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Antioxidants Released from *Cichorium pumilum* Jacq. Amendment Mitigate Salinity Stress in Maize

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Abstract

Amending soil with weeds, among the organic farming practices, is an innovative approach to improve crop yield and recycle nutrients. A pot experiment was carried out to investigate the impact of amending soil with phenolic rich *Cichorium pumilum* Jacq. leaves (0.0, 10 and 20 g powdered dry matter kg^{-1} soil) on maize responses to salt stress (0.0, 100 and 200 mM NaCl). Generally, soil amendment enhanced the growth of maize and alleviated the negative stress impact. Amendment at 10 g powered dry leaves of *C. pumilum* kg^{-1} soil enhanced the total chlorophyll in severe stressed plant, compared with that grown in unamended stressed soil. Antioxidants such as flavonoids content were increased in all stressed and unstressed samples grown in the amended soil. Consequently, soil amending also induced less oxidative damage (hydrogen peroxide and lipid peroxidation) in stressed plants. Reduced oxidative damage was also associated with the increased catalase and peroxidase activities, and these increases were higher in root explaining the more enhancement in root growth (Pearson correlation). Results indicated that using *C. pumilum* Jacq. amendment, as a way of organic farming, mitigated the harmful effect of salinity on maize plants via its antioxidative potential. Weed amendment, as one of the organic farming methods, contributed via its ability to release antioxidant phenolic compounds.

Keywords: Antioxidant, Organic farming, Phenolic compounds, Salt stress

1. Introduction

Cichorium pumilum Jacq. (synonym: *C. endivia* subsp. *Divaricatum* (Schousb.) P.D. Sell, *C. divaricatum* Schousb., *C. endivia* subsp. *Pumilum* (Jacq.) Coutinho, *C. intybus* subsp. pumilum (Jacq.) Ball) (http://www.theplantlist.org) belongs to family Asteraceae. It is one of the annual wild plants growing in Egypt. It also grows as one of the undesired weeds infecting Egyptian fields as well as other fields in some of the Mediterranean countries (Abu-Irmaileh, 1982; Boulet *et al.*, 2002; Gervilla *et al.*, 2019; Qasem, 1992). Previous studies stated that genus *Cichorium* is well defined with the presence of polyphenols including phenolic acids and flavonoids in addition to the presence of sesquiterpenoids (El-Shafey and AbdElgawad, 2012; Kisiel and Michalska, 2006). Also, the antioxidant and antiradical activities of the genus *Cichorium* have been proved (Ghanaatiyan and Sadeghi, 2017; Sahan *et al.*, 2017).

Organic farming as an alternative to conventional agriculture offers solutions to the environmental problems created by some of the practices such as using industrial fertilizers and pesticides. Organic farming is one of the fastest growing agriculture practices over the world, due to providing soil with long-term fertility, supplementing crop with nutrients and reducing adverse impacts of farming systems on the natural habitats and environment (Hassan *et al.*, 2018; Padel and Lampkin, 1994; Peigné *et al.*, 2016). Amending soil with organic wastes and plant residues comes among organic farming practices to enhance soil fertility, recycling nutrients and biodiversity and microbial populations (Lim *et al.*, 2015). In addition, organic amendment contributes to supply nutrients and enhance plant productivity depending on the quality and quantity of the amendment (Diallo *et al.*, 2006; Roy *et al.*, 2010). Investigating the influence of weed residues on crop productivity has attracted attention, since removing weeds manually, leaving to dry and mixing with soil or ploughing them directly with soil is already still among farming practices (Batish *et al.*, 2007; Hassan *et al.*, 2018). Previous studies recommended utilizing weed residues to enhance crop biomass and yield (Awodun and Ojeniyi, 1999; Awopegba *et al.*, 2016; Falade and Ojeniyi, 1997).

Salt stress is among the main constraints that cause a great loss in crop production. It severely affects plant via inducing osmotic stress and ion toxicity. Moreover, salinity disturbs redox homeostasis in plant cells, causing the burst of reactive oxygen species (ROS). These ROS result in oxidation of lipids, proteins and nucleic acids, leading to membrane damage, enzymatic inhibition and metabolic dysfunction and finally plant death (Hasanuzzaman *et al.*, 2013; Liang *et al.*, 2018). Parallel to production of ROS generally produced from electron transport systems, plant evolved enzymatic antioxidant defense systems including superoxide dismutase (SOD) catalase (CAT) and peroxidase (POD) in addition to nonenzymatic ones such as ascorbate, glutathione, tocopherol, flavonoids and polyphenolic compounds (Liang *et al.*, 2018; Parida and Das, 2005). Plants with more effective employment of their enzymatic and non-enzymatic antioxidants exhibit more enhanced salt

tolerance, as ROS scavenging is very important in salt tolerance (Chinnusamy *et al*., 2005). There is a worldwide demand for inexpensive and environmentally safe technologies to overcome salinity problem, increase plant tolerance and enhance soil. This makes amending soil with weeds, particularly those rich in phenolics, one of the options that may secure most of these demands.

In the present study, our hypothesis is that *C. pumilum* amendment enhances maize plant growth, especially under stress conditions. To test this hypothesis, the antioxidative potential of *C. pumilum* amendment (10 and 20 g powered dry leaves kg^{-1} soil) and its impact on maize growth under different levels of salt stress (0.0, 100 and 200 mM NaCl) were investigated. The biochemical basis of the main effects of amendment (10 g powered dry leaves kg^{-1} soil), salinity and their interaction on maize plant were also studied.

2. Materials and Methods

Fresh leaves of the flowering plants of *Cichorium pumilum* Jacq. were collected during winter season from clover and wheat fields in Beni-Suef Governorate, Beni-Suef, Egypt. Samples were identified as reported previously (El-Shafey and AbdElgawad, 2012).

2.1. Extraction of phenolic compounds

The collected leaves were washed under running tap water and then washed three times using distilled water. Leaves were air dried at room temperature and then grinded to fine powder. For preparation of the aqueous extract, about 5 g of the powdered dry leaves were soaked in 100 ml dist. water and agitated on the orbital shaker for 24 hours at 110 rpm and 60°C. The extract was centrifuged at 4000 rpm, filtered through a muslin cloth and then through filter paper Whatman No. 1 by using vacuum and pressure pump (AP-9925 Auto Science). The filtrate was concentrated by using a rotary evaporator (Shanghai Senco Technology Company, Shanghai, China) under reduced pressure at 45°C. Finally, the residue was collected and used for HPLC analysis.

2.2. Analysis of free phenolic compounds

After drying, the residue of aqueous extract was dissolved in HPLC grade MeOH to give 1000 ppm, then 20 µl of methanol dissolved sample were injected into HPLC system (Shimadzu class-LC 10 AD chromatograph supplied with shimadzu SPD-10 AUV-VIS). Phenomenex C18 column (25cm*4.6mm i.d, 5Mm particle size) was used as a stationary phase for HPLC determinations. The retention times of twenty-five highly purified phenolic compounds (Sigma-Aldrich Laborchemikalien, Germany) as well as our sample were detected at 254 nm. Quantitative determinations were carried out using calibration curves of the standards, and values of phenolic compounds were expressed as µg g^{-1} dry weight.

2.3. Treatments and plant growth analysis

Experiment was repeated twice, giving similar trends, and results of one are shown. Before sowing, pots (15 cm diameter and 15 cm depth) were filled with sandy clay soil (1:3), and the dry powder of *Cichorium* leaves was applied as amendment at 0.0, 10 and 20 g powered dry leaves kg^{-1} soil (mixed with 2 cm depth of the surface soil layer). Ten grains of maize (single cross 10; Sc10) were sown per each pot in a random way. Pots with amended and unamended soil were irrigated with tap water and kept in net house at the Botanical Garden, Botany and Microbiology Department, Faculty of Science, Beni-Suef University, where average high and low temperatures were 32-35 and 20-22°C respectively, during May and June. After emergence, the growing seedlings were thinned to 4 seedlings. After two weeks of sowing, pots were irrigated with salt solutions (0.0, 100 and 200 mM NaCl) and permanently kept at its field capacity level. The experiment was designed in a split-plot design, where the amendment treatments were the main plots and salinity levels were subplots. After two weeks of salinity application, the plants were harvested and used to determine the growth parameters (lengths, and fresh and dry weights of both root and shoot). The biochemical analyses were performed in fresh and dry samples of plants grown in both amended (10 g powered dry leaves kg^{-1} soil) and unamended soils.

2.4. Photosynthetic pigments and flavonoids

The photosynthetic pigments were extracted and estimated according to Lichtenthaler (1987). Approximately 0.5 g of fresh leaves was homogenized in 5 ml cold acetone (80%) and the extract was centrifuged for ten minutes at 4000 rpm and 4°C. The optical density of the extract was measured at 663.2, 646.8 and 470 nm using 80% acetone as a blank. The amounts of chlorophyll (Chl) a, Chl b and carotenoids were calculated in µg per ml extract by using the equations given below, and expressed as µg g^{-1} dry weight.

Concentration of Chl a = $12.25\ A_{663.2} - 2.79\ A_{646.8}$

Concentration of Chl b = $21.50\ A_{646.8} - 5.10\ A_{663.2}$

Concentration of carotenoids = $(1000\ A_{470} - 1.82$ Chl a $- 85.02$ Chl b$) / 198$

Total flavonoids were extracted in 80% methanol (Sayed *et al*., 2017). The mixture was agitated overnight at 100 rpm on an orbital shaker and then centrifuged at 6000 rpm at room temperature. Content of total flavonoids was determined in the supernatant by aluminum chloride (Zhishen *et al*., 1999). The absorbance was read at 510 nm. Quercetin standard curve was used to estimate the concentration of total flavonoids that was expressed as µg g^{-1} dry weight.

2.5. Enzymatic antioxidants

Frozen samples were homogenized in cold phosphate buffer (67 mM and pH 7.0). The homogenate was centrifuged at 10,000 rpm for 15 min at 4ºC, and the supernatant was used as a crude enzyme. Activity of SOD (EC 1.15.1.1) was quantified based on the competitive inhibition of nitroblue tetrazolium chloride (NBT) reduction by the superoxide radical (Beyer, 1987). One unit of SOD activity was calculated as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction. The activity of catalase (CAT; EC 1.11.1.6) was assayed by following the decomposition of H_2O_2 as a decline in the absorbance at 240 nm (Kato and Shimizu, 1987). Catalase activity was calculated using the extinction coefficient (40 mM^{-1} cm^{-1} at 240 nm) and expressed as µM H_2O_2 destroyed min^{-1} g^{-1} fresh weight. Peroxidase (POD; EC 1.11.1.7) activity was estimated following the change of catechol absorbance at 430 nm due to oxidation by H_2O_2 (Kar and Mishra, 1976). The enzyme activity was expressed as the change in optical density of catechol min^{-1} g^{-1} fresh weight.

2.6. Hydrogen peroxide (H2O2) and lipid peroxidation (LPO)

One gram of frozen plant material was homogenized with 10 ml of 0.1% trichloroacetic acid (TCA) and the homogenate was centrifuged at 10,000 rpm for 15 min. The supernatant was used for assaying H_2O_2 and lipid peroxidation. Hydrogen peroxide was estimated by potassium iodide and the absorbance was recorded at 390 nm (Velikova *et al.*, 2000). The concentration of H_2O_2 was calculated from its standard curve and expressed as µM g^{-1} fresh weight. Lipid peroxidation (LPO) was determined by measuring the absorbance of the colored complex formed due to reaction of malondialdehyde (MDA; a principal product of lipid peroxidation) with thiobarbituric acid (TBA) at 532 nm (Jambunathan, 2010). The amount of MDA was calculated using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} fresh weight.

2.7. Statistical analysis

All statistical analyses were performed using SPSS 16.0 program for Windows (SPSS, Chicago, IL, USA). Split-plot ANOVA was applied to investigate the effect of amendment, salinity and their interaction. Differences among means were established using Duncan test ($P < 0.05$), and Pearson correlations were determined among variables.

3. Results

3.1. Free phenolic compounds in C. pumilum

Nine free phenolic compounds, most of which are phenolic acids, were detected and quantified in the aqueous extract of *C. pumilum* (Table 1). Among these, caffeic acid (18.9%), gallic acid (16.2%), pyrogallic acid (14.2%), vannilic acid (14.0%) and cinnamic acid (13.6%) were the most abundant phenolic compounds detected in *C. pumilum* aqueous extract. Also, ferulic acid with the proportion 10.7% of the total detected compounds was found in the aqueous extract. Protocatechuic acid, coumarin and apigenin were found in lower proportion (3.0-5.3%).

Table 1. HPLC analysis of phenolic compounds in aqueous extract of *C. pumilum* Jacq. leaves

Standard phenolic compounds	Retention time min		Concentration
	Standard	Sample	µg g^{-1} dry weight
Gallic acid	8.2	8.2	151.1
Pyrogallic acid	9.2	9.3	132.2
Protocatechuic acid	12.7	12.8	28.0
Caffeic acid	18.0	18.1	176.4
Vanillic acid	18.0	18.1	130.4
Coumarin	22.2	21.6	38.6
Ferulic acid	24.9	25.0	99.6
Cinnamic acid	36.1	36.0	126.7
Apigenin	38.0	38.2	49.0
Total			932.0

3.2. Plant growth

Salinity adversely affected growth parameters of maize, while amendment enhanced these parameters and alleviated their loss exerted by salinity as compared with control (Table 2 and Figure 1). As interaction (salinity×amendment) was significant for shoot and root lengths as well as root fresh weight ($P < 0.001$ and $P < 0.05$, respectively; Table 2), the amendment-induced enhancement in these parameters was more pronounced under stress conditions, particularly the severe one (Figure 1a-b, d). Amendment significantly increased means of shoot ($P < 0.001$; Table 2) and root ($P < 0.05$) dry biomass of both salt stressed and unstressed maize (Figure 1e-f). In most cases, there was no significant difference between the influence of 10 g and that of 20 g of *C. pumilum* amendment; both significantly enhanced shoot and root biomass. However, the dose 10 g of amendment was the most effective in mitigating the adverse effect of salinity on plant growth. For example, shoot and root dry weights treated with 10 g amendment amplified by 2 and 5.7 fold, respectively (Figure 1e-f) as compared with those grown under severe stress without amendment.

Table 2. Mean Squares of main effects and interaction for morphological and physiological criteria of maize treated with *C. pumilum* amendment and salinity.

Source	df	Shoot length	Root length	Shoot FW	Root FW	Shoot DW	Root DW
Salinity	2	603***	82***	3.3***	0.022	0.049***	0.002
Amendment	2	1498***	172***	5.5***	0.375*	0.036***	0.01*
Salinity× Amendment	4	134***	34***	0.097	0.113*	0.003	0.002
		Chl a	Chl b	Carotenoids	Total chlorophyll		Chl a/b
Salinity	2	29333	46243***	1812	149173*		0.032
Amendment	1	10850	159795***	1205	87368		2.107***
Salinity× Amendment	2	78504	12330*	9890**	153009*		0.079
		Shoot flav	Root flav	Shoot SOD	Root SOD	Shoot CAT	Root CAT
Salinity	2	648***	305***	29114***	554085*	1802***	6575***
Amendment	1	624***	624***	6277	485253	632**	5036***
Salinity× Amendment	2	10.7	0.106	7230***	357811*	62.3	1044***
		Shoot POD	Root POD	Shoot H_2O_2	Root H_2O_2	Shoot LP	Root LP
Salinity	2	7484***	9603***	3485***	4409*	2066***	3803***
Amendment	1	1661*	2150***	2892***	8916*	3562***	11769***
Salinity× Amendment	2	287*	2176***	14.8	866	99.6	417**

*, **, *** < significant at 0.05, 0.01, and 0.001 probability levels, respectively, Chl a; chlorophyll a, Chl b; chlorophyll b, flav; flavonoids, SOD, superoxide dismutase, CAT; catalase, POD; peroxidase, LP; lipid peroxidation.

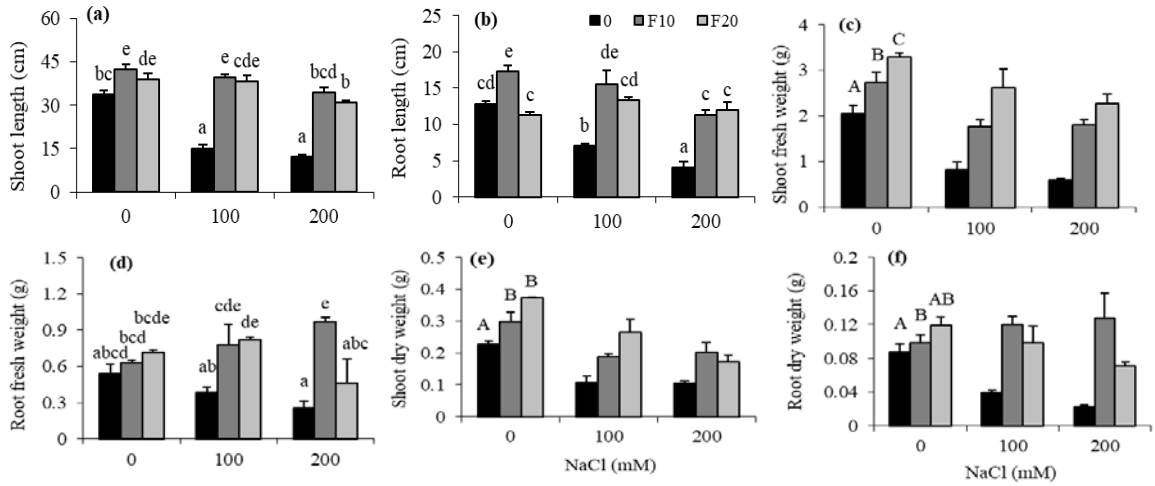

Figure 1. Effect of *C. pumilum* amendment on shoot and root lengths (a-b), fresh weights (c-d) and dry weights (e-f) of maize under different levels of salt stress (0.0, 100 and 200 mM NaCl). Treatments of 0, F10 and F20 are 0.0, 10 and 20 g amendment kg^{-1} soil. Values are means of 5 replicates ± SE. Values with at least one similar letter are non-significantly different at *P*=0.05. Capital letters are for main effect of amendment, while small letters are for multiple comparison in case of a significant interaction.

3.3. Photosynthetic pigments and flavonoids

Under severe stress, both Chl a and carotenoids as well as total chlorophyll declined below control (Table 3), but that decline was alleviated by applying amendment making the levels of these parameters approach that of control. Amendment mainly augmented Chl b content leading to a significant decrease (Table 2; $P < 0.001$) in Chl a/b ratio. Salinity×amendment interaction was significant for Chl b and total Chlorophyll ($P < 0.05$), as well as for carotenoids ($P < 0.01$). In all these traits, amendment effectively enhanced their levels and alleviated the loss imposed in them by severe salt stress (Table 3). Means comparison of flavonoids, one of the non-enzymatic antioxidants, revealed a significant decline as affected by salinity ($P < 0.001$; Table 2) and was more apparent at the highest level of stress, while the main effect of amendment significantly increased means of flavonoids of all the stressed and unstressed shoot and root ($P < 0.001$; Table 2-3). Although there was no significant interaction for this parameter in both organs, the increase in flavonoids value as affected with amendment was more pronounced in the stressed organs, particularly roots.

Table 3. Effect of *C. pumilum* amendment (10 g powered dry leaves kg^{-1} soil) on photosynthetic pigments and contents of shoot and root flavonoids in maize plants under different levels of salt stress (0.0, 100 and 200 mM NaCl).

Treatments	Chl a (µg g^{-1} dry wt)	Chl b (µg g^{-1} dry wt)	Carotenoids (µg g^{-1} dry wt)	Total chlorophyll (µg g^{-1} dry wt)	Chl a/b	Shoot flavonoids (µg g^{-1} dry wt)	Root flavonoids (µg g^{-1} dry wt)
C	592 ± 0	432 ± 18c	217 ± 4c	1024 ± 24b	1.37 ± 0.04	38.5 ± 1.5	31.19 ± 0.7
F	425 ± 4	570 ± 3d	146 ± 20ab	995 ± 30b	0.75 ± 0.07	53.37 ± 0.5	42.73 ± 1.5
100	506 ± 134	313 ± 87b	224 ± 57c	819 ± 221b	1.62 ± 0.02	29.61 ± 1.1	21.9 ± 0.8
F-100	311 ± 98	447 ± 22c	170 ± 17abc	758 ± 120b	0.69 ± 0.18	39.74 ± 0.8	33.65 ± 0.7
200	267 ± 31	184 ± 12a	124 ± 14a	451 ± 43a	1.45 ± 0.08	20 ± 0.7	16.87 ± 0.5
F-200	481 ± 262	477 ± 57c	201 ± 61bc	958 ± 319b	0.96 ± 0.44	30.33 ± 1.0	28.94 ± 0.6

Values are means of 3 replicates ± SE. Values with at least one similar letter are non-significantly different at *P*=0.05, while those with no letters are incase nonsignificant interaction. F; Samples amended with 10 g powered dry leaves per kg soil.

3.4. Enzymatic antioxidants

The effect of salt stress on SOD activity was contrasting to that on CAT and POD enzymes. While salinity amplified the activity of SOD, those of CAT and POD were decreased by increasing NaCl-concentration in both shoot and root of maize (Figure 2). Moreover, amending soil with *C. pumilum* dry powdered leaves significantly inhibited SOD, but stimulated CAT and POD in both parts. Interaction (salinity×amendment) was significant on SOD of both shoot and root ($P < 0.001$ and 0.05, respectively; Table 2). At 200 mM NaCl, amendment kept the activity of shoot SOD nonsignificantly changed relative to the nonamended samples, while declined that of root SOD to the level of control (Figure 2a-b). Salinity dimensioned CAT and POD activities in shoots grown in both amended and nonamended soils. In all the investigated salinity levels, CAT activity of the amended shoots was higher than that of the nonamended ones, whereas the alleviating effect of amendment on shoot POD was significant only under severe stress ($P > 0.05$; Table 2). Although salinity induced a dramatic decline in CAT and POD of roots grown in the nonamended soil, amendment significantly stimulated these enzymes at 100 mM NaCl to a level over control ($P < 0.001$; table 2, Figure 2d, f). Interestingly, the response of those enzymes to amendment at 200 mM was different, as amendment attenuated the activity of both enzymes.

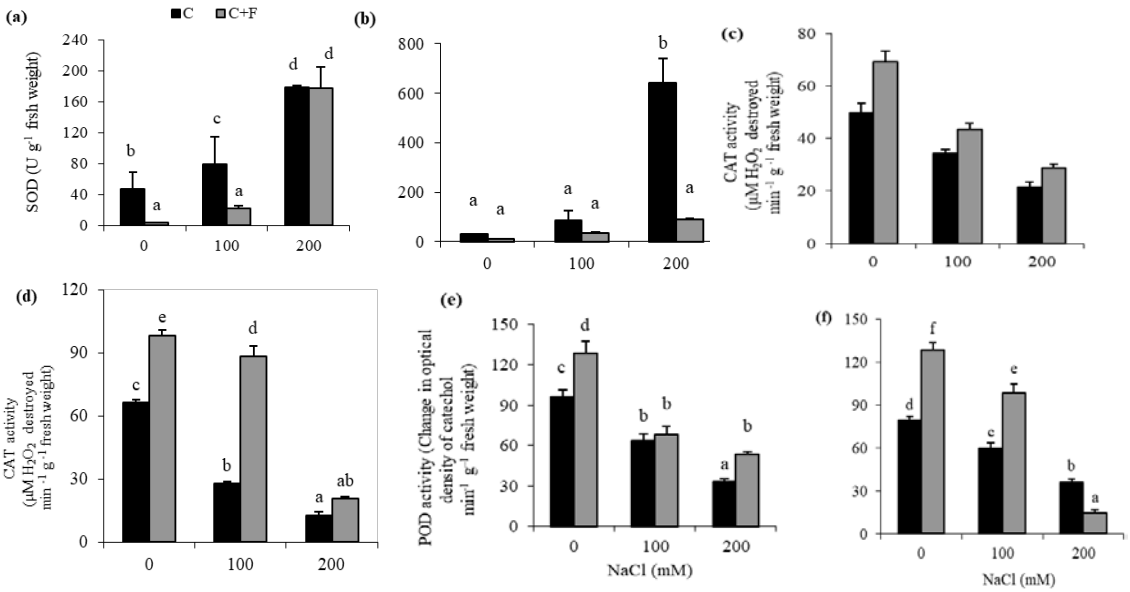

Figure 2. Effect of *C. pumilum* amendment (10 g powered dry leaves kg^{-1} soil) on SOD; superoxide dismutase (a-b), CAT; catalase (c-d) and POD; peroxidase (e-f) activities in shoot and root of maize plants grown under different levels of salt stress (0.0, 100 and 200 mM NaCl). Treatments of C and C+F; 0.0 and 10 g amendment kg^{-1} soil. Values are means of 3 replicates ± SE. Values with at least one similar letter are non-significantly different at P=0.05, while those with no letters are in case of no significant interaction.

3.5. Hydrogen peroxide (H_2O_2) and lipid peroxidation (LPO)

Salinity main effect significantly increased the accumulation of H_2O_2 in both shoot ($P < 0.001$; Table 2) and root ($P < 0.05$) grown in the amended and nonamended soils as well, and the accumulation was lower in the amended samples across all salinity levels (Figure 3a-b). Similar effect to that of salinity and amendment on H_2O_2 accumulation was imposed on LPO in shoot (Figure 3c). The decline in lipid peroxidation due to treatment with amendment was more pronounced in stressed roots than stressed shoots. Amendment effectively declined LPO value to the level of control ($P < 0.01$; Table 2, Figure 3d), although it was increased to 1.8-fold of control at severe concentration.

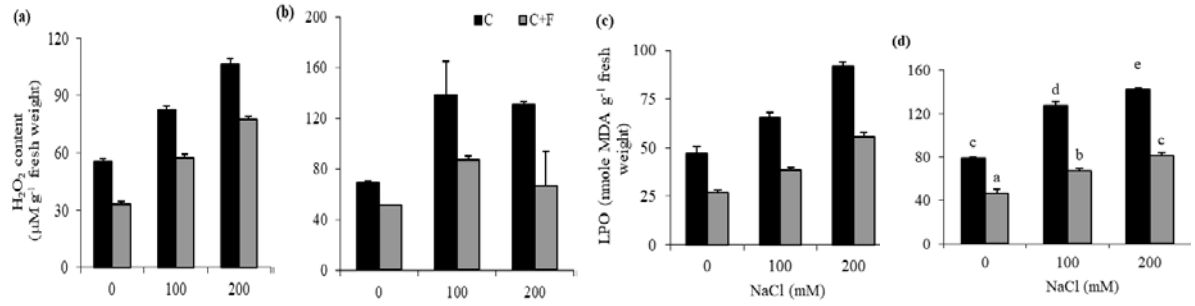

Figure 3. Effect of *C. pumilum* amendment (10 g powered dry leaves kg^{-1} soil) on H_2O_2; hydrogen peroxide (a-b) content and LPO; lipid peroxidation (c-d) in shoot and root of maize plants grown under different levels of salt stress (0.0, 100 and 200 mM NaCl). Treatments of C and C+F; 0.0 and 10 g amendment kg^{-1} soil. Values are means of 3 replicates ± SE. Values with at least one similar letter are non-significantly different at P=0.05, while those with no letters are in case of no significant interaction.

4. Discussion

Organic amendment is known to supply nutrients to soil and enhance its fertility and physical properties which leads in turn to improvement of plant performance (Ali *et al.*, 2017; Roy *et al.*, 2010). In the current study, amending soil with *C. pumilum* dry powdered leaves enhanced fresh and dry biomass as well as the length of both stressed and unstressed maize plants, and the influence was more pronounced in the stressed samples, particularly root ones. Similar enhancement of maize growth was also reported after applying weed residues to soil (Awodun and Ojeniyi, 1999; Falade and Ojeniyi, 1997; Jabeen and Ahmed, 2009). The positive effect on growth that detected in the present study by applying amendment may be due to the antioxidative stimulatory and/or protective effect of the components released from *C. pumilum* amendment. Applying weed amendment, particularly that rich in phenolics, may add more advantages to the employment of organic amendment. Recently, it was reported that the productivity of bean plants was increased when soil was amended with the dry residues of the phenolic-rich weed; *Sonchus oleraceus* (Hassan *et al.*, 2018). Most of the phenolic compounds that showed abundancy in *C. pumilum* leaves were documented as potential antioxidants (Hussain and Reigosa, 2011; Li *et al.*, 2013; Wan *et al.*, 2014). These phenolic compounds, when leached from *C. pumilum* leaves to soil, could work in a synergistic or antagonistic way that they positively affected the growth and the physiological processes of maize plants. They

might also supply the plant with the defenses effectively employed by the root to counteract the adverse effects of salinity, leading to more pronounced enhancement of root than shoot. The ability of the exogenously applied natural phenolics to improve plant defense mechanisms was documented (El-Shafey and AbdElgawad, 2012; Mohamed *et al.*, 2017).

Both phenolics and salt-stress are known to impact photosynthetic pigments concentration (Hussain and Reigosa, 2011; Khodary, 2004). Due to the imbalance between the generation and detoxification, salt stress excessively generates ROS such as superoxide radicals and singlet oxygen, naturally produced in chloroplasts within the electron transfer chain of photosystem II (PSII) and photosystem I (PSI) (Foyer and Shigeoka, 2011). Thus, presence of integrative and effective enzymatic and nonenzymatic antioxidative system is necessary for maintaining efficient photosynthetic machinery. In addition to dismutases and peroxidases, plant utilizes some antioxidants as carotenoids, flavonoids, ascorbates and glutathione to quench and scavenge the excess generated singlet oxygen, superoxides and hydrogen peroxide (Agati *et al.*, 2007; Gururani *et al.*, 2015). Treating maize plants with 10 g of *C. pumilum* amendment in combination with 200 mM NaCl increased the concentration of Chl a, Chl b, carotenoids and total chlorophyll as compared with plants grown under salt-stress without amendment. Our results support those reported on syringic acid and apigenin, when applied on cowpea (Alsaadawi *et al.*, 1986) and rice (Mekawy *et al.*, 2018), respectively. The positive effect of *C. pumilum* amendment was more obvious in case of Chl b than Chl a, leading in turn to a decline in Chl a/b ratio in all samples that have been subjected to the amendment in presence or absence of salinity. The lower a/b ratio is linked with the increase in size of light-harvesting chlorophyll a/b-binding proteins associated with PSII (LHCII) antenna (Taiz and Zeiger, 2010). Enlargement of the LHCII antenna size may cause excess excitation of chlorophyll which, if not dissipated via non-photochemical quenching (NPQ), may cause excess generation and accumulation of superoxides and H_2O_2; these could cause photoinhibition and damage of PII when not scavenged effectively. It is difficult to precisely explain how amendment enhanced Chl b content, probably by decreasing conversion of Chl b into Chl a, but we believe that photoinhibition did not happen in our investigated plants grown in amended soil. The reason is that amendment alleviated the loss in carotenoids which play an essential role in photoprotection and antioxidant defense (Pessarakli, 2016) and increased their concentration over those in the leaves of nonamended plants under salt stress. Additionally, amending soil with phenolic-rich residues was reported to increase the concentration of phenolics in plant (Batish et al., 2007; Hassan et al., 2018). *Cichorium pumilum* is known to contain high concentrations of not only phenolics (Table 1), but also quinones and quinone precursors (Threlfall and Whistance, 1970). These components, when taken up, could work as antioxidants or photochemical or nonphotochemical quenchers and hence improve photosynthesis, mainly under stressful conditions (Bukhov *et al.*, 2003; Kościelniak *et al.*, 2011; Zhao and Zou, 2002). Unfortunately, the quenching of chlorophyll inflorescence was not measured in the current study. Nevertheless, exogenous phenolics were found to enhance photochemical efficiency of photosystem PII (F_v/F_m & F_m/F_0), photochemical quenching (qP) and net photosynthetic rate (P_N) and lowered NPQ leading to enhanced photosynthesis (Zhao and Zou, 2002). This role was found to be accomplished via scavenging superoxide species and protecting photosynthetic machinery from the induced oxidative damage. Similar results were reported on salicylic acid when applied to cucumber leaves under high temperature and strong light stress (Sun *et al.*, 2006), but cinnamic acid applied to *Lactuca sativa* at high concentration (1.5 mM) induced oxidative stress. It reduced plant biomass and decreased photochemical efficiency of photosystem PII as well as qP and NPQ (Hussain and Reigosa, 2011). Hence, it is important to take in consideration that the effect of the phenolic compound that would be exogenously applied to plant under stressful condition would be varied according to its structure and concentration. In their study, Bukhov *et al.* (2003) discovered that exogenous artificial quinones could work as photochemical and non-photochemical quenchers of energy in PSII, mimicking the properties of the endogenous plastoquinones that act as an electron carrier between PSII and the cytochrome b6f complex.

Concerning flavonoids, Agati *et al.* (2007) provided a strong evidence that flavonoids located in chloroplast, likely associated with its envelope, have the potential to scavenge singlet oxygen *in vivo*. The increased flavonoids content, in absence or presence of salt stress, might be linked with the amendment-mediated triggering of the biosynthesis of these compounds and/or the uptake by roots of the exogenous flavonoids released from the surrounding amended rhizosphere. The up-regulation of phenylpropanoids pathway, in response to exogenously applied phenolics, resulting in enhanced accumulation of flavonoids was discussed in literatures (El-Soud *et al.*, 2013; Hassan *et al.*, 2018). In the present work, flavonoids were found to be positively correlated (r = 0.754** and 0.787**) with both of fresh and dry weights, respectively (Table 4), while high negative correlations were scored between flavonoids and H_2O_2 (r = -0.964**) as well as LPO (r = -0.908*). These correlations indicated that the augmented flavonoids in the salt-stressed samples due to amendment was directly linked with the enhanced tolerance of maize plants, and that enhancement was accomplished by protecting membranes against the stress-induced H_2O_2 and other ROS and their subsequent LPO and membrane damage. In the same context, accumulation of flavonoids due to apigenin application was found to be related to the increased salt tolerance of rice seedlings (Mekawy *et al.*, 2018).

Table 4. Correlations among various parameters of maize plants cultivated in soil amended with (0.0 and 10 g powered dry leaves kg^{-1} soil) *C. pumilum* powdered dry leaves under different levels of salt stress (0.0, 100 and 200 mM NaCl).

Criteria	Correlation Coefficient (*r*)						
	Fresh weight	Dry weight	Flavonoids	Catalase	Peroxidase	Superoxide dismutase	Hydrogen peroxide
Dry weight	0.986**						
Flavonoids	**0.754****	**0.787****					
Catalase	**0.807***	**0.817***	0.959**				
Peroxidase	**0.830***	**0.868***	0.852*	0.920*			
Superoxide dismutase	-0.390	-0.480	-0.659*	-0.590*	-0.530		
Hydrogen peroxide	-0.921*	**-0.862***	**-0.964****	**-0.865***	**-0.590***	**0.640***	
Lipid peroxidation	-0.770**	-0.838**	**-0.908***	**-0.578***	**-0.628***	**0.711****	**0.974****

** Correlation is significant at 0.01 level. * Correlation is significant at 0.05 leve1.

Generating more ROS, such as superoxides and H_2O_2, is among the dangerous effects exerted by salinity on plant cells. Plants utilize SOD to dismutate superoxide radicals and form H_2O_2 and O_2 molecules (Parida and Das, 2005). The scavenging of H_2O_2 in cells is critical to avoid oxidative damage (Yamasaki *et al.*, 1997), as it can generate ˙OH, a highly dangerous ROS, via Fenton reaction (Gill and Tuteja, 2010). In the existing work, increasing salt stress stimulated maize SOD, while inhibited CAT and POD enzymes, and their inhibition could lead to the accumulation of H_2O_2 causing peroxidation of macromolecules and dysfunction of membranes (Parida and Das, 2005). The positive correlation (r = 0.974**) that was found between H_2O_2 and LPO and the negative one (r = -0.862*) existing between H_2O_2 and dry weight (Table 4) may confirm the harmful effect of the accumulated H_2O_2 in the stressed maize tissues. Compared with the plants grown in nonamended soil, those grown in the amended one exhibited a lower activity of SOD in absence as well as presence of NaCl, indicating lower *de novo* synthesis of SOD that was likely associated with less generation of superoxide radicals or better scavenging with other mechanisms. That situation was obviously exhibited at 200 mM NaCl in maize roots. Meanwhile, at lower salinity levels CAT and POD exhibited higher activity in response to *C. pumilum* amendment. The role of enzymatic antioxidants at lower salt stress appeared more effectively in root, linked with its more enhancement in fresh (r = 0.807* and 0.830*) and dry (r = 0.817* and 0.868*) weights (Table 4).

Since root was the main part that is close to soil, it was reasonable to exhibit a more noticeable inhibition in its dry weight by salinity alone and more alleviation of that inhibition by adding *C. pumilum* amendment than that of shoot (Figure 1e-f). The negative correlation (r = -0.659*) that was detected between flavonoids and SOD and the higher increase of root flavonoids at severe salt stress may indicate that flavonoids could have a direct role in scavenging the superoxide radicals induced under salinity. These findings are in parallel with the study of Mekawy *et al.* (2018) who found that pretreatment of rice seeds with apigenine, a flavone aglycone, enhanced seedling growth under salt stress. That enhancement was associated with the increased activities of CAT and ascorbate peroxidase (APX) in root and the accumulation of carotenoids and flavonoids in shoot. Besides flavonoids, other nonenzymatic antioxidants could be involved in alleviation of oxidative stress as a result of amending soil with *C. pumilum*. For example, organic amendment was found to alleviate salinity-induced oxidative damage in tomato via maintaining the redox states of ascorbate and glutathione, increasing ascorbate (ASC) and glutathione (GSH) concentrations and enhancing the enzymatic antioxidant defenses and photosynthetic machinery (Tartoura *et al.*, 2014). In the present study, *Cichorium pumilum* amendment might directly help via its released antioxidants, or indirectly, by motivating maize antioxidant defenses, in scavenging some of salinity-generated ROS to the level that made increasing SOD activity unrequired, and at the same time enough to trigger the *de novo* synthesis of CAT and POD enzymes. Consequently, all lead to less accumulated H_2O_2 and MDA, more stability and integrity of membranes and macromolecules in the amended samples and more tolerance to salt stress. Confirming this, positive correlations were detected between SOD (r = 0.640* and 0.711**), and negative ones between CAT (r = -0.865* and -0.578*) or POD (r = -0.590* and -0.628*) and both of H_2O_2 and MDA, respectively (Table 4). The results of the current study support the positive correlation reported previously between the ability of exogenously applied phenolics to alleviate stress-induced harmful effects on plant and the enhancement of antioxidant defenses (El-Soud *et al.*, 2013; Saleh and Madany, 2015; Wan *et al.*, 2014). In the same regard, *Corchorus olitorius* and *Urtica pilulifera* seed extract alleviated the oxidative damage induced by copper stress on tomato seed germination. The extracts lowered the levels of MDA and H_2O_2 and enhanced the activities of CAT and ascorbate peroxidase (APX) in the stressed seedlings (İşeri *et al.*, 2018).

In conclusion, amending soil with powdered dry *C. pumilum* leaves, as a way of organic farming, mitigated salt stress in maize via scavenging the evolved ROS. That scavenging came directly by the antioxidants released from the amendment or indirectly by enforcing the enzymatic antioxidant defenses or the non-enzymatic ones, particularly flavonoids, leading consequently to lower level of lipid peroxidation and enhanced productivity. However, prospective large-scale investigations are still needed to validate the application of *C. pumilum* amendment in field and to confirm its positive potential on maize grain yield.

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References

Abu-Irmaileh BE. 1982. **Weeds of Jordan (weeds of crop fields)**, Amman University, Amman, Jordan.

Agati G, Matteini P, Goti A and Tattini M. 2007. Chloroplast-located flavonoids can scavenge singlet oxygen. *New Phytol*, **174**: 77-89.

Ali S, Rizwan M, Qayyum MF, Ok YS, Ibrahim M, Riaz M, Arif MS, Hafeez F, Al-Wabel MI and Shahzad AN. 2017. Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. *Environ Sci Pollut R*, **24**: 12700-12712.

Alsaadawi IS, Al-Hadithy SM and Arif MB. 1986. Effects of three phenolic acids on chlorophyll content and ions uptake in cowpea seedlings. *J Chem Ecol*, **12**: 221-227.

Awodun M and Ojeniyi S. 1999. Use of weed mulches for improving soil fertility and maize performance. *Appl Trop Agric*, **2**: 26-30.

Awopegba M, Awodun M and Oladele S. 2016. Maize (*Zea mays*) biomass and yield as influenced by leguminous and non-leguminous mulch types in southwestern Nigeria. *Bulg J Soil Sci*, **1**: 154-169.

Batish D, Lavanya K, Singh H and Kohli R. 2007. Phenolic allelochemicals released by *Chenopodium murale* affect the growth, nodulation and macromolecule content in chickpea and pea. *Plant Growth Regul*, **51**: 119-128.

Beyer W. 1987. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Anal Biochem*, **161**: 559-566.

Boulet C, Labrousse P, Arnaud MC, Zehhar N and Fer A. 2002. Orobanche -weeds relationships: an important aspect of broomrape control. Proceedings of the meeting. Integrated control of brromrape, COST 849 Parasitic Plant Management in Sustainable Agriculture. Obermarchtal, Germany.

Bukhov NG, Sridharan G, Egorova EA and Carpentier R. 2003. Interaction of exogenous quinones with membranes of higher plant chloroplasts: modulation of quinone capacities as photochemical and non-photochemical quenchers of energy in Photosystem II during light–dark transitions. *Biochim Biophys Acta (BBA)-Bioenergetics*, **1604**: 115-123.

Chinnusamy V, Jagendorf A and Zhu J-K. 2005. Understanding and improving salt tolerance in plants. *Crop Sci*, **45**: 437-448.

Diallo MD, Duponnois R, Guisse A, Sall S, Chotte JL and Thioulouse J. 2006. Biological effects of native and exotic plant residues on plant growth, microbial biomass and N availability under controlled conditions. *Eur J Soil Biol*, **42**: 238-246.

El-Shafey N and AbdElgawad H. 2012. Luteolin, a bioactive flavone compound extracted from *Cichorium endivia* L. subsp. *divaricatum* alleviates the harmful effect of salinity on maize. *Acta Physiol Plant*, **34**: 2165-2177.

El-Soud WA, Hegab MM, AbdElgawad H, Zinta G and Asard H. 2013. Ability of ellagic acid to alleviate osmotic stress on chickpea seedlings. *Plant Physiol Biochem*, **71**: 173-183.

Falade I and Ojeniyi S. 1997. Effect of siam weed mulch on soil nutrient contents and maize performance. *Appl Trop Agric*, **2**: 100-113.

Foyer CH and Shigeoka S. 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiol*, **155**: 93-100.

Gervilla C, Rita J and Cursach J. 2019. Contaminant seeds in imported crop seed lots: a non-negligible human-mediated pathway for introduction of plant species to islands. *Weed Res*, **59**: 245-253.

Ghanaatiyan K and Sadeghi H. 2017. Differential responses of chicory ecotypes exposed to drought stress in relation to enzymatic and non-enzymatic antioxidants as well as ABA concentration. *J Hortic Sci Biotechnol*, **92**: 404-410.

Gill SS and Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem*, **48**: 909-930.

Gururani Mayank A, Venkatesh J and Tran LSP. 2015. Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Mol Plant*, **8**: 1304-1320.

Hasanuzzaman M, Nahar K and Fujita M. 2013. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmad P, Azooz M and Prasad M (Eds.), **Ecophysiology and Responses of Plants under Salt Stress**. Springer, New York, pp. 25-87.

Hassan MO, Saleh AM and AbdElgawad H. 2018. *Sonchus oleraceus* residue improves nutritive and health-promoting value of common bean (*Phaseolus vulgaris* L.): A metabolic study. *J Agr Food Chem*, **66**: 2092-2100.

Hussain MI and Reigosa MJ. 2011. A chlorophyll fluorescence analysis of photosynthetic efficiency, quantum yield and photon energy dissipation in PSII antennae of *Lactuca sativa* L. leaves exposed to cinnamic acid. *Plant Physiol Biochem*, **49**: 1290-1298.

İşeri ÖD, Körpe DA, Sahin FI and Haberal M. 2018. *Corchorus olitorius* and *Urtica pilulifera* extracts alleviate copper induced oxidative damage and genotoxicity in tomato. *Acta Biol Hung*, **69**: 300-312.

Jabeen N and Ahmed M. 2009. Possible allelopathic effects of three different weeds on germination and growth of maize (*Zea mays*) cultivars. *Pak J Bot*, **41**: 1677-1683.

Jambunathan N. 2010. Determination and detection of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage in plants. In: Sunkar R (Ed.) **Plant Stress Tolerance: Methods and Protocols**. Humana Press, Totowa, NJ, pp. 291-297.

Kar M and Mishra D. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant Physiol*, **57**: 315-319.

Kato M and Shimizu S. 1987. Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in higher pants. VII. Chlorophyll degradation in senescing tobacco leaves: Phenolic-dependent peroxidative degradation. *Can J Bot*, **65**: 729-735.

Khodary S. 2004. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. *Int J Agric Biol*, **6**: 5-8.

Kisiel W and Michalska K. 2006. Sesquiterpenoids and phenolics from roots of *Cichorium endivia* var. crispum. *Fitoterapia*, **77**: 354-7.

Kościelniak J, Ostrowska A, Biesaga-Kościelniak J, Filek W, Janeczko A, Kalaji HM and Stalmach K. 2011. The effect of zearalenone on PSII photochemical activity and growth in wheat and soybean under salt (NaCl) stress. *Acta Physiol Plant*, **33**: 2329-2338.

Li D-M, Nie Y-X, Zhang J, Yin J-S, Li Q, Wang X-J and Bai J-G. 2013. Ferulic acid pretreatment enhances dehydration-stress tolerance of cucumber seedlings. *Biol Plant*, **57**: 711-717.

Liang W, Ma X, Wan P and Liu L. 2018. Plant salt-tolerance mechanism: A review. *Biochem Biophys Res Commun*, **495**: 286-291.

Lichtenthaler HK. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In: **Methods in enzymology**. Academic Press, pp. 350-382.

Lim SL, Wu TY, Lim PN and Shak KPY. 2015. The use of vermicompost in organic farming: overview, effects on soil and economics. *J Sci Food Agric*, **95**: 1143-1156.

Mekawy AMM, Abdelaziz MN and Ueda A. 2018. Apigenin pretreatment enhances growth and salinity tolerance of rice seedlings. *Plant Physiol Biochem*, **130**: 94-104.

Mohamed MSM, Saleh AM, Abdel-Farid IB and El-Naggar SA. 2017. Growth, hydrolases and ultrastructure of *Fusarium oxysporum* as affected by phenolic rich extracts from several xerophytic plants. *Pestic Biochem Physiol*, **141**: 57-64.

Padel S and Lampkin N. 1994. **The Economics of Organic Farming: An International Perspective**, CAB International.

Parida AK and Das AB. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol Environ Saf*, **60**: 324-349.

Peigné J, Casagrande M, Payet V, David C, Sans FX, Blanco-Moreno JM, Cooper J, Gascoyne K, Antichi D, Bàrberi P, Bigongiali F, Surböck A, Kranzler A, Beeckman A, Willekens K, Luik A, Matt D, Grosse M, Heß J, Clerc M, Dierauer H and Mäder P. 2016. How organic farmers practice conservation agriculture in Europe. *Renew Agr Food Syst*, **31**: 72-85.

Pessarakli M. 2016. (Ed.) **Handbook of Photosynthesis**, CRC press, Boca Raton.

Qasem J. 1992. Nutrient accumulation by weeds and their associated vegetable crops. *J Hortic Sci*, **67**: 189-195.

Roy S, Arunachalam K, Dutta BK and Arunachalam A. 2010. Effect of organic amendments of soil on growth and productivity of three common crops viz. *Zea mays*, *Phaseolus vulgaris* and *Abelmoschus esculentus*. *Appl Soil Ecol*, **45**: 78-84.

Sahan Y, Gurbuz O, Guldas M, Degirmencioglu N and Begenirbas A. 2017. Phenolics, antioxidant capacity and bioaccessibility of chicory varieties (*Cichorium spp*.) grown in Turkey. *Food Chem*, **217**: 483-489.

Saleh AM and Madany MMY. 2015. Coumarin pretreatment alleviates salinity stress in wheat seedlings. *Plant Physiol Biochem*, **88**: 27-35.

Sayed M, Khodary SEA, Ahmed ES, Hammouda O, Hassan HM and El-Shafey NM. Elicitation of flavonoids by chitosan and salicylic acid in callus of *Rumex vesicarius* L. *Acta Horticulturae*, **1187**: 165-176.

Sun Y, Xu W and Fan A. 2006. Effects of salicylic acid on chlorophyll fluorescence and xanthophyll cycle in cucumber leaves under high temperature and strong light. *Ying yong sheng tai xue bao= The Journal of Applied Ecology*, **17**: 399-402.

Taiz L and Zeiger E. 2010. **Plant Physiology**, 5th ed. Sinauer Associates, Sunderland, Massachusetts U.S.A.

Tartoura KAH, Youssef SA and Tartoura E-SAA. 2014. Compost alleviates the negative effects of salinity via up-regulation of antioxidants in *Solanum lycopersicum* L. plants. *Plant Growth Regul*, **74**: 299-310.

Threlfall D and Whistance G. 1970. Biosynthesis of ubiquinone—a search for polyprenyl phenol and quinone precursors. *Phytochemistry*, **9**: 355-359.

Velikova V, Yordanov I and Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci*, **151**: 59-66.

Wan Y-Y, Chen S-Y, Huang Y-W, Li X, Zhang Y, Wang X-J and Bai J-G. 2014. Caffeic acid pretreatment enhances dehydration tolerance in cucumber seedlings by increasing antioxidant enzyme activity and proline and soluble sugar contents. *Sci Hortic*, **173**: 54-64.

Yamasaki H, Sakihama Y and Ikehara N. 1997. Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H_2O_2. *Plant Physiol*, **115**: 1405-1412.

Zhao H and Zou Q. 2002. Protective effects of exogenous antioxidants and phenolic compounds on photosynthesis of wheat leaves under high irradiance and oxidative stress. *Photosynthetica*, **40**: 523-527.

Zhishen J, Mengcheng T and Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*, **64**: 555-559.