RESEARCH ARTICLE

Alleviated Effect of Salinity Stress by Exogenous Application of Ascorbic Acid on the Antioxidant Catalase Enzymes and Inorganic Mineral Nutrient Elements Contents on Tomato Plant

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ABSTRACT

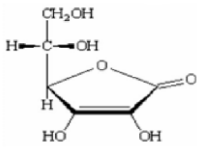
The aim of the study to explain the role of ascorbic acid (AsA) for alleviates the effect of salinity stress on special cultivar (cv. Bonus F1) of tomato plant. The tomato seeds (cv. Bonus F1) soaked in AsA (0.75 mM) before germinated for 12 hours in the dark. The seeds planted in trays of cork contain 218 eyes for 14 days, seedlings plant transplanted to plastic containers containing a mixture of sand/peat-moss (1:2). Each pot contained 7 seedling plants were irrigated using different concentrations (1,500 - 3,000 - 4,500 & 6,000 ppm) NaCl. The data explained that the catalase enzyme activity increased significantly in the present of AsA more than in the absent of AsA under NaCl salinity stress compared with control. Also, the results indicated that the contents of macro and micro nutrient mineral elements (N, P, K, Ca, Mg, Mn, Fe, Cu, Z, B, Na and Cl) increased significantly more under salinity stress in the presence more in the absence of ascorbic acid compared with the control. The data provide strong support to the hypothesis that exogenous of ascorbic acid (AsA) reduces the harmful effects of salinity and increases resistance to salinity in tomato plant.

Keywords: Salinity, Ascorbic acid, Catalase, Nutrient mineral elements, Tomato

INTRODUCTION

Tomato, one of the important and widespread crops in the world, is sensitive to moderate levels of salt in the soil. Tomato plant belongs to the *Solanaceae* family and rich in minerals, vitamins, essential amino acids, sugars and dietary fibres, vitamin B and C, iron and phosphorus. Tomatoes are a major source of lycopene, carotenoid with a notable capacity to eliminate (AOS). In particular, carotenoid intake reduces the risk of certain types of cancer, cardiovascular pathologies, and xerophthalmia. Phenolic compounds and ascorbic acid represent the main water-soluble antioxidants in tomatoes. Several epidemiological studies have shown the importance of vitamins in preventing cancer

and heart diseases (Rao *et al*., 1998; Khachik *et al*., 2002; Donaldson, 2004; Blum *et al*., 2005; Houston, 2005; Toor and Savage, 2005). Many studies have reported large variation among tomato genotypes in their response to salinity (Ben Ahamed *et al.,* 2009). In addition to its economic importance, tomato consumption has recently been demonstrated to be beneficial to human health, because of its content of phytochemicals such as lycopene, *β*-carotene, flavonoids, vitamin C and many essential nutrients. (Beutner *et al*., 2001; Palop *et al*., 2010).



Ascorbic acid (vitamin C) is water - soluble vitamin. It occurs as a white or slightly yellow crystal or powder with a slight acidic taste. The chemical name of ascorbic acid (vitamin C) is L-ascorbic acid. The empirical formula is $C_6H_8O_6$, and the molecular weight is 176.13. The biochemical functions of ascorbate have been divided into four categories, (1) enzyme cofactor for hydroxylase enzymes involved in the synthesis of hydroxyproline-rich glycolproteins, cell wall structural proteins (Carpita and Gibeaut, 1993); (2) antioxidant, regenerates the lipophilic antioxidant α-tocopherol, vitamin E (Asada, 1994); (3) electron transport, acts as an in vitro electron donor and acceptor in transmembrane electron transport (Asard *et al*., 1995); and (4) oxalate and tartarate synthesis (Saito, 1996).

El Sayed *et. al.* (2015 a & b) reported that the ascorbic acid (75 mM) increased the total organic components for both shoot and roots of the tomato plant and mitigate the impact of salinity inhibitory to the plant metabolism. Whereas, the ascorbic acid (AsA 75 mM) tended to reduce the harmful effects of salinity and increases resistance to salinity in tomato plant. Ascorbic acid (AsA) is one of the most powerful antioxidants; the supply of ascorbic acid (vitamin C) to tomato seedling might decrease the synthesis of active oxygen species (AOS) and thereby increase resistance to salt stress. It is involved in removing AOS and regenerating R-tocopherol, an important antioxidant in the lipid phase (Shalata and Neumann, 2001; Navari-Izzo *et al.,* 2002).

Ascorbic acid (AsA) is associated with chloroplasts and apparently plays a role in ameliorating the oxidative stress of photosynthesis. In addition, AsA has a number of other roles in cell division and protein modification. Plants appear to be able to make ascorbate by at least one other biochemical route that is different from the major route in animals, although precise details remain unknown. Improved understanding of ascorbate concentration in plants will lead to the possibility of increasing ascorbate concentration in plants by genetic manipulation. This will have benefits for tolerance of plants to oxidative stresses (Smirnoff, 1996) and wheat plants El Sayed and Mujahed, (2016 a & b).

Salinity is one of the major abiotic stresses that reduce plant growth and crop productivity in many vegetable production areas of the world. Several investigations reported that AsA plays important roles in enhancing the salt tolerance of different plants (Barakat, 2003; Athar *et al*., 2008; Colla *et al*., 2010; El-Hariri *et al*., 2010; Paital and Chainy, 2010).

Salinity stress effects on the plants in different ways such as osmotic effects, specific-ion toxicity and/or nutritional disorders. The extent by which one mechanism affects the plant over the others depends upon many factors including the species, genotype, plant age, ionic strength, composition of the salinizing solution and the organ in question (Läuchli and Epstein, 1990). Rapid growth of human population has led to rising demand for food and usage of saline soils or water for crop production. Successful plant production under salt stress conditions requires an adequate understanding of how salts affect soil characteristics and plant performance (Chartzoulakis *et al*., 2002). Salinity is an important abiotic stress factor seriously affecting plant growth, productivity, fruit yield and survival. Abiotic stresses such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and the natural status of the environment. Salinization is rapidly increasing on a global scale and currently effect more than 10% of arable land, which results in a decline of the average yields of major crops greater than 50% found that the present out of 1.5 billion hectares of cultivated land around the world, about 77 million hectares is affected by excess salt content (Wang *et al*., 2003; Parida and Das, 2005; Eker *et al*., 2006; Evelin *et al.,* 2009; Jadhav *et al*., 2010).

The objective of this study aimed to explain the role of ascorbic acid (AsA) for reducing the effect of salinity stress on special cultivar of tomato plant on catalase enzyme activity and micro and micro mineral nutrient elements.

MATERIAL AND METHODS

Nutrient Solutions and Salinity Treatments: The base nutrient solution used was similar to that applied by Hoagland and Arnon (1950). The solution was held at pH 6 throughout the experiment.

Salinity Concentrations (NaCl): A molar solution was prepared of NaCl was added to the Hoagland solutions to give four concentrations of salinity as follows: Control (Hoagland), 1,500 - 3,000 - 4,500 - 6,000 ppm NaCl salinity**.**

The Soil Used: The soil used for cultivated tomato plant was the ratio between the sand and peat-moss (1:2 – v: v), added in each pot (diameter 16 cm and depth of 16 cm), by the same ratio of the soil of the volume**.**

Plant Material and Growth Conditions: Selected the seeds of Tomato Plant (*Solanum lycopersicum* L.) intact, homogeneous in size and free from wrinkles to plant tomatoes cultivar (Bonus F1). Then soaked the seeds for 12 hours in the dark using the following solutions where seeds were divided into 2 groups as follows: First group (1): seeds soaked in distilled water. The second group (2); seed soaked in a solution of AsA concentration 0.75 mM.

The seedling plant transplanted after germinated (14 days) in trays of cork (39 cm × 67 cm), which containing 218 tray diameter eye (3cm and depth 6.5 cm). The tray eyes containing an equal amount of peat-moss only mixture thoroughly with water so distributed one seed in each eye tray and left the seeds to grow under greenhouse conditions at temperature of 18^{o}C ± 1^{o}C (night) 22^{o}C ± 2^{o}C (day) and relative humidity varied between 60 - 70%. The tomato seeds watering using distilled water until the true leaf appearance then transferred to another pots (diameter 16 cm and depth of 16 cm) which containing the sandy soil washed by diluted hydrochloric acid (1N HCl) and washed thoroughly with distilled water more five times. Used the same pots, and each pot containing the same volume of washing sandy soil and peat moss, (1: 2 - v: v). The sand culture technique and nutrient solution were similar to those adopted by Hewitt (1952); Hoagland and Arnon (1950) respectively.

Seedling of tomato plant was transferred from cork trays to plastic pots, each pot containing 7 transplanting (seedling plant) then left the seedling for one days and then irrigated using NaCl salinity with different concentrations (1500; 3000; 4500; 6000 ppm) in Hoagland solution (nutrient solution) and using a Hoagland solution as control in the presence or absent the AsA.

Irrigation process four NaCl salinity (1,500 - 3,000 - 4,500 - 6,000 ppm) concentrations in addition to Hoagland solution (nutrient solution) by using a hand spray control the distribution of salt and avoid the accumulation of salts in one place of pot, irrigated plants on average once every two days with a fixed amount of each concentration brines by 400 ml.

Estimation of Catalase Enzymes:
Extraction of soluble proteins by a frozen sample of 0.5 g of 14- d old tomato seedling was homogenized in 8 ml of 50 mM cold phosphate buffer at pH 7 (modified from Beauchamp and Fridovich, 1971). The homogenates were centrifuged at 4000 rpm for 20 min and the supernatant was used as a crude extract for enzymatic assay.

Assay of catalase:
Principle:

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

Catalase was assayed (Kato and Shimizu, 1987) by measuring the initial rate of disappearance of H_2O_2. A sample of 3ml of reaction mixture contained 0.1 M sodium phosphate buffer (pH 7), 2 Mm H_2O_2 and 0.1 ml enzyme extract. The decrease in H_2O_2 was followed as a decline in absorbance at 240 nm and the activity was calculated using the extinction coefficient (40mM^{-1} cm $^{-1}$ at 240 nm) for H_2O_2. The activity was expressed in units of mM of the substrate converted to min.$^{-1}$ g.$^{-1}$ Fresh Weight.

Mineral Composition:
Cation contents of the milled samples were estimated following the "wet ashing procedure" of the powdered samples as described by Richards, (1954). The acid

digests of the oven dried samples were analyzed for sodium, potassium, calcium, magnesium and determinations. Sodium, potassium and calcium contents were determined photometrically using a corning- 400 flam photometer (Johnson and Utrich, 1959; Allen *et al.,* 1974). The levels of magnesium, phosphorus, manganese, iron, zinc and copper contents were determined using an atomic absorption spectrophotometer.

The mixed- acid digestion method was used in preparing the sample solution for determination of element content. Phosphorus was estimated by the Molybdenum-blue method (Allen *et al.,* 1974), while nitrogen was estimated by the Automatic MicroKjeldahl method (Allen *et al*., 1974).
Automatic MicroKjeldahl consists of:

1. Digestion system.
2. Kjeltec Distillation system.

Procedure: A 250 mg oven dry plant materials together with a tablet of mercuric chloride and 6 ml concentrated H_2SO_4 were placed in tubes in digestion system unit the temperature reached 420ºC. After ½ h. the tubes were removed cooled and 25 ml distilled water added. Concentrated NaOH was added to make the solution alkaline and then the mixture was distilled, volatile nitrogenous, materials being trapped in a boric acid solution. The latter was subsequently titrated against 0.1 N HCl, using universal indicator (end point from blue to pink), and the total nitrogen calculated from the equation:

$$\text{Total Nitrogen (\%)} = \frac{(\text{ml acid- ml blank}) \times 0.1 \times 14.007 \times 100}{\text{Wt. sample (mg)}}$$

0.1 = Normality of acid.
14.007 = Atomic wt. of nitrogen.

Chlorides were determined by the $AgNO_3$ titration method as described by Jackson and Thomas (1960). Measurements were carried out extraction with 0.1 nitric acid of ashed (powdered) milled samples at 500ºC obtained after combustion in muffle furnace.

Boron was determined by the ICP – AES technique, measurements being carried out after extraction from the ashed milled samples at 550ºC in a muffle furnace with HCl. The equipment involved Phillips PV 8490 Plasma Source Unit Linked to a spectraspan III Echelle spectrometer controlled by an Apple ll_e microcomputer. The inductively Coupled Plasma Atomic Emission Spectrometer technique (*ICP - AES*) involves a microcomputer controlled inductively coupled plasma emission spectrometer. The computer also stores the corrected intensity values from the samples and then calculates the concentration of analytic with reference to the calibration graph.

The computer find the best – fit line using least squares analysis, it calculates the intensity which should occur for each concentration using the specific time calculated concentration in ppm.

1. Measured standards.
2. Measured Samples.
3. Calibration Procedure.

Statistical Analysis:
Statistical analyses of the data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using mean and standard deviation or standard error of mean for normally distributed data. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparisons between different groups were analyzed using *F*-test (*ANOVA*). Find the effects between growth stages, AsA. (mM) and NaCl ppm and their interactions two way *ANOVA* was used. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level (Leslie *et at*., 1991; Kirkpatrick and Feeney, 2013).

RESULTS AND DISCUSSION

Exogenous Application of AsA on Catalase Enzyme Content (H_2O_2/g F.Wt. Protein/min) in Shoots of Tomato Plant:

Data presented in Fig. (1) and Table (1), indicated that the impact of AsA on catalase enzyme content in leaves of tomato plant tended to increased significantly ($p \leq 0.001$) under NaCl salinity stress with different concentrations (1500, 3000, 4500 & 6000 ppm), compared to non-stressed treatment. The catalase enzyme content increased significantly ($p \leq 0.001$)

more under high salinity level. Also, the catalase enzyme content increased significantly ($p \leq 0.001$) in the present of AsA as compared to the absent of AsA under saline and non-saline stressed conditions. Soaking seeds of tomato plant with concentration (75 mM) of AsA was more effective by increasing catalase enzyme content. In all cases, the interactions between salinity levels and ascorbic acid (AsA) resulted an increased significantly ($p \leq 0.001$) the catalase enzyme content compared to the non-saline stressed treatment. Generally, in the present of AsA (75 mM) indicated that the catalase enzyme content increased more with all NaCl concentrations compared to control. Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at growth stage (42 days) indicated that the *F* test highly significant at $P \leq 0.001$.

Table (1): Effect of Exogenous Application of AsA (0.75 mM) on Catalase Enzyme (H_2O_2/g F. Wt. Protein/min) of Tomato Plant Shoot Grown for 42 Days under Salinity Stress (0.000; 1,500; 3,000; 4,500 & 6,000 ppm NaCl). Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

AsA. (mM) / NaCl (ppm)	*Shoot* Catalase			
	0.00	0.75	F_1	*p*
Control	6.33 ± 0.34	16.71 ± 0.69	157.047*	<0.001*
1500	10.67 ± 0.91	20.76 ± 0.38	84.751*	<0.001*
3000	12.09 ± 0.35	24.98 ± 0.42	232.468*	<0.001*
4500	14.51 ± 0.65	29.16 ± 0.37	282.329*	<0.001*
6000	17.39 ± 0.57	31.28 ± 0.79	185.321*	<0.001*
F_2	142.842*	343.144*		
p	<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		*F* = 950.508*	*p* <0.001*
	AsA (mM) Conc.		*F* = 862.814*	*p* <0.001*
	NaCl ppm Conc. x AsA (mM)		*F* = 5.876*	*p* <0.001*

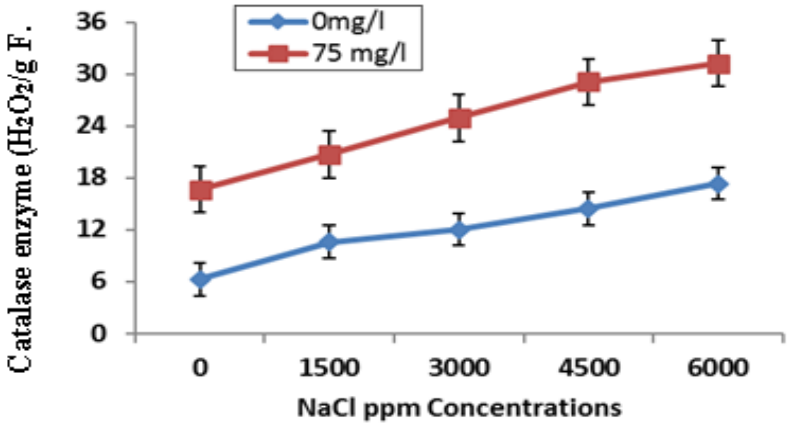


Fig. (1): Effect of Exogenous Application of AsA (0.75 mM) on Catalase Enzyme (H_2O_2/g F. Wt. Protein/min) of Tomato Plants Shoot Grown for 42 Days under Salinity Stress (0.000; 1,500; 3,000; 4,500 & 6,000 ppm NaCl). Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

The present results were agree with data obtained by Dolatabadian *et al*. (2008), they found that the exogenous application of ascorbic acid (AsA) induces activation of antioxidant enzyme system in canola (*Brassica napus* L.) resulting in reduction of detrimental effects of salinity. The data obtained by Mandhania *et al*. (2012) they found that the activities of CAT and APX increased with increasing the salt stress in both salt tolerant and salt sensitive wheat cultivars. Also, confirmed our findings Barakat (2011) where he noted that the wheat plant activity levels of antioxidant enzymes as CAT , POD and APX showed progressive significant increases with increasing concentration of NaCl specially at high salinity level

(150 and 200 mM NaCl) compared to control plant (0.0 mM NaCl). Also, The result obtained by Lee *et al*. (2001) they observed that salt stress increased the activities of leaf mitochondrial and chloroplastic antioxidant enzymes, which are considered the primary scavenger in the detoxification of active oxygen species in plants and converts superoxide to H_2O_2 and O_2, and offers protecting cells against superoxide induced oxidative stress.

The present results also agree with the result by Dolatabadian and Jouneghani (2009) they found that the catalase activity in bean plant increased with increasing salt stress. However, there was not significant effect in non-stressed plant, total catalase (CAT) activity mostly increased in salt stressed plants, but it was higher at 400 mM NaCl than at 100 mM NaCl. Also, the activities of all antioxidant enzymes SOD, POD and CAT increased in two wheat cultivars, S-24 (salt tolerant) and MH-97 (moderately salt sensitive) due to the imposition of salt stress. Similarly, the results obtained by Alscher *et al.* (2002) they found that the explained the scavenging of ROS by increased activation of antioxidant enzymes can improve salt tolerance. However, foliar applied of AsA spray with varying levels (0; 50; 100 mg L^{-1}) caused a further increase in SOD activity of salt stressed plants of MH-97, whereas it remained unchanged in the salt stressed plants of S-24 (Khan *et al*., 2006).

A relationship between salt tolerance and increased activation of antioxidant enzymes under salt stress has been demonstrated in pea (Hernandez *et al*., 2000), onion (*Allium cepa*) (Abd El-Baky *et al*., 2003), mulberry (Harinasut *et al*., 2003), common bean (Jebara *et al.,* 2005), tomato; maize (Azevedo – Neto *et al*., 2006), soybean (Cicek and Cakirlar, 2008) and in sorghum (Costa *et al*., 2005; Heidari, 2009). In general, the activities of antioxidant enzymes were increased in the root and shoot under saline stress. But the increase was more significant and consistent in the root (Kim *et al*., 2005). Also, the data obtained by Chookhampaeng (2011) he found that the activities of CAT increased with the increase of the concentration of NaCl in shoots and roots of pepper plant.

Effects of Applications of AsA on Inorganic Mineral Nutrient Elements:

Unless stated to the contrary, inorganic mineral nutrient element contents increased in shoot and root of tomato plants at growth stages (42 days) under saline and non-saline stress in the present or in the absent of AsA concentration. From the data presented by Tanji (1995) he found that the salinity is defined as the presence of high levels of minerals (cations K^+, Mg^{2+}, Ca^{2+}, Na^+ and anions NO^{-3}, HCO^-, SO^{-2}, Cl^-) in water and soil. Heimann and Ratner (1966), they reported that the relative quantities of ions were more decisive than their absolute quantity in determining the growth and life limits of plants under saline condition. They found that in spite of high salinity, good crops may be obtained if K^+ was present in appreciable concentrations in relation to Na^+

Nitrogen Content (mg/100g Dry Weight):

Increasing NaCl salinity concentrations resulted an increasing the levels of nitrogen (N^{+3}) at (42 days) in the presence or absence of AsA with concentration (0.75 mM) as shown in Fig. (2 a & b) and Table (2). The results indicated that the uptake of N^{+3} in shoot and root of tomato plants increased significantly ($p \leq 0.001$) with increasing NaCl salinity (1500; 3000' 4500 & 6000 ppm) in the present or in the absent of AsA with concentration (0.75 mM) under saline and non-saline stress compared with control. In the present of AsA increasing the N^{+3} content in shoot and root of tomato plant at (42 days). Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at 42 days indicated that the *F* test highly significant at $P \leq 0.001$.

Potassium Content (mg/100g Dry Weight):

Overall, potassium contents (K^+) in shoot and root of tomato plant increased significantly ($p \leq 0.001$) gradually with increasing NaCl salinity concentrations (1500, 3000, 4500 & 6000 ppm) at (42 days) compared with control as shown in Fig. (3 a & b) and Table (3). Whereas, in the present of AsA with concentration (0.75 mM) resulted a significantly ($p \leq 0.001$) increased in K^+ contents under saline stress in shoot and root compared to tomato plant in the absent of AsA and control at (42 days). General potassium content was higher in shoot and root of tomato plant in the present of AsA compared to control, the K^+ content increased in shoot more than the root especially in the present of AsA with increasing NaCl salinity concentrations. Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at (42 days) indicated that the *F* test highly significant at $P \leq 0.001$.

Table (2): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Nitrogen (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA.(mM) / NaCl (ppm)	*Shoot (a)*				*Root (b)*			
	0.00	**0.75**	F_1	***p***	**0.00**	**0.75**	F_1	***p***
Control	10.31 ± 0.37	14.80 ± 0.28	55.240*	<0.001*	7.30 ± 0.38	10.91 ± 0.54	23.875*	<0.001*
1500	11.71 ± 0.38	18.30 ± 0.20	66.026*	<0.001*	8.98 ± 0.35	13.99 ± 0.47	40.101*	<0.001*
3000	12.81 ± 0.32	19.99 ± 0.35	91.165*	<0.001*	10.13 ± 0.30	17.31 ± 0.54	74.141*	<0.001*
4500	13.90 ± 0.47	20.33 ± 0.39	62.286*	<0.001*	11.21 ± 0.48	19.71 ± 0.37	112.897*	<0.001*
6000	14.71 ± 0.44	23.29 ± 0.82	48.887*	<0.001*	12.07 ± 0.39	22.01 ± 0.51	166.684*	<0.001*
F_2	18.975*	45.194*			23.604*	82.418*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		*F* = 276.685*	*p* <0.001*	NaCl ppm Conc.		*F* =614.671*	*p* <0.001*
	AsA(mM) Conc.		*F* = 536.197*	*p* <0.001*	AsA(mM) Conc.		*F* =657.822*	*p* <0.001*
	NaCl ppm Conc. x AsA(mM)		*F* = 0.496*	*p* = 0.944	NaCl ppm Conc. x AsA(mM)		*F* =1.950*	*p* = 0.024*

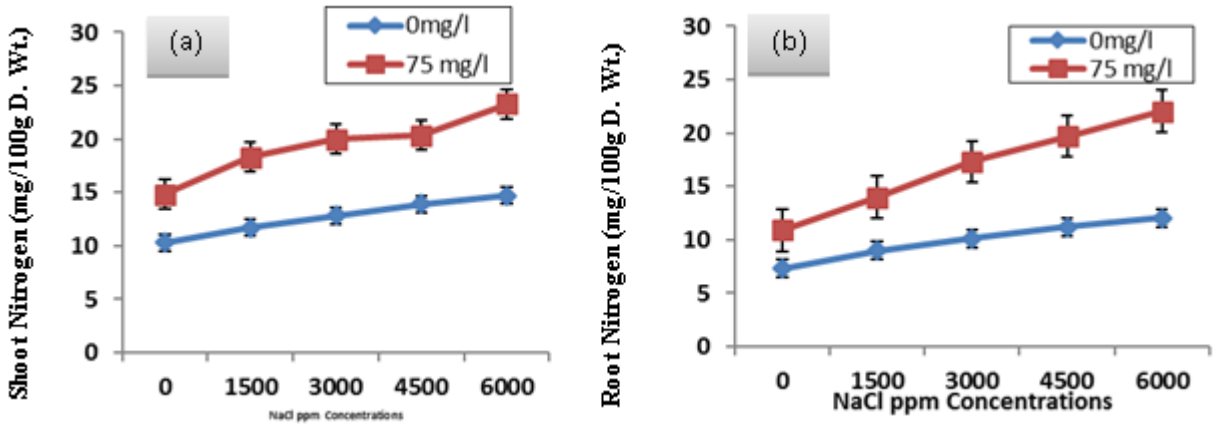


Fig. (2 a& b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Nitrogen (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

These results have varied with the findings of the Babu *et al.* (2012) where found that the potassium content was found in leaves and tomato fruits to be decreasing with increase in salt stress. The results of this study agree with the findings by Shawky (2003) he found that the ascorbic acid increased the potassium concentration in shoot and root of sweet pepper plants growing under normal and saline conditions. In this respect the protection of sweet pepper plants against salt stress by an exogenous supply of AsA is believed to be caused indirectly as a result of its effect on K^+ uptake which plays an essential role in many metabolic processes; may be induce the synthesis of stress proteins as a "messenger" (chitinase, glucanase, peroxidase, peroxidismutase) in general is it the induction of PR-proteins. Potassium (K^+) content increased with application of AsA as compared to non-salinized plants. In most cases, pre-soaking with AsA at 100 ppm was the most effective in increasing K^+ concentration in the shoots and roots. However, K^+ concentration decreased in the root system more than shoot. Whereas, salinity and AsA increased K^+

Table (3): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Potassium (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA. (mM) / **NaCl (ppm)**	*Shoot (a)*				*Root (b)*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	5.78 ± 0.05	10.21 ± 0.18	342.759*	<0.001*	4.18 ± 0.64	8.01 ± 0.08	48.282*	<0.001*
1500	7.10 ± 0.24	14.81 ± 0.09	522.660*	<0.001*	5.83 ± 0.05	8.81 ± 0.06	1027.332*	<0.001*
3000	8.89 ± 0.14	15.83 ± 0.11	539.296*	<0.001*	7.31 ± 0.07	10.38 ± 0.05	741.960*	<0.001*
4500	10.21 ± 0.18	18.20 ± 0.18	824.956*	<0.001*	8.98 ± 0.04	12.90 ± 0.05	1246.647*	<0.001*
6000	12.30 ± 0.13	20.13 ± 0.09	214.397*	<0.001*	10.01 ± 0.09	15.01 ± 0.06	134.119*	<0.001*
F_2	253.748*	769.693*			65.483*	2323.264*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		*F* = 2562.535*	*p*<0.001*	NaCl ppm Conc.		*F* = 2630.566*	*p*<0.001*
	AsA(mM) Conc.		*F* = 2793.961*	*p*<0.001*	AsA(mM) Conc.		*F* = 2169.061*	*p*<0.001*
	NaCl ppm Conc.xAsA (mM)		*F* = 13.302*	*p*<0.001*	NaCl ppm Conc. X AsA (mM)		*F* = 4.947*	*p*<0.001*

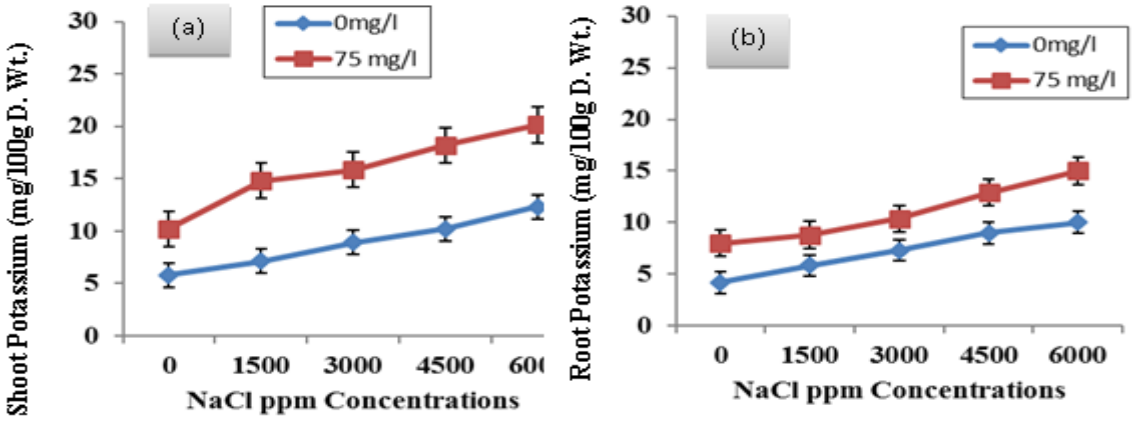


Fig. (3 a& b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Potassium (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

concentration as compared to salinity stress. The results obtained by Khafagy *et al.* (2009) they found that the significant decrease in K^+ concentration occurred with increasing salinity levels in sweet pepper plants. In this respect, high salinity level (6000 ppm) NaCl (11.88 dsm^{-1}) was the most effective in this concern as compared to control.

Phosphorous Content (mg/100g Dry Weight):
Increasing NaCl salinity resulted a marked increase in phosphorous contents (P^{+3}) in shoot and root of tomato plant compared with control at (42 Days) as shown in Fig. (4 a & b) and Table (4). Generally, the P^{+3} contents in shoot and root significantly increased ($p \leq 0.001$) in tomato plant especially at moderate and high salinity concentration.

Whereas, in the present of AsA with concentration (0.75 mM) under the various of NaCl salinity caused an increased significantly ($p \leq 0.001$) in P^{+3} contents in shoot and root of tomato plant compared with control under saline and non-saline stress. In the present of AsA the shoot and root of tomato plant

significantly increased the P^{+3} contents at lowest NaCl salinity concentration (1500 ppm), compared with control. Also AsA concentration increased P^{+3} contents in shoot and root under moderate and high NaCl salinity concentrations (3000 – 4500 – 6000 ppm) at (42 days) compared with control. Maximum increase in P^{+3} contents was shown at AsA concentration (0.75 mM) at NaCl concentration (6000 ppm). Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at (42 days) indicated that the *F* test highly significant at $P \leq 0.001$.

Table (4): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Phosphorus (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA. (mM) / NaCl (ppm)	*Shoot (a)*				*Root (b)*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	3.72 ± 0.03	7.61 ± 0.18	55.240*	<0.001*	1.12 ± 0.20	4.61 ± 0.42	63.323*	<0.001*
1500	4.81 ± 0.13	8.94 ± 0.24	149.576*	<0.001*	1.89 ± 0.11	5.81 ± 0.14	138.674*	<0.001*
3000	6.31 ± 0.11	12.01 ± 0.35	186.098*	<0.001*	2.57 ± 0.18	7.30 ± 0.25	126.538*	<0.001*
4500	7.98 ± 0.20	15.30 ± 0.17	422401*	<0.001*	3.03 ± 0.16	9.10 ± 0.17	167.357*	<0.001*
6000	9.01 ± 0.28	17.80 ± 0.20	592.866*	<0.001*	3.98 ± 0.24	10.30 ± 0.21	118.066*	<0.001*
F_2	159.290*	321.343*			35.986	82.322*		
p	<0.001*	<0.001*			<0.001*			
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		*F* = 2318.342*	*p* <0.001*	NaCl ppm Conc.		*F* = 759.933*	*p* <0.001*
	AsA(mM) Conc.		*F* = 3128.093*	*p* <0.001*	AsA(mM) Conc.		*F* = 1123.842*	*p* <0.001*
	NaCl ppm Conc. x AsA(mM)		*F* = 4.510*	*p* <0.001*	NaCl ppm Conc. x AsA(mM)		*F* = 1.293	*p* = 0.217

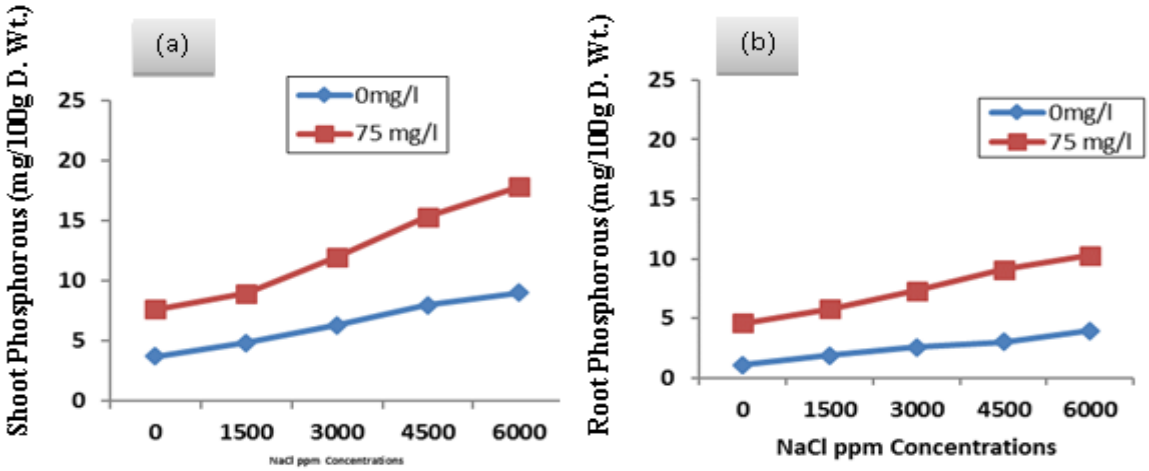


Fig. (4 a& b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Phosphorous (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

The results of this study agree with results obtained by Talaat (2003) on sweet pepper where found detect that foliar application of ascorbic acid increased the macronutrient elements (N; P and K) content. Whereas, the results obtained by Soltani Nezhad *et al.* (2011) they found that the effects of different concentrations of NaCl (0, 60, 90, 120 and 150 mM NaCl) on phosphorus content in tomato (*Lycopersicon peruvianum* L) plant decreased was increased at 150 mM NaCl. Whereas, with increasing salinity levels, the phosphorus content of roots decreased in all NaCl salinity concentrations. So, up to 120 mM NaCl salinity there was no significant difference in phosphorus content between untreated and treated plants. However a significant decrease between, 150 mM NaCl and other salt treated plants was observed. In contrast, by increasing of salt concentration in the culture medium, phosphorus content decreased significantly in roots compared to untreated plants.

Calcium Content (mg/100g Dry Weight):

The calcium contents (Ca^{+2}) in shoot and root of tomato plant increased gradual with increasing NaCl salinity concentrations (1500; 3000; 4500 & 6000 ppm) at (42 days) compared with control as shown in Fig. (5 a & b) and Table (5). Whereas, in the present of AsA with concentration (0.75 mM) as shown that the increasing of Ca^{+2} content in shoot and root more than in the absent of AsA under NaCl salinity stress compared to control. In the present of AsA (+AsA) the increased significantly ($p \leq 0.001$), the uptake of Ca^{+2} content under both saline and non-saline conditions at (42 days). So, the uptake of Ca^{+2} content was enhanced in tomato under saline and non-saline conditions. The concentration (0.75 mM) of AsA was effective an increased significantly ($p \leq 0.001$). Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at (42 days) indicated that the *F* test highly significant at $P \leq 0.001$.

The present results agree with the results presented by McLaughlin and Wimmer (1999) they reported that the despite its obvious importance, the low mobility of Ca^{2+} make the rates of its uptake and distribution limiting processes for many key plant functions. Furthermore, the general lack of recognition of the limiting role of Ca^{2+} is due in part to the fact that some important plant functions are controlled by changes in very small physiologically active pools of Ca^{2+} within the cytoplasm. As such, whole-leaf Ca^{2+} levels might not reflect any potential limitations

Table (5): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Calcium (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA. (mM) / NaCl (ppm)	***Shoot (a)***				***Root (b)***			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	19.63 ± 0.57	26.57 ± 0.50	49.165*	<0.001*	13.71 ± 0.48	18.01 ± 0.53	38.364*	<0.001*
1500	22.36 ± 0.58	28.09 ± 0.60	29.339*	<0.001*	14.22 ± 0.48	19.98 ± 0.45	57.399*	<0.001*
3000	24.99 ± 0.55	32.01 ± 0.38	66.152*	<0.001*	16.08 ± 0.42	21.31 ± 0.37	63.223*	<0.001*
4500	27.02 ± 0.59	35.09 ± 0.51	75.991*	<0.001*	18.04 ± 0.37	23.91 ± 0.59	40.314*	<0.001*
6000	28.21 ± 0.55	39.01 ± 0.42	134.332*	<0.001*	19.98 ± 0.51	27.80 ± 0.51	74.992*	<0.001*
F_2	37.486*	107.958*			32.897*	58.962*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		F = 649.500*	p<0.001*	NaCl ppm Conc.		F =350.679*	P <0.001*
	AsA(mM) Conc.		F = 511.550*	p<0.001*	AsA(mM) Conc.		F = 392.690*	P <0.001*
	NaCl ppm Conc. x AsA(mM)		F = 1.515	p=0.109	NaCl ppm Conc. x AsA(mM)		F = 1.026	P =0.437

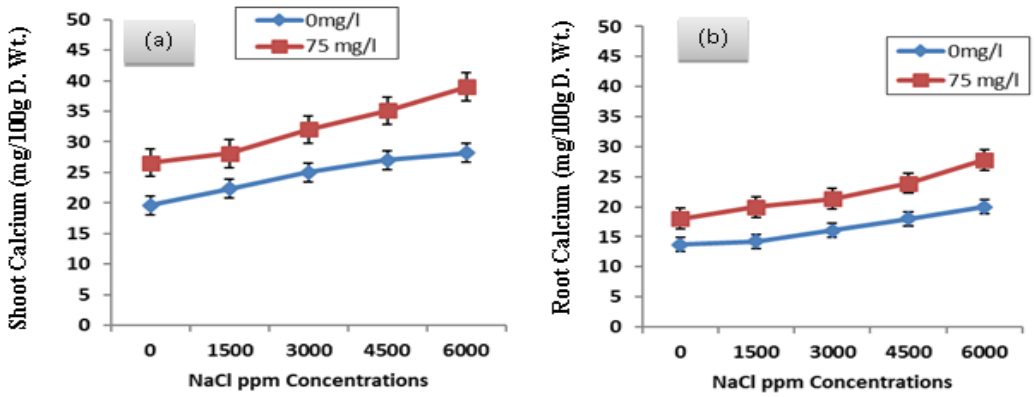


Fig. (5 a& b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Calcium (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

Magnesium Content (mg/100g Dry Weight):

The magnesium content (Mg^{+2}) increased significantly ($p \leq 0.001$) in shoot and root of tomato plant with increasing NaCl salinity concentrations (1500; 3000; 4500 & 6000 ppm NaCl) compared to control as shown in Fig.(6 a & b) and Table (6). The clearly increased observed under high salinity level compared to control. Whereas, in the present of AsA with concentration (0.75 mM) tended to increasing Mg^{+2} content in both shoot and root of tomato plant growing under saline and non-saline conditions. The more effective in the present of AsA concentration (0.75 mM) with NaCl salinity concentrations (4500 – 6000 ppm NaCl) tended to increasing Mg^{+2} contents in the both shoot and root of tomato at (42 days) compared to control. The results indicating a positive impact to use AsA with concentration (0.75 mM) tended to increase the Mg^{+2} content in shoot and root with increasing NaCl salinity concentration. Mg^{+2} content tended to increase with the application of AsA by soaking seeds of tomato in concentration (AsA 0.75 mM) compared with control. As a matter of fact, that Mg^{+2} uptakes were positive affected with increasing

Table (6): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Magnesium (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA. (mM)	***Shoot (a)***				***Root (b)***			
NaCl (ppm)	**0.00**	**0.75**	***F1***	***p***	**0.00**	**0.75**	***F1***	***p***
Control	33.84 ± 0.59	39.10 ± 0.56	20.298*	<0.001*	23.01 ± 0.98	29.17 ± 0.59	18.456*	<0.001*
1500	36.67 ± 0.59	40.89 ± 0.53	14.231*	<0.001*	25.71 ± 0.56	31.30 ± 0.55	21.576*	<0.001*
3000	39.40 ± 0.61	46.01 ± 0.59	37.313*	<0.001*	27.01 ± 0.62	33.10 ± 0.60	36.678*	<0.001*
4500	40.88 ± 0.59	47.01 ± 0.62	36.645*	<0.001*	29.89 ± 0.51	36.30 ± 0.57	33.340*	<0.001*
6000	42.87 ± 0.65	48.98 ± 0.60	26.162*	<0.001*	30.33 ± 0.61	37.90 ± 0.62	38.575*	<0.001*
F_2	34.210*	52.536*			19.987*	37.092*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (ANOVA	NaCl ppm Conc.		F = 397.494*	p<0.001*	NaCl ppm Conc.		F = 302.559*	p<0.001*
	AsA(mM) Conc.		F = 236.538*	p<0.001*	AsA(mM) Conc.		F = 245.510*	p<0.001*
	NaCl ppm Conc. x AsA(mM)		F = 0.463	p=0.959	NaCl ppm Conc. x AsA(mM)		F = 0.589	p=0.885

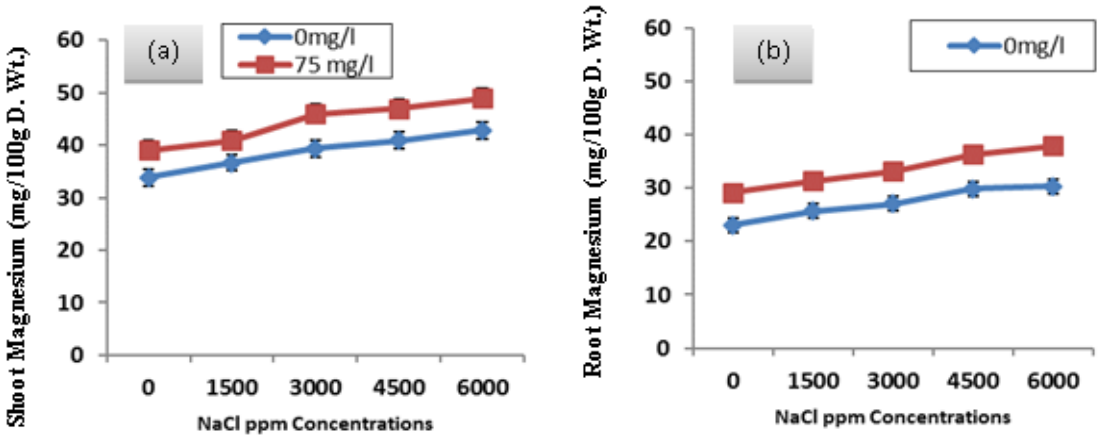


Fig. (6 a& b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Magnesium (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

concentrations of both AsA and NaCl salinity at (42 days) of tomato plant. Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at (42 days) indicated that the *F* test highly significant at $P \leq 0.001$. The results of the present study agree with the results obtained by Farahat *et al.* (2013) they found the highest nitrogen in shoot resulted from ascorbic acid (200 ppm) and salinity (3000 ppm) level. Barakat (2003) found that the ascorbic acid has effects on many physiological processes including the regulation of growth and metabolism of plants under saline conditions and increasing physiological availability of water and nutrient

Manganese Content (mg/100g Dry Weight):

Manganese content (Mn^{+2}) in shoot and root of tomato plant increased significantly ($p \leq 0.001$) with increasing NaCl salinity concentrations Fig. (7 a & b) and Table (7).

Table (7): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Manganese (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA. (mM)	*Shoot (a)*				*Root (b)*			
NaCl (ppm)	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	0.277 ± 0.002	0.398 ± 0.004	66.539	<0.001*	0.201 ± 0.008	0.230 ± 0.008	5.926*	0.010*
1500	0.333 ± 0.003	0.433 ± 0.005	160.983*	<0.001*	0.270 ± 0.009	0.301 ± 0.008	4.967*	0.018
3000	0.350 ± 0.004	0.493 ± 0.002	126.575*	<0.001*	0.290 ± 0.012	0.390 ± 0.001	50.578*	<0.001*
4500	0.390 ± 0.002	0.504 ± 0.007	426.071*	<0.001*	0.337 ± 0.008	0.403 ± 0.004	22.705*	<0.001*
6000	0.430 ± 0.005	0.591 ± 0.009	363.609*	<0.001*	0.358 ± 0.010	0.417 ± 0.002	15.212*	<0.001*
F_2	278.412*	150.873*			42.901*	208.916*		
p	<0.001*	<0.001*			<0.001*			
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		F = 503.800*	*p* <0.001*	NaCl ppm Conc.		F = 700.286*	*p* <0.001*
	AsA(mM) Conc.		F = 825.556*	*p* <0.001*	AsA(mM) Conc.		F = 91.657*	*p* <0.001*
	NaCl ppm Conc. x AsA(mM)		F = 4.775*	*p* <0.001*	NaCl ppm Conc. x AsA(mM)		F = 4.021*	*p* <0.001*

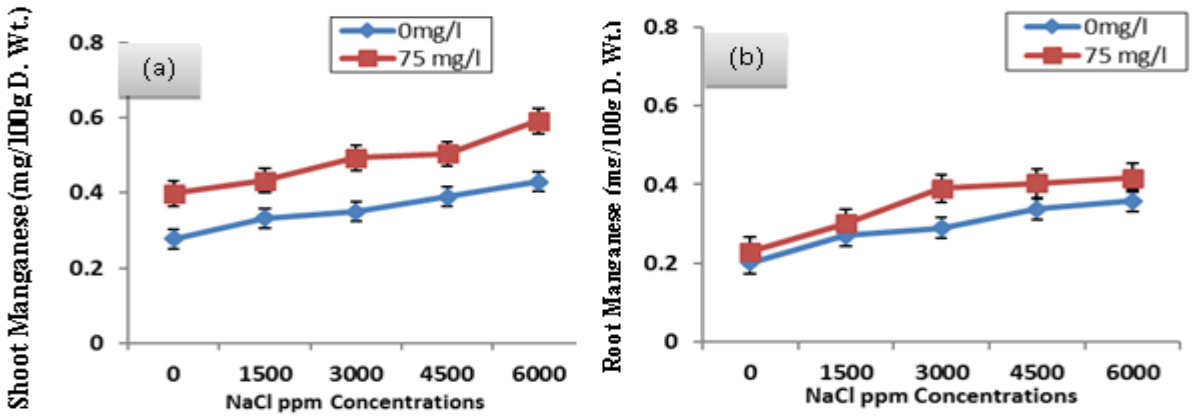


Fig. (7 a& b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Manganese (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

In the present of AsA with concentration (0.75 mM) tended to increase Mn^{+2} content in both shoot and root of tomato plant compared with control under saline and non-saline stress. The result indicated that the uptake of Mn^{+2} content in shoot and root of tomato plant increased by increasing NaCl salinity from (1500; 3000; 4500 & 6000 ppm) compared to control. Under NaCl salinity treatments the Mn^{+2} content increased more in the present than in the absent of AsA with concentration (0.75 mM) at 42 days. Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in concentration of AsA (0.75 mM) at growth stage (42 Days) indicated that the *F* test highly significant at $P \leq 0.001$.

Copper Content (mg/100g Dry Weight):

The copper content (Cu^{+2}) increased significantly ($p \leq 0.001$) with increasing NaCl salinity concentrations particularly in the present of AsA concentration (0.75 mM) at (42 days) as shown in Fig. (8a & b) & Table (8).

Table (8): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Copper (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA. (mM) / NaCl (ppm)	*Shoot (a)*				*Root (b)*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	9.71 ± 0.04	11.09 ± 0.08	64.952*	<0.001*	4.41 ± 0.04	5.03 ± 0.07	156.648*	<0.001*
1500	10.02 ± 0.05	12.90 ± 0.09	185.383*	<0.001*	4.53 ± 0.05	5.63 ± 0.38	16.237*	<0.001*
3000	10.19 ± 0.05	13.90 ± 0.13	427.709*	<0.001*	4.76 ± 0.02	6.35 ± 0.09	280.345*	<0.001*
4500	10.29 ± 0.12	15.01 ± 0.11	786.672*	<0.001*	4.88 ± 0.04	7.33 ± 0.16	177.988 *	<0.001*
6000	10.70 ± 0.09	15.98 ± 0.10	802.975*	<0.001*	5.01 ± 0.09	8.01 ± 0.03	342.176*	<0.001*
F_2	22.268*	339.952*			20.245*	40.198*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		*F* = 220.117*	*p* <0.001*	NaCl ppm Conc.		*F* = 901.236*	*p* <0.001*
	AsA(mM) Conc.		*F* = 316.977*	*p* <0.001*	AsA(mM) Conc.		*F* = 832.661*	*p* <0.001*
	NaCl ppm Conc. x AsA(mM)		*F* = 3.364*	*p* <0.001*	NaCl ppm Conc. x AsA(mM)		*F* = 10.237*	*p* <0.001*

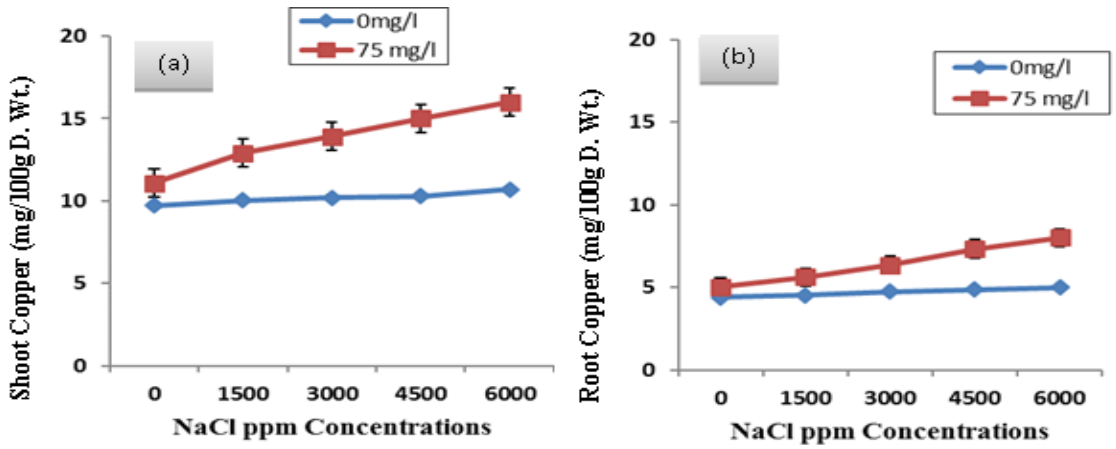


Fig. (8 a & b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Copper (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

The Cu^{+2} content tended to increased significantly ($p \leq 0.001$) in the present of AsA with concentration (0.75 mM) at both shoot and root of tomato plant under saline and non-saline conditions at (42 days) . On other hand, moderate and high NaCl salinity levels (3000; 4500; 6000 ppm) tended to increased Cu^{+2} content in both shoot and root of tomato plant at AsA concentration (0.75 mM) compared to control. Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in concentration of AsA at (42 days) indicated that the *F* test highly significant at $P \leq 0.001$

Zinc Content (mg/100g Dry Weight):

Zinc content (Zn^{+2}) increase significantly ($p \leq 0.001$) with NaCl salinity increased particularly in shoot and root of tomato plant in the present of AsA with concentration (0.75 mM) at (42 Days) compared with control as shown in Fig. (9 a & b) and Table (9).

Table (9): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Zinc (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA. (mM) / NaCl (ppm)	*Shoot (a)*				*Root (b)*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	0.187 ± 0.003	0.293 ± 0.004	542.975*	<0.001*	0.169 ± 0.001	0.233 ± 0.004	72.532*	<0.001*
1500	0.219 ± 0.004	0.249 ± 0.001	11.212*	0.001*	0.183 ± 0.003	0.275 ± 0.004	6.085*	0.010*
3000	0.279 ± 0.004	0.383 ± 0.004	12.103*	0.001*	0.189 ± 0.001	0.310 ± 0.004	262.819*	<0.001*
4500	0.305 ± 0.003	0.438 ± 0.005	89.690*	<0.001*	0.195 ± 0.002	0.390 ± 0.005	284.380*	<0.001*
6000	0.337 ± 0.010	0.503 ± 0.009	23.867*	<0.001*	0.205 ± 0.005	0.405 ± 0.008	330.987*	<0.001*
F_2	127.058*	396.199*			23.635*	219.020*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		*F* = 1299.034*	*p* <0.001*	NaCl ppm Conc.		*F* = 318.804*	*p* <0.001*
	AsA(mM) Conc.		*F* = 234.152*	*p* <0.001*	AsA(mM) Conc.		*F* = 396.233*	*p* <0.001*
	NaCl ppm Conc. x AsA(mM)		*F* = 4.026*	*p* <0.001*	NaCl ppm Conc. x AsA(mM)		*F* = 10.002*	*p* <0.001*

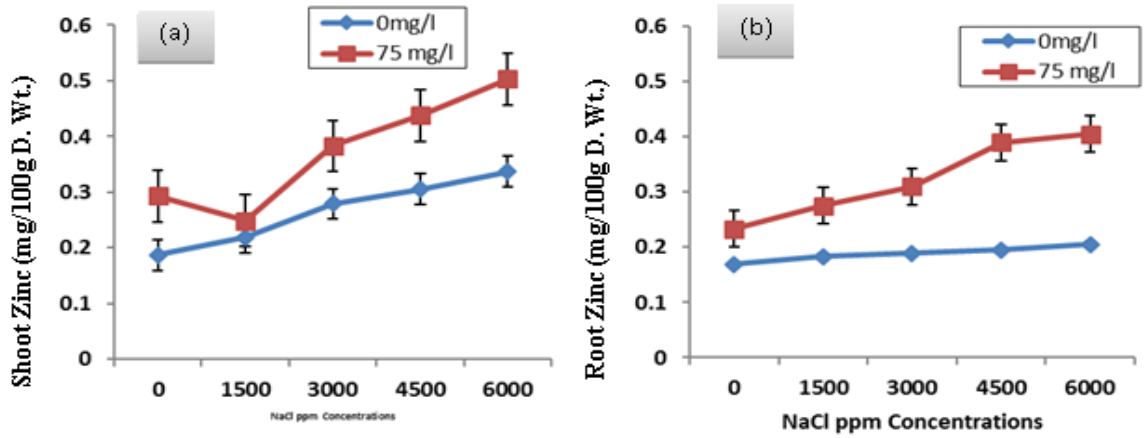


Fig. (9 a & b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Zinc (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

Using different concentrations of NaCl caused a significantly ($p \leq 0.001$) increased of Zn^{+2} content in both shoot and root of tomato plant, the maximum Zn^{+2} content at NaCl salinity concentrations (4500 & 6000 ppm) compared with control at (42 days). While, in the present of AsA the Zn^{+2} content in shoot and root of tomato plant tended to increased. Highest content of Zn^{+2} recorded in shoot and root of tomato plants at concentration of AsA (0.75 mM) under NaCl salinity concentrations (4500- 6000 ppm) compared with control at (42 days). Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at (42 days) indicated that the *F* test highly significant at $P \leq 0.001$.

3.1.1. Boron Content (mg/100g Dry Weight):

Overall, Boron content (B^{+2}) increased significantly ($p \leq 0.001$) in both shoot and root of tomato plant with increasing NaCl salinity concentrations at (42 Days) as shown in Fig.(10 a & b) and Table (10).

Table (10): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Boron (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA. (mM) NaCl (ppm)	*Shoot (a)*				*Root (b)*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	0.021 ± 0.001	0.070 ± 0.001	737.220*	<0.001*	0.007 ± 0.00	0.015 ± 0.001	8.912*	0.002*
1500	0.053 ± 0.00	0.101 ± 0.002	710.500*	<0.001*	0.010 ± 0.002	0.021 ± 0.00	12.947*	0.001*
3000	0.090 ± 0.001	0.137 ± 0.001	582.095*	<0.001*	0.013 ± 0.001	0.023 ± 0.002	14.716*	<0.001*
4500	0.107 ± 0.002	0.207 ± 0.001	1670.254*	<0.001*	0.018 ± 0.001	0.027 ± 0.002	12.221*	0.001*
6000	0.135 ± 0.001	0.230 ± 0.001	1885.705*	<0.001*	0.021 ± 0.004	0.031 ± 0.002	2.297	0.130
F_2	1276.606	2498.321*			8.018*	13.189*		
p	<0.001*	<0.001*			0.004*	0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		*F* = 27786.467*	*p* <0.001*	NaCl ppm Conc.		*F* = 78.426*	*p* <0.001*
	AsA(mM) Conc.		*F* = 11640.962*	*p* <0.001*	AsA(mM) Conc.		*F* = 51.193*	*p* <0.001*
	NaCl ppm Conc. x AsA(mM)		*F* = 92.926*	*p* <0.001*	NaCl ppm Conc. x AsA(mM)		*F* = 0.217	*p* = 0.999

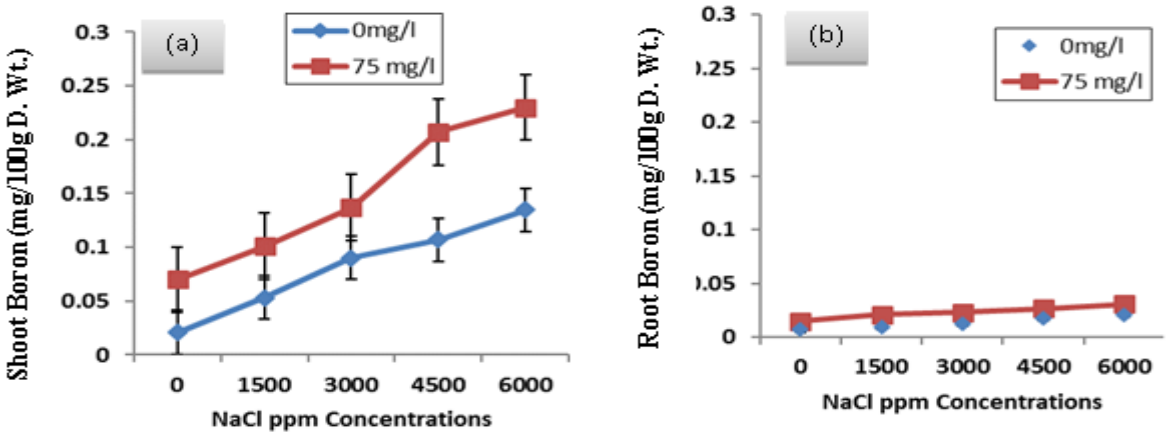


Fig. (10 a & b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Boron (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

The results indicated that the application of NaCl salinity stress caused a significantly increased ($p \leq 0.001$) B^{+2} content in shoot and root of tomato plants compared to control. While, in the present of AsA with concentration (0.75 mM) tended to increasing the B^{+2} content in both shoot and root under different levels of NaCl salinity stress compared with control. Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at growth stage (42 days) indicated that the *F* test highly significant at $P \leq 0.001$.

Iron Content (mg/100g Dry Weight)

Generally, the iron content (Fe^{+3}) increased significantly ($p \leq 0.001$) in both shoot and root of tomato plant with increasing NaCl salinity concentrations at (42 days) as shown in Fig. (11 a & b) and Table (11).

Table (11): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Iron (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA. (mM) / NaCl (ppm)	*Shoot (a)*				*Root (b)*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	0.12 ± 0.03	0.27 ± 0.03	15.409*	<0.001*	0.15 ± 0.02	0.23 ± 0.03	22.070*	<0.001*
1500	0.17 ± 0.01	0.36 ± 0.02	52.653*	<0.001*	0.23 ± 0.01	0.28 ± 0.01	23.393*	<0.001*
3000	0.25 ± 0.03	0.45 ± 0.02	68.219*	<0.001*	0.27 ± 0.03	0.40 ± 0.02	29.674*	<0.001*
4500	0.37 ± 0.03	0.54 ± 0.02	39.159*	<0.001*	0.30 ± 0.02	0.44 ± 0.03	41.461*	<0.001*
6000	0.44 ± 0.04	0.61 ± 0.02	21.912*	<0.001*	0.37 ± 0.02	0.53 ± 0.02	58.577*	<0.001*
F_2	66.221*	120.893*			51.881*	106.885*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		F = 515.754*	p <0.001*	NaCl ppm Conc.		F = 245.518*	p <0.001*
	AsA(mM) Conc.		F = 231.363*	p <0.001*	AsA(mM) Conc.		F = 82.376*	p <0.001*
	NaCl ppm Conc. xAsA(mM)		F = 0.625	p = 0.857	NaCl ppm Conc. xAsA(mM)		F = 0.947	p = 0.520

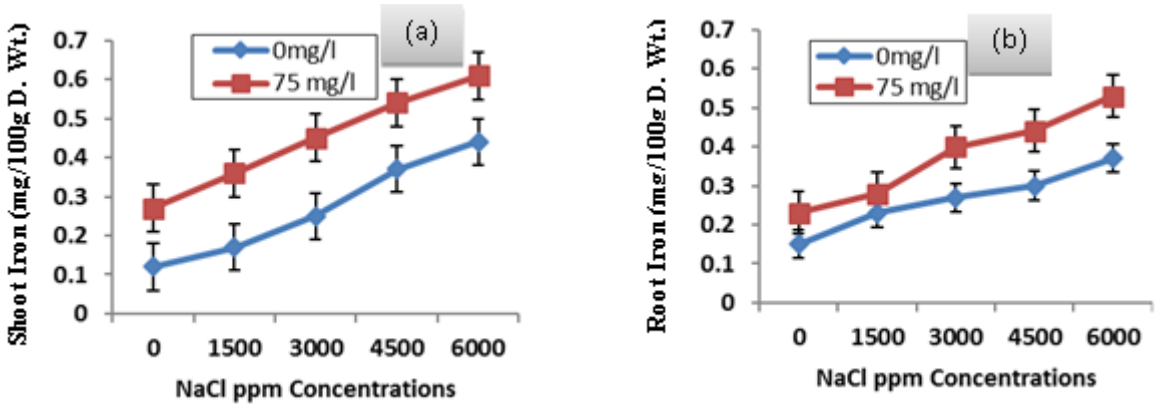


Fig. (11 a & b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Iron (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

The results showed that the Fe^{+3} content in both shoot and root of tomato plants were high under NaCl salt stress treatments compared to control. Increasing NaCl salinity concentrations (1500; 3000; 4500 & 6000 ppm) tended to significantly increased ($p \leq 0.001$) the Fe^{+3} content in both shoot and root. While, in the present of AsA with concentration (0.75 mM) tended to increase Fe^{+3} content in both shoot and root of tomato plant under different levels of NaCl salinity concentrations compared with control. Generally, it can be said that the AsA more effective for improving plant performance under saline and non-saline stress. Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at growth stage (42 days) indicated that the *F* test highly significant at $P \leq 0.001$. The present results agree with the results finding by Farahat *et al.* (2013) they reported that the all of nitrogen (N); phosphorus (P) and potassium (K) contents in both shoots and roots increased gradually with increasing the levels of ascorbic acid. So, the results obtained by Shao *et al*. (2008) they found that the ascorbic acid (AsA) protect metabolic processes against H_2O_2 and other toxic derivatives of oxygen affected many enzyme activities, minimize the damage caused by oxidative processes through synergistic function with other antioxidants and stabilize membranes.

The results presented here agreed with results obtained by Farouk (2011) he reported the application of antioxidants, especially ascorbic acid, significantly increased potassium, calcium and magnesium in wheat flag leaf, because th application of antioxidants, especially ascorbic acid, partially reversed the negative effects of salinity in this respect. While, the findings not consistent with that potassium, magnesium and calcium contents decreased with increasing salinity levels up to 11 dsm^{-1}. The results obtained by Bassuony *et al.* (2008) they found that the content of K^{+}; Ca^{+2} and Mg^{+2} in *Zea mays* plant decreased significantly under salinity stress, compared with control. While, application of 100 ppm from vitamins C (ascorbic acid) resulted significantly increases of K^{+}, Ca^{+2} and Mg^{+2} contents compared with controls. Also, the results obtained by Flores *et al.* (2001) they found that the salt stress inhibits the uptake and transport of potassium, calcium and phosphorus, we predict that sodium chloride will inhibit growth in our tomato plants.

Sodium Content (mg/100g Dry Weight):

Overall, the sodium contents (Na^{+}) in shoot and root of tomato plant increased significantly ($p \leq 0.001$) with increasing NaCl salinity concentrations (1500, 3000, 4500 & 6000 ppm NaCl) at (42 days) compared with control as shown in Fig.(12 a & b) and Table (12). Whereas, in the present of AsA and in the absent of AsA with concentration (0.75 mM) resulted an increased significantly the sodium contents in shoot and root under saline and non-saline conditions at (42 days). Generally, the Na^{+} content was higher in shoot and root of tomato plant compared to control, the Na^{+} content increased in shoot more than the root especially in the present of AsA with increased the NaCl salinity concentrations. Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at growth stage (42 days) indicated that the *F* test highly significant at $P \leq 0.001$.

Table (12): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Sodium (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (*P = 0.05*).

AsA. (mM) / NaCl (ppm)	*Shoot (a)*				*Root (b)*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	73.35 ± 0.37	86.19 ± 0.42	105.941*	<0.001*	56.81 ± 0.43	67.42 ± 0.24	372.805*	<0.001*
1500	84.62 ± 0.42	92.13 ± 0.53	54.683*	<0.001*	63.19 ± 0.13	70.13 ± 0.47	299.235*	<0.001*
3000	86.19 ± 0.49	96.81 ± 0.43	71.068*	<0.001*	72.81 ± 0.39	79.01 ± 0.32	117.454*	<0.001*
4500	92.86 ± 0.41	105.01 ± 0.31	230.319*	<0.001*	77.91 ± 0.14	87.01 ± 0.36	380.806*	<0.001*
6000	99.63 ± 0.64	112.81 ± 0.92	95.096*	<0.001*	83.88 ± 0.11	93.18 ± 0.12	1454.534*	<0.001*
F_2	428.319*	345.854*			1557.993*	1148.022*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		*F* = 3427.248*	*p*<0.001*	NaCl ppm Conc.		*F* = 249.800*	*p*<0.001*
	AsA(mM) Conc.		*F* = 416.901*	*p*<0.001*	AsA(mM) Conc.		*F* = 96.103*	*p*<0.001*
	NaCl ppm Conc. x AsA(mM)		F =16.991*	*p*<0.001*	NaCl ppm Conc. x AsA(mM)		*F* = 4.549*	*p*<0.001*

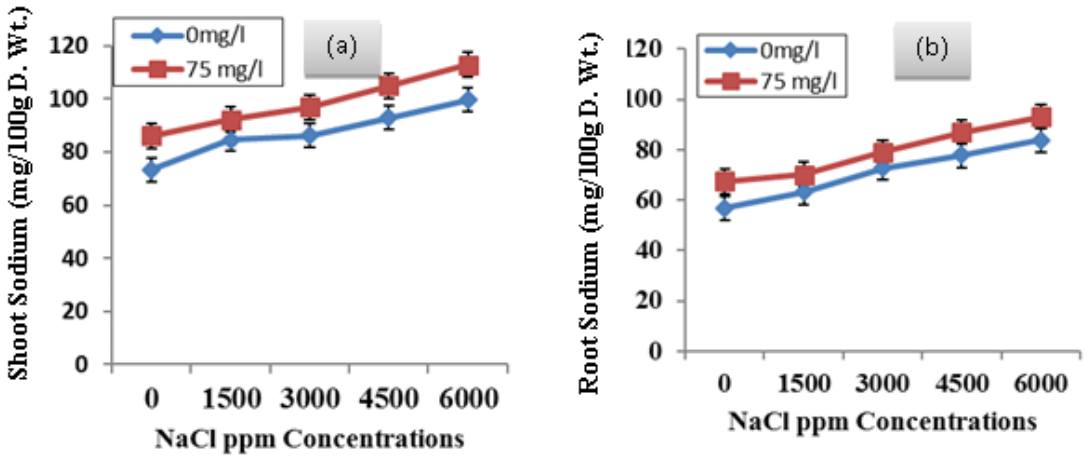


Fig. (12 a & b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Sodium (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

The present results of this study agree with the resulted by Babu *et al.* (2012) they found that the tomato cultivar PKM 1 were subjected to 25, 50, 100, 150 and 200 mM NaCl stress, showed a significant elevation in the levels of sodium ion concentration. Similar studies were carried out and same outcomes were found by some other authors (Hu and Schmidhalter, 1997; Sagi *et al.*, 1997; Bagci *et al.*, 2003; Beck *et al.*, 2004; Netondo *et al.*, 2004; Akram *et al.*, 2007; Loukehaich *et al.*, 2011).

Similar results agree with the obtained by Arab and Ehsanpour (2006) they explained that the increasing in sodium (Na) content of stem-leaf and roots of alfalfa (*Medicago sativa* L.) due to increasing NaCl in the medium, whereas, the results disagree with our results in the present of AsA (added ascorbic acid at 2.0 mM) in the medium decreased the level of Na^+ content of stem-leaf and roots ($p<0.05$) significantly. The results presented here agree with results reported by Shiwani *et al*. (2010) they found that the

plants growing under saline conditions accumulate more of sodium resulting in ionic imbalance which has an adverse effect on plant metabolism. Accumulation of Na^+ was observed in leaves of both the cultivars WH-542 and KRL-19. Also, Ali *et al.* (2011) found tomato cultivars showed significant variation in their sodium uptake. NaCl stress increases Na^+ contents in dry tissues from 191.828 to 436.17 µM Na^+ g^{-1} dry weights. Among cultivars, significantly higher sodium absorption was recorded in cultivar Rio Grande (365.645 µM Na^+ g^{-1} dry weight).

Chloride Content (mg/100g Dry Weight)

Overall, the chloride (anion) content (Cl^-) in shoot and root of tomato plant increased significantly ($p \leq 0.001$) with increasing NaCl salinity concentrations at (42 Days) as shown in Fig. (13 a & b) and Table (13).

Table (13): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Chloride (mg/100g D. Wt.) Contents of Tomato (*Solanum lycopersicum*, L.) Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (p = 0.05).

AsA. (mM) / NaCl (ppm)	*Shoot (a)*				*Root (b)*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	27.07 ± 0.51	29.33 ± 0.50	4.625*	0.023*	17.56 ± 0.56	19.38 ± 0.54	3.028	0.071
1500	32.62 ± 0.50	34.88 ± 0.53	4.035*	0.033*	20.69 ± 0.61	25.81 ± 0.53	39.686*	<0.001*
3000	36.64 ± 0.42	39.01 ± 0.59	9.778*	0.002*	22.74 ± 0.57	28.98 ± 0.44	30.624*	<0.001*
4500	41.07 ± 0.55	45.89 ± 0.50	17.928*	<0.001*	26.16 ± 0.62	30.01 ± 0.59	16.692*	<0.001*
6000	43.40 ± 0.48	47.17 ± 0.54	17.645*	<0.001*	27.59 ± 0.48	32.12 ± 0.59	22.542*	<0.001*
F_2	173.812*	198.920*			50.861*	84.546*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		F = 1697.612*	*p* <0.001*	NaCl ppm Conc.		F = 706.717*	*p* <0.001*
	AsA(mM) Conc.		F = 90.187*	*p* <0.001*	AsA(mM) Conc.		F = 156.492*	*p* <0.001*
	NaCl ppm Conc. x AsA(mM)		F = 0.709	*p* =0.779	NaCl ppm Conc. x AsA(mM)		F = 0.537	*p* = 0.921

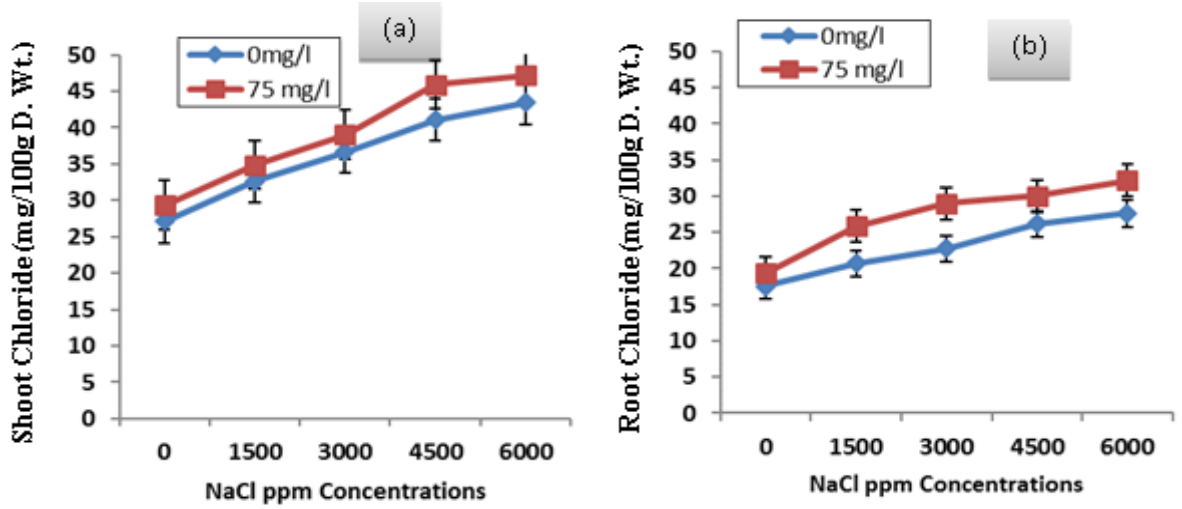


Fig. (13 a & b): Effect of Exogenous Application of AsA (0.75 mM) on Shoot (a) and Root (b) Chloride (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (*P = 0.05*).

The change in Cl^- content in both shoot and root of tomato plant was affected by NaCl salinity with different concentrations compared to control, Cl^- content increased significantly ($p \leq 0.001$) in shoot and root of tomato at all NaCl salinity concentrations. Maximum increased in chloride content was shown at high NaCl salinity concentrations (6000 ppm). While as, in the present of AsA with concentration (0.75 mM) caused an increase in chloride content in shoot and root of tomato plant at (42 Days) compared with control. The results showed that the concentration (0.75 mM) of AsA tended to increasing the Cl^- content in both shoot and root under different concentrations of NaCl as compared with control. But maximum increase in Cl^- content in shoot and root were determined at NaCl concentrations (4500-6000ppm) and AsA concentration (0.75 mM). Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl in each concentration of AsA at (42 Days) indicated that the *F* test highly significant at $P \leq 0.001$.

The results of this study agree well with the data obtained by Gunes *et al.* (2007), they revealed that ascorbic acid stimulated N, Mg, Fe, Mn and Cu concentrations of salt stressed maize plants. These results suggest that ascorbic acid could be used as a potential growth regulator to improve plant salinity stress resistance. As well as, Abd El-Aziz *et al*. (2007) found that the N, P and K contents in *Syngonium podophyllum*, (L) increased gradually by increasing the concentration of AsA to 100 ppm compared with the untreated plants. Whereas, previous workers found that salinity increases Na^+ and Cl^- and decreases K^+, Ca^{2+} in plant leaves (Porcelli *et al.,* 1995; Saghir *et al.,* 2002; Hosseini and Thengane, 2007; Taffouo *et al.,* 2010a.) Our current findings agree with the data obtained by Taffouo *et al.* (2010 b) they found that the salt treatments (50, 100 and 200 mM) increased significantly Na^+ contents in roots, stems and leaves of plants. Whereas, no agree with K^+ and Ca^{2+} concentrations of plants were decreased in all tomato cultivars.

The increment in N concentration due to AsA treatments could be explained by the findings of Talaat (2003) who showed that accumulation of nitrate by AsA foliar application may be due to the positive effect of AsA on root growth which consequently increased nitrate absorption. In this context the increase in P concentration by thiamine and AsA treatments may be attributed to the postulation. The results obtained from this study agree by Hussein *et al.* (2011) they reported that the interaction effect between ascorbic acid (AsA) spraying rate from 0 to 200 ppm and salinity irrigation, in the present of ascorbic acid increased the uptake of essential nutrients N, P, K, Ca and Mg of wheat, but did not agree with where observed decreased the Na and Cl uptake so the ascorbic acid played an important role of decreasing effects of saline conditions. Many studies reported that the optimal concentration of vitamin C exhibited beneficial effect on growth and yield of some crop plants grown under saline conditions. The ascorbic acid can play an inductive role in alleviating the adverse effect of salinity on plant growth and metabolism in many plants. So, the investigate inductive role of 100 ppm vitamin C solution either before (seed soaking) or after (shoot spraying) cultivation on seed germination, growth, water status, antioxidant enzymes and protein patterns of *Silybum marianum* (L) Gaertner plants under irrigation with diluted NaCl (Azooz, 2004; Khan *et al.,* 2006; Bassuony *et al.,* 2008).

CONCLUSION

Generally, this study concluded that the catalase enzyme activity increased significantly in the present of AsA (75 mM) more than in the absent of AsA under NaCl salinity stress. Also, the results indicated that the contents of macro and micro nutrient mineral elements (N, P, K, Ca, Mg, Mn, Fe, Cu, Z, B, Na and Cl) increased significantly under salinity stress in the presence or absence of (AsA) compared with the control. In general, the presence of (AsA) was one of the main mechanisms used by the plant to raise its efficiency to bear the salt stress for growth stages compared to the control

Conflicts of interest: The authors stated that no conflicts of interest.

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