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Amelioration of The Adverse Effects of Salinity Stress By Using Compost, *Nigella Sativa* Extract or Ascorbic Acid in Quinoa Plants

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Abstract : Compost can enhance water holding capacity and fertility of soil and thus increase soil water availability and nutrient uptake by plants, but it is not clear whether it can also improve the ability of plants to recover after salinity stress. Quinoa plant was grown in soil with or without compost either with foliar spray *Nigella sativa* extract (25 & 50%) or ascorbic acid (200 mg/l). Irrigation plant with saline water with different concentrations (0.0, 4000 and 8000 mg/l). Salinity stress led to decreases in growth parameters, yield components, photosynthetic pigments and carbohydrate constituents. Meanwhile, salinity caused significant increases in some osmoprotectants as (free amino acids and proline) and some antioxidant enzyme activities. The cultivation of quinoa plant in the presence of compost and either ascorbic acid or *Nigella sativa* extract led to increases in growth parameters, yield components, photosynthetic pigments and carbohydrate constituents. More accumulation of the tested organic solutes of leaves (TSS, free amino acids and proline) and antioxidant enzyme activities. As a conclusion, , the nutritional values of the yielded seeds of quinoa were improved when cultivated in the presence of compost and sprayed with 25% of *Nigella sativa* extract.
Key words: Antioxidant enzymes, Ascorbic acid, Compost, Quinoa, *Negella sativa* extract, Yield.

Introduction

Salinity is a one of the most important problem in arid and semi-arid regions especially with low amounts of fresh water, high evapotranspiration rate and lack of precipitation, which has a harmful effect on crop production. In Egypt, the large scale land reclamation demands large amounts of water for irrigation in order to guarantee potent plant growth and high yield. This has made it necessary to use various sources of irrigation water, which often have relatively high salinity levels such as well water. [1]Sairam and Tyagi reported that salt stress is considered one of the most important abiotic stress limiting plant growth and productivity throught the increase in reactive oxygen species (ROS). The ROS may cause oxidative stress, resulting in cellular damage by oxidation of lipids, proteins and nucleic acids[2]. Plant cells have evolved a complex antioxidant system to reduce the effect of oxidative salt stress. The antioxidants are composed of low molecular

mass antioxidants as well as ROS-scavenging enzymes[3]. Inducing oxidative stress tolerance would be to increase the cellular levels of antioxidants (vitamins) such as ascorbic acid and α-tocopherol.

Recently, the use of natural antioxidants, such as tocopherols, flavonoids and plant extracts protect plant against oxidative stress. Plants, including spices and herbs, have many phytochemicals which are potential sources of natural antioxidants. Blackseed (*Nigella sativa* L.) commonly known as Habbat El Baraka in the Arab world, is a spices of family Ranunculaceae which can be applied as a source of antioxidant. At present, this plant residue not being used for any other purposes and are mostly dumped as solid waste at large expense. It is thus imperative and even essential to find applications for this plant residue as they can contribute to real environmental problems.

Another approach to recycle and maximizing the benefits of plant wastes is too converted into compost composting processes can be ameliorated by using specific microorganisms. [4]Lampkin reported that, composting of agricultural residues by supplying the natural microbial flora present on them, with their requirements of inorganic nutrients such as phosphorus and nitrogen and applying a proper moistening and turning resulted in the final product with high capacity to improve soils and enhance plant growth and yield. Composts are used in agriculture and horticulture to improve soil fertility and quality because they can increase organic matter content, especially in sandy soils which have low organic matter contents and low water holding capacity [5]. By increasing soil organic matter content, composts improve soil physical properties such as structural stability[6], total porosity, hydraulic conductivity and water holding capacity [7].

It is vital to compare the individual pure antioxidants with antioxidant activities of plant extracts, which may contain more than one antioxidant component, in order to determine possible synergistic interaction among the antioxidants. To avoid oxidative damage and in order to deal with stress, plants develop a serious of enzymatic and non enzymatic antioxidant systems. Amongst, ascorbic acid (vitamin C) is an abundant small molecule in plants. Ascorbic acid reacts non–enzymatically with superoxide, hydrogen peroxide and singlet oxygen[8]. Ascorbic acid plays multiple roles in plant growth, such as in cell-cycle progression, cell wall expansion, and gene expression, synthesis of many hormones, anthocyanin, flavonoids and other developing processes[9]. In plants Ascorbic acid (AsA) is an important antioxidant that increased as an adaptive mechanism to environmental stress such as water stress. In addition, ascorbic acid is a key substance in the network of plant antioxidant, that detoxify H_2O_2 to counteract oxygen radicals[10]**.** [11] El Hariri *et al.* reported that, exogenous application of AsA improves salt tolerance of flax cultivars in a number of ways.

Quinoa (*Chenopodium quinoa* Willd) is a newly introduced food crop can refill part of food gap in the developing countries. Because of its high nutritive value seeds can be utilized for human food, in flour production and in animal feedstock[12]. Quinoa seeds was recognized as high-quality protein seeds, especially rich in essential amino acids, minerals, carbohydrates, antioxidant compounds as carotenoids, flavonoids, vitamin C and dietary fiber) compared to that of cereals such as corn, oat, rice and wheat[13]. In addition, as being gluten-free and highly nutritious i.e. did not contain anti-nutritional factors quinoa seeds have enormous potential in the food industry[14]. Quinoa is considered as a multipurpose crop which can grow in arid and semiarid regions and can tolerate different forms of a biotic stresses like salinity and drought which reduce crop production[15]**.** [16]illustrated that; quinoa seems to use several mechanisms in order to acclimate to a saline environment may be due to improved metabolic control based on osmolyte accumulation, ion absorption and finally osmotic adjustment[17].

So in the present study, we will focus to further characterizing the biological (antioxidant) activities of the aqueous extract of the waste of *Nigella sativa* plant and ascorbic acid in the presence or absence of compost on alleviating the adverse effect of saline water on the performance of quinoa plant in Egyptian land.

Materials and Methods

Plant material and growth conditions:

The experimental plant used in the present work was quinoa (*Chenopodium quinoa Willd.*). Quinoa cultivar was obtained from Agricultural Research Centre Giza, Egypt tested for its sensitivity towards salinity stress. The applied substance, ascorbic acid used in the present work was supplied from Sigma Chemical Company, St. Louis, MO, USA. The current study was carried out to elucidate the roles of *Nigella saliva*

extract and ascorbic acid (AsA) in the presence or absence of compost on growth, some physiological parameters, yield and chemical composition of the yielded seeds of quinoa under different salinity levels. The compost used during this study was prepared by El Sebai et al., 2104 and its physic-chemical properties are presented in table (2).

A pot experiment was carried out in two successive seasons in the screen greenhouse of National Research Centre, Dokki, Giza, Egypt. The salt type used in irrigation was mainly the chloride mixture suggested by [18]. The salt components of salt mixture are shown in Table (1).

Table 1. The component of salt mixture used for chloride salinization expressed as % of total salt content.

$MgSO_4$	$CaSO_4$	NaCl	$MgCl_2$	$CaCO_3$
10	1	78	2	9

The component of specific anions and cations in chloride mixture expressed as percentage of total mill equivalents.

Na^+	Mg^{+2}	Ca^{+2}	SO^{-2}	Cl^-	CO_3^{-2}
38	6	6	5	40	5

Seeds were grown in Pots (diameter $50cm^2$); filled with equal amounts of homogenous clay and sand (2:1) after the inoculation the soil by the recommended amount of compost.*Nigella sativa* extract concentrations (25 and 50%) compared with one concentration of Ascorbic acid (200 mg/l) were sprayed twice after 21and 28 days of cultivation. The fertilization with super phosphate (5 g / pot), potassium sulfate (25 g / pot) and urea (6 g / pot) were used.

Extraction of Plant Material:

Negilla sativa wastes herb (shoot system) collected from plants cultivated in the Experimental Farm of the National Research Centre, Nobaria, El-Bihara Governrate (150 Km Northern South of Cairo), Egypt, The collected leaves was air-dried, powdered and kept for extraction. The resulting powder (500 g) was extracted with 2L of distilled water and left to stand for 48 hours at room temperature. The extract was centrifuged at 4500 rpm for 10 min. After centrifugation the residue was reextracted twice with water as described above. The crude aqueous extract was concentrated using rotary evaporator under reduced pressure at 45^oC then the concentrated extracts were lyophilized and kept at - 20°C.

Table 2. Physic-chemical properties of compost used through this study

Character	Inoculated with mutant strain
pH (1:10)	7.80
E.C. (1:10) ds/ml	3.23
Organic matter (%)	32.18
Organic carbon (%)	18.66
C/N ratio	11.24
Total Nitrogen (%)	1.66
Total Phosphorus (P_2O_5%)	0.73
Total Potassium (K_2O%)	1.38
Asch (%)	67.82

Adopted from El Sebai et al., [19]

The pots were divided into two main groups; the first group was without compost and the second inoculation with compost. Each group was divided into three subgroups according to irrigation with different levels of saline solutions by using Stroganov nutrient solutions at 0.0, 4000 and 8000 mg/l which equal to (**EC of 0.03, 2.1, 5.0 and 9.0 dSm^{-1}**), respectively. Each of the previous subgroups were divided into four groups were sprayed twice with ascorbic acid concentration (200 mg/l) and two concentrations *of Nigella sativa* extract

(NSE). Every treatment consisted of 5 replicates distributed in a completely randomized design system. Quinoa seeds were sown on November in both seasons.

The seedlings were irrigated with equal volume (one liter/pot) of different salt solutions for three times, whereas tap water was used for the forth one to prevent the accumulation of salts around root system. The seedlings were left under the following natural growth conditions: 12h light period, 65%- 70% relative humidity, day/night temperatures of 24/16°C. The Plant samples were taken after 60 days from sowing for estimation of some growth parameters as plant height (cm), fresh &dry weight of shoot/plant (g), fresh & dry weight of root/plant (g), chemical analysis of photosynthetic pigments, total soluble sugar, polysaccharide, total carbohydrates, proline, total free amino acid and the antioxidant enzyme. At harvest stage the following characters were recorded on random samples of 5 plants in each treatment to measure plant height (cm), number of fruiting branches/plant, dry weight of plant (g) and seed weight/plant as well as nutritive value of the yielded seeds as total carbohydrates%, protein%, oil%, nitrogen, phosphorus, potassium contents and flavonoids content, in addition to antioxidant activities %.

Biochemical analysis:

Photosynthetic pigments: Total chlorophyll a and b and carotenoids contents in fresh leaves were determined using the method of[20]. Total soluble sugars (TSS), were extracted and analyzed according to[21] and [22]. Determination of total carbohydrates was carried out according to [23]. Proline was assayed according to the method described by[24]**.** Free amino acid was determined with the ninhydrin reagent method [25]. The antioxidant enzyme (Peroxidase. (POX, EC 1.11.1.7) activity was spectrophotometrically assayed by the method of[26]. Superoxide dismutase. (SOD, EC 1.12.1.1) activity was spectrophotometrically assayed at 560 nmby nitro-blue-tetrazolium(NBT) reduction method[27]. Catalase. (CAT, EC 1.11.1.6) activity was determined spectrophotometrically by following the decrease in absorbance at 240 nm[27]. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined as described by [28]. Total phenol content, measured as described [29]. Total protein concentration of the supernatant was determined according to the method described by [30]. Total N was determined by using micro-Kjeldahl method as described in[31]. Seed oil content was determined using soxhlet apparatus and petroleum ether (40-60 ^{o}C) according to[32]. Macroelement contents of the yielded grains were determined according to [33]. Phosphorus was determined using a Spekol spectrocolorimeter (VEB Carl Zeiss; Jena, Germany, while, estimation of K+ contents were done using a flame photometer. Total flavonoids were determined using the method reported by[34]. The antioxidant activity (DPPH radical scavenging) was determined using the method of [35].

Statistical analysis

The data were statistically analyzed according to[36]. Combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5% levels of probability.

Results:

Growth parameters:

The effect of of *Nigella sativa* extract (NSE) concentrations (25% and 50%) compared with one concentration of ascorbic acid (200 mg/l) on growth parameters of quinoa plants and irrigation with different levels of salinity in soils amended with compost Table (3). When compared to the control plants the irrigation of plants with 4000 and 8000 mg/l leads to a marked decrease in all morphological parameter studied (plant height, fresh and dry weight of shoots and roots). While the plants cultivated in the presence of compost led to progressive increases as compared to the corresponding treatment plants cultivated without compost. Quinoa plant treats with ascorbic acid or NSE (25% and 50%) increased all growth parameters in the presence and absence of compost under different salinity levels. The most pronounced increases in all the growth parameters were obtained by using 25% NSE in the presence of compost as compared with the corresponding salinity level.

Table 3: Effect of ascorbic acid (AsA) or *Nigella sativa* extract (NSE) on morphological criteria of quinoa plants and irrigation with different levels of saline solution (at 60 days from sowing) in absence (-) and presence (+) of compost.

Salinity (mg/l)	Treatment		Shoot length (cm)		Shoot FW (gm)		Shoot DW (gm)		Root FW (gm)		Root DW (gm)	
			-	+	-	+	-	+	-	+	-	+
0	Control		16.98	22.75	7.47	9.93	0.69	0.97	0.60	0.99	0.55	0.52
	AsA	200 mg/l	21.50	37.13	12.32	15.21	1.50	1.90	1.36	1.68	0.58	0.68
	NSE	25%	25.60	39.00	14.50	17.78	1.73	2.10	1.68	1.72	0.70	0.72
		50%	20.88	29.00	10.89	13.05	1.38	1.71	1.20	1.49	0.50	0.62
4000	Control		13.01	15.75	3.26	4.26	0.39	0.42	0.34	0.57	0.20	0.46
	AsA	200 mg/l	15.33	19.75	5.90	7.83	0.45	0.78	0.48	0.86	0.31	0.46
	NSE	25%	16.50	21.50	6.30	8.22	0.60	0.90	0.55	0.90	0.38	0.47
		50%	15.88	20.04	5.56	7.51	0.53	0.69	0.50	0.80	0.26	0.30
8000	Control		11.37	13.30	2.86	3.87	0.30	0.35	0.33	0.46	0.06	0.10
	AsA	200 mg/l	12.80	17.30	4.30	6.60	0.43	0.65	0.47	0.60	0.20	0.22
	NSE	25%	14.01	19.40	5.88	7.35	0.54	0.75	0.50	0.65	0.19	0.28
		50%	13.43	17.80	4.63	6.38	0.45	0.47	0.40	0.45	0.14	0.19
LSD at 5%			1.11		0.84		0.55		0.01		0.04	

Photosynthetic pigments contents:

Quinoa plants irrigated with saline water (4000 and 8000 mg/l) caused gradual significant decreases in chlorophyll a, chlorophyll b, carotenoid and total pigments contents as compared with the control Table (4). The results also, observed that plants grow in the presence of compost led to an increase in photosynthetic pigments at the different levels of salinity stress compared to the corresponding treatment of plants grown in the absence of compost. Results also observed that, plants treated with either ascorbic acid or NSE, under normal condition or under salinity stress in the presence or absence of compost led to significant increase in all photosynthetic pigment contents in response to as compared with control plants and the corresponding salinity levels. The maximum increases of the photosynthetic pigments were obtained by foliar application with 25% of NSE and amended with compost. As the percentage of increases in response to 25% NSE reached to 101% by 45%, 45% and 42% as compared with the control salinity level without compost at (0.0, 4000 and 8000 mg/l) respectively.

Table 4: Effect of ascorbic acid (AsA) or *Nigella sativa* extract (NSE) on photosynthetic pigments as (µg/g fresh weight) of quinoa plants and irrigation with different levels of saline solution (at 60 days from sowing) in absence (-) and presence (+) of compost.

Salinity (mg/l)	Treatment		Chlorophyll a		Chlorophyll b		Carotenoids		Total Pigment	
	Material		-	+	-	+	-	+	-	+
0	Control		17.3	18.86	3.61	3.99	4.58	5.14	25.49	27.99
	AsA	200 mg/l	20.02	21.21	5.10	5.76	6.23	7.34	31.35	34.31
	NSE	25%	20.98	22.64	5.89	6.27	7.61	7.99	34.48	36.90
		50%	18.95	20.20	4.33	5.09	5.78	6.30	29.06	31.59
4000	Control		15.01	15.48	2.12	2.98	2.45	2.75	19.58	21.21
	AsA	200 mg/l	16.3	17.56	3.00	3.58	3.54	3.88	22.84	25.02
	NSE	25%	17.01	18.70	4.43	4.89	3.99	4.75	25.43	28.34
		50%	16.75	16.86	3.01	3.83	3.87	5.09	23.63	25.78
8000	Control		13.2	13.44	1.59	2.36	1.99	2.89	16.78	18.69
	AsA	200 mg/l	14.02	14.33	2.30	3.56	3.23	4.29	19.55	22.17
	NSE	25%	14.88	15.50	2.75	3.79	3.33	4.51	20.96	23.80
		50%	14	14.30	2.10	2.60	2.76	3.88	18.86	20.78
LSD at 5%			1.41		0.55		0.45		1.02	

Changes in carbohydrate constituents:

Treatments of quinoa plants with different concentrations of salinity decreased significantly total soluble sugars, polysaccharides and total carbohydrates contents as compared with the control plants (without salt) (Table 5). Data showed that, compost addition to soil increased significantly TSS, polysaccharides and total carbohydrates of wheat plants as compared with those of the corresponding treatments in absence of compost. Data also show that, total soluble sugars, polysaccharides and total carbohydrate contents of quinoa plant significant increases when will be treated with different concentrations of AsA or NSE. Application of 25% of NSE in the soil amended with compost were the most effective treatment as it increased TSS by 28%, 13% & 8%, polysaccharides by 21%, 11% & 14% and total carbohydrates 21%, 11% & 13% as compared with control without compost at 0.0, 4000 and 8000 mg/l respectively.

Table 5: Effect of ascorbic acid (AsA) or *Nigella sativa* extract (NSE) on carbohydrate constituents as (mg/ 100g dry) weight of quinoa plants and irrigation with different levels of saline solution (at 60 days from sowing) in absence (-) and presence (+) of compost.

Salinity (mg/l)	Treatment		Total soluble sugars		Polysachharides		Total carbohydrates	
			-	+	-	+	-	+
0	Control		1538	1638	14719	14813	16256	16451
	AsA	200	1639	1839	15487	16467	17126	18306
	NSE	25%	1776	1976	16657	17763	18433	19739
		50%	1611	1711	15331	16370	16942	18081
4000	Control		1397	1437	13441	13981	14838	15418
	AsA	200	1427	1576	14515	14715	15941	16290
	NSE	25%	1482	1584	14648	14949	16130	16533
		50%	1409	1520	14415	14613	15823	16133
8000	Control		1383	1403	12751	12789	14134	14192
	AsA	200	1417	1447	13209	13452	14626	14899
	NSE	25%	1468	1498	13941	14532	15409	16030
		50%	1400	1420	13068	13134	14467	14554
LSD at 5%			67.35		106		175	

Free proline and free Amino acids

Data recorded in Table 6, showed that salt stress induced accumulated of proline and free amino acid in plant with increasing salt stress. Table 6 clearly shows that foliar application of either ascorbic acid or NSE induced an additive accumulation of proline and free amino acids contents in presence and absence of compost as compared with those of the corresponding salinity level. Application of 50 % of NSE in the soil amended with compost were the most effective treatment as it increased proline by 62%, 35% & 42% and total free amino acid by 31%, 36%, & 39% in presence of compost as compared of the (control without compost) at 0.0, 4000 and 8000 mg/l salinity level respectively.

Table 6: Effect of ascorbic acid (AsA) or *Nigella sativa* extract (NSE) on proline and free amino acids as (mg/ 100g dry weight) of quinoa plants and irrigation with different levels of saline solution (at 60 days from sowing) in absence (-) and presence (+) of compost.

Salinity (mg/l)	Treatment		proline		Total Amino acids	
			-	+	-	+
0	Control		14.9	17.0	236	263
	AsA	200 mg/l	17.3	20.5	274	301
	NSE	25%	16.3	19.5	268	290
		50%	19.3	24.1	290	308
4000	Control		25.3	27.0	324	354
	AsA	200 mg/l	29.5	32.1	371	388
	NSE	25%	28.4	30.8	361	410
		50%	32.1	34.2	395	441
8000	Control		30.5	32.3	352	377
	AsA	200 mg/l	34.3	38.4	410	416
	NSE	25%	32.4	36.4	395	429
		50%	40.2	43.3	450	491
LSD at 5%			1.89		22.35	

Antioxidant enzymes

The changes in the activities of the various enzymes in response to salinity stress either alone or in combination with each of the NSE or ascorbic acid in absence and presence of compost are illustrated Table 7. Results indicated that, superoxide dismutase (SOD) and APX were significantly increased under stress conditions. The magnitude of enhanced was increased with increasing salinity level and in presence more than in absence of compost. In response of (CAT) results observed that, there were no significant increased under stress conditions. While, POX was significantly decreased under stress conditions. The magnitude of reduction was increased with increasing salinity level and in presence more than in absence of compost. Treatment of quinoa seeds with NSE or ascorbic acid in absence and presence of compost improve stress tolerance by the increase in SOD, CAT, POX, and APX activities as compared with corresponding salinity level. The higher activities were recorded at 50% NSE in presence of compost as it increased SOD by 38%, 25%, 32%, CAT by 6%, 7%, 17%, POX by 31%, 21%, 43% and APX by 9%, 12%, 13% in presence of compost as compared of the control without compost at salinity level 0.0, 4000 and 8000 mg/l respectively.

Table 7: Effect of ascorbic acid (AsA) or *Nigella sativa* extract (NSE) on antioxidant enzyme as (µ g-1 FW) of quinoa plants and irrigation with different levels of saline solution (at 60 days from sowing) in. absence (-) and presence (+) of compost

Salinity (mg/l)	Treatment		SOD		CAT		POX		APX	
			-	+	-	+	-	+	-	+
0	Control		19.78	21.4	59.56	60.54	33.99	36.15	12.7	13.0
	AsA	200 mg/l	20.48	23.14	61.04	61.86	39.12	40.52	13.6	13.7
	NSE	25%	22.38	25.96	60.42	61.56	36.98	38.31	13.4	13.3
		50%	23.33	27.34	61.98	63.01	42.54	44.55	13.6	13.9
4000	Control		25.94	26.63	60.93	61.925	30.86	32.98	13.4	13.8
	AsA	200 mg/l	28.19	30.4	62.03	63.14	33.63	36.13	14.1	14.8
	NSE	25%	28.35	30.21	61.96	62.84	31.56	34.12	13.7	13.9
		50%	30.36	32.54	63.00	65.34	35.86	37.45	14.3	15.0
8000	Control		32.33	36.05	61.02	62.71	20.71	23.18	14.5	15.3
	AsA	200 mg/l	37.77	40.12	66.56	68.31	23.11	26.36	15.3	16.0
	NSE	25%	37.48	39.69	66.61	68.24	23.12	25.38	15.1	15.9
		50%	39.89	42.54	68.65	71.3	23.09	29.14	15.8	16.2
LSD at 5%			1.35		2.66		2.25		0.9	

Yield Components*:*

Results in Table (8a) illustrate the effect of foliar application of AsA or NSE with or without compost on quinoa plant grown under different salinity levels (0.0, 4000 and 8000 mg/l) on yield parameters. Increasing salinity level effect resulted in gradual reduction of yield such as shoot length, fruiting branches number /plant and shoot weight seed weight/ plant. The inoculation with compost showed that an increase in all yield components. Appling AsA or NSE of quinoa plant showed that, both treatment increased yield components of the quinoa as compared with the corresponding salinity levels particularly in the presence of compost. The NSE at (25%) was the most effective increased in yield components under different salinity levels as it increased seeds weight/plant by 95%, 65%, 154% in presence of compost as compared to control without compost at 0.0, 4000 and 8000 mg/l salinity level respectively.

Table 8a: Effect of ascorbic acid (AsA) or nigella extract (NSE) on yield of quinoa plants and irrigation with different levels of saline solution in **absence (-) and presence (+) of compost.**

Salinity (mg/l)	Treatment		Plant height		No of branches /plant		Shoot weight		Seed weight /Plant	
	Material		-	+	-	+	-	+	-	+
0	Control		45.2	46.3	12.0	14.3	7.80	8.02	4.00	4.80
	AsA	200 mg/l	49.9	53.3	17.3	19.0	10.00	11.83	5.50	6.33
	NSE	25%	55.4	59.0	18.2	19.3	9.98	12.03	7.00	7.80
		50%	48.7	50.3	17.0	18.2	9.03	10.00	4.99	5.88
4000	Control		38.2	40.3	10.2	11.3	4.18	7.70	2.55	3.16
	AsA	200 mg/l	42.3	44.9	11.4	12.9	5.75	6.24	3.78	3.90
	NSE	25%	43.9	45.7	12.3	13.5	6.21	6.99	4.00	4.22
		50%	41.0	44.3	11.0	12.8	5.01	5.88	2.78	3.89
8000	Control		22.0	26.7	8.3	9.5	3.10	4.58	1.58	2.97
	AsA	200	30.0	32.5	10.0	10.6	4.89	5.95	2.75	3.32
	NSE	25%	35.5	39.7	10.6	11.5	5.30	6.00	3.33	4.01
		50%	25.3	31.3	10.0	10.6	4.08	5.03	2.77	3.13
LSD at 5%			3.21		0.75		0.77		0.25	

Changes in carbohydrate, protein and oils contents in yielded seeds:

Data in (Table 8b) showed that, resulted a gradual reduction in carbohydrates, protein % and oil% of quinoa yielded seeds in all salinity levels. Moreover, the quinoa plants sowing in the soil amended with compost in different salinity levels increased significantly carbohydrates, protein % and oil% of quinoa yielded seeds as compared with those in absence of compost. Data also show significant increases in carbohydrates, protein % and oil% of quinoa seeds treated with different concentrations of ascorbic acid or *N sativa*. Application of 50 % of nigella extract in the soil amended with compost the most effective treatment as it increased carbohydrates % by 12%, 12% & 13%, protein and oil % by 48%, 49%, & 38% in presence of compost as compared to control without compost at salinity level(0.0, 4000 and 8000 mg/l) respectively.

Table 8b: Effect of ascorbic acid (AsA) or *Nigella sativa* extract (NSE) on Carbohydrate %, Protein % and Oil %in the yielded seeds of quinoa plants and irrigation with different levels of saline solution in absence (-) and presence (+) of compost.

Salinity (mg/l)	Treatment		Carbohydrate %		Protein %		Oil %	
			-	+	-	+	-	+
0	Control		58.35	60.64	11.85	13.91	6.23	6.64
	AsA	200	62.18	63.25	15.77	16.75	6.98	7.65
	NSX	25%	60.33	61.75	13.54	14.98	6.54	6.83
		50%	63.21	65.32	17.01	18.55	7.94	9.25
4000	Control		51.02	52.3	10.33	11.51	5.00	5.85
	AsA	200	53.87	55.36	14.53	15.23	6.32	6.83
	NSE	25%	51.87	53.89	12.33	14.5	6.4	6.67
		50%	55.32	57.25	15.9	16.23	6.95	7.45
8000	Control		46.12	48.98	9.55	10.33	4.75	4.98
	AsA	200	49.33	50.87	11.35	12.01	5.44	5.9
	NSE	25%	47.03	49.32	10.02	10.98	5.00	5.21
		50%	50.21	52.30	12.61	13.54	6.01	6.54
LSD at 5%			2.05		0.85		0.12	

Changes in macronutrient contents in yielded seeds:

Data in (Table 8c) show that, all salinity levels resulted in a gradual reduction in nitrogen, phosphorus and potassium percentage in the absence and presence of compost as compared with the corresponding salinity level. Data also showed that, compost amended in soil increased significantly seeds contents of nitrogen, phosphorus and potassium as compared with those in absence of compost. Foliar spraying of wheat plants with ascorbic acid or NSE stimulated nitrogen, phosphorus and potassium contents of quinoa seeds compared with the corresponding control plant. Data also show that foliar treatment of NSE at 50 % to quinoa plant and addition of compost was more effective as it gave the highest contents of nitrogen by 83%, 81%, 93, phosphorus by 102%, 105%, 81% and potassium by 80%, 86%, 60% as compared to control without compost at 0.0, 4000 and 8000 mg/l salinity level respectively.

Table 8c: Effect of ascorbic acid (AsA) or nigella extract (NSE) on nitrogen, phosphorus and potassium as (%/ g dry weight) of quinoa plants and irrigation with different levels of saline solution in absence (-) and presence (+) of compost.

Salinity (mg/l)	Treatment		N%		P%		K%	
			-	+	-	+	-	+
0	Control		2.27	3.61	0.243	0.420	0.354	0.521
	AsA	200 mg/l	2.78	4.10	0.354	0.480	0.444	0.619
	NSE	25%	2.68	3.92	0.275	0.460	0.408	0.558
		50%	2.81	4.15	0.370	0.491	0.460	0.637
4000	Control		2.16	3.45	0.220	0.386	0.318	0.563
	AsA	200 mg/l	2.63	3.87	0.331	0.462	0.406	0.586
	NSE	25%	2.52	3.79	0.260	0.436	0.360	0.566
		50%	2.79	3.91	0.350	0.451	0.412	0.593
8000	Control		2.01	3.23	0.202	0.322	0.301	0.344
	AsA	200 mg/l	2.47	3.56	0.240	0.359	0.321	0.477
	NSE	25%	2.22	3.55	0.237	0.355	0.311	0.462
		50%	2.63	3.88	0.255	0.365	0.344	0.483
LSD at 5%			0.08		0.013		0.011	

Total flavonoids contents in yielded seeds:

Data in (Table 8d) reveal that, non significantly increased in flavonoid content in all salinity levels of quinoa yielded seeds. Data in (Table 8d) showed that, compost addition to soil increased significantly total flavonoids content of quinoa yielded seeds as compared with those in absence of compost. Data also show significant increases in total flavonoid contents of quinoa yielded seeds treated with ascorbic acid or NSE. Foliar spraying of quinoa planted in pots amended with compost with 25% NSE was the most effective treatment as compared with of the corresponding salinity level (control without compost).

Antioxidant activity in yielded grains:

Table (8d) observed that, salinity levels resulted in a gradual reduction in antioxidant activity (as DPPH- radical scavenging capacity). Data also showed that amended soil with compost caused significant increases in antioxidant activity of quinoa as compared with untreated soil. Ascorbic acid at 200 mg/l or NSE (25 and 50%) caused gradual increases antioxidant activity as compared with the corresponding control plants. Higher content of antioxidant activity was obtained with 25% NSE application in the presence of compost.

Table 8d: Effect of ascorbic acid (AsA) or *nigella sativa* extract (NSE) on Flavenoids % and antoxidant activity as (% DPPH- radical scavenging capacity) of quinoa plants and irrigation with different levels of saline solution in absence (-) and presence (+) of compost.

Salinity (mg/l)	Treatment		Flavenoids %		DPPH	
			-	+	-	+
0	Control		63.22	64.42	47.13	49.87
	AsA	200	65.14	66.13	50.14	52.12
	NSE	25%	66.15	68.47	51.33	52.98
		50%	64.15	65.44	48.88	51.22
4000	Control		63.84	65.74	44.65	45.61
	AsA	200	67.75	69.93	45.78	46.85
	NSE	25%	71.17	74.33	47.14	48.35
		50%	65.32	67.33	43.27	45.02
8000	Control		64.14	65.01	40.35	42.33
	AsA	200	66.02	67.14	42.00	45.45
	NSE	25%	66.89	69.42	44.23	46.33
		50%	64.57	66.13	41.13	43.44
LSD at 5%			2.60		1.89	

Discussion

Growth parameters

The response of quinoa plant to salinity stress caused gradual significant decreases in all growth parameters (Table 3). These results might be due to the inhibitory effect of salinity through reduced water absorption, metabolic activities due to Na+ and Cl- toxicity and nutrient deficiency caused by ion interference. The obtained results are in good agreement with previous ones reported by[37] on sunflower plants. In this connection, [38] cleared that, under salinity stress, plant growth decreased and accumulates osmolytes in cells in order to maintain against dehydration. Moreover, [39] reported that the effect of different salinity levels on growth criteria of canola were due to the effects of salt stress on plant cell functions including the functions of different enzymes, metabolism of the cell. The inhibition of growth under salinity stress reduced photosynthesis (Table 4) which in turn limited the supply of carbohydrate needed for growth (Table 5) which is in agreement with previous study[40].

Compost amendment to agricultural soils influences plant growth and soil quality throught (i) direct effects such as supplying the plants with its required nutrients, increasing soil quality and fertility, soil organic matter and also it works as soil conditioning and (ii) indirect effects, since the compost contains abundances of

microorganisms that play a key role in improving nutrient availability such as phosphorus, sulfur, manganese and micronutrients. Also compost contains several microorganisms that exudates several substances and metabolites that act as phytophormonses and as plant growth promoting. In particular, a mutant of *Penicillium sp*. was used to enhance composting processes during preparing this compost that used throughout this study. The positive effect of compost on plant growth (Tables 3) is most likely due to increased nutrient availability N, P and K (Table 8c) which is in agreement with previous study [41]. The role of compost in increasing quinoa growth may be via endogenous growth promoters producing by mutant *penicillium* which enhance the mobilization of nutrient towards the buds through increasing cell divisions and / or increasing the differentiation of the vascular connection between the axillary buds and the main stem. Moreover, [42] reported that under salinity stress the mutant *penicillium* producing auxin in which IAA-producing fungi enhanced the growth of rice plant.

The exogenous application of ascorbic acid and /or *Nigella sativa* extract (containing large amount of antioxidants) mitigated partially the adverse effects of salt stress on growth parameters [11] and [43]. Ascorbic acid have effects on many physiological processes including the regulation of growth, differentiation and metabolism of plants under salinity stress and increasing physiological availability of water and nutrients [44]. In addition, ascorbic acid protect metabolic processes against H_2O_2 and other toxic derivatives of oxygen, enhanced many enzyme activities (Table 7), reduce the damage caused by oxidative processes through synergic function with other antioxidants and stabilize membranes[45].

Photosynthetic pigments

Data in Table (4) show the inhibitory effect of salinity stress on the photosynthetic pigments of quinoa plant. It may be due to the effect of salinity on the activities of photosynthetic enzymes and stress leads to an increase in free radicals in chloroplasts and destruction of chlorophyll molecules by ROS, which results in reduction of photosynthesis and growth[46].

The compost inoculation enhanced the photosynthesis rate than without compost, which is consistent with compost effects on stomatal opening. Moreover, the increases in chlorophyll contents as a result of compost, could be attributed to the compost containing mutant *peniccillium* ameliorated the adverse effects of salinity stress[47]. It is worthy to mention that, carotenoids content was significantly higher in quinoa plants under treatment with compost in combination with different concentrations of salt as compared to the corresponding treatment without compost. Carotenoids might play a role as a free radical scavenger. Therefore, increasing of carotenoids in quinoa with compost amended in soil could enhance their capacity to reduce the damage caused by ROS, which in turn increased chlorophyll content of such plants. The same findings were reported by[38].

Exogenous application of ascorbic acid or *N sativa* extract increased chlorophyll contents, protected photosynthesis and growth in quinoa plant grown under salinity stress. The above protective effects of ascorbic acid could be related to the improved activities of key antioxidant enzymes (Table 4), and thereby their free radical scavenging in the stressed plants[48].

The effect of *N sativa* extract on the biosynthesis of chlorophyll may be attributed to its activation of enzymes that regulate photosynthetic carbon reduction, which are potential sources of natural antioxidants such as tocopherols, flavonoids and phytochemical[43]**.**

Change in carbohydrate contents:

Effect of foliar application with different concentrations of ascorbic acid or *N sativa* extract in absence and presence of compost at different salinity levels are presented in (Table 4). Data show that, addition of compost increased TSS, polysaccharides and total carbohydrates contents of quinoa plant as compared with those in absence of compost. The processes involved of compost lead to increased rates of photosynthesis and of carbon compounds to the plants [49]**.** In addition, the increase in total soluble sugars adjust the osmotic balance and increase the contents of chlorophyll which increase the rate of photosynthesis and carbohydrate synthesis [50].

Data also indicated increases in carbohydrates constituents as affected by ascorbic acid or *N sativa* extract treatments. Those obtained data are in good agreement of those obtained by[51]. The enhancement effect of ascorbic acid on photosynthetic pigments as shown in Table (4) reflected on total carbohydrate contents (Table, 5). [52]Khan et al. reported that application of ascorbic acid enhanced synthesis of chlorophyll that

involved in increases of photosynthetic metabolites, which lead to the accumulation of different fractions of soluble sugar contents in plant tissues under saline conditions or this could perhaps alleviate the inhibitory effects of salinity on glucose incorporation to cell wall polysaccharides.

Proline contents and total amino acid contents:

In the present work, salinity stress caused significant increases of proline contents and free amino acids in quinoa plants (Table 6). These results are in agreement with the results observed by[53] on sunflower plant, where, they concluded that salinity stress were capable of acting as activators of free amino acids accumulation. Moreover, accumulations of compatible osmolytes at high concentrations causing the osmotic adjustment in plants under salinity stress[38]. Proline has vital roles in osmotic adjustment, stabilization and protection of enzymes, proteins and membranes from damaging effects of drought-osmotic stresses[54]. Also, reducing oxidation of lipid membranes[55]. Furthermore, Asc or *Nigella S* extract additive increased of free amino acids and proline in quinoa plants. These results added support to the results obtained by[56]. Addition the compost with salinity stress additive increased in proline and free amino acids levels in quinoa plant (Table 6). [57] stated that, total amino acid concentration was higher in leaves of mustard plants treatment with compost.

Antioxidant enzymes

Superoxide dismutase, catalase, peroxidase and ascorbate peroxidase are enzymes that responsible for ROS-scavenging. Salinity exhibited increased SOD, CAT and APX activities in quinoa leaves as compared to control plant (Table 7). Peroxidase activity showed a gradual decrease with the increase in salinity level in water of irrigation of the quinoa plant Table (7). These results are in agreement with by[53] .These increases in the activities of antioxidative enzymes under salt stress could be a protective mechanism to reduce oxidative damage triggered by stress which considered as an indicative of the increased production of ROS. Superoxide dismutase (SOD) is the first defense enzyme that converts superoxide to H_2O_2, which can be scavenged by catalase (CAT) and different classes of peroxidases (POX) and ascorbate peroxidase. These results are in agreement with the results observed by[56]**.** In addition, ascorbic acid decreases the damage of many enzyme activities which induced by oxidative process [8].

Foliar application of ascorbic acid or *N sativa* extract in general, significantly increased the all enzyme activities (SOD, CAT, POX and APX) as compared to the corresponding salinity levels. Our obtained results are agreement with those obtained by [58] on faba bean and [56] on flax. Ascorbic acid acts as a primary substrate in cyclic pathway for enzyme detoxification of hydrogen peroxide [59].

Moreover, [60]confirmed the similar results on sweet basil. It was found that growth of bean plants in extract of *N. sativa* significantly decreased the inhibitory effect of salt on the activities of SOD, POD, and CAT. These suggested that extract of *N. sativa* effectively controlled the activities of antioxidant enzymes only in stressed plants[61].

Concerning the effect of applying the compost on increasing the antioxidant enzyme activities obtained in this study some investigators recorded increments in peroxides activity as a result of inoculation the plants with *Pseudomonas* strains[62] on *Triticum aestivum*. [63]Stainer *et al* reported that when inoculated wheat plants with *Azotobacter* increased the catalase enzyme activity. In the present investigation, plants treated with compost led to marked increases in the enzymes SOD, CAT, POX and APX. These responses may be attributed as an attempt of the plant to overcome the adverse conditions of some elements required for growth and development of sorghum plants.

Yield and its components:

Increasing salinity level resulted in gradual reduction of yield such as shoot length, fruiting branches number /plant, shoot weight and seed weight/ plant. These results were agreement with those obtained by [64] on wheat. It could be concluded that these reduction may be attributed to the inhibitory effect of salinity on growth (Table 3), chemical composition of plant, such variation were reflected in the produced yield. Furthermore, the reduction of wheat yield / plant due to salinity stress might be due to the harmful effect of salt on growth as well the disturbance in mineral uptake[65].

The use of compost can be an excellent opportunity to decrease the negative effect of abiotic stresss, such as salinity, on crop yield. The term "plant-growth promoting- fungi" was established to designate some rhizosphere fungi able to promote a direct effect on plant growth upon root colonization or by the treatment with their metabolites[66]. [67]Gomaa *et al* found that, the soil impact with compost significantly increased the yield component of maize plant under water stress.

The foliar treatment of ascorbic acid or *N sativa* extract could be mitigated the adverse effects of salt stress on yield and yield components of the quinoa plant as compared with the corresponding salinity levels under test. In addition, these changes may be attributed to the increase in nutrients uptake and assimilation. Thus, it can be concluded that the increment of seed yield/ plant, in response to the applied treatments is mainly due to the increases in the number of branches/plant which increases the fruits number/plant. Moreover, the increase in yield and its components might be due to the effect of antioxidants role on enhancing protein synthesis (Table 8b) and delaying senescence[68].

Changes in carbohydrate, protein and oils contents:

The different treatments of ascorbic acid and /or *N sativa* extract in the present and absent of compost effectively increased the total carbohydrate, protein and oil percentage of yielded quinoa seeds. Similar finding were obtained in sunflower plant in response to biofertilizer application[69] and [70],who found that, total carbohydrate, protein and oil % were increased in mycorrhizal wheat and Acacia saligna plants respectively. Moreover**,** Inoculation with microbien or compost in *Schefflera arboricola* L. increased total carbohydrate percentage compared with control plants[71]**.** **[67]**Gomaa *et al* found that, the amended of compost on maize under water stress condition protein content (%) increased .However, [72] and [56] reported that ascorbic acid significantly increased oil percentage and protein of sunflower seeds and Flax cultivars respectively.

Changes in mineral contents in in yielded seeds:

It increases availability of plant nutrients, improves physical properties, and stimulates biological activities by amendment the soil with compost is beneficial to soil quality. The present study indicated that application of salt induced significant decreases in N, P and K levels in the yielded seeds in absence of composts. High salt competes with the uptake of other nutrient ions, especially K+, causing deficiency in K+ and other ions. The ion deficiencies develop a nutritional imbalance[73]. The present study showed that, addition of compost containing mutant *peniccillium* to soil provided higher total N, K and available P contents than control of quinoa plant (Table 8c) our obtained data are in harmony with those of by [74] found that the organic compost applications increased the uptake of N,P and K of *Narcissus Tazetta, L.*.

Furthermore, ascorbic acid and /or *N. sativa* extract in generally encouraged the increases of total nitrogen, phosphorus and potassium contents in quinoa plant. Several reports indicated that ascorbic acid application increased minerals contents[58]. It could be concluded that the simulative effect of ascorbic acid to increase nitrogen content is through enhancing the biosynthesis of free amino acids (Table 5) and their combination I nto protein (Table 8 c)[75]. Positively charged macronutrients such as potassium (K^+) are required in relatively large amount for plant growth and development. Thus, the above mentioned results are consistent with the results of growth parameters (Table 2) and also with pigments (Table 4).

Total flavonoids content in yielded seeds:

Data represented in Table (8d) indicated that ascorbic acid and *N sativa* extract foliar application had non significant effect on flavonoids in absence of compost but induced significant increase in total flavonoids content in the presence of compost in different salinity levels. This increase may be returned to the inoculation of compost with mutant *peniccillium.* In this connection, [76]suggested that the microbial (bacteria or fungi) ameliorate the abiotic stress by the expression of enzymes involved in flavonoids biosynthesis. Flavonoids have high antioxidant activity[77] which play a number of important roles in stress protection of plants[78], whereas ascorbic acid is a strong antioxidant, application of this vitamin prevents increase of flavonoid concentration through scavenging of ROS.

Antioxidant activity in yielded seeds:

Data in Table (8d) showed that (compost) addition to soil caused significant increases the antioxidant activity (as DPPH- radical scavenging capacity) of quinoa seeds. Also, foliar treatment of quinoa plant with ascorbic acid and *N sativa* extract at different salinity levels caused increases in the antioxidant activity as compared with the corresponding control plants. The antioxidant activities have been detected in quinoa indicating that quinoa may serve as an excellent dietary source of natural antioxidants for disease prevention and health promotion[79]. The increase in the scavenging activity can be considered an advantage of treatment used. The antioxidant activity increases may be returned to the increases in total phenols and total flavonoids [80] and[70].

Conclusion

This paper summarizes effects of ascorbic acid or *Nigella sativa* extract in the presence of the compost containing mutant peniccillium ameliorated the adverse effects of salinity stress and improved quinoa plant growth by influencing biosynthesis of the plant's bioactive compounds such as osmoliytes (total soluble sugars, proline, total free amino acid), antioxidant compounds (carotenoids, flavonoids) and antioxidant enzyme activities (superoxide dismutase, catalase, peroxidase, ascorbate peroxidase). Moreover, quinoa plant cultivated in soil amended with compost and ascorbic acid or *Nigella sativa* extract gave higher nutritional value of macronutrients (N, P, K,), carbohydrate%, protein %, total flavonoids, antioxidant activity in yielded seeds.

Compost addition can increase soil nutrient viability and thereby nutrient uptake by the plants. This effect can be direct effects are via nutrients added with the compost whereas indirect effects are via increased microbial activity, improved soil structure or nutrient and water retention. Microbial activity can increase nutrient mobilization and producing bioactive substances such as phytohormons and mitigate salinity stress.

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