Physiological role of ascobin on 1uality and productivity of sunflower plants irrigated with sodium chloride solution

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Abstract: Ascobin had a promotion effect on growth and active constituents' compounds of various plants under normal and stressed conditions. The physiological response of sunflower plant to foliar application of ascobin treatments (200, 400, 600 ppm) was investigated either under normal or salinity stressed conditions (5000 ppm NaCl solution) in pot experiments at the wire-house of the National Research Centre, Dokki, Cairo, Egypt. Data revealed that salinity stress caused significant decreases in shoot height, leaf area chlrorophyll *b*, carotenoids, total photosynthetic pigments, seed yield, yield components, oil and protein content of the yielded seeds relative to control. The decrease in oil percentage was more obvious by salinity than protein percentage. Since salinity caused decreases in oil % by 10.42% and decreases in protein % by 3.44% relative to control. Meanwhile, salinity stress caused significant increases in H_2O_2, MDA and activity of antioxidant enzymes (CAT, SOD, APX and GR) as well as total soluble carbohydrate, phenolic content, proline, free amino acids. Salinity stress caused significant increases in sum of stearic acid and palmitic acid accompanied by significant decreases in sum of oleic and linoleic acids as well as ratio of oleic/linoleic and total unsaturated fatty acids/total saturated fatty acids. On the other hand, ascobin treatments caused significant increases in most of growth parameters and activity of all antioxidant enzymes under investigation accompanied by significant decreases in H_2O_2 and MDA under normal and stressed conditions relative to corresponding controls. Ascobin treatment at 400 ppm showed significant increases in all components of photosynthetic pigments under normal condition relative to control. Meanwhile ascobin treatments significantly decreased total soluble carbohydrate, phenolic content and increased proline and free amino acids. It was noted that all treatments alleviate the harmful effect of salinity stress on sunflower yield and yield components. Since all treatments caused significant increases in yield and yield components as well as oil and protein percentages. Ascobin treatment at 400 ppm was the most optimum treatments in increasing seed yield/plant by 44.78% under normal condition and by 45.43% under stressed conditions relative to corresponding controls. Oleic acid and linoleic acid significantly increased by ascobin treatment at 400 ppm leading to a non-significant decrease in total saturated fatty acid and significant increase in total unsaturated fatty acids.

Keywords: *Helianthus annuus* L, ascorbic acid, citric acid, seed quality, antioxidant enzymes

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1 Introduction

Sunflower (*Helianthus annuus* L.) could be cultivated in different types of soils and climate conditions (Osman and Awed, 2010), moderately sensitive to soil salinity and can tolerate salinity up to EC equal to 1.7 dsm^{-1} (Katarji et al., 2003). It fits well in existing cropping systems and can be grown without replacing any major crop because it can be grown twice a year and has a short duration crop (90-120 days). Sunflower oil is quite palatable and considered as an important source of edible vegetable oil throughout the world because of its high monounsaturated (C18:1) and polyunsaturated (C18:2) fatty acids with low saturated fatty acids (C16:0 and C18:0).

Salinity is one of the most severe environmental stresses that affect a multitude of plant physiological

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activities and caused a significant decrease in crop yields in many areas of the world, especially in arid and semi-arid area (Raza et al., 2006). The deleterious effects of salinity on plant growth are associated with low osmotic potential of the soil solution, nutritional imbalance, specific ion effect, hormonal imbalance and induction of oxidative stress, or a combination of these factors (Rahnama et al., 2010). High salt concentration in the soil solution is bound to create high osmotic pressure in the root zone and reduce availability of water and nutrients to plants and trigger to the formation of reactive oxygen species (ROS) such as superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), and singlet oxygen ($1O_2$), (Gong et al., 2006) which can damage mitochondria and chloroplasts (Mittler,2002) and oxidizing photosynthetic pigments, membrane lipids, proteins, and nucleic acids (Reddy et al. 2004).

Ascorbic acid is water-soluble antioxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide and has a supplementary role in protecting or regenerating oxidized carotenoids or tocopherols (Shao et al., 2006). Ascorbic acid has been shown to play multiple roles in plant growth, such as regulation of cell elongation (Yabuta et al., 2004), cell division, cell wall expansion, and other developmental processes (Pignocchi, and Foyer, 2003). Moreover, ascorbic acid can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce H_2O_2 to water via ascorbate peroxidase reaction (Noctor and Foyer, 1998). Ascorbic acid plays an important role in minimizes the damage caused by oxidative process through synergic function with other antioxidants (Foyer and Noctor, 2005). Several investigations reported that application of ascorbic acid through the rooting medium, foliar spray or as seed priming plays important roles in enhancing the salt tolerance of different plants (Athar et al., 2008; Paital and Chainy, 2010).

Citric Acid (CA) is an organic compound belongs to carboxylic acids group. It is one of a series of compounds involved in the physiological oxidation of fats, proteins and carbohydrates to CO_2 and water. Citric acid is an important substrate in Krebs cycle and plays an important role in stimulating biosynthesis processes (Abd El-Al, 2009). Citric acid is considered as one of non-enzymatic antioxidants which act to eliminate free radicals produced in plants under stress (Yan-Lin and Soon, 2001). Citric acid also induced defense mechanisms by increasing the activities of antioxidant enzymes (Sun and Hong, 2011).

Ascorbic and citric acids as natural organic antioxidants compounds have an auxinc action and synergistic effect on improving fruit retention (Ahmed et al., 2002).Ascobin (ascorbic acid and citric acid in a ratio of 2:1), had a promotion effect on growth and active constituents compounds of various plants under normal or stressed conditions (Sheteawi, 2007; Sadak et al., 2013). Sheteawi (2007) stated that ascobin reduced harmful effect of NaCl (50 and 100 mM NaCl) on carbohydrates, lipids, proteins, N, P and K of the yielded soybean. Moreover, ascobin treatment stimulated growth parameters, endogenous growth hormones, carbohydrate constituents and wheat grain yield under normal and salinity conditions (Sadak et al., 2013).

This work aimed to study physiological role of ascobin treatments (200, 400 and 600 ppm) on quality and quantity of sunflower plants irrigated with sodium chloride solution (5000 ppm)

2 Materials and Methods

Two pot experiments were conducted during two successive summer seasons (2014 and 2015) at wire house of National Research Centre, Egypt. Sunflower seeds (Giza 102 cultivar) were obtained from Agricultural Research Centre, Giza, Egypt.

The sunflower seeds were sown 2 cm deep during first of June, and grown under the average maximum and minimum temperature of 35.5°C±1°C and 18.5°C±1°C. The pots had a40 cm diameter and 40 cm height contained equal amounts of sieved soil. The chemical analysis of the experimental soil was determined according to Chapman and Pratt (1978) and included the following characters: pH7.8, organic matter 0.21%, $CaCo_3$ 1.0%, E.C. 0.5 mhos cm^{-3} and available total N, P, K were 0.10, 3.20, 20.0 ppm respectively. To reduce compaction and improve drainage, the soil was mixed with sand in a proportion of 2:1 (v:v). Nitrogen fertilizer was applied at a rate of 72 units of (N) ha^{-1} and

Phosphorus fertilizer was applied at a rate of 24 units of (P_2O_5) ha^{-1} to each pot. These N and P fertilizers were mixed into the soil in each pot immediately before sowing. Thinning of sunflower was done after 2 weeks from sowing leaving two homogeneous seedlings per pot. The seedlings were sprayed with 200, 400, 600 ppm ascobin (2:1; ascorbic acid: citric acid) at 30 and 45 days old. The plants were watered with an equal volume of saline solution (5000ppm NaCl) 30 days after sowing until the end of experiment. The soil water capacity was estimated by saturating the soil in each pot with water and weighting the soil after the soil had drained for 48 h. Soil water capacity were maintained at approx. 90% of the pot water capacity. Level of soil moisture was calculated by weighting each pot and any loss of water supplemental daily. The pots were distributed in a complete randomized design. Each treatment was represented by 10 pots. Samples of sunflower plant were collected at 60 days old to determine some growth parameters (shoot height, leaves number, fresh and dry weight of leaves and stem/plant, leaf area) photosynthetic pigments, total soluble carbohydrates, phenolic contents, proline and total free amino acids as well as some antioxidant enzymes (catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in the leaves. At harvest, sunflower plants were collected to determine head diameter and head weight. Heads were air dried and threshed to determine seeds weight/plant, and 100-seeds weight as well as oil, protein content of the yielded seeds and fatty acid composition.

Chemical analysis of sunflower plants: Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in the fresh sunflower leaf was determined as the method described by Moran (1982). The method used for extracting the enzyme was that of Mukherjee and Choudhuri (1983). Catalase (CAT, EC 1.11.1.6) activity and Superoxide dismutase (SOD, EC 1.12.1.1) activity were assayed according to the method of Chen and Wang (2006). The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was assayed according to Chen and Asada (1992). The activity of glutathione reductase (GR, EC 1.8.1.7) was assayed according to Rao et al., (1996). The H_2O_2 level was calorimetrically measured as described by Jana and Choudhuri (1981). The intensity of yellow color of supernatant was measured at 410 nm. H_2O_2 level was calculated using the extinction coefficient 0.28 μmol^{-1} cm^{-1}. Lipid peroxidation was determined by measuring the malondialdehyde (MDA) content following the method of Dhindsa et al. (1982). Total soluble carbohydrates were determined in the dry leaf using the colorimetric method described by Dubois et al. (1956). Total phenolic compounds were determined colorimetrically according to the method defined by Snell and Snell (1953) using Folin Ciocalteu phenol reagent. Proline was estimated according to Bates et al. (1973). Total free amino acids were determined according to Muting and Kaiser (1963).

The oil content of the seeds was determined according to the procedure reported by AOAC (1990). The defatted meals were used for determination of the protein content by microkjeldahl method according to AOAC (1990). As the quality of the oil depends on the proportion of different fatty acids, their composition was determined quantitatively by Gas Liquid Chromatography according to the method described by Fedak and De La Roche (1977). Methyl esters of fatty acids were prepared from an aliquot of total lipid according to Harborne (1984). Fatty acid composition was determined quantitatively by gas liquid chromatography of methyl ester using a "HEWLETT PACKARD HP 6890 series GC system" instrument equipped with a flame ionization detector (FID). The capillary column "HP-INNOWAX polyethylene glycol, length (30 m); diameter (530 mm)and film thickness (1 μm). Two injections were made from each sample. The operating conditions were: initial temperature 120ºC; final temperature 240ºC and detector temperature 300ºC. The nitrogen, hydrogen and air flow rates were 30, 30and 300 ml min^{-1} respectively.

Statistical analysis: The averages of two growing seasons were statistically analyzed as a randomized complete block design system according to Snedecor and Cochran (1980). The Duncan multiple range test was used to compare the treatment means at 5% levels of probability (1955). The MSTATC (1989) program was used in this connection.

3 Results and Discussion

3.1 Growth parameters

Salinity stress caused non-significant decreases in all investigated growth parameters except shoot height and leaf area that showed significant decreases relative to control (Table 1). The decreases in growth parameters of sunflower plants under effect of salinity might be due to reduced water absorption and metabolic activities as well as nutrient deficiency (De Lacerda et al., 2003).

On the other hand, ascobin treatments caused significant increases in most of growth parameters under normal and stress conditions relative to corresponding control (Table 1). Ascobin treatment at 400 ppm was the most optimum treatments. The positive effect of ascobin (ascorbic acid + citric acid, 2:1) on growth parameters may be attributed to the fact that both organic acids act as antioxidant under salinity stress and enhanced salt tolerance. Ascobin application exert a promotional effect on various vegetative growth and yield characters of different plants e.g. on grapevine (Fayed, 2010) and wheat (Sadak et al., 2013).

Table 1 Effect of ascobin treatments on some growth parameters of sunflower plants irrigated with saline solution

Treatments		Shoot height, cm	Leaves number/plant	Leaves fresh wt. /plant, g	Stem fresh wt. /plant, g	Shoot fresh wt. /plant, g	Leaves dry wt. /plant, g	Stem dry wt. /plant, g	Shoot dry wt. /plant, g	Leaf area, cm^2
Ascobin, ppm	Salinity, ppm									
0	0	69.33a±7.09	16.00ab±1.73	5.823abc±1.00	13.80ab±1.21	19.62abc±0.26	1.063abc±0.24	2.550ab±0.33	3.613abc±0.09	85.39d±2.30
200	0	71.67a±5.69	16.33ab±1.15	6.130abc±0.80	15.68a±3.58	21.81ab±3.68	1.330a±0.21	2.573ab±0.38	3.903ab±0.48	99.75b±2.02
400	0	76.00a±3.61	17.33a±2.08	6.917a±0.48	16.13a±0.73	23.05a±1.19	1.347a±0.12	2.917a±0.10	4.263a±0.05	108.0a±1.83
600	0	69.00a±1.73	16.00ab±1.73	6.533ab±0.77	13.18ab±1.97	19.71abc±2.67	1.093abc±0.13	2.270ab±0.23	3.363bc±0.31	90.04c±0.13
0	5000	56.67b±3.21	14.00b±1.00	4.843c±0.33	10.94b±2.64	15.78c±2.40	0.993bc±0.07	2.167b±0.56	3.160c±0.62	77.87ef±0.12
200	5000	64.67ab±6.51	14.00b±1.00	5.160c±0.57	12.74ab±2.63	17.90bc±2.20	1.160ab±0.21	2.403ab±0.21	3.563bc±0.36	79.38e±1.24
400	5000	68.33a±7.77	15.00ab±2.65	5.747abc±0.58	15.34ab±1.26	21.08ab±1.68	1.250ab±0.11	2.570ab±0.38	3.820abc±0.31	81.65de±1.50
600	5000	65.33ab±6.43	14.33ab±0.58	5.237bc±0.33	13.91ab±2.50	19.15abc±2.72	0.843c±0.10	2.353ab±0.25	3.197c±0.24	74.60f±4.61

3.2 Photosynthetic pigments

Salinity stress caused non-significant decreases in chlorophyll *a* and significant decreases in chlrorophyll *b*, carotenoids, chlorophyll *a*+*b* and total photosynthetic pigments relative to control (Table 2). Salinity stress adversely affects photosynthetic rate and limits photosynthetic capacity (Ashraf and Harris, 2004) may be due to an increase in free radicals in chloroplasts and destruction of chlorophyll molecules by ROS (Dolatabadian and Jouneghani, 2009).

Ascobin treatments caused non-significant increases in all components of photosyythetic pigments either under normal or stressed conditions except ascobin treatment at 400 ppm showed significant increases in all components of photosyythetic pigments under normal condition relative to control (Table 2). Ascobin as antioxidant mitigated harmful effect of salinity on photosynthetic pigments may be due to its role in decreasing the rate of photochemical reduction (Kumar et al., 1988), stabilization and protection of photosynthetic pigments and the photosynthetic apparatus from oxidative damage (Hamada,1998). Ascorbate is also an important co-factor of some enzymes or protein complexes that are involved in the regulation of photosynthesis (Davey et al., 2000).

Table 2 Effect of ascobin treatments on photosynthetic pigments of sunflower plants irrigated with saline solution

Treatments		Chlorophyll A, mg g^{-1} fresh wt.	Chlorophyll B, mg g^{-1} fresh wt.	Chlorophyll A+B, mg g^{-1} fresh wt.	Carotenoid, mg g^{-1} fresh wt.	Total pigments, mg g^{-1} fresh wt.
Ascobin, ppm	Salinity, ppm					
0	0	1.747bcd±0.13	1.077bc±0.17	2.823bcd±0.14	0.653b±0.05	3.477bc±0.16
200	0	2.053b±0.11	1.133b±0.05	3.187b±0.16	0.763ab±0.04	3.950b±0.20
400	0	2.457a±0.21	1.380a±0.11	3.837a±0.31	0.907a±0.08	4.743a±0.39
600	0	1.957bc±0.24	1.083bc±0.14	3.040bc±0.38	0.730ab±0.10	3.770b±0.48
0	5000	1.513d±0.18	0.860d±0.11	2.373d±0.29	0.380c±0.28	2.753d±0.34
200	5000	1.503d±0.12	0.890cd±0.05	2.393d±0.16	0.573bc±0.03	2.967cd±0.19
400	5000	1.757bcd±0.09	0.953bcd±0.07	2.710bcd±0.16	0.647b±0.04	3.357bcd±0.19
600	5000	1.670cd±0.16	1.007bcd±0.18	2.677cd±0.33	0.660b±0.11	3.337bcd±0.44

3.3 H_2O_2, MDA, Antioxidant enzymes

Salinity stress caused significant increases in H_2O_2, MDA and activity of antioxidant enzymes (CAT, SOD, APX and GR) relative to control (Table 3). Enzymatic antioxidant defense systems regulated endogenous ROS concentrations throughout plant development; however, oxidative damage may occur when ROS formation and antioxidant defenses become unbalanced (Mahalingam and Fedoroff, 2003). Salinity stress accumulates H_2O_2 in plant cells and an increased level of lipid peroxidation (MDA). The antioxidant enzymes such as catalase and peroxidases are involved in elimination of H_2O_2 from stressed cells (Kim et al., 2005) and the increase in their activities lead to neutralizing H_2O_2 from the oxidative salt stress (Shalata and Neumann, 2001). The SOD removes superoxide anion -accompanied by formation of H_2O_2- which is then detoxified by CAT and POX (Sudhakar et al., 2001). In the ascorbate-glutathione cycle, Ascorbate peroxidase is one of the most important enzymes playing a crucial role in eliminating toxic H_2O_2 from plant cell (Foyer et al., 1993). Hernandez et al. (2000) mentioned that activities of antioxidative enzymes increased under salt stress in wheat.

Ascobin treatments caused significant increases in all antioxidant enzymes under investigation accompanied by significant decreases in H_2O_2 and MDA either under normal or stressed conditions (Table 3). Application of ascorbic acid alleviates reactive oxygen species and increased plant tolerance to oxidative stresses (Dolatabadian et al., 2009). Moreover, Ebrahimian and Bybordi (2012) conclude that ascorbic acid decreased adverse effects of water stress and improved plant growth via increase of water deficit resistance. Sheteawi (2007) stated that ascorbic and citric acids appeared to act in a concert which indicates a complete set of antioxidant defense system, rather than protection by a single antioxidant under stressful conditions.

Table 3 Effect of ascobin treatments on H_2O_2, MDA and activity of some antioxidant enzymes of sunflower plants irrigated with saline solution

Treatments		CAT	SOD	APX	GR	H_2O_2	MDA
Ascobin, ppm	Salinity, ppm	Unit /min /g fresh wt				μmol g^{-1} fresh wt	
0	0	31.83h±0.31	21.44g±1.09	0.468e±0.005	0.530f±0.006	3.707d±0.025	8.980e±0.03
200	0	33.01g±0.60	24.86f±0.35	0.492d±0.010	0.570e±0.002	2.587f±0.055	8.997e±0.01
400	0	43.17e±1.04	35.69d±0.52	0.530c±0.007	0.627c±0.006	2.420g±0.01	8.167g±0.14
600	0	37.42f±0.72	29.36e±1.05	0.513c±0.003	0.602d±0.009	2.480fg±0.05	8.597f±0.15
0	5000	51.88d±0.36	42.56c±1.25	0.622b±0.010	0.631c±0.001	5.730a±0.09	16.22a±0.05
200	5000	58.21c±0.01	42.22c±0.91	0.647a±0.005	0.652b±0.002	5.080b±0.04	12.47b±0.04
400	5000	66.76a±1.55	48.46a±0.15	0.661a±0.001	0.685a±0.003	3.167e±0.045	10.02d±0.13
600	5000	62.67b±0.54	45.52b±0.31	0.645a±0.007	0.686a±0.005	4.417c±0.095	11.08c±0.06

3.4 Total soluble carbohydrates

It is obvious that salinity stress significantly increased the total soluble carbohydrate of sunflower leaf; meanwhile ascobin treatments significantly decreased total soluble carbohydrate under normal and stressed conditions as compared to the corresponding controls (Table 4). Adaptation to water stress is associated with metabolic adjustment that leads to the accumulation of several organic solutes as sugars to prevent the water loss from the cell and protect the cellular proteins. Bartels and Sunkar (2005) reported that the increase in total soluble sugars under salinity stress was considered protective and adaptive functions of soluble carbohydrates.

In contrast, decrease of soluble sugars by ascobin treatments under salinity stress conditions can be related to neutralizing adverse effect of water stress by this antioxidant and alleviated its harmful effect.

3.5 Proline

Proline content significantly increased by both salinity stress and ascobin treatments either under normal or stressed conditions (Table 4). Thus, It could be suggested that salt tolerance was manifested via activated proline synthesis and hydrolysis of protein into free amino acids to act as osmoprotectant to contribute in osmotic adjustment at the cellular level and stabilizing the structure of macro-molecules (Mahajan et al., 2008).

Ascobin treatments alleviated the inhibitory effect of salt stress on the sunflower plant through increasing proline synthesis and/or enhancing the biosynthesis of other amino acids. These results are in agreement with Sheteawi (2007) who reported that foliar treatment of ascobin increased free proline under two salinity levels.

Table 4 Effect of ascobin treatments on some compatible solutes and phenolic content of sunflower plants irrigated with saline solution

Treatments		Soluble sugar. %	Free amino acid. mg 100 g^{-1}	Proline. mg 100 g^{-1}	Phenolic content. %
Ascobin, ppm	Salinity, ppm				
0	0	2.700b±0.04	56.63c±3.30	19.37g±0.80	0.9600c±0.01
200	0	2.323c±0.07	61.27c±2.32	23.07g±2.28	0.7800f±0.02
400	0	2.283c±0.05	67.37c±1.89	30.77f±1.89	0.8433e±0.01
600	0	2.607b±0.04	66.80c±5.26	37.73e±2.76	0.6967g±0.02
0	5000	3.080a±0.03	87.03b±3.21	44.83d±4.64	1.120a±0.01
200	5000	2.357c±0.05	118.3a±7.51	64.13c±2.22	1.047b±0.01
400	5000	2.303c±0.12	123.9a±11.31	83.27a±5.47	0.903d±0.06
600	5000	2.633b±0.13	121.5a±19.68	77.70b±2.21	0.803ef±0.02

3.6 Free Amino Acids

Salinity stress induced significant accumulation of free amino acids of the sunflower (Table 4). The increases in amino acids may be attributed to the decrease in protein synthesis and/or to the increase in its degradation leading to lowering osmotic potential in plant tissues exposed to stress and acting as a putative osmoprotective solute (Sadak et al., 2010).

Ascobin treatments caused non-significant increases in free amino acids under normal conditions but significant increases appeared under stressed condition. These results are in agreement with those reported by Murakeozy et al. (2003) on pea and black cumin. Sheteawi (2007) mentioned that salt tolerance of soybean was increased by ascobin treatment by enhancing the accumulation of non-toxic metabolites (free amino acids) as a protective adaptation.

3.7 Total phenolic content

Total phenolic content of sunflower leaf significantly increased with salinity, meanwhile, ascobin treatments significantly decreased total phenolic content under normal and stressed conditions as compared to the corresponding controls (Table 4). The levels of total phenolic compound are enhanced as a response to biotic and abiotic stresses because of its role in the defense mechanism of plants (Dudjak et al., 2004). In this connection, the accumulation of phenolic compounds would be beneficial to protect stressed cells from oxidative damage and increase stability of cell membrane (Burguieres et al., 2006).

3.8 Yield and yield components

The inhibitory effect of salinity on vegetative growth (Table 1) and photosynthetic pigments (Table 2) led to decreasing in seed yield (Table 5). This reduction may be attributed to the disturbance in mineral uptake and/or enhancement of plant respiration or due to inhibitory effects of salinity on many metabolic processes including, activity of mitochondria and chloroplasts (Singh and Dubey, 1995). Taffouo et al. (2009) attributed significant reductions in cowpea seed yield to a significant reduction of chlorophyll contents (more than 50%).

Regarding ascobin treatments, it was noted that all treatments alleviate the harmful effect of salinity stress on sunflower yield and yield components. Since all treatments caused significant increases in yield and yield components either under normal or stressed conditions relative to corresponding controls (Table 5). Ascobin treatment at 400 ppm was the most optimum treatments in increasing seed yield/plant by 44.78% under normal condition and by 45.43% under stressed conditions relative to corresponding controls.

Fawy and Atyia (2012) showed that increasing application rates of citric acid from 100 up to 300 ppm increased wheat yield parameters. Abdelgawad (2014) indicated that ascobin treatments significantly increased yield and yield parameters of cawpea plants at normal and salinity stress conditions. These results are in good agreements with those obtained by Fayed (Fayed, 2010) on grapevine, Sheteawi (2007) on soybean and Sadak et al. (2013) on wheat.

Table 5 Effect of ascobin treatments on seed yield, yield components, oil and protein content of the yielded seeds of sunflower plants irrigated with saline solution

Treatments		Head diameter, cm	Head weight, g	Seeds weight/plant, g	100 Seed weight, g	Oil %	Protein %
Ascobin, ppm	Salinity, ppm						
0	0	7.100b±0.2	14.98d±0.16	10.07c±0.92	2.25c±0.25	31.75bc±0.26	14.24e±0.101
200	0	8.55a±0.45	18.59b±0.63	14.18ab±1.12	3.10b±0.20	32.14b±0.19	15.74c±0.040
400	0	8.90a±0.1	20.48a±0.48	14.58a±0.42	3.70a±0.20	32.64a±0.23	18.24a±0.100
600	0	7.05b±0.25	17.47c±0.56	13.25b±0.09	3.50a±0.30	31.75bc±0.24	17.49b±0.050
0	5000	4.55e±0.35	10.97f±0.64	6.09e±0.14	0.85e±0.05	28.44e±0.20	13.75f±0.041
200	5000	5.30d±0.3	11.88e±0.07	8.147d±0.77	1.60d±0.20	30.57d±0.39	14.91d±0.288
400	5000	6.75b±0.55	12.41e±0.41	8.857d±0.15	1.90cd±0.10	31.49c±0.36	15.74c±0.100
600	5000	6.05c±0.35	12.28e±0.32	7.860d±0.65	1.80d±0.10	30.18d±0.18	13.91f±0.145

3.9 Seed nutritive value

Oil and protein content of the yielded seeds was significantly decreased by salinity stress relative to control (Table 5). The decrease in oil % was more obvious by salinity than protein percentage. Since salinity caused decreases in oil % by 10.42% and decreases in protein % by 3.44% relative to control. Flagella et al. (2004) mentioned that salt stress might have caused a shortening of the lipid accumulation phase and some damages to all enzymatic activities, including that of oleate desaturase. Moreover, they observed a significant reduction in seed oil yield of sunflower with increasing salinity level. The reduction in protein content under salinity stress may be due to the disturbance in nitrogen metabolism or inhibition of nitrate absorption or decrease availability of amino acids and the denaturation of enzymes involved in amino acid and protein synthesis (El-Mashad and Mohamed, 2012).

Oil and protein percentages were significantly increased by ascobin treatments either under normal or stressed conditions except 200 and 600 ppm ascobin caused non-significant increases in oil percentage under normal condition (Table 5). It is worthy to mention that a 400 ppm acscobin treatment was the most optimum treatments. The positive effect of ascobin on the oil percentage either under normal or salinity stressed conditions may be due to the role of ascorbic acid as antioxidant besides stimulating several physiological activities (Gamal El-Din, 2005; Sadak and Dawood, 2014). Ascorbic acid prevented lipid and protein oxidation and degradation as well as maintained plasma membrane permeability due to its effective role in scavenging reactive oxygen species as reported by Dolatabadian et al. (2010).

Talaat (2003) showed that ascorbic acid had a positive effect on root growth and increased nitrate absorption and N concentration. Sheteawi (2007) mentioned that ascobin treatment ameliorated the salinity effects on seed carbohydrates, lipids, proteins, N, P and K and explained that the ameliorative effect of ascobin (ascorbic acid + citric acid) on plant yield quality and quantity comes from the fact that they act as an antioxidant and mitigated salinity effects by enhancing salt tolerance through interaction of antioxidant response and protection of membranes.

Ascorbic and citric acids as natural and organic antioxidants compounds have an auxinc action and synergistic effect on improving fruit retention and quality (Ahmed et al., 2002). Maksoud et al. (2009) indicated that sole application of either ascorbic acid or citric acid 2000 ppm improved yield and fruit quality of olive trees. Citric acid is involved in Kerbs cycle and had a beneficial effect on enhancing the biosynthesis of organic foods as well as its action as natural auxins (Elade, 1992).

3.10 Fatty acid composition

The genotype is the most important factor that defines the fatty acid composition and the water stress also affects the fatty acid composition of sunflower oil (Petcu et al., 2001). The stearic acid concentration significantly increased under saline conditions and palmitic acid concentration decreased under the same conditions. Salinity stress caused a significant decrease in oleic acid and non-significant decrease in the content of linoleic acid (Table 6). Salinity stress caused significant increases in sum of stearic acid and palmitic acid accompanied by significant decreases in sum of oleic and linoleic acids as

well as ratio of oleic/linoleic and total unsaturated fatty acids/total saturate fatty acids (Table 6). Regarding unsaturated fatty acids, oleic acid (C18:1) was more affected than linoleic acid in response to salinity levels. Salinity stress caused decreases in oleic acid and linoleic acid by 10.14% and 1.80% respectively as compared with untreated unstressed plants. Water stress caused a significant reduction (~15%) in the oleic acid concentration in standard hybrid as reported by Baldini et al. (2002). In addition, Mansour and Salama (2004) stated that NaCl induced relative compositional changes in fatty acids and decreased unsaturated/saturated ratio. As salinity levels increased, marked increases in total saturated fatty acids accompanied by decreases in total unsaturated fatty acids occurred (Sadak and Dawood, 2014).

Regarding ascobin treatments, it was noted that oleic acid and linoleic acid significantly increased by ascobin treatments relative to corresponding control (Table 6). 400 ppm ascobin treatment caused non-significant decrease in total saturated fatty acid and significant increase in total unsaturated fatty acids. Joshi et al. (2004) detected that vitamin treatments might be accelerated the biosynthetic pathway of linolenic acid. Moreover, ascorbic acid treatment decreased saturated fatty acids (palmitic and stearic) as mentioned by Emam et al. (2011). In addition, 2.27 mM ascorbic acid caused marked increases in unsaturated fatty acids and counteracted the effect of salinity as compared to control plants (Sadak and Dawood, 2014).

Table 6 Effect of ascobin treatments on fatty acid composition (%) of the yielded oil of sunflower plants irrigated with saline solution

Treatments		Palmitic	Stearic	Oleic	Linoleic	Stearic + Palmitic	Oleic + Linoleic	Oleic/ Linoleic	Unsaturated/ Saturated
Ascobin, ppm	Salinity, ppm								
0	0	8.367bc±0.37	6.677c±0.48	51.26b±2.10	31.13b±1.88	15.04b±0.84	82.38b±0.22	1.654a±0.17	5.489a±0.32
400	0	9.827a±0.68	5.167d±0.17	54.15a±0.02	33.17a±0.97	14.99b±0.84	87.32a±0.95	1.633ab±0.05	5.840a±0.39
0	5000	7.727c±0.58	10.31a±0.76	46.06c±0.90	30.57b±2.42	18.03a±1.34	76.63c±3.32	1.511c±0.09	4.274b±0.50
400	5000	9.250ab±0.75	8.317b±0.19	49.06b±0.94	31.63b±1.57	17.57a±0.57	80.68b±0.63	1.555bc±0.11	4.596b±0.11

4 Conclusion

Ascobin treatments caused significant increases in most of growth parameters and activity of all antioxidant enzymes under investigation accompanied by significant decreases in H_2O_2 and MDA under normal and stressed conditions relative to corresponding controls. Meanwhile ascobin treatments significantly decreased total soluble carbohydrate, phenolic content and increased proline and free amino acids. All treatments caused significant increases in yield and yield components as well as oil and protein percentages. Oleic acid and linoleic acid significantly increased by ascobin treatment at 400 ppm leading to non-significant decrease in total saturated fatty acid and significant increase in total unsaturated fatty acids.

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