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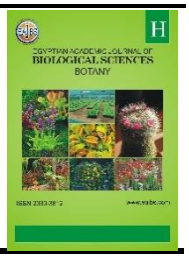

Differential Responses of Common Bean, (*Phaseolus vulgaris* L.) to the Interactive Effects of Ascorbic Acid and *Trichoderma harzianum* under Salinity Stress

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ABSTRACT

Recent ecological perturbations are presumed to be minimized by the application of biofertilizers as a safe alternative to chemical fertilizers. The current study aims to use biofertilizers (*Trichoderma harzianum*) and non-enzymatic antioxidants (ascorbic acid) in combination with NaCl on growth and some metabolic activities as well as esterase enzyme in *Phaseolus vulgaris* cv Bronko. Results showed that NaCl inhibited both fresh and dry weights, shoot and root lengths. Treatment of plants with either ascorbic acid or *Trichoderma* enhanced these parameters. All pigments (Chl.a, Chl.b, Chl.a+b and carotenoids) were enhanced at lower and higher salinity levels. Plants treated with either ascorbic acid or *Trichoderma* promoted carotenoids at moderate and higher levels of salinity. Soluble proteins, soluble carbohydrates and amino acids were increased in shoots and decreased in roots with increasing salinity, treatment of plants with either ascorbic acid *or Trichoderma* reduced these components in shoots. Treatment of plants with either ascorbic acid or *Trichoderma* decreased MDA and significantly increased proline in both shoots and roots. Salinity increased the DPPH% activity in both shoots and roots, and treatment with either ascorbic acid or *Trichoderma* significantly enhanced the scavenging activity of DPPH in both organs. Plants treated with either ascorbic acid or *Trichoderma* exhibited a reduction in phenols and increment in flavonoids, especially with *Trichoderma.* The assessment of esterase isozyme profiles on 7.5% polyacrylamide gel revealed the presence of 6 isoforms in response to water presoaking and treatments with either ascorbic acid or *Trichoderma* under different concentrations of NaCl with different density bands.

INTRODUCTION

Plants undergo various biotic and abiotic stresses during their life cycle among many abiotic stresses, drought, heavy metal, and oxidative stress, salinity is the most typical abiotic stress and also plays a vital role as a growth-limiting factor for most non-halophytic plants which impaired physiological and biochemical processes that has a direct impact on the yield and productivity of crops (Mahajan and Tuteja, 2005, Arzani and Ashraf, 2016, Ma *et al.*, 2020 and Hafeez *et al.*, 2021). The ability to tolerate salinity differs among plant species and ultimately affects many aspects of plant metabolism (Munns and Tester 2008). Many studies have shown that salinity stress reduced plant growth, leaf area, fresh and dry weights of the shoot system (Taffouo *et al.*, 2010, Helaly *et*

al., 2017). Salinity stress-induced reduction in growth parameters undergoes osmotic regulation and affected many biochemical processes such as photosynthetic pigments and protein content (Kapoor and Srivastava, 2010, Rady *et al.,* 2019). Proline is a compatible solute that accumulated in salt-tolerant plants under stress conditions and acts as an osmoprotectant (Munns and Tester 2008). Salt stress causes an imbalance in cellular ions, releasing ion toxicity which leads to ROS overproduction. This causes damage to DNA, lipids, and proteins, concurrently with chlorophyll degradation and membrane lipid peroxidation, decreasing membrane fluidity and selectivity (Rady *et al.,* 2018). To overcome these ROS effects, plants have developed various antioxidant defense systems including non-enzymatic (e.g., proline, AsA, carotenoids, etc.) and enzymatic (e.g. SOD, CAT, POD, etc.) antioxidants (Hodges *et al.,* 1997). Plants with high levels of constitutive and/or induced antioxidants have been reported to have greater capacity tolerance to oxidative damage. It has been suggested that the extent of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of their antioxidant systems (Rady *et al.,* 2018).

Biofertilizers such as plant growth-promoting rhizobacteria (PGPR), *Trichoderma* played a very important role in yield improvement. The use of PGPR and *Trichoderma harzianum* biofertilizers may be useful in developing strategies to facilitate plant growth in saline soils (Shaharoona *et al.***,** 2007, Abd El-Baki and Mostafa, 2014, Zhang, *et al.,* 2019, Niu *et al.,* 2020 and Negi *et al.,* 2021). Plant beneficial fungi of the genus *Trichoderma* have been successfully used worldwide both to control fungal diseases and to promote plant growth. *Trichoderma spp*. represent a fundamental component of the rhizosphere microbiome because these fungi help plants to overcome numerous environmental constraints by stimulating defense responses including the secretion of antimicrobial reactive oxygen species (ROS), the production of secondary metabolites (Shoresh *et al.,* 2010) and improving fitness and development (Hermosa *et al.,* 2013). These abilities have supported the application of *Trichoderma* strains as biocontrol agents or plant biostimulants in agriculture.

The effect of ascorbate on plant growth has been extensively studied by Niakan *et al.* (2012). Ascorbate is an important antioxidant in plants and much literature showed that it has an essential role in several physiological processes in plants, including growth, differentiation, and metabolism (Noctor & Foyer, 1998 and Horemans *et al.,* 2000). Ascorbate functions as a reductant for many free radicals because of its ability to donate electrons in a number of enzymatic and non-enzymatic reactions (Gill & Tuteja, 2010). Ascorbate enhances plant tolerance to environmental stressors such as saline stress (Khan *et al.,* 2011; Ejaz *et al.,* 2012, Abbasi and Faghani, 2015; Cai *et al.,* 2016). Among the most important Fabaceae crops, the common bean (*Phaseolus vulgaris L*.) is a legume food that produces a significant amount of seeds rich in the protein content needed for human nutrition (Rady *et al.,* 2013). The common bean plant is classified as salt-sensitive, suffering yield losses at soil salinity of less than 2 dS m^{-1} (Pessarakli, 1999).

The understanding of physiological responses of common bean (*Phaseolus vulgaris* cv Bronko) under salinity may help in programs that aimed to improve the grain yield under salinity stress. Therefore, the aim of this study was to assess the effect of biofertilizers and ascorbic acid as presoaking integrated with foliar spray on the changes in growth and green yield, physio-biochemical components, and the antioxidant defense systems of common bean plants grown under salt stress.

MATERIALS AND METHODS

Seeds of common bean (*Phaseolus vulgaris* cv. Bronko) were obtained from the Seed Center affiliated with the Directorate of Agriculture in Minia. Seeds were selected for

uniformity by choosing those of equal size and with the same color. The selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water. The seeds were left to dry at room temperature at (28-30 ^{0}C) on filter paper for about two days and then divided into three groups, one of them was soaked in water for three hours, and the second group was soaked in ascorbic acid (200 ppm) also for three hours, the third group was coated with *Tricoderma harzianum* (T24) during sowing. Five uniforms air-dried common bean seeds (*Phaseolus vulgaris* cv. Bronko) were sown along a centre row in each pot at 30-mm depth in plastic pots, each filled with about 4 kg mixed (clay sandy soil) within a proportion of 2:1 (v:v), respectively in order to reduce compaction and improve drainage. Plants were left to grow for 3 weeks and then treated with different concentrations of NaCl (0.0, 50, 100, 150 and 200 mM of NaCl) and irrigated with tap water, according to its measured field capacity with top irrigation, then left to grow further for 60 days from sowing.

Plant samples were collected after 60 days from sowing for measurement of some growth parameters i.e. (leaf area, plant height, root length, fresh and dry weights of shoots and roots) photosynthetic pigments, carotenoids, soluble sugars, soluble proteins, total free amino acids, MDA, proline, total phenolics, total flavonoids, DPPH% activity, Ascorbic acid, Esterase profile. Chlorophyll a, chlorophyll b and carotenoids were determined using the spectrophotometric method described by Lichtenthaler, (1987). Total free amino acids were determined according to the method of Moore and Stein (1948), soluble sugars by anthrone sulphuric acid method which was carried out by Fales (1951) and Schlegel (1956) and adopted by Badour (1959. The soluble proteins were determined according to the method adopted by Lowery *et al.* (1951). Leaf areas were carried out according to McKee, (1974). The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content. MDA is a product of lipid peroxidation following the method of Heath and Packer (1968), expressed as n mol (MDA) g^{-1} (fresh weight). Total phenolic contents according to Reis *et al*, 2012, and Total flavonoids according to Jia *et al.*, 1999, 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH$^{\cdot}$) were assayed according to Yi et al. 2008 and ascorbic acid was assayed according (Ismail *et al.*, 2014). Preparation of polyacrylamide gel electrophoresis (PAGE) and staining of esterases, the plant tissue was homogenized in buffer (0.1 M Tris-HCl with 2 mM EDTA pH7.8) and centrifuged at 13000 g for 15 min. Esterase (EST, EC 3.1.1.x) isoforms were detected by staining with α-naphthyl acetate/Fast blue RR according to Tanksley and Orton (1983).

Statistical analysis:

The data of all experiments were subjected to a one-way analysis of variance and means were compared using the least significant difference test (L.S.D.) using a statistical program (Sta. Base. Exe.) on the computer (Steel and Torrie 1960).

RESULTS

The data in Figure (1) showed the differential effects of NaCl on (fresh matter, dry matter and plant length) in both shoot and root of *Phaseolus vulgaris* cv Bronko presoaked in either water or ascorbic acid and others inoculated with *Trichoderma harzianum* (T24). In water presoaked plants salinity had a remarkable inhibitory effect only at 200 mM NaCl which reduced fresh, dry and shoot length to (56.57%, 47.96% and 88.64%) and (66.72%, 61.54%, 76.19%) in both shoot and root respectively compared to absolute control. However lower and moderate salinity levels had a slight promoting effect on these parameters. Treatment of plants with either ascorbic acid or *Trichoderma* enhanced these parameters which reached (119.40% and 114.44%) with *Trichoderma* at 50 mM NaCl in shoot fresh and dry matter respectively compared to absolute control. In roots

treated with ascorbic acid or inoculated with *Trichoderma* the promotion of these parameters was pronounced at almost salinity levels used.

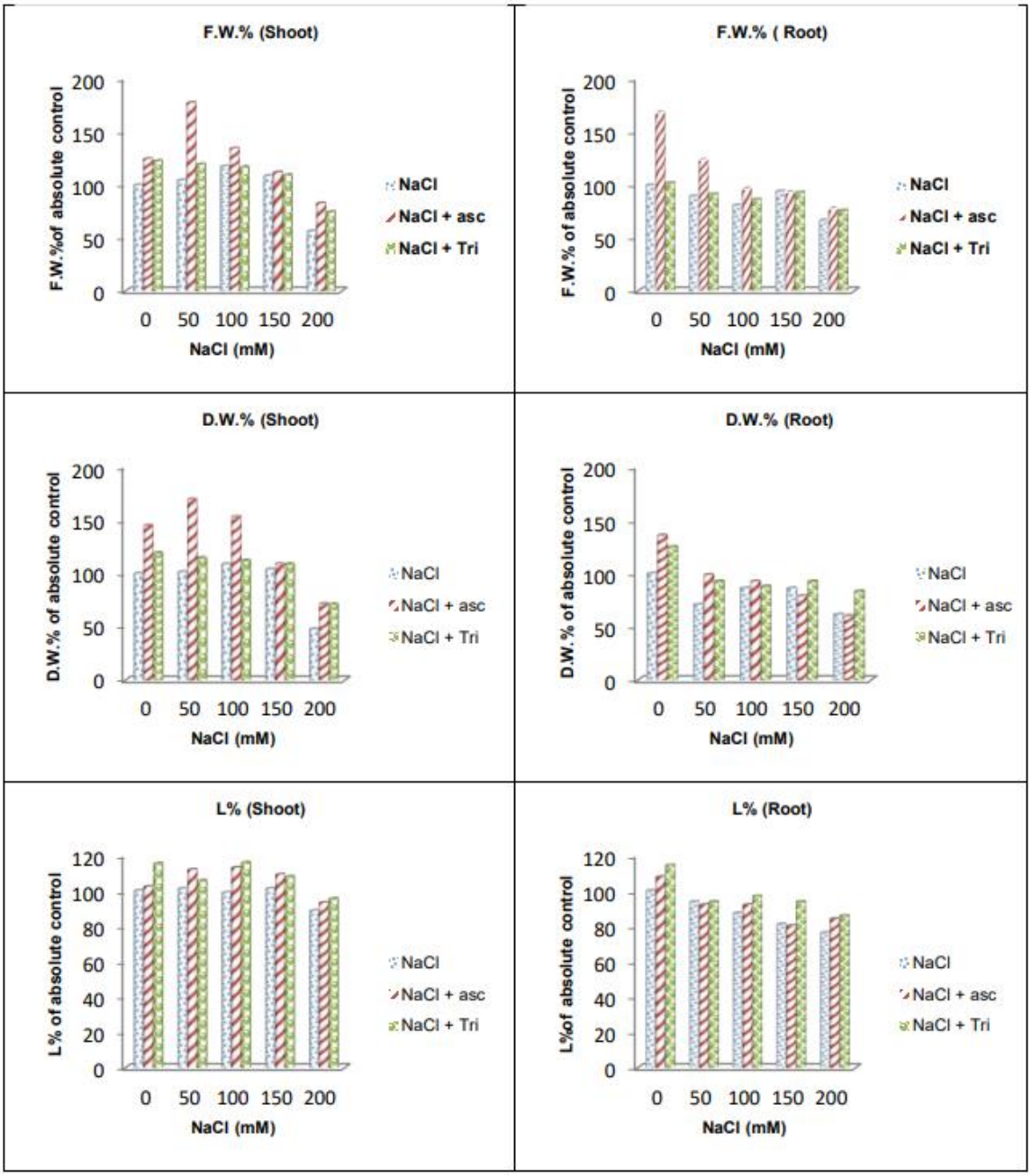

Fig. (1): Fresh (F.W. %) and dry (D.W. %) matter (g), length (L %) of shoots and roots of *Phaseolus vulgaris* cv Bronko presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data mean of 3 replications.

The data presented in Figure (2) concluded that all pigment contents (Chl.a, Chl.b, Chl.a+b and carotenoids) were enhanced at lower and higher salinity levels in plants presoaked in water, however moderate salinity levels keep the values near to absolute control. At lower salinity levels 50 mM NaCl, the significant enhancement reached to 149.13%, 140%, 147.77% and 139.37% in both (Chl.a, Chl.b, Chl.a+b and carotenoids) respectively compared with absolute control. At higher salinity levels 200 mM NaCl all pigment fractions gained higher values reached to (114.24%, 108.86%, 113.41% and 110.78%) respectively comparable with absolute control. The treatment of plants with

either ascorbic acid or *Trichoderma* did not affect the pigment contents whatever the treatment used or pigment fraction analyzed except for carotenoids which exhibited a slight promotion at moderate and higher levels of salinity with either ascorbic or *Trichoderma*. This reflects the protecting role of this fraction under stress conditions. It is worth mentioning that the promotion effect of *Trichoderma* is better than ascorbic acid, especially at moderate salinity levels used.

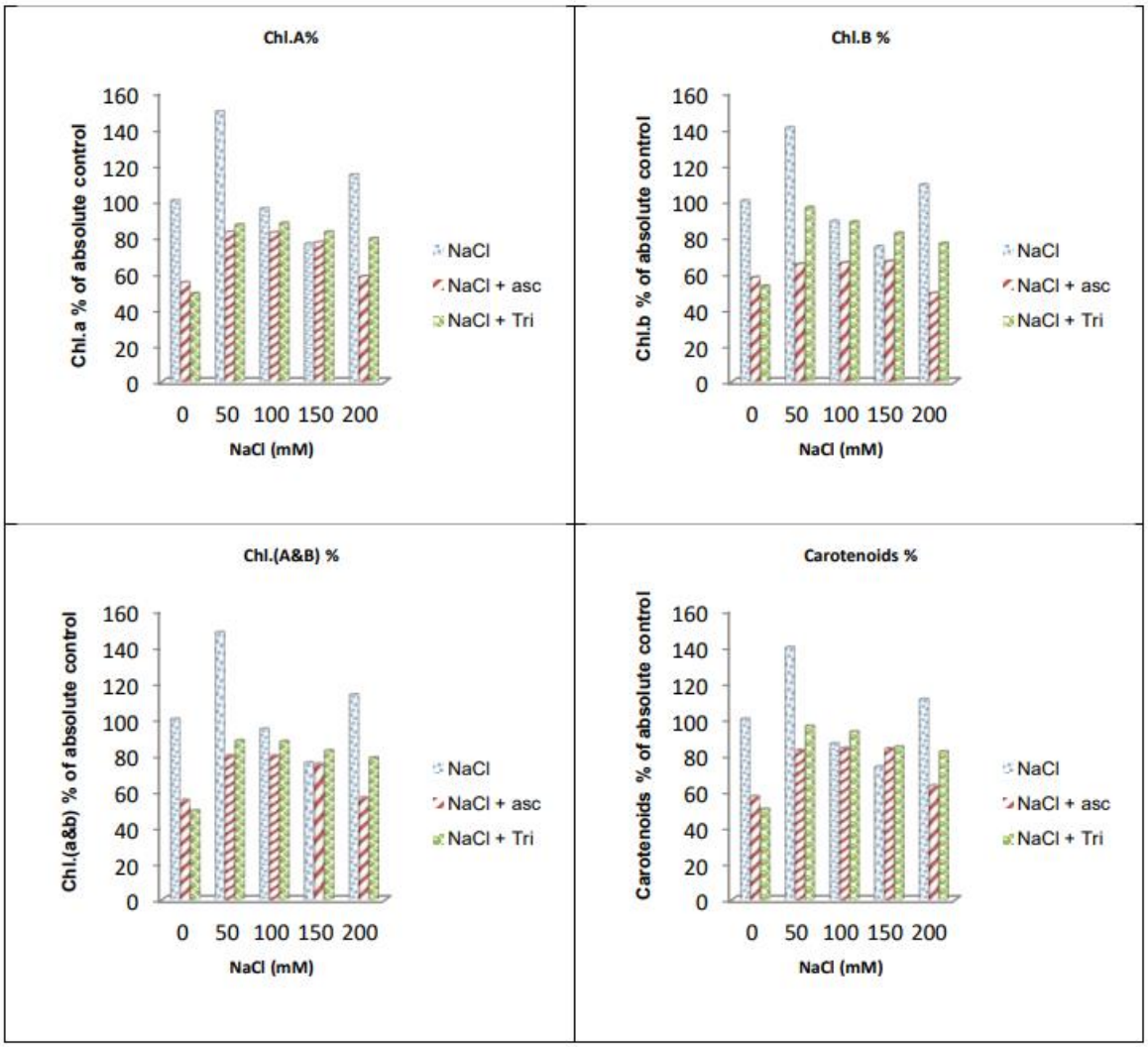

Fig. (2): Pigment contents (mg/g dry matter) of *Phaseolus vulgaris* cv Bronko presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data mean of 3 replications.

The data in Table (1) revealed the effect of presoaking with water or ascorbic acid and others inoculated with *Trichoderma* on water content and leaf area. In water presoaked plants, salinity had various effects on both shoot and root water content, increasing it slightly in shoots and pronouncedly enhancing it in roots reached to 112.54% from absolute control at 200 mM NaCl. The leaf area remained unchanged at lower and moderate levels of salinity but reduced sharply at higher levels of salinity in plants presoaked in water. Treatment of plants with ascorbic acid enhanced leaf area only at a higher salinity level (200 mM NaCl) compared with reference control values.

Table 1: leaf area (cm^2) and water content of shoots and roots of *Phaseolus vulgaris* cv Bronko presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data mean of 3 replications.

	NaCl (mM)	L.A	%	W.C Shoot	%	W.C Root	%
Absolute	**0**	**33.39**	**100**	**84.82**	**100**	**78.93**	**100**
Reference	50	33.63	100.72	86.04	101.43	80.74	102.30
	100	33.62	100.69	86.74	102.26	84.60	107.18
	150	26.32	78.83	87.22	102.82	89.38	113.24
	200	18.71	56.03	86.71	102.22	88.82	112.54
Ascorbic	0	27.98	83.80	85.33	100.60	85.47	108.29
	50	28.01	83.89	86.88	102.43	85.99	108.94
	100	25.97	77.78	86.77	102.29	87.90	111.37
	150	25.46	76.25	87.48	103.13	88.50	112.13
	200	24.74	74.09	88.36	104.17	86.14	109.13
Trichoderma	0	28.62	85.71	87.47	103.12	88.13	111.66
	50	24.87	74.48	88.38	104.19	89.62	113.54
	100	27.78	83.20	88.34	104.15	90.65	114.85
	150	21.71	65.02	88.49	104.32	91.47	115.89
	200	15.65	46.87	88.79	104.68	89.17	112.98
LSD at 5%		**1.70**		**0.43**		**1.5**	
LSD at 1%		**2.5**		**0.64**		**2.2**	

The data in Figure (3) illustrated soluble proteins, soluble carbohydrates and amino acids. All fractions were increased in shoots and decreased in roots with increasing salinity except for soluble proteins which remained more or less unchanged along the salinity levels used. Treatment of plants with either ascorbic acid *or Trichoderma* resulted in a reduction of these components in shoots except for soluble proteins which increased slightly with *Trichoderma* treatments. In roots, both soluble proteins and amino acids were increased, however, soluble carbohydrates were decreased under the same treatments. It is worth mentioning that the amount of soluble carbohydrates in shoots is much higher than that of roots especially plants presoaked in water compared to others treated with *Trichoderma* or ascorbic acid.

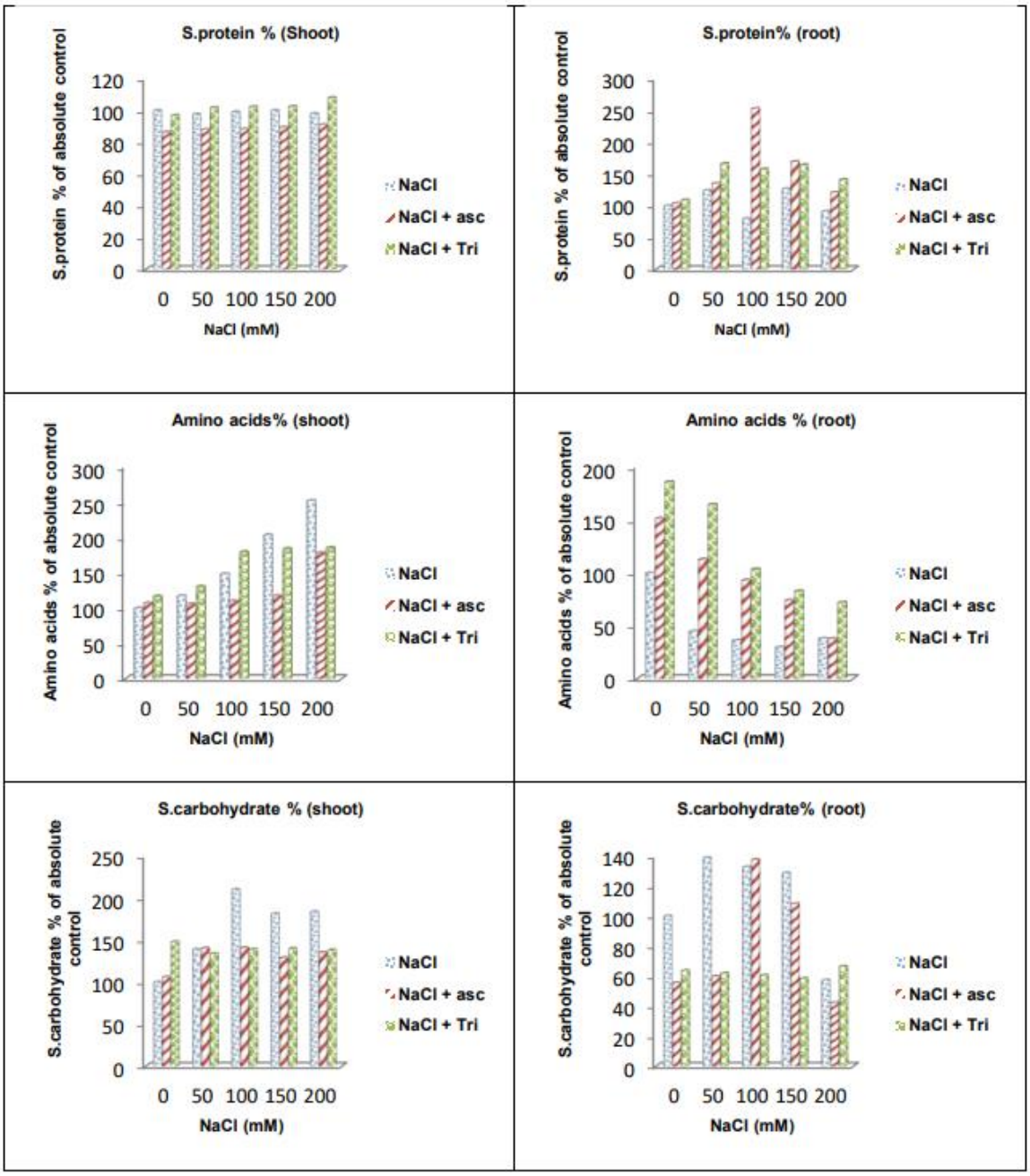

Fig. (3): Soluble proteins, Amino acids and Soluble carbohydrates (mg/g dry matter) in both shoots and roots of *Phaseolus vulgaris* cv Bronko presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data mean of 3 replications.

The data in Table (2) showed the effect of salt stress and combination with ascorbic acid or *Trichoderma* on MDA and proline contents in both plant organs of *Phaseolus vulgaris* cv Bronko. The data clearly demonstrated that both proline and MDA were increased with increasing salinity in both shoots and roots of water-presoaked plants. Treatment of plants with either ascorbic acid or *Trichoderma* decreased MDA and significantly increased proline in both shoots and roots. It is worthy to mention that, proline accumulated with higher content in shoots with either ascorbic or *Trichoderma* reaching 286.9% and 320.1% at 200 mM NaCl with ascorbic or *Trichoderma* respectively comparable with absolute control values.

Table 2: MDA content (n mol g^{-1} Fw) and Proline (mg/gm DW) of shoots and roots of *Phaseolus vulgaris* cv Bronko presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data mean of 3 replications.

	NaCl (mM)	Shoot				Root			
		MDA	%	Proline	%	MDA	%	Proline	%
Absolute	**0**	**144.10**	**100**	**0.42**	**100**	**227.85**	**100**	**0.15**	**100**
Reference	50	183.42	127.28	0.52	123.30	225.70	99.06	0.18	115.25
	100	182.90	126.93	0.61	142.47	245.93	107.94	0.23	148.59
	150	171.50	119.01	0.95	225.26	257.30	112.93	0.23	149.15
	200	158.10	109.72	1.13	267.94	297.08	130.39	0.17	111.86
Ascorbic	0	136.40	94.66	0.66	156.08	107.90	47.36	0.28	182.49
	50	121.93	84.62	0.61	144.12	111.08	48.75	0.29	188.70
	100	110.57	76.73	0.80	189.69	112.11	49.20	0.24	153.67
	150	106.40	73.84	0.98	232.37	128.13	56.23	0.26	168.93
	200	98.17	68.12	1.21	286.91	123.48	54.19	0.21	139.55
Trichoderma	0	132.70	92.09	0.55	130.52	73.37	32.20	0.25	163.28
	50	146.73	101.83	0.55	129.48	76.46	33.56	0.27	178.53
	100	153.97	106.85	1.13	267.94	82.15	36.05	0.26	168.93
	150	113.15	78.52	1.25	296.39	97.13	42.63	0.24	153.11
	200	112.63	78.16	1.35	320.10	98.17	43.08	0.20	127.12
LSD at 5%		**4.6**		**0.14**		**3.1**		**0.06**	
LSD at 1%		**6.7**		**0.2**		**4.4**		**0.09**	

Table 3: Antioxidant activity (DPPH%) of shoots and roots of *Phaseolus vulgaris* cv Bronka presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data mean of 3 replications.

	NaCl (mM)	DPPH %			
		Shoot	%	Root	%
Absolute	**0**	**74.00**	**100**	**36.36**	**100**
Reference	50	76.27	103.07	43.69	120.14
	100	76.61	103.54	44.31	121.85
	150	77.49	104.72	44.07	121.18
	200	78.53	106.13	40.78	112.15
Ascorbic	0	76.79	103.77	31.82	87.50
	50	78.80	106.49	45.58	125.35
	100	80.10	108.25	48.73	134.01
	150	90.58	122.41	48.99	134.72
	200	86.74	117.22	49.24	135.41
Trichoderma	0	84.64	114.39	32.95	90.63
	50	87.26	117.92	43.69	120.14
	100	84.29	113.91	46.21	127.08
	150	84.99	114.86	41.16	113.19
	200	83.51	112.85	56.44	155.21
LSD at 5%		**5.39**		**7.44**	
LSD at 1%		**7.81**		**10.78**	

The data in Figure (4), is concerned with phenols, flavonoids and ascorbic acid in both shoots and roots of common bean plants. Phenols and flavonoids increased in both shoots and roots with increasing salinity, especially at lower and moderate levels of salt, however, ascorbic acid remained more or less unchanged with all treatments in reference plants but exhibited a remarkable increase in both organs with *Trichoderma* treatments reached to 116.67% and 108.3% in both shoots and roots respectively at 200 mM NaCl. Treatment of plants with either ascorbic acid or *Trichoderma* exhibited a reduction in phenols and increment in flavonoids, especially with *Trichoderma* at all salinity levels used.

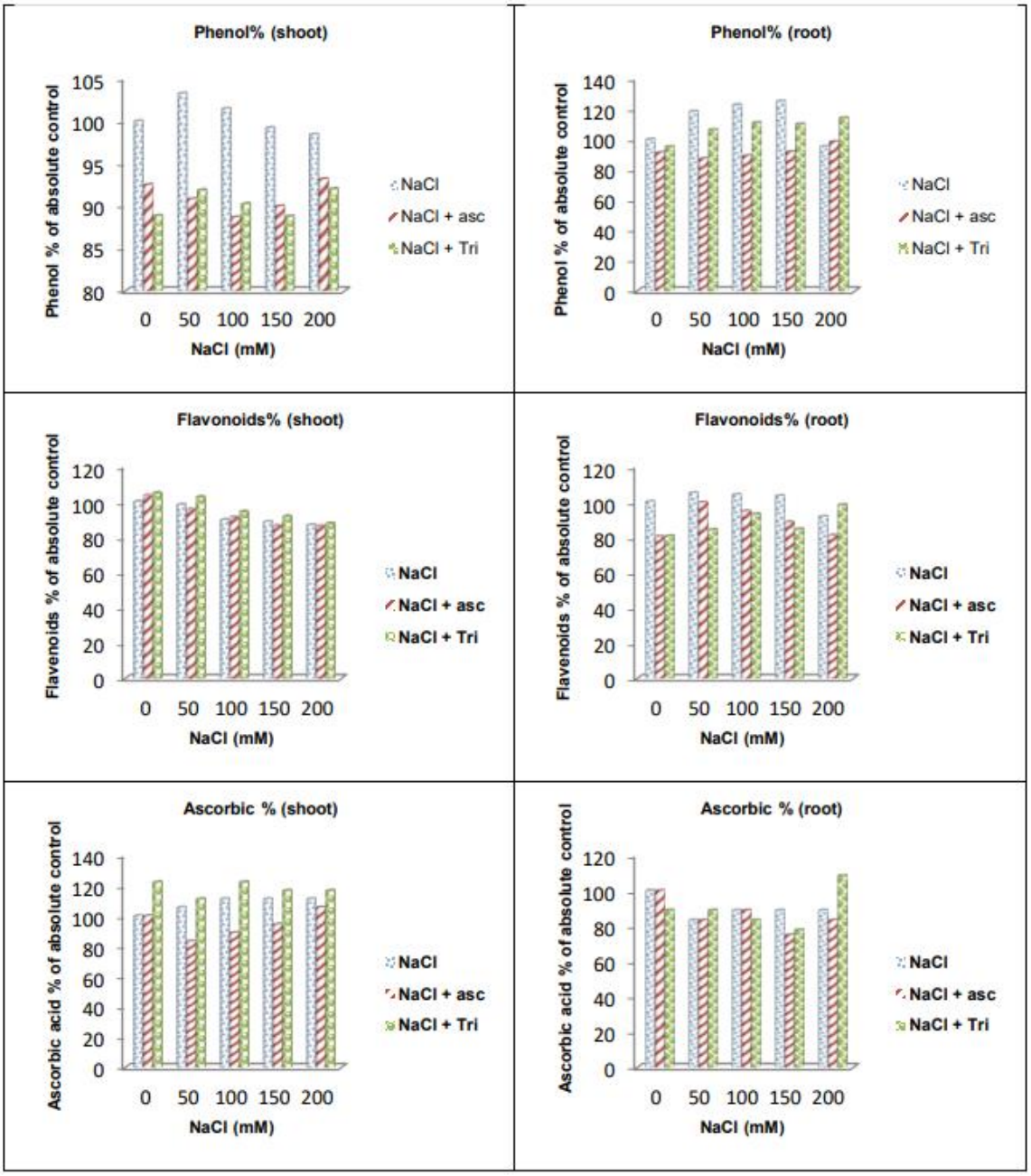

Fig. (4): Total phenolics (mg Ga /g DW), flavonoids (mg Qu /g DW) and ascorbic acid (mg/gm DW) of shoots and roots of *Phaseolus vulgaris* cv Bronko presoaked in water, ascorbic acid and coated with *Trichoderma* under different concentrations of NaCl. Data mean of 3 replications.

The data in **Table (3**) showed the effect of salt treatment on plants presoaked in water or ascorbic acid and others inoculated with *Trichoderma* on the percentage activity of DPPH in both organs. Salinity increased the DPPH activity in both organs shoots and roots at all salinity levels used, however treatment with either ascorbic acid or *Trichoderma* significantly enhanced the scavenging activity of DPPH% in both organs along the salt levels used.

The assessment of esterase isozyme profiles on 7.5% native polyacrylamide gel revealed the presence of 6isoforms in *Phaseolus vulgaris* cv Bronko in response to water presoaking and treatments with either ascorbic acid or *Trichoderma* under different concentrations of NaCl Figure (5). The increase of band intensity, the appearance of new

bands and the disappearance of esterase bands may be an indication of an increase in activity response or tolerant strategy to treatments with ascorbic acid or *Trichoderma* under different concentrations of NaCl. The difference in density and number of bands are more pronounced under different concentrations of salinity which is more obvious under treatments of ascorbic acid and *Trichoderma*, however, *Trichoderma* was more effective than all salinity levels. The presence of 6 isozymes under control and treated plants reflects the role of these enzyme isoforms in salt stress resistance.

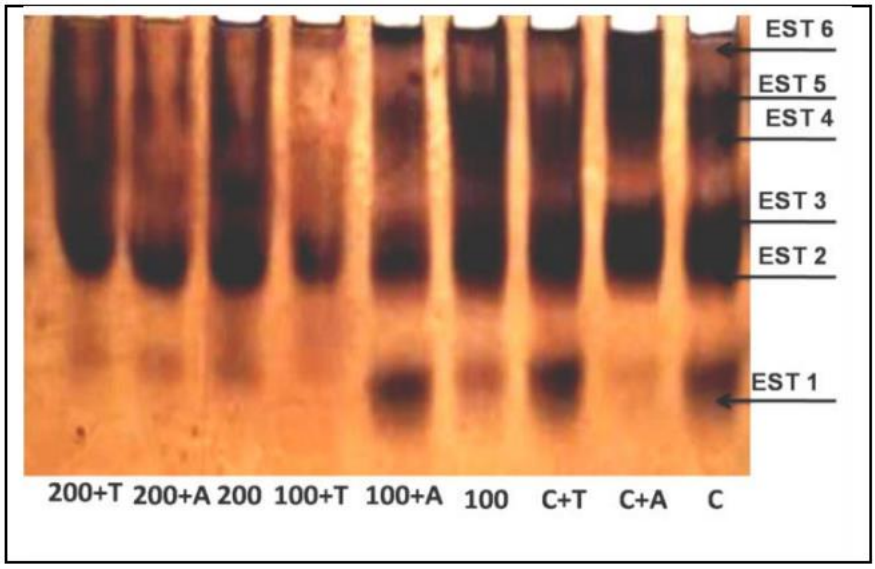

Fig. (5): Esterase profile showing different esterase isozymes C: control, C+A: (Control+Ascorbic), C+T: (Control+ *Trichoderma*), 100: (100 mM NaCl), 100+A: (100 mM NaCl +Ascorbic acid), 100+T: (100 mM NaCl+ *Trichoderma*), 200: (200 mM NaCl), 200+A: (200 mM NaCl+Ascorbic acid) and 200+T (200 mM NaCl +*Trichoderma*).

DISCUSSION

Various morphological and physiological factors in common bean *Phaseolus vulgaris* cv Bronko were negatively affected by salinity stress, while treatment with either ascorbic acid or *Trichoderma harzianum* (T24) mitigated these effects. Both fresh, dry, as well as shoot and root lengths, were decreased with increasing salinity. The reduction in this parameter reached 56.57%, 47.69%, 88.64%, 66.72%, 61.54 and 76.19 at 200 mM NaCl in both shoot and root respectively compared with absolute control. Treatment with either ascorbic acid or *Trichoderma* enhanced all these parameters. The enhancement with *Trichoderma* reached to 74.63%, 71.12%, 95.45%, 76.03%, 83.08% and 85.71% at 200 mM NaCl respectively in both shoot and root compared with absolute control. Salinity stress induces osmotic and ionic stress that leads to retarded growth in terms of both shoot and root length, fresh and dry weight, reduced pigment content and hampers uptake of mineral elements (Ahmad *et al.,* 2014; Ma *et al.,* 2020). In the same manner, Younis *et al.* (2010) reported that the growth reduction caused by salinity stress is due to inhibition of apical growth in plants as well as an imbalance of endogenous hormones. The enhanced production of ROS during salinity stress leads to progressive oxidative damage and ultimately cell death and growth suppression (Ruiz-Lozano *et al.,* 2012).

AsA treatment increases the content of IAA, which stimulates cell division and/or cell enlargement and this, in turn, improves plant growth (Khan *et al.* 2011). Working with bean plants treated with *Trichoderma velutinum*, Mayo *et al.,* (2016) found that plants inoculated with this fungus showed a significant increase in dry weight in both shoots and roots by inducing the expression of defense-related genes. The enhancement of

plant biomass by promoting the growth of lateral root has been observed in many plant species treated with *Trichoderma* spp. and this effect has also been related to the production of indole-3-acetic acid (IAA) or auxin analogues Vinale *et al.,* (2012). According to Ahmad *et al.* (2015), the application of *T. harzianum* restored mustard plant height. Similar results were obtained by Hashem *et al.* (2011), who mentioned that stigmasterol caused stimulation of plant height and leaf area.

All pigment fractions (Chl.a, Chl.b, Chl.a+b and carotenoids) were enhanced at lower and higher salinity levels in plants presoaked in water, however moderate salinity levels keep the values near-absolute control. The treatment of plants with either ascorbic acid or *Trichoderma* enhanced carotenoids at moderate and higher levels of salinity which synergistically function with ascorbic acid to provide an effective barrier against oxidation under salinity stress. The reduction of chlorophylls under salinity stress was attributed to salt-induced acceleration of enzymes responsible for chlorophyll degradation and the instability of the pigment-protein complex (Hernandez and Almansa 2002, Duarte *et al.,* 2013). Directly or indirectly the reduction of photosynthesis affects the pigments and chlorophyll contents of plants (Parida and Das 2005). Among the positive effects of ascorbic acid in the counteraction of the adverse effects of salt, and stress is the stabilization and protection of the photosynthetic pigments and the photosynthetic apparatus from oxidization damage (Khan *et al.,* 2011). Application of *Trichoderma harzianum* has restored the chlorophyll and carotenoids content to appreciable levels in the present study and the results corroborate with the findings of Zhang *et al.* (2013). *Trichoderma harzianum* increases the uptake of essential elements especially Mg2+ that are negatively affected by NaCl stress; hence the chlorophyll synthesis increases in *Trichoderma* inoculated plants especially we found an increment in Mg^{+2} contents in our results data not shown.

Water content increased in both shoots and roots with increasing salinity however, treatments with either ascorbic acid or *Trichoderma* enhanced the water content . Zhang *et al.,* 2016, postulated that wheat seeds treated with *Trichoderma longibrachiatum* (T6) increased relative water content in the leaves and roots. In the same context, Khafagy *et al.* (2009), and Azzedine *et al.* (2011) reported that ascorbate mitigated the inhibitory effect of salt stress on plant growth due to increased leaf area, improved chlorophyll and carotenoid contents, and enhanced antioxidant accumulation. The primary plant response to salinity is a reduced leaf surface expansion, followed by a diverse closure of stomata as the stress intensifies and finally leads to photosynthesis inhibition (Parida and Das, 2004).

Soluble proteins, soluble carbohydrates and amino acids were increased in shoots and decreased in roots with increasing salinity. Treatment of plants with either ascorbic acid or *Trichoderma* resulted in a reduction of these components in shoots except for soluble proteins. In roots, both soluble proteins and amino acids were increased, however, soluble carbohydrates were decreased under the same treatments. Accumulation of soluble carbohydrates, proteins and amino acids might have a physiologically important role in energy supply, osmotic adjustment to maintain leaf water potential, increase stress tolerance and decrease osmotic shock (Babaeian *et al.,* 2011; Ma *et al.,* 2020). Ejaz *et al.* (2012) stated that sugarcane soluble protein contents were increased by salt stress. Ebrahim, 2005 found that salinity decreased the soluble protein content of *Vicia faba* and sugar beet exposed to salt stress. According to the plant species or cultivar, the stress conditions make changes in soluble protein contents due to the cell structural modifications Wimmer *et al.,* 2003.

Proline increased significantly with increasing salinity in both shoots and roots of water-presoaked plants however MDA increased in both shoots and roots at all salinity levels used compared with absolute control. Treatment of plants with either ascorbic acid or *Trichoderma* decreased MDA in both shoots and roots and increased proline in the same

organs. Malondialdehyde content can serve as an indicator of the rate of oxidative processes in cells. In contrast, salinity stress increased malondialdehyde whether in leaf tissue or root. Malondialdehyde results indicated that lipid peroxidation started to increase at 100 mM NaCl in roots. Accumulation of malondialdehyde was reported in a number of salt-sensitive plants (Luna et al., 2002, Gehlot *et al.,* 2003). In this study, proline content increased in NaCl-treated plants and reached 267.94% in shoots at 200 mM NaCl compared to absolute control confirming its contribution to osmotic adjustment (Delauney and Verma, 1993, Mervat *et al.,* 2013). Proline protects membranes and proteins against the adverse effects of high concentrations of inorganic ions and temperature extremes (Rudolph et al., 1986; Santoro et al., 1992; Ma et al., 2020). Proline may also function as a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989)). Proline is also an important osmolyte that maintains the cell osmoregulation in NaCl stress (Rasool *et al.,* 2013 and Yan *et al.,* 2017).

Phenols and flavonoids increased in both shoots and roots, with increasing salinity, especially at lower and moderate levels of salt, however ascorbic acid remained more or less unchanged with all treatments in reference plants but exhibited a remarkable increase in both organs with *Trichoderma* treatment. We found differences in the accumulation of flavonoids, phenols and ascorbic acid in both plant parts depending on the interaction conditions used either ascorbic or *Trichoderma*. Plant with a higher amount of ascorbic acid content showed better protection against oxidative stress, influencing many enzyme activities, and minimizing the oxidative damage through synergetic function with other antioxidants, Foyer and Noctar, (2005). Flavonoids protect plants against various biotic and abiotic stresses, playing an important role in the interaction between the plant and the environment [Pourcel, *et al.,* 2007]. The differences in the pattern of flavonoid accumulation observed in the metabolome of the bean could reflect the different metabolic pathways induced by the beneficial fungus *Trichoderma* or treatment with ascorbic acid. We found that the application of ascorbic acid or *Trichoderma* at lower salinity levels of 50 mM NaCl increased total phenolics and total flavonoids. These results confirmed those of Jaleel *et al.,* 2009 and Salama *et al.,* 2014 in *Phaseolus vulgaris* under salt stress. Application of ascorbic acid at a dose of 200 or 400 mg via foliar spray significantly increased total phenolics, total flavonoids, and total tannins in *Phaseolus vulgaris* under water stress conditions Alaa *et al.* (2020). In general, the production of phenol compounds in plants under abiotic stress depended on the type of stress, stress intensity, stress duration, plant development stages and plant part type, i.e., whole seedlings or plant parts, namely roots or leaves Weidner, *et al.,* (2011).

Antioxidant activity (free radical scavenging activity by DPPH%) increased in both organs shoots and roots at all salinity levels used, however treatment with either ascorbic acid or *Trichoderma* increased the scavenging activity of DPPH% in both organs along the salt levels used. The decrease in absorbance of the DPPH% radical caused by antioxidants can be ascribed to the action between an antioxidant molecule and the radicle. In addition, the further increase in DPPH activity and endogenous ascorbic acid was a result of strengthening the antioxidative defense system followed by an increased tolerance of bean plants to salt stress. Ascorbic acid may be participated in the up-regulation of soluble sugars and proline synthesis to enhance tolerance mechanisms under salt stress. The increase in the antioxidant activity was attributed to the increases of secondary metabolites (phenolics and flavonoids) which are considered a tolerance advantage of biotic stresses as reported by El-Amier *et al.* (2019).

The presence of 6 esterase isoforms in *Phaseolus vulgaris* cv Bronko presoaked in water or treated with either ascorbic acid or *Trichoderma* under different concentrations of NaCl, the increase of band intensity, the appearance of new bands and disappearance of other esterase bands may be an indication of an increase in activity response to presoaking

in water or treatment with either ascorbic acid or inoculation with *Trichoderma* under various NaCl concentrations. Gigova. *et al*. (2012) reported the isoenzyme pattern and changes in the esterase activity of Synechocystis under different growth conditions four clearly visible bands and eight weaker bands of EST activity were detected by gels analysis. *Phaseolus vulgaris* cv Bronko contains several forms of esterase with a broad range of functions, hydrolyzing ester bonds in different types of metabolites. Multiple forms of esterase and their differential expression during stress conditions suggested their important role in several physiological processes Coppens and Dewitte (1990). Hydrolytic enzymes such as esterase participate in altering cell walls Cosgrove, (2001)**.** The role of esterase in many cases is associated with cell wall metabolism but little is known about their intracellular function. One of their most interesting functions in the cytoplasm is the metabolism of many pesticides and pollutants entering the cell Cummins *et al*, (2001). Mukherjee *et al.* (2004) reported that esterase variation of *Lemna minor* is a potential biomarker of heavy metal pollution. Precise localization, isolation and characterization of *Trichoderma* or ascorbic acid-induced esterase isozymes are required for the understanding of their role during salt stress. These results are in agreement with Hassanein (1999), who found that salinity increases esterase isozymes and the highest numbers of esterase isozymes were detected under the highest NaCl concentration. Also, El-Sayed *et al.* (2007) found that salinity and gamma rays caused the appearance and disappearance of bands in two wheat cultivars. These results are similar to Mohamed (2005) who found that under salt stress, 150 mM NaCl caused enhancement of the esterase isozyme bands in shoots and roots of maize plants.

Conclusion:

Most of the studies emphasize the role of plant growth-promoting rhizofungi against biotic and abiotic stresses. Nevertheless, the current study revealed the synergistic effects between rhizofungi *Trichoderma harzinum* and non-enzymatic antioxidant (ascorbic acid) in mitigation stress in *Phaseolus vulgaris* cv Bronko which proved to be beneficial in most cases in imparting resistance through improved uptake of essential elements, increased carotenoids which played a protective role against stress, modulation of osmolytes (sugars, proteins and proline), antioxidants, enhanced scavenging activity and also esterase isozymes which played a vital role under salt stress conditions. Also, water presoaking is an efficient mechanism to promote some physiological activities parallel to *Trichoderma* and ascorbic acid in our results.

REFERENCES

Abbasi M. and Faghani E. (2015). Role of salicylic acid and ascorbic acid in the alleviation of salinity stress in wheat (*Triticum aestivum* L.). *Journal Biological and Environmental Science*, 6: (2) 107-113. [31].

Abd El-Baki G. K. and Mostafa D. (2014). The potentiality of *Trichoderma harzianum* in alleviation the adverse effects of salinity in faba bean plants. *Acta Biologica Hungarica*, 65 (4), pp. 451–468.

Ahmad P, Abeer H, Elsayed FAA, Alqarawi AA, Riffat J, Dilfuza E (2015). Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (Brassica juncea L.) through antioxidative defense system. *Frontiers in Plant Science,* 6: 868, 1-15.

Ahmad P., Ozturk M.,Sharma S. and Gucel S.(2014). Effect of sodium carbonate induced salinity alkalinity on some key osmoprotectants, proteinprofile, antioxidant tenzymes, and lipid peroxidation in two mulberry (Morusalbal) cultivars. *Journal of plant interaction*, 9(1) 460-467.

Alaa A. Gaafar, Sami I. Ali, Mohamed A. El-Shawadfy, Zeinab A. Salama, Agnieszka S˛ekara, Christian Ulrichs and Magdi T. Abdelhamid (2020). Ascorbic Acid Induces the Increase of Secondary Metabolites, Antioxidant Activity, Growth,and Productivity of the Common Bean under Water Stress Conditions. *Plants*, 9 (5), 627.

Arzani, A.and Ashraf, M., (2016). Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. Critical Reviews in Plant Sciences, 35, 146–189.

Azzedine, F., Gherroucha, H. and Baka, M. (2011). Improvement of salt tolerance in durum wheat by ascorbic acid application. *Journal of stress physiology and Biochemstry*,7: 27–37.

Babaeian, M., Tavassoli, A., Ghanbari, A., Esmaeilian, Y. and Fahimifard, M., (2011). Effects of foliar micronutrient application on osmotic adjustments, grain yield and yield components in sunflower (Alstar cultivar) under water stress at three stages. *African Journal of Agricultural Research*, 6, 1204–1208.

Badour S.S.A. (1959). Analitisch–chemische Untersuchung des Kaliummangels bei Chlorella in Vergleich mit anderen Mangel-Zustanden. Ph.D. Dissertation, Göttingen University, Germany.

Cai. X., Zhang C., Shu W., Ye. Z and Li. H. (2016). The transcription factor lD of 22 involved in ascorbate accumulation and salinity stress in tomato. *Biochemical and Biophysical Research Communications;* 474, 736-741.

Coppens, L. and Dewitte, D. (1990). Esterase and peroxidase zymograms from barley (*Hordeum vulgare* L.) callus as a biochemical marker system of embryogenesis and organogenesis. *Plant Science*, 67, 97–105.

Cosgrove, D.J. (2001): Wall structure and wall loosening. A look backwards and forwards. *Plant Physiology*, 125, 131–134.

Cummins, I., Burnet, M. and Edwards, R. (2001). Biochemical characterization of esterases active in hydrolysing xenobiotics in wheat and competing weeds. *Physiologia plantarum*, 113, 477–485.

Delauney, A.J., Verma, D.P.S., (1993). Proline biosynthesis and osmoregulation in plants. *The Plant Journal*, 4, 215–223.

Duarte B., Santos D., Marques J.C. and Cacador I.(2013). Ecophysiological adaptations of two halophytes to salt stress: Photosynthesis, PS II photochemistry and antioxidant feedback - Implications for resilience in climate change. *Plant Physiology and Biochemistry*, 67:178-88.

Ejaz B., Sajid Z. and Aftab F. (2012). Effect of exogenous application of ascorbic acid on antioxidant enzyme activities, proline contents, and growth parameters of Saccharum spp. hybrid cv. HSF-240 under salt stress. *Turkey Journal of Biology;* 630–640.

El-Amier, Y.A.; Elhindi, K.; El-Hendawy, S.; Al-Rashed, S. and Abd-Elgawad, A.(2019). Antioxidant System and Biomolecules Alteration in Pisum sativum under Heavy Metal Stress and Possible Alleviation by 5-Aminolevulinic Acid. *Molecules*, 24, 4194.

El-Sayed O.E., Rizkalla A.A. and Sabri S.R.S. (2007). *In vitro* mutagenesis for genetic improvement of salinity tolerance in wheat. *Research Journal of Agriculture* and *Biological Sciences*, 4(5):377-383.

Fales F. W. (1951). The assimilation and degradation of carbohydrates by yeast cells. *Journal of Biological Chemistry;* 193, 113–124.

Foyer, C.H. and G. Noctor (2005). Redox homeostis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell, 17*: 1866-1875.

Gehlot, H.S., Dinesh, R., Choudhary, R., Joshi, S., Sankhla, N. (2003). In vitro studies on chickpea seedlings: Effect of salt on growth and antioxidant activity. *Proceeding Plant Growth Regulation Society of America,* 30, 119–121.

Gigova L, Gacheva G, Ivanova N and Pilarski P. (2012). Effects of temperature on Synechocystis sp. R10 (Cyanoprocaryota) at two irradiance levels. I affect on the growth, biochemical composition and defense enzyme activities. *Genetic Plant Physiology*, 2(1–2): 24–37.

Gill S.and Tuteja N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry,* 48, 909–930.

Hafeez, M.B.; Raza, A.; Zahra, N.; Shaukat, K.; Akram, M.Z.; Iqbal, S.; Basra, S.M.A. (2021). Gene regulation in halophytes in conferring salt tolerance. In Handbook of Bioremediation; Elsevier: Amsterdam, The Netherlands; Academic Press: Cambridge, MA, USA; pp. 341–370.

Hashem, H.A., Bassuony, F.M., R.A. Hassanein, D.M. Baraka and R.R. Khalil, (2011). Stigmasterol seed treatment alleviates the drastic effect of NaCl and improves quality and yield in flax plants. *Australian Journal of Crop Science,* 5(13):1858-1867.

Hassanein A.M. (1999). Alteration in protein and esterase of peanut in response to salinity stress. *Biologia Plant,* 42:241-248.

Heath R. L. and Packer L. (1968). Photoperoxidation in isolated chloroplast. 1. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics,* 125, 189–198.

Helaly, M.N., Z. Mohammed, N. I. El-Shaeery, A. A. Abdelaal Kh. and I.E. Nofal, (2017). Cucumbe rgrafting onto pumpkin can represent an interesting tool to minimize salinity stress.Physiological and anatomical studies. *Middle East Journal of Agriculture Research,* 6(4):953-975

Hermosa R., Belén Rubio M., Cardoza RE., Nicolás C., Monte E and Gutiérrez S. (2013). The contribution of Trichoderma to balancing the costs of plant growth and defense. *International Microbiology,* 16, 69–80.

Hernandez J.A. and Almansa M.S. (2002). Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. *Physiologia Plantarum,* 115:251–257.

Hodges, D.M., Andrews, C.J., Johnson, D.A. and Hamilton, R.I., (1997). Antioxidant enzyme responses to chilling stress in differentially sensitive inbred maize lines. *Journal of Experimental Botany,* 48, 1105–1113.

Horemans N., Foyer C.H. and Asard H. (2000); Transport and action of ascorbate at the plant plasma membrane. *Trends in Plant Science,* 5, 263–267.

Ismail M., Ali S. and Hussain M.; (2014); Quantitative Determination of Ascorbic Acid in Commercial Fruit Juices by Redox Titration. *International Journal of Pharmaceutical Quality Assurance;* 5(4); 22-25.

Jaleel, C.A.; Manivannan, P.; Wahid, A.; Farooq, M.; Somasundaram, R. and Panneerselvam, R. (2009). Drought stress in plants: A review on morphological characteristics and pigments composition. *International Journal of Agriculture Biology,* 11: 100–105.

Jia Z., Mengcheng T.and Jianming W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry;* 64:555-9.

Kapoor, K., Srivastava, A., (2010). Assessment of salinity tolerance of *Vinga mungo* var. Pu-19 using ex vitro and in vitro methods. *Asian Journal of Biotechnology,* 2, 73–85.

Khafagy, M.A., Arafa, A.A. and El-Banna, M.F. (2009). Glycinebetaine and ascorbic acid alleviate the harmful effects of NaCl salinity in sweet pepper, *Australian Journal of Crop Science,* 3, 257–267.

Khan T. M. and Mazid F, Mohammad. (2011). A review of ascorbic acid potentialities against oxidative stress induced in plants. *Journal of Agrobiology;* 28: 97-111.

Lichtenthaler H. K. (1987); Chlorophylls and carotenoids; pigments of photosynthetic biomembranes. *Methods in Enzymology;* 148: 350-382.

Lowery O. H., Rosebrough N. H., Farr A. L., Randall R. J. (1951). Protein measurements with the folin phenol reagent. *Journal of Biological Chemistry,* 193, 291–297.

Luna, C., Luca, M.D. and Taleisnik, E. (2002). Physiological causes for decreased productivity under high salinity in bom tetraploid *Chloris gayan*a cultivar II. Oxidative stress. *Australian Journal of Agricultural Research,* 53, 669–674.

Ma, Y., Dias, M.C. and Freitas, H. (2020). Drought and salinity stress responses and microbe-induced tolerance in plants. *Frontiers in Plant Science,* 11, 591911.

Mahajan, S. and Tuteja, N. (2005). Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics,* 444, 139–158.

Mayo, S.; Cominelli, E.; Sparvoli, F.; González-López, O.; Rodríguez-González, A.; Gutiérrez, S.; Casquero, P.A. (2016): Development of a qPCR strategy to select bean genes involved in plant defense response and regulated by the *Trichoderma velutinum*–Rhizoctonia solani interaction.*Frontiers in Plant Science,* 7, 1109.

McKee G. W. (1974). A coefficient for computing leaf area in hybrid corn. *Agronmy Journal;* 56; 240-241.

Mervat, S. S. and M.A.E. Ebtihal, (2013). Physiological response of flax cultivars to the effect of salinity and salicylic acid. *Journal of Applied Sciences Research;* 9(6):3573-3581.

Mohamed A.A. (2005). Two-dimensional electrophoresis of soluble proteins and profile of some isozymes isolated from maize plant in response to NaCl. *Reseach Journal of Agriculture and Biological Science,* 1(1):38-44.

Moore S., Stein W. (1948). Partition chromatography of amino acids on starch. *Annals of the New York Academy of Sciences;* 49, 265–278.

Mukherjee, S., Bhattacharyya, P. and Duttagupta, A.K. (2004). Heavy metal levels and esterase variations between metal-exposed and unexposed duckweed Lemna minor: field and laboratory studies. *Environment Interactions,* 30, 811–814.

Munns, R., Tester, M., (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology,* 59, 651–681.

Negi, S.; Bharat, N.K.; Kumar, M. (2021). Effect of seed biopriming with indigenous PGPR rhizobia and trichoderma sp. On growth, seed yield and incidence of diseases in french bean (*Phaseolus vulgaris* L.). *Legume Research,* 44, 593–601.

Niakan M., Malekian A., Norinia A. (2012). The effect of exogenous ascorbate and canola aquatic extracts on growth parameters and photosynthetic apparatus of soybean. *Plant and Ecosystem;* 7, 19–32.

Niu, B.;Wang,W.; Yuan, Z.; Sederoff, R.R.; Sederoff, H.; Chiang, V.L.; Borriss, R. (2020). Microbial InteractionsWithin Multiple-Strain Biological Control Agents Impact Soil-Borne Plant Disease. *Frontiers Microbiology,* 11, 2452.

Noctor G. and Foyer C. H.; Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology,* (1998); 49, 249–279.

Parida A.K. and Das A.B. (2004). Effects of NaCl stress on nitrogen and phosphorous metabolism in a true mangrove *Bruguiera parviflora* grown under hydroponic culture. *Journal Plant Physiology,* 161:921–928.

Pessarakli, M., (1999). Handbook of Plant and Crop Stress. Second edition. Marcel Dekker, New York, USA. *Physiological Biochemistry,* 67:178–88.

Pourcel, L., Routaboul, J.M., Cheynier, V., Lepiniec, L. and Debeaujon, I. (2007). Flavonoid oxidation in plants: From biochemical properties to physiological functions. *Trends in Plant Sciences,* 12, 29–36.

Rady, M.M., Taha, S.S. and Kusvuran, S. (2018a). Integrative application of cyanobacteria and antioxidants improves common bean performance under saline conditions. *Scientia Horticulturae,* 233, 61–69.

Rady, M.M.; Talaat and N.B. (2019). Abdelhamid, M.T.; Shawky, B.T.; Desoky, E.-S.M. Maize (*Zea mays* L.) grains extract mitigates the deleterious effects of salt stress on common bean (*Phaseolus vulgaris* L.) growth and physiology. *Journal of Horticulure Science and Biotechnology,* 94, 777–789.

Rady,M.M., Varma, B.C. and Howladar, S.M. (2013). Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. *Scientia Horticulturae*, 162, 63–70.

Rasool S, Ahmad A, Siddiqi TO, Ahmad P. (2013). Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Physiolgia Plantarum,* 35:1039-1050.

Reis F. S., Stojkoviæ D. and Sokoviæ M. (2012). Chemical characterization of *Agaricus bohusii*, antioxidant potential and antifungal preserving properties when incorporated in cream cheese. *Food Research International,* 48: 620-626.

Rudolph, A.S., Crowe, J.H., Crowe, L.M., (1986). Effects of three stabilizing agents proline, betaine and trehalose on membrane phospholipids. *Archives of Biochemistry and Biophysics,* 245, 134–143.

Ruiz-Lozano J., Porcel R., Azco´n C. and Aroca R. (2012). Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. *Journal of Experimental Botany,* 63(11):4033–4044.

Salama, Z.A.; El-Nour, A.A.; El-Fouly, M.M. and Gaafar, A.A. (2014). Ascorbic acid foliar spray counteracting effect of salinity on growth, nutrients concentration, photosynthesis, antioxidant activities and lipid peroxidation of bean (*Phaseolus vulgaris* L.) cultivars. *American Journal of Agriculture and Biological Science,* 9, 384–393.

Santoro, M.M., Liu, Y., Khan, S.M.A., Hou, L.X., Bolen, D.W., (1992). Increased thermal stability of proteins in the presence of naturally occurring osmolytes. *Biochemistry,* 31, 5278–5283.

Schlegel H.G. (1956). The recovery of organic acid by Chlorella in the light. *Planta;* 47, 510–526.

Shaharoona B., Arshad M., Khalid A. (2007). Differential response of etiolated pea seedling to 1-aminocyclopropane-1- carboxylate and/or l-methionine utilizingrhizobacteria. *Microbiology,* 45:15–20.

Shoresh M., Harman G.E. and Mastouri F. (2010). Induced systemic resistance and plant responses to fungal biocontrol agents. *Annual Review of Phytopathology;* 48, 21–43.

Smirnoff, N., Cumbes, Q.J., (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry,* 28, 1057–1060.

Steel R.G.D and Torrie J.H. (1960). Principles and procedures of statistics, with special reference to biological science McGraw –Hill Book Company, New york.

Taffouo, V.D., Wamba, O.F., Yombi, E., Nono, G.V., (2010). Growth, yield, water status and ionic distribution response of three bambara groundnut (*Vigna subterranea*n

(L.) verdc.) landraces grown under saline conditions. *International Journal of Botany*, 6, 53–58.

Tanksley, S. D., Orton, T. J. (eds) (1983). Isoenzymes in plant genetics and breedin*g.* Part A, Elsevier Amsterdam, New York.

Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.L.; Ruocco, M.; Woo, S. and Lorito, M. (2012). *Trichoderma* secondary metabolites that affect plant metabolism. *Natural Production Communications*, 7, 1545–1550.

Weidner, S.; Karolak, M.; Karama´c, M.; Amarowicz, R. (2011). Phenolic compounds and properties of antioxidants in grapevine roots (*Vitis vinifera* L.) under drought stress followed by recovery. *Acta Societatis Botanicorum Poloniae*, 78, 97–103.

Yan, K., Shijie Zhao, S., Bian, L. and Chen, X. (2017). Saline stress enhanced accumulation of leaf phenolics in honeysuckle *(Lonicera japonica* Thunb.) without induction of oxidative stress. *Plant Physiology and Biochemistry*, 112, 326–334.

Yi Z., Yu Y., Liang Y. and Zeng B. (2008). In vitro antioxidant and antimicrobial activities of the extract of Pericarpium Citri Reticulatae of a new Citrus cultivar and its main flavonoids. *Lebensmittel-Wissenschaft Technologie Food*, 17(9): 11281–11291.

Younis M.E., Hasaneen M.N.A. and Kazamel A.M.S. (2010). Exogenously applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in *Vicia faba* seedlings. *Protoplasma;* 239: 39-48.

Zhang F., Wang Y., Liu C., Chen F., Ge H., Tian F., Yang T., Ma K. and Zhang Y.(2019). Trichoderma harzianum mitigates salt stress in cucumber via multiple responses. Ecotoxicol. *Environmental Safety*, 170, 436–445.

Zhang F., Yuan J., Yang X., Cui Y., Chen L. and Ran W. (2013). Putative *Trichoderma harzianum* mutant promotes cucumber growth by enhanced production of indole acetic acid and plant colonization. *Plant Soil;* 368:433-444.

Zhang S, Xu B and Gan Y. (2016). Application of Plant-Growth-Promoting Fungi *Trichoderma Longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Frontiers in Plant Science*,1–11.

ARABIC SUMMARY

الإستجابات التفاضلية لنبات الفاصوليا (*Phaseolus vulgaris* L.)للتأثيرات التفاعلية لحمض الأسكوربيك والترايكودرما هارزيانم *Trichoderma harzianum*تحت اجهاد الملوحه

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من المفترض أن يتم التقليل من الاضطرابات البيئية الحديثة باستخدام الأسمدة الحيوية كبديل آمن للأسمدة الكيماوية. تهدف الدراسة الحالية إلى استخدام الأسمدة الحيوية (*Trichoderma* harzianum) ومضادات الأكسدة غير الأنزيمية (حمض الأسكوربيك) مع إجهاد الملح (NaCl) على النمو وبعض الأنشطة الأيضية بالإضافة إلى إنزيم الإستريز في *Phaseolus vulgaris* cv Bronko الذي تم نموه لمدة 60 يومًا من الزراعة في تربة طينية رملية (2: 1 .V: V). أظهرت النتائج أن إجهاد الملوحة يثبط كلاً من الأوزان الرطبة والجافة للنبات وأطوال السيقان والجذور والمحتوى المائي. عززت معالجة النباتات بحمض الأسكوربيك أو الترايكوديرما من هذه المعايير. تم تحسين محتوى الاصباغ (Chl.a و Chl.b و Chl.a + b والكاروتينات) عند مستويات الملوحة المنخفضة والمرتفعة في النباتات المنقوعة مسبقًا في الماء ، ومع ذلك فإن النباتات المعالجة إما بحمض الأسكوربيك أو *Trichoderma* عززت الكاروتينات بمستويات معتدلة وأعلى من الملوحة. ظلت مساحة الاوراق دون تغيير عند المستويات المنخفضة والمتوسطة من الملوحة ولكنها انخفضت بشكل حاد عند المستويات الأعلى، ومعالجة النباتات بحمض الأسكوربيك حسنت فقط عند مستويات الملوحة العاليه. ازدادت البروتينات الذائبة والكربوهيدرات الذائبه والأحماض الأمينية في المجموع الخضرى وتناقصت في المجموع الجذرى مع زيادة الملوحة، وقد أدت معالجة النباتات بحمض الأسكوربيك أو الترايكوديرما إلى تقليل هذه المكونات في المجموع الخضرى. أدت معالجة النباتات بحمض الأسكوربيك أو *Trichoderma* إلى خفض MDA وزيادة البرولين بشكل ملحوظ في كل من المجموع الخضرى والجذرى. زادت الملوحة من نشاط DPPH في كل من ا لمجموع الخضرى والجذرى، وقد عززت المعالجة بحمض الأسكوربيك أو *Trichoderma* بشكل كبير من نشاط لـ DPPH٪ في كلا العضوين. أظهرت النباتات المعالجة إما بحمض الأسكوربيك أو الترايكوديرما انخفاضًا في الفينولات وزيادة في مركبات الفلافونويدات خاصة مع الترايكوديرما في جميع مستويات الملوحة المستخدمة. أظهر التفريد الكهربى على 7.5٪ بولي أكريلاميد جل وجود 6 أشكال متشابهه من esterase isozyme استجابة للنقع المسبق بالماء والمعالجات إما بحمض الأسكوربيك أو *Trichoderma* تحت تركيزات مختلفة منNaCl . يعكس وجود 6مشابهات إنزيميه للاستيريز فى الكنترول والنباتات المعالجة دور هذا الإنزيم في مقاومة الإجهاد الملحي.