassium were decreased in response to salinity treatments. On the other hand, calcium, magnesium, sodium and chloride percentages were increased under different concentrations of salinity. There are 12 main soluble salts made up of cations (Na^+, Ca^{2+} and Mg^{2+}) and anions (CO_3^{2-}, HCO_3^-, Cl^- and SO_4^{2-}) in saline soils [50]. Uptake and accumulation of certain toxic ions from the saline soil or irrigation water can lead to toxicity. These toxic constituents include mainly sodium, chloride and sulphate [51]. Concentrations of Na^+ and Cl^- in cotton roots, xylem sap and leaf were increased with increasing concentration of NaCl in the soil environment [52]; a large quantity of Na^+ and Cl^- poured into the cells destroys the ion balance in the cytoplasm, particularly the Ca^{2+} balance. Salinity may result in the disturbance of uptake and utilization of essential nutrients due to competition and interactions of soluble salts with mineral nutrients [53]. Ionic imbalance occurs in the cells due to over accumulation of Na^+ and Cl^- and reduced uptake of other mineral nutrients, such as K^+, Ca^{+2}, Mg^{+2}, N^+ and Mn^{2+} leading to the suppression in growth [54-56]. According to Brugnoli and Bjorkman [57], nitrogen content in cotton leaves decreased with increasing salt concentration. Moreover, Martinez and Läuchli [58] found that under low P conditions, salt stress inhibited the absorption of phosphorus in cotton seedlings.

In this regard, foliar application of Put and HA (Table 2) indicated that the percentages of the elements N, P and K^+ were slightly increased as a result of foliar application of either HA or Put under different levels of salinity. The maintenance of a high K^+ content may be crucial to sustaining the integrity of the photosynthetic system under high salinity [59, 60] and thus an important pre-requisite for salinity tolerance. In this regard, calcium, magnesium, sodium and chloride. The application of Put and HA affect the percentages of these elements compared to unsprayed plants under all different levels of salinity. Slight decreases were recorded in these nutrients in response to all foliar applications of Put and HA under different levels of salinity. It is noticeable that the lowest values of these elements resulted from 2 ppm Put treatment. Le Chang *et al.* [61] reported that, nitrogen content in leaves was remarkably enhanced by HA. Moreover, Asik *et al*. [62] pointed that, the lowest doses of both soil and foliar application of humic substances increased the nutrient uptake of wheat. On the other hand, Liu [63] pointed that the application of humic acid during salinity stress did not increase the uptake of N, P, K^+ or Ca^{+2}. David *et al*. [64] reported promoting growth and nutrient uptake of plants due to the addition of humic substances. The plants take more mineral elements due to better-developed root systems. In addition, the stimulation of ions uptake under the applications of humic materials led many investigators to proposing that these materials affect membrane permeability [65]. This is related to the surface activity of humic substances resulting from the presence of both hydrophilic and hydrophobic sites [66]. Therefore, the humic substances may interact with the phospholipids structures of the cell membranes and react as carriers of nutrients through them. These results were coincided with those reported by Tattini *et al.* [67] who found that the humates can stimulate the uptake of

Table 2: Effect of spraying with putrescine (Put) and humic acid (HA), salinity treatments and their interaction on nitrogen, phosphorus, potassium, calcium,magnesium, sodium and chloride percentage in shoots of cotton Giza 90 at 100 days DAP during 2011 and 2012 seasons.

Spray Salinity ppm	C	Put 1ppm	Put 2ppm	HA 1%	HA 2%	Mean A
			Nitrogen percentage			
C	3.63	3.70	3.80	3.79	3.84	3.62
3000	3.14	3.24	3.46	3.37	3.49	3.24
6000	2.90	2.98	3.10	3.00	3.16	2.89
9000	2.55	2.61	2.75	2.67	2.82	2.60
Mean B	3.06	3.13	3.28	3.21	3.33	--
			Phosphorus percentage			
C	0.565	0.578	0.588	0.584	0.594	0.582
3000	0.511	0.522	0.545	0.535	0.550	0.533
6000	0.469	0.482	0.494	0.486	0.498	0.486
9000	0.414	0.427	0.447	0.438	0.452	0.436
Mean B	0.490	0.503	0.519	0.511	0.524	--
			Potassium percentage			
C	3.80	3.89	3.97	3.93	3.95	3.91
3000	3.60	3.73	3.84	3.77	3.87	3.76
6000	3.22	3.29	3.44	3.38	3.53	3.37
9000	3.01	3.10	3.22	3.15	3.28	3.15
Mean B	3.41	3.50	3.62	3.56	3.66	--
			Calcium percentage			
C	2.43	2.32	2.16	2.26	2.09	2.25
3000	2.78	2.69	2.51	2.57	2.50	2.61
6000	3.04	2.92	2.79	2.85	2.74	2.87
9000	3.46	3.33	3.13	3.22	3.08	3.25
Mean B	2.93	2.82	2.65	2.73	2.61	--
			Magnesium percentage			
C	0.51	0.49	0.42	0.45	0.39	0.45
3000	0.64	0.61	0.54	0.57	0.55	0.58
6000	0.78	0.74	0.69	0.69	0.67	0.71
9000	1.04	0.97	0.85	0.88	0.80	0.91
Mean B	0.74	0.71	0.62	0.65	0.61	--
			Sodium percentage			
C	0.40	0.36	0.31	0.29	0.31	0.33
3000	0.56	0.52	0.43	0.47	0.45	0.49
6000	0.70	0.67	0.58	0.60	0.61	0.63
9000	0.89	0.85	0.75	0.81	0.71	0.80
Mean B	0.64	0.60	0.52	0.54	0.52	--
			Chloride percentage			
C	3.02	2.92	2.86	2.89	2.85	2.91
3000	3.26	3.18	3.09	3.14	3.08	3.15
6000	3.53	3.49	3.32	3.36	3.28	3.39
9000	3.81	3.75	3.60	3.62	3.76	3.71
Mean B	3.41	3.34	3.22	3.26	3.24	--

C: Control , A= Salinity, B= Spray, A*B= Interaction

macro-and microelements. Also, Yildirim, [68] and Karakurt *et al.* [69], reported that the foliar sprays of these substances also promote growth and increased yield and quality in a number of plant species at least partially through increasing nutrient uptake, serving as a source of mineral plant nutrients and regulator of their release [70]. According to many researchers, humic substances may enhance the uptake of some nutrients, reduce the uptake of toxic elements and improve the plant response to salinity. Such positive response might reflect, the humic acids are especially beneficial in freeing up nutrients in the soil so that they are made available to the plant as needed [70, 71]. It is possible that the enhancement in growth of okra seedlings, after incorporation of HA and

K into the plant growth medium, could be attributed at least partially to the increased nutrient uptake of plants. HA have been reported to enhance mineral nutrient uptake by plants, increasing the permeability of membranes of root cells [72].

Regarding to Put treatment, recently, evidence indicated that Put plays an important role in plant responses to environmental stresses. This is consistent with other reports that suggested that exogenously applied PAs could regulate ion homeostasis and absorption and translocation of toxic ions in crops such as Put in bean [73], Put and spermidine (Spd) in wheat [74]. Moreover, Sharma *et al*. [75] worked on Citrus Rootstock Karnakhatta (*Citrus karma* Raf.) found that the minimum Na^{+} and Cl^{-} accumulation was recorded with the treatment of 50 mg/L Put. Also, they found that the leaf K and Ca contents were increased when the plants were sprayed with 50 mg/L Put alone. Polyamine-induced repression of Na+ influx into roots and prevention of K^{+} loss from shoots improved K^{+}/Na^{+} homeostasis in barley seedlings and tolerance to high salinity [76]. Regulation of ion channels by polyamines is confined within the multiple and versatile mechanisms through which polyamines participate in the stress response. For instance, micromolar concentrations of polyamines block both inward and outward currents through the non-selective cation channels (NSCCs) in pea mesophyll protoplasts, thus assisting the adaptation to salinity by reducing the uptake of Na^{+} and leakage of K^{+} from mesophyll cells [77]. From a practical point of view, polyamines are ideally suited as physiological channel blockers, since they are the only organic polycations present in sufficient quantities to perform the role of channel blockage, without compromising cell metabolism. Inorganic polycations are also efficient channel blockers, but most of them are highly toxic and, hence, cannot be accumulated in the cytosol at the required concentrations for a “safe control” of cellular homeostasis. Regarding the molecular mode of action of polyamines in ion channels, evidences point to specific polyamine-binding proteins in cytoplasmic [78] and plasma membrane fractions [79] that might mediate regulatory effects of polyamines on ion channel activities. Phosphorylation and dephosphorylation of ion channel proteins are closely related to their activities [80, 81]. Thus, polyamines could also affect protein kinase and/or phosphatase activities to regulate ion channel functions. Yamaguchi *et al*. [82] proposed that the protective role of Spm against high salt and drought stress is a consequence of altered control of Ca^{2+} allocation through regulating Ca^{2+}-permeable channels, including CAXs. The increase in cytoplasmic Ca^{2+} results in prevention of Na^{+}/K^{+} entry into the cytoplasm, enhancement of Na^{+}/K^{+} influx to the vacuole or suppression of Na^{+}/K^{+} release from the vacuole, which in turn increases salt tolerance [82, 83].

Data presented in Table 3 revealed that chlorophyll a, b, total chlorophyll and carotenoids decreased in response to salt stress. The data showed increasing salinity levels up to 9000 ppm decreased of all previous characters with increasing. These results are in agreement with those obtained by Qadir and Shams [84] on cotton, Ganieva *et al.* [85] and Noor-e-Saba *et al.* [86]. They also suggested that the reduction of chlorophyll concentration could be used for screening of plants for salinity tolerance. Moreover, the results in Table 3 revealed that leaf chlorophyll of cotton plants was enhanced with Put and HA foliar applications. The highest chlorophyll concentration in shoot was noticed in plants treated with2 ppm Put followed by 1% HA level. The results obtained are in agreement with those reported by Cavalcante *et al*. [87], Ferrara and Brunetti [88] they suggested that even one application of humic substances was able to increase chlorophyll content in grape leaves and/or they delayed chlorophyll degradation. In addition, Tahir *et al*. [89] pointed that higher leaf chlorophyll associated to humic substances could be related to increased cell membrane permeability by these substances, thus promoting greater efficiency in the absorption of nutrients, especially nitrogen a nutrient with direct relation with leaf chlorophyll concentration. Tattini *et al*. [67] found that the humates can stimulate the uptake of macro and microelements. Also, Yildirim [68] and Karakurt *et al*. [69], reported that the foliar sprays of these substances also promote growth and increased yield and quality in a number of plant species at least partially through increasing nutrient uptake, serving as a source of mineral plant nutrients and regulator of their release [70, 90]. Application of Put resulted in total chlorophyll content higher than their unsprayed controls. Zeid [73] also reported improvement in photosynthetic pigment by the application of Put. There is an increase in chlorophyll a, chlorophyll b and total chlorophyll content was recorded when salinized plants were treated with 50 mg/L Put alone followed by the combined treatment of 500 mg/L paclobutrazol (PBZ) and 50 mg/L Put [91].

Organic Components: Data presented in Table 4 indicated that an increase on total sugars, total free amino acids, total soluble phenols and proline in shoots of cotton plants grown under different levels of salinity.

Table 3: Effect of spraying with putrescine (Put) and humic acid (HA), salinity treatments and their interaction on chlorophyll a, b, total chlorophyll and total carotenoids concentrations of cotton Giza 90 at 100 days DAP during 2011 and 2012 seasons.

Spray Salinity ppm	C	Put 1ppm	Put 2ppm	HA 1%	HA 2%	Mean A
			Chlorophyll a (mg/g F.W)			
C	1.04	1.05	1.06	1.06	1.05	1.05
3000	1.04	1.05	1.07	1.07	1.04	1.05
6000	0.97	1.00	1.02	1.01	0.98	0.99
9000	0.89	0.90	0.95	0.94	0.90	0.92
Mean B	0.99	1.00	1.03	1.02	0.99	--
LSD 5%	A=N.S		B=N.S		A*B=N.S	
			Chlorophyll b (mg/g F.W)			
C	0.64	0.67	0.72	0.70	0.66	0.68
3000	0.65	0.70	0.74	0.74	0.69	0.70
6000	0.46	0.50	0.56	0.55	0.50	0.51
9000	0.39	0.45	0.49	0.43	0.42	0.44
Mean B	0.54	0.58	0.63	0.61	0.57	--
LSD 5%		A=N.S	B=N.S		A*B=N.S	
			Total chlorophyll (mg/g F.W)			
C	1.69	1.72	1.77	1.86	1.69	1.74
3000	1.71	1.75	1.83	1.78	1.67	1.75
6000	1.45	1.49	1.60	1.60	1.49	1.53
9000	1.29	1.35	1.43	1.42	1.32	1.36
Mean B	1.54	1.58	1.66	1.66	1.54	--
LSD 5%	A=0.024		B=0.027		A*B=0.054	
			Total carotenoids (mg/g F.W)			
C	0.50	0.53	0.58	0.56	0.52	0.54
3000	0.49	0.57	0.62	0.62	0.55	0.57
6000	0.41	0.42	0.47	0.45	0.43	0.44
9000	0.37	0.39	0.44	0.42	0.37	0.40
Mean B	0.44	0.48	0.53	0.51	0.47	--
LSD 5%	A=0.023		B=0.026		A*B=N.S	

C: Control , A= Salinity, B= Spray, A*B= Interaction

Table 4: Effect of spraying with putrescine (Put) and humic acid (HA), salinity treatments and their interaction on total sugars, total soluble phenols, total free amino acids and praline concentrations in shoots of cotton Giza 90 at 100 days DAP during 2011 and 2012 seasons.

Spray Salinity ppm	C	Put 1ppm	Put 2ppm	HA 1%	HA 2%	Mean A
			Total sugars (mg/g F.W)			
C	11.60	12.30	19.66	11.99	14.73	14.06
3000	17.14	14.82	16.47	12.93	11.01	14.47
6000	13.59	16.21	17.88	8.61	16.98	14.65
9000	19.60	12.30	9.34	12.11	12.58	13.19
Mean B	15.48	13.90	15.84	11.41	13.82	--
LSD 5%	A=0.513		B=0.573		A*B=1.147	
			Total soluble phenols (mg/g F.W)			
C	3.37	3.72	4.17	4.11	3.57	3.79
3000	4.13	4.35	4.87	4.56	4.27	4.44
6000	4.72	5.30	4.79	4.66	4.75	4.85
9000	4.75	4.89	5.17	5.13	5.24	5.03
Mean B	4.25	4.57	4.76	4.61	4.46	--
LSD 5%	A=0.168		B=N.S		A*B=N.S	

Table 4: Continue

Spray Salinity ppm	C	Put 1ppm	Put 2ppm	HA 1%	HA 2%	Mean A
			Total free amino acids (mg/g F.W)			
C	7.89	8.80	9.38	8.97	8.32	8.67
3000	8.21	8.77	9.66	9.02	8.55	8.84
6000	8.37	8.92	9.83	9.41	8.65	9.03
9000	8.68	9.32	10.11	9.92	9.13	9.43
Mean B	8.29	8.95	9.75	9.33	8.66	
LSD 5%	A=N.S	B=0.205	A*B=N.S			
			Proline (mg/g D.W)			
C	1.17	1.16	1.11	1.13	1.09	1.13
3000	1.28	1.26	1.21	1.23	1.19	1.23
6000	1.41	1.39	1.34	1.37	1.32	1.37
9000	1.53	1.51	1.42	1.50	1.40	1.47
Mean B	1.35	1.33	1.27	1.31	1.25	--

C: Control , A= Salinity, B= Spray, A*B= Interaction

Increase of free amino acids and proline in response to salt stress was investigated previously by Sharma *et al.* [75].Foliar applications of Put and HA affect positively the concentration of the previous organic constitutes. Application of 2 ppm Put followed 1% HA recorded the highest values of free amino acids, total soluble phenols and proline. Proline is the key osmolyte contributing to osmotic adjustment [92]. It can also improve stress tolerance by protecting and stabilizing membranes and enzymes during stress conditions [93]. Increasing levels of proline by the application Put provided better osmotic adjustment and also helped stabilize membrane and enzymes during stress conditions. Bagga *et al.* [94] suggested that under salt stress Put catabolism (via diamine oxidase) could contribute to proline accumulation. Similar results were also obtained by Verma and Mishra [95], who had also reported that the application of Put increased the activities of antioxidant enzymes, thereby decreasing lipid peroxidation under salt stress. Moreover, Hanafy Ahmed *et al*. [96] showed that, putrescine is a diamine involved in important biological processes such as ionic balance and DNA, RNA and protein stabilization, hence, leading to the enhancement of free amino acids synthesis and accumulation under salinity stress of *Myrtus communis* plants. Also, Ozturk and Demir [97] demonstrated that putrescine application on Spinach leaves increased polyphenol oxidase activity (PPO) and catalase activity (CAT) and proline content, on the other hand, it decrease peroxidase (POD).To elucidate the effects of humic substances, several hypotheses suggesting the formation of a complex between these substances and mineral ions, their involvement in the enhancement of enzyme catalysis, their influence of stimulating respiration, photosynthesis and nucleic acid metabolism and their hormonal activity have been reported Dell'Agnola and Nardi [98]; Nardi *et al*. [99]; Muscolo *et al*. [100]; Serenella *et al*. [101].

Concerning total sugars the data revealed that, increasing salinity level increased the concentrations of the total sugar compared with the unstressed control plants, this finding was obtained under all salinity levels except 6000 ppm which showed a decrease in total sugars. The accumulation of total sugars in salt stressed cotton leaves are in agreement with those reported by Carvajal *et al.* [102] on tomato, Hanafy Ahmed *et al.* [103] on wheat and Hanafy Ahmed *et al.*[97] on *Myrtus communis.* They mentioned that, total sugars concentration was significantly increased with increasing salinity. The reduction of total sugars which noticed at highest salinity level was observed by Trivedi *et al.* [104] and Gadallah [105] on wheat plants who found the same reduction of total sugars under salt stress conditions. These reductions resulted in losses in the photosynthetic activity with a drop in the net formation of carbohydrates. In general, the increment in soluble components among which total sugars due to saline conditions might in turn plays an important role in increasing the osmotic pressure of the cytoplasm. This conclusion is in accordance with the results obtained by Greenway and Munns [106], who stated that, these organic molecules act as osmotica and plays an important role in osmotic adjustment in non-halophytes, moreover, sugars as osmolytes enable plants to keep better water relation under salt stress condition. They also suggested that sugar concentration may be an indicator to the osmoprotectant levels in wheat plant and may contribute to salt tolerance in this system. Also, Kaouther *et al.* [107] concluded that the synthesis of compatible organic solutes occurs in response to salt stress and that these organic solutes could be used as biochemical marker for assessing increased salt tolerance in pepper genotypes.

Foliar applications with Put or HA increased total sugars under different levels of salinity compared to control plants, but the increased in total sugars was not found in all salinity levels. In this respect, plant treated with 1 ppm Put accumulated more total sugars with 3000 and 6000 ppm, while decreased with 9000 ppm. In this regard, the plants sprayed with 2 ppm Put, produced the least sugars concentration under all salinity levels compared with unstressed plants. However, foliar application with 1% HA, it was found that total sugars concentration slightly increased in plants grown under 3000 ppm salinity while it was decreased in plants were subjected under 6000 ppm salinity. Again the sugars concentration increased in response to the highest level of salt stress. In this regard to plants sprayed with 2% HA decreased concentration of sugars in cotton plants grown under all salinity levels, except those grown under 6000 ppm which show an increase in sugars concentration. The only promoting effect of the foliar application on sugar concentration was recorded when the plants treated with 1 or 2 ppm Put and 2% HA subjected under 6000 ppm salinity. This increase, however, disappeared in plants subjected to 3000 and 9000 ppm in previously mentioned treatments. Concerning, application of 1% HA decreased total sugars concentration in cotton plants leaves. The decrease was noticed under all salinity levels compared to unsprayed plants. It is also worth mentioning that with all foliar applications, plants subjected at 9000 ppm salinity failed to accumulate more sugars than unsprayed plants. In this regard, it was found that salinity induced generation of ROS such as the super oxide radical and hydrogen peroxide (H_2O_2) are deleterious to the cellular constituent and further potentate production of hydroxy radicals [108]. The free radicals disrupt normal metabolism through peroxidating lipids, denaturing proteins and nucleic acids [109,110]. Lipid peroxidation causes degradation and impairment of structural components [111]. This leads to change in selective permeability of bio-membranes [112] and thereby membrane leakage and change in activity of enzymes bound to membrane [111]. These hasten the loss of membrane integrity and cell metabolites such as sugar, protein, phenols [113].The controlling of free radicals level are considered to be the way of plant to tolerate the stress [110, 114, 115]. Hence, to explore the potential of chemical(s) to control the free radical generation and thereby membrane damage could be a way to salt stress management in plants. The polyamines (PAs) including Spm (a tetramine), Spd (a triamine) and their obligate precursor Put (a diamine) are implicated in induction of plant adaptation to stresses [116,117]. It has been suggested that PAs may plays an important role in antioxidative system and protect membrane from peroxidation in *in vitro* cultures [118]. Plant protection from oxidative damage by PAs has been considered Chattopadhyay *et al*.[4]. In addition, PAs in general and Put in specific is considered as an antistress and nitrogen source for salt stressed plants [119]. On the other hand, there is a report that different nitrogen sources may act on antioxidative system differentially in different plants [120]. Humic acid as additional N source may play an important role as an anti-stress and nitrogen source for salt stressed plants [119].

Concerning total soluble phenols and total free amino acids the data reveled that, increasing salinity level increased the concentrations of total soluble phenols and total free amino acids compared with the unstressed control plants. This finding was obtained under all levels salinity, when plants sprayed with Put and HA resulted in produced more total soluble phenols and total free amino acids under different salinity levels compared control plants. Similar results was obtained by Hanafy Ahmed *et al*. [96, 121] on *Myrtus communis* and wheat and Verma and Mishra [95] on Indian mustard (*Brassica juncea* L.), they reported that under salinity stress conditions the total soluble phenols and total free amino acids increased with increasing salinity, while treated plants with PAs lead to increase of these constituents in comparison to control plants. Radi *et al.* [122] pointed out the accumulation of the carbohydrate and protein fractions plays an important role in osmotic adjustment in plants under salt stress. Galston [123] mentioned that conjugated PAs are known to be associated with the physiology of flowering, metabolite synthesis and response of the plant to viral infections. Consequently, it can be assumed that the favorable effects of Put on plant growth and yield might be attributed to increases in the concentration of conjugated Put. Similar conclusion was also reported by Bais *et al.* [124] who inferred that Put treatment played an important role in chicory root growth and development and in turn also influenced the production of coumarins and Put conjugates. Hanafy Ahmed *et al.*[125] on snap bean (*Phaseolus vulgaris*, L. cv. Paulista) plants humic acid (20 g/l) as foliar application, reported that adding of 20 g/l HA as foliar application significantly increased total free amino acids and total soluble phenols concentration in shoots of snap bean plants comparison with control plants. Thenmozhi *et al.* [126] declared that, application of HA in combination with inorganic fertilizer increased the quality parameters (shelling percentage, 100-kernel weight, crude protein and oil contents) of groundnut. In addition, physiological mechanisms through which humic substances exert their effects may depend on hormones and, in particular, on the presence of auxin or auxin like components in their structure and, consequently its effect on plant growth and development [36].

Components and Quality of the Yield: Data in Tables 4, 5 and 6 represented the yield and yield components of cotton plants grown under different levels of salinity and spraying with different levels of Put and HA. The results revealed that number of total, open and closed bolls/plant, seed cotton yield/plant and seed index were decreased when subjected under different levels of salinity, except lint percentage it was increased with increasing salinity level. Similar results were obtained by Rezaei *et al.* [127] and El-Dahan [128] on cotton (*Gossypium hirsutum* L.) who found soil that growth and production were different between non-saline and saline soil, as well as the production decreased when growing cotton under saline soil. Seed cotton yield and its components were insignificantly affected by Put and HA influence, except seed index was significantly increased. Generally, it is clear from Tables 5 and 6 that yield and its components reached to highest value when sprayed with 2 ppm Put, 1% HA, 1 ppm Put and 2% HA, respectively. This promoting effect of these previously mentioned foliar applications was found under all different levels of salinity. In this regard, the quality of cotton fiber e.g. fiber length, fiber strength and fineness in response to salt stress as well as foliar applications, the results indicated that, these quality traits were decreased with increasing salinity levels, while there was an enhancement of fiber quality by foliar applications treatments. Here this increase, however, was non-significant except fibre fineness which increased significantly compared with control. It worth to mention that, though there was enhancement in yield components in response to foliar applications, this increase was non-significant. The results are in accordance with those obtained by Govindasmy and Chandresakaran [129] who mentioned that sprayed humic acids extracted from lignite onto sugarcane improved sugar yield. Also, Emara and Hamoda

Table 5: Effect of spraying with putrescine (Put) and humic acid (HA), salinity treatments and their interaction on number of total bolls, open bolls and closed bolls per plantof cotton Giza 90 during 2011 and 2012 seasons.

Spray Salinity ppm	C	Put 1ppm	Put 2ppm	HA 1%	HA 2%	Mean A
			Number of total bolls/plant			
C	8.83	9.50	12.50	11.00	9.50	10.27
3000	7.17	8.50	9.34	9.00	7.67	8.33
6000	6.33	7.17	7.50	7.33	6.84	7.03
9000	1.67	2.00	2.67	2.17	1.84	2.07
Mean B	6.00	6.79	8.01	7.38	6.46	--
LSD 5%	A=N.S		B= N.S		A*B=N.S	
			Number of open bolls/plant			
C	7.84	8.50	11.33	9.50	8.34	9.10
3000	6.00	6.50	7.67	7.34	6.33	6.77
6000	4.84	5.17	5.84	5.67	5.00	5.30
9000	0.67	1.17	2.17	1.84	1.00	1.37
Mean B	4.84	5.34	6.75	6.09	5.17	--
LSD 5%	A= N.S		B= N.S		A*B=N.S	
			Number of closed bolls/plant			
C	1.00	1.00	1.17	1.50	1.17	1.17
3000	1.17	2.00	1.67	1.67	1.33	1.57
6000	1.50	2.00	1.67	1.67	1.84	1.73
9000	1.00	0.84	0.50	0.33	0.84	0.70
Mean B	1.21	1.46	1.25	1.29	1.29	--
LSD 5%	A=N.S		B= N.S		A*B=N.S	

C: Control , A= Salinity, B= Spray, A*B= Interaction

Table 6: Effect of spraying with putrescine (Put) and humic acid (HA), salinity treatments and their interaction on boll weight, seed cotton yield/plant, seed index and lint percentage of cotton Giza 90 during 2011 and 2012 seasons.

Spray Salinity ppm	C	Put 1ppm	Put 2ppm	HA 1%	HA 2%	Mean A
			Boll weight (g)			
C	2.07	2.20	2.39	2.35	2.18	2.24
3000	2.11	2.13	2.19	2.19	2.13	2.15
6000	1.81	1.98	2.05	2.02	1.88	1.95
9000	1.13	1.22	1.73	1.69	1.45	1.44
Mean B	1.78	1.89	2.09	2.06	1.91	--
LSD 5%	A=N.S		B= N.S		A*B=N.S	
			Seed cotton yield/plant(g)			
C	16.15	18.57	26.99	22.31	18.10	20.43
3000	12.55	13.81	16.77	16.03	13.39	14.51
6000	8.65	10.19	11.85	11.42	9.29	10.28
9000	0.90	1.79	3.64	3.07	1.45	2.17
Mean B	9.57	11.09	14.82	13.21	10.56	--
LSD 5%	A=N.S		B= N.S		A*B=N.S	
			Seed index (%)			
C	10.05	10.12	10.24	10.21	10.08	10.14
3000	9.84	10.07	10.11	10.10	10.05	10.03
6000	9.61	9.81	9.92	9.87	9.79	9.80
9000	8.83	9.48	9.71	9.61	9.35	9.40
Mean B	9.58	9.87	10.00	9.95	9.82	--
LSD 5%	A= 0.048		B= 0.054		A*B=0.108	
			Lint percentage (L%)			
C	35.66	34.72	33.76	34.08	35.30	34.70
3000	35.97	35.84	35.73	35.76	35.70	35.80
6000	36.51	36.45	36.08	36.22	36.51	36.35
9000	37.41	37.07	36.68	36.85	36.97	37.00
Mean B	36.39	36.02	35.56	35.73	36.12	--
LSD 5%	A=N.S		B= N.S		A*B=N.S	

C: Control , A= Salinity, B= Spray, A*B= Interaction

Table 7: Effect of spraying with putrescine (Put) and humic acid (HA), salinity treatments and their interaction on fibre length, fibre strength and fineness (Micronaire value) of cotton Giza 90during 2011 and 2012 seasons.

Spray Salinity ppm	C	Put 1ppm	Put 2ppm	HA 1%	HA 2%	Mean A
			Fibre length (mm)			
C	31.091	31.413	31.713	31.655	31.250	31.424
3000	30.101	30.200	30.550	30.472	30.206	30.306
6000	28.932	29.000	29.246	29.145	29.000	29.65
9000	28.110	28.250	28.452	28.374	28.203	28.278
Mean B	29.559	29.716	29.990	29.912	29.665	--
LSD 5%	A=0.650		B=N.S		A*B=N.S	
			Fibre strength (g/tex)			
C	34.315	34.532	34.821	34.753	34.499	34.584
3000	31.634	31.746	32.023	31.953	31.697	31.811
6000	28.841	29.200	29.533	29.417	29.012	29.201
9000	26.773	27.000	27.250	27.180	26.950	27.031
Mean B	30.391	30.620	30.907	30.826	30.540	--
LSD 5%	A=0.394		B=N.S		A*B=N.S	
			Fineness (Micronaire value)			
C	3.847	3.900	4.001	3.950	3.875	3.915
3000	3.751	3.800	3.840	3.835	3.778	3.801
6000	3.663	3.700	3.730	3.722	3.686	3.700
9000	3.500	3.580	3.635	3.615	3.540	3.574
Mean B	3.690	3.745	3.802	3.781	3.720	--
LSD 5%	A=0.006		B=0.007		A*B=0.014	

C: Control , A= Salinity, B= Spray, A*B= Interaction

[130] on Egyptian cotton Giza 86, who reported that humex treatments had significant affects on No. of open bolls/plant, boll weight, seed cotton yield/plant and seed cotton yield/fed in the two seasons, while L% and seed index were non-significantly affected by the tested treatments. On contrary, Beheary *et al.* [131] pointed out that added humic acid on soil or foliar applications improved the yield, yield components and fibre quality under different irrigation intervals. Moreover, Gupta and Gupta [132] showed a promoting effect of putrescine foliar application on growth and yield components of wheat plants under non-stressed as well as water stressed conditions. In soybean, foliar spray of 10^{-3} M polyamines at 50% increased number of pods/plant, 100- seed weight, seed and oil yield [133, 134]. The relationship between PAs and reproductive development has long been established due to the significant increase in their concentrations as plants transition from their vegetative to reproductive stage of growth [135]. Kloareg *et al.* [136] indicated that PAs are indispensable to plants at the time of flowering and early fruit development. Polyamines have been implicated in flower induction [137,138], flower initiation [139], pollination [140], fruit growth and ripening [135]. Sexual differentiation of tissues appears to be dependent on PAs biosynthesis and catabolism, as well as their free or conjugated forms [141]. The importance of polyamines in reproductive development had the attention of many researchers to exogenously applying polyamines in an effort to enhance fruit development. Research for apricot (*Prunus armeniaca* L.), Alburquerque *et al.* [142] showed improved fruit retention and yield with exogenous PAs, the exogenous application of Put on flowers increased the percentage of functional ovules about 16%. Also, exogenous application of Put has been shown to improve yield in litchi (*Litchi chinensis* L.) Stern and Gazit [143].Galston and Kaur-Sawhney [144] reported that the application of exogenous PAs to plants produced visible effects such as the prevention of senescence and the formation of embryos or floral primordial in certain otherwise vegetative tissue. The plant growth regulator BM86, an alternative to PAs, was found to increased cotton yield [145].

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