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ARBUSCULAR MYCORRHIZAL FUNGI ENHANCE BASIL TOLERANCE TO SALT STRESS THROUGH IMPROVED PHYSIOLOGICAL AND NUTRITIONAL STATUS

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Abstract

Pot experiments were conducted to evaluate the influence of salinity on some physio-biochemical traits in sweet basil (*Ocimum basilicum* L.) cultivars with contrasting salt stress tolerance and to determine the role of arbuscular mycorrhizal fungi (AMF) in ameliorating the salt stress in plant. Salt stress (250 mM NaCl) reduced the colonization potential of AMF and inhibited photosynthetic pigments, chlorophyll and carotenoids in plant tissue. AMF inoculated plants contained higher level of cholorphyll pigments. Salt stressed plants showed increased lipid peroxidation, antioxidant enzyme activities like superoxide dismutase (SOD), ascorbate peroxidase (APX) and peroxidase (POD). Plants inoculated with AMF showed lower lipid peroxidation and enhanced antioxidant enzyme activities. Moreover, the content of lipids, proline, and soluble sugars in basil plants was improved with AMF inoculation. AMF inoculation reduced accumulation of Na^+ and improved nutrient acquisition. In conclusion, AMF were capable to reduce oxidative stress via supporting of the antioxidant system. Salt tolerant cultivar showed higher antioxidant enzyme activity and accumulation of osmolytes.

Key words: Arbuscular mycorrhizal fungi; Salinity; *Ocimum basilicum*; Defense system.

Introduction

The various environmental factors like salinity and water stress have hostile impact on plant growth (Alqarawi *et al.,* 2014a; Abd_Allah *et al.,* 2015a; Hashem *et al.*, 2015). The plants have evolved many tolerance mechanisms to cope with adverse conditions so that physiology and growth processes remain maintained. Sequestering the toxic ions into less susceptible organelles or spaces has an immense role in imparting salt tolerance (Wu *et al.,* 2014; Ahanger *et al*., 2014). High levels of salinity cause damage to important structures like membranes, proteins, lipids and nucleic acids. Peroxidation of membrane lipids results in the leakage of cellular constituents and hence curtails growth (Abd_Allah *et al*., 2015a,b; Alqarawi *et al*., 2014a,b). One of the important reasons for this is enhanced production of toxic free radicals like superoxide, hydrogen peroxide, peroxides etc. Increased production of radicals alters normal growth by initiating chain of radical producing reactions resulting in production of peroxyl radicals that ultimately damage cellular functioning (Unal *et al*., 2014; Wu *et al*., 2014). Cells have antioxidant defence system to maintain the levels of free radicals within the normal range. Antioxidant may be enzymatic as well as non enzymatic (Wu *et al*., 2014; Ahanger *et al*., 2015). In addition, an increased accumulation of osmolytes also contribute for better adaptations to stressed conditions through favoring the maintenance of tissue water potential (Tang *et al*., 2009; Masood *et al*., 2013). Alqarawi *et al*. (2014a) have reported reduced growth, increased free radical production and upregulation of antioxidant system in *Ephedra aphylla* due to salinity stress.

Besides having inherent mechanisms, plant tolerance can be enhanced by using Arbuscular mycorrhizal fungi (AMF) that have mutualistic relationships with many plants and have the potential to improve plant growth under normal as well as stressed environmental conditions (Alqarawi *et al*., 2014a; Hashem *et al*., 2015; Abd_Allah *et al.,* 2015a,b). AMF act as bio-ameliorators of stress and help plants in alleviating stress induced damage (Wu *et al.,* 2014; Hameed *et al.,* 2014). Plants colonized with AMF have been reported to show enhanced growth and vigour (Ahanger *et al.,* 2014; Abd_Allah *et al.,* 2015a). Morphological, nutritional and physiological changes induced in plants colonized with AMF contribute to their enhanced resistance to abiotic stresses. In addition, AMF modifies root architecture for ensuring the increased water uptake (Hashem *et al*., 2014a; Alqarawi *et al*., 2014a; Abd_Allah *et al*., 2015a).

Ocimum basilicum, a culinary herb commonly called as basil or sweet basil is an important medicinal plant of the family Lamiaceae. Sweet basil has been widely known for its several therapeutic uses like lowering blood pressure, blood glucose level, cholesterol level, anti-inflammatory and antistress etc (Tomar *et al*., 2010; Tewar *et al*., 2012). Chemical constituents present within the different *Ocimum* species determine their flavor and smell. In this study we investigated the impact of salt stress on some physiological and biochemical attributes in sweet basil (*Ocimum basilicum* L.) cultivars with contrasting salt tolerance and determined the role of arbuscular mycorrhizal fungi (AMF) in ameliorating the salt stress in plant.

Materials and Methods

Plant inoculation: The seeds of basil (*Ocimum basilicum* L. cv. cinnamon and thyrsiflorum) were kindly provided by ARC, Giza, Egypt. The seeds were germinated on blotter after surface disinfection using NaOCl (5 %, v/v for three min). Hoagland's full strength solution supplemented with NaCl (250 mM/ L) was used for irrigation. Experiments were carried using sandy soil collected from Derab Agricultural Research Station, Riyadh, Saudi Arabia with following properties (%): sand (73.6); moisture content, 4.15; organic carbon, 0.14; total nitrogen, 0.006; (EC) = 7.12 dS m^{-1}; and pH 7.8. The soil was autoclaved for 3 h at 121°C, cooled and then divided among plastic pots (1 kg capacity). The mycorrhizal fungi (*F. mosseae* (syn. *G. mosseae*); *R. intraradices* (syn. *G. intraradices*) and *C. etunicatum* (syn. *G. etunicatum*) used in the present study were provided kindly by Dr. Abeer Hashem (Hashem *et al*., 2014a). Fungal inoculum potential was determined by the most probable numbers method (Alexander, 1982), and each trap culture contained 10.2 x 10^3 propagules per pot. Fungal inoculums consisted of spores, hyphae and colonized root fragments with mycorrhizal fungi. The mycorrhizal inoculum was added to the experimental soil as 10 g of trap soil culture (approx. 100 spores/g trap soil, M = 80%)/ pot (1Kg). Non-mycorrhizal soil was used as reference.

Pot experiment: The germinated seeds were sown into pots and pots were arranged in complete randomized block design with five replications. The plants were grown in greenhouse at 25°C under 12 h light (750 µmol m^{-2} S^{-1}) and 12 h dark photo-cycle, and RH of 72-75% for ten weeks. After the plants were uprooted, the soil was carefully washed off the roots with water carefully, thereafter were separated into shoots and roots. The samples were dried at 70°C for 48 h for estimation of dry weight, crude fiber, total lipids, sugars and mineral elements. Photosynthetic pigments, malondialdehyde contents, proline and enzymes activities were estimated in fresh tissue. The fresh root samples were used for mycorrhizal studies.

Determination of arbuscular mycorrhizal colonization: Mycorrhizal spores were extracted from the experimental soil of each treatment by wet sieving and decanting procedure described by Daniels & Skipper (1982) and modified by Utobo *et al*. (2011). Plant roots were washed carefully in ice-cold water (4°C) to remove the soil and were cleaned with KOH (10%) and thereafter stained with trypan blue in lactophenol as described by Phillips & Hayman (1970). Stained root segments were examined under light microscope at 400x magnification. Degree of fungal infection (mycelium, vesicles and arbuscules) and development within the infected regions of the roots were calculated according to the following formula:

$$\% \text{ Colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of segments studied}} \times 100$$

Nutritional values: Nutritional values (total lipids, crude protein, ash content, moisture content, total carbohydrates and fiber content) of shoot system was estimated according to Anon. (1995).

Determination of photosynthetic pigments: Leaves of tomato plants were extracted in dimethyl sulfoxide (DMSO) as described by Hiscox & Israelstam (1979) and absorbance of homogenate was determined spectrophotometrically at 480, 510, 645, 663 nm (T80 UV/VIS Spectrometer, PG Instruments Ltd, USA), DMSO was used as blank.

Determination of proline: Proline was estimated following the method given in Sadasivam & Manickam (1996). Briefly, 0.5g plant sample was extracted in 3% (w/v) aqueous sulphosalicylic acid. After centrifugation at 3000 xg for 20 min supernatant was collected and mixed with acetic acid and ninhydrin. Reaction was initiated by heating the mixture for 1 hour and thereafter proline was separated using toluene and absorbance was read on spectrophotometer at 520 nm using toluene as blank.

Determination of malondialdehyde: Fresh leaves were extracted in 1% trichloroacetic acid (TCA) and the homogenate was centrifuged at 10,000 rpm for 5 minutes. 1.0 ml of supernatant was taken and 4.0 ml of 0.5% (w/v) thiobarbituric acid (TBA) was added and heated at 95°C for 30 min. After that samples were cooled on ice bath and were again centrifuged at 5000 rpm for 5 min for clarification. Absorbance of the supernatant was taken at 532 and 600 nm (Heath & Packer, 1968). Calculation of MDA was done using following equation:

MDA equivalents (µg/ g fresh wt) = 1000[(Abs $_{523}$- Abs $_{600}$nm)]/155

Assay of antioxidant enzymes: Fresh plant tissue was extracted in phosphate buffer (0.1 M, pH 7.5) containing EDTA (0.5 mM) and ascorbic acid (1 mM) [for APX]. SOD activity was assayed following the method of Dhindsa *et al*. (1981). One unit of SOD activity was considered equivalent to amount of enzyme causing 50 % nitro blue tetrazolium chloride (NBT) photoreduction (Singh *et al*., 2015). Ascorbate peroxidase (APX) was assayed by observing the change in ascorbate at 290nm (Nakano & Asada, 1981). The reaction mixture contained potassium phosphate buffer (50 mM, pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 0.1 mM H_2O_2 and 0.1 ml enzyme. Guaiacol peroxidase was assayed by measuring the formation of tetraguaiacol from guaiacol at 470 nm in the presence of H_2O_2 as described by Castillo *et al*., (1984) and Rao *et al*. (1996). Activity of each enzyme was expressed as EU mg^{-1} protein.

Estimation of ions: The dry weight of leaf samples was acid digested and sodium, potassium, phosphorus, magnesium and calcium were estimated according to the method of Wolf (1982) using Jenway Flame Photometer, Bibby Scientific Ltd-Stone-Staffs-St15 0SA–UK. Phosphorus (P^{3+}) was extracted by nitric-perchloric acid digestion and measured using the vanado-molybdophosphoric colorimetric method.

Statistical analysis: Data was subjected to Duncan's Multiple Range Test was performed using one way ANOVA for a completely randomized design by SPSS-21 software and the differences in means were determined by the least significant differences (LSD) (p=0.05) test.

Results

The structural colonization of AMF in roots of sweet basil (*Ocymum basilicum*) is shown in Fig. 1 (A-F). AMF colonized the roots as different structural forms as mycelium (1A), spores (1B, C), vesicles (1D), vesicles and subtending hyphae (1E) and trunk and arbuscules (1F). In our results salt tolerant cultivar maintain relatively higher number of spores, mycelium, vesicles and arbuscules. Percent reduction in number of spores, mycelium, vesicles and arbuscules in susceptible cultivar was 91.7%, 68.5%, 75.08% and 50.7% respectively as compared to tolerant cultivar that showed 49.14%, 25.18%, 15.3% and 21.05% reduction (Table 1).

The content of chlorophyll a, b and total cholorphyll was reduced by 33.5%, 47.13% and 37.5% in susceptible cultivar, whereas it was increased by 16.6%, 51.4% and 26.6% in tolerant cultivar under salinity, respectively. In tolerant cultivar, AMF caused 29.3%, 18.5% and 12.2% increase in chlorophyll a, b and total cholorphyll while as in susceptible cultivar their content increased by 8.9%, 13.7% and 10.6% under non saline condition, respectively (Table 2). The total carotenoid content increased by 54.8% in tolerant cultivar, and reduced by 57.1% in susceptible cultivar due to salinity (Table 2). AMF inoculation increased carotenoid content by 23.07% and 30.9% in tolerant and susceptible cultivar, respectively.

Effects of salinity and AMF on proline content are shown in Fig. 2A. AMF increased proline content by 60.2% and 11.7% in tolerant and susceptible cultivar under non saline condition. Proline accumulation was higher in tolerant cultivar as compared with susceptible cultivars. The increase in proline content due to salinity stress was 338.9% and 169.4% for tolerant and susceptible cultivar, respectively (Fig. 2A). AMF inoculation reduced lipid peroxidation, measured in terms of MDA content in both cultivars (Fig. 2B). Salinity caused considerable increase in MDA content by 72.7% and 106.7% in tolerant and susceptible cultivar, respectively (Fig. 2B). In contrast salinity stressed AMF inoculated plants showed only 14.4% and 29.5% increase in MDA accumulation (Fig. 2B).

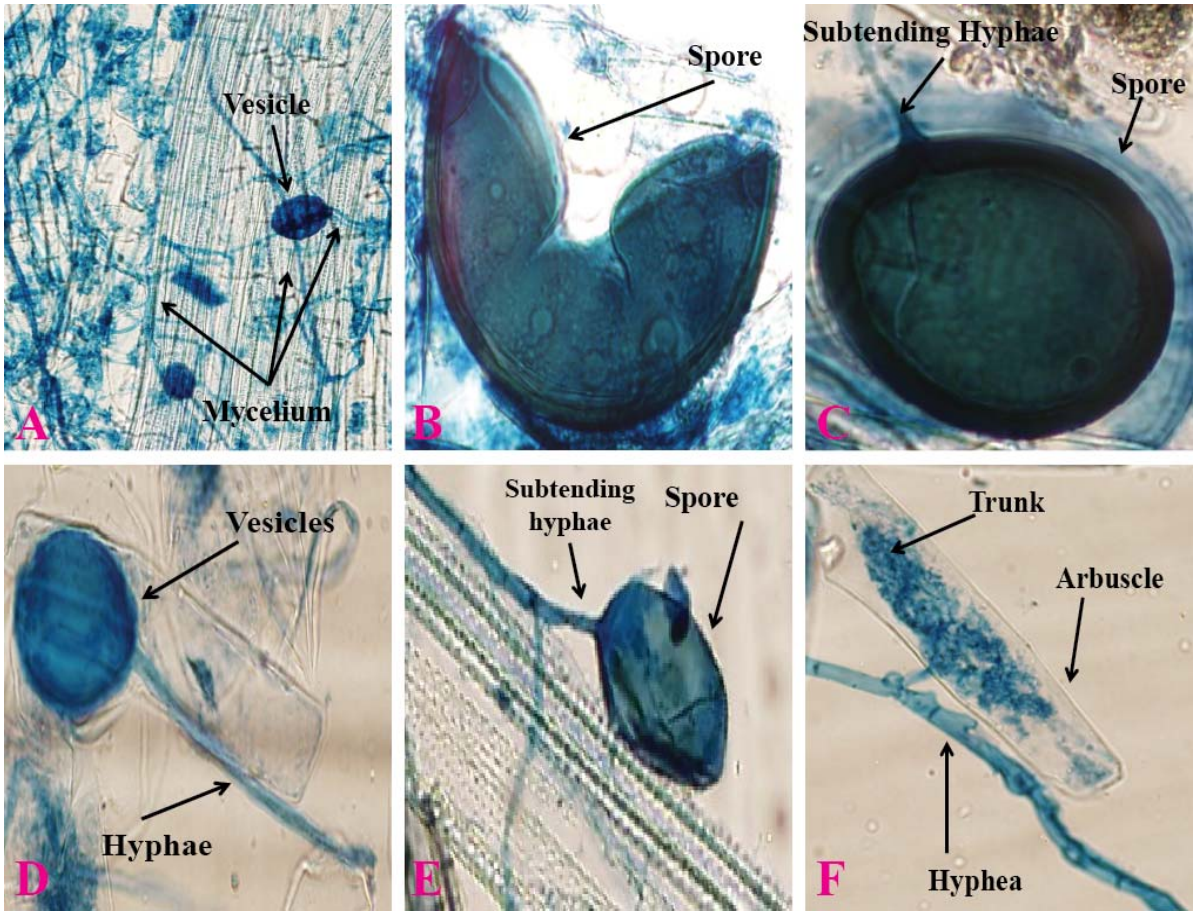

Fig. 1. A-F: The structural colonization of AMF in roots of sweet basil (*Ocymum basilicum*). [A]: vesicle and mycelium occurrence of the AMF-colonized root sections (arrow), [B]: Spore morphology of AMF (arrow), [C]: Intact mycorrhizal spores and subtending hyphae (arrow), [D]: vesicles and subtending hyphae of AMF (arrow), [E]: vesicles and subtending hyphae (arrow), [F]: Trunk, arbuscle and hyphae of AMF (arrow).

Table 1. Effect of salinity (250 mM NaCl) on mycorrhizal status (total spores; mycelium; vesicles and arbuscules) of sweet basil (*Ocymum basilicum*) cultivars. Data presented are the means ± SE (n = 5).

Treatments		Mycorrhizal status			
		Total spores (Spores/ 100 g soil)	Mycelium (%)	Vesicles (%)	Arbuscules (%)
AMF	Tolerant	136.3	79.8	46.8	73.9
	Susceptible	334.3	48.2	35.7	20.1
Salinity	Tolerant	69.3	59.7	39.6	58.3
	Susceptible	27.6	15.2	8.9	9.9
LSD at: 0.05		32.4	8.1	3.7	8.2

AMF: Arbuscular mycorrhizal fungi

Table 2. Effect of salinity (250 mM NaCl) in presence and absence of AMF on chlorophyll pigments (chlorophyll a, chlorophyll b and total chlorophylls) and carotenoid content (mg/g fresh weight) of sweet basil (*Ocymum basilicum*) cultivars. Data presented are the means ± SE (n = 5).

Treatments	Photosynthetic pigment (mg/ g fresh weight)									
	Chlorophyll a		Chlorophyll b		Chl a/ Chl b		Carotenoid		Total pigments	
	T	S	T	S	T	S	T	S	T	S
Control	1.41	1.18	0.45	0.35	3.09	3.30	0.10	0.064	1.97	1.60
AMF	1.54	1.28	0.54	0.40	2.86	3.15	0.12	0.084	2.21	1.77
250 mM NaCl	1.64	0.78	0.69	0.18	2.38	4.15	0.16	0.027	2.50	1.00
250 mM NaCl + AMF	1.76	0.96	0.85	0.29	2.06	3.31	0.19	0.049	2.81	1.30
LSD at: 0.05	0.11	0.09	0.07	0.02	0.14	0.02	0.01	0.01	0.15	0.08

T: Tolerant; S: Susceptible; AMF: Arbuscular mycorrhizal fungi

Salinity stress resulted in increased activity of antioxidant enzymes studied (Figs. 3A, B, C). In stressed plants, guaiacol peroxidase (GPX), superoxide dismutase (SOD) and ascorbate peroxidase (APX) showed an enhancement of 76.1%, 65.5% and 87.4% in tolerant cultivar while as susceptible cultivar showed only 106.1%, 97.6% and 108.8% increase in activity of GPX, SOD and APX respectively (Figs. 3A, B, C). Percent increase in GPX, SOD and APX due to AMF inoculation was 14.5%, 15.9% and 29.02% in tolerant and 11.6%, 8.4% and 35.95% in susceptible cultivar, respectively. In AMF inoculated stressed plants, percent increase in activities of GPX, SOD, APX was 47.5%, 34.1%, 53.3% and 70.8%, 68%, 69.3% in tolerant and susceptible cultivars, respectively (Figs. 3A, B, C).

The results on the sugar content affected by salinity and AMF inoculation are shown in Table 3. Total soluble sugars increased by 72.8% and 115.2% in tolerant and susceptible cultivars due to salt stress, however, AMF inoculated salt stressed plants showed only 4.4% and 75.2% increase in tolerant and susceptible cultivars (Table 3). The sugar content increased in AMF inoculated plants by 53.4% and 6.8% in tolerant and susceptible respectively (Table 3).

Salt stress resulted in reduced uptake of K, Ca, Mg, and P while an increase of Na considerably (Table 4). In tolerant cultivar reduction in K, Ca, Mg and P was 42.03%, 23.8%, 34.23% and 18.2% while as in susceptible cultivar 38.4%, 35.7%, 46.5% and 47.4% respectively (Table 4). AMF inoculation increased K, Ca, Mg, and P content by 34.8%, 26.0%, 20.0%, 99.0% in tolerant and 24.01%, 7.57%, 20.3% 36.3% in susceptible cultivar, respectively (Table 4). AMF inoculation reduced sodium content by 12.9% and 8.2% in tolerant and susceptible genotypes respectively (Table 4).

Salinity stressed caused an increase in fiber by 13.8%, 23.7%, ash content 10.3%, 7.02% in tolerant and susceptible genotype respectively (Table 5). Slight reduction of 10.3% and 7.02% was observed due to AMF inoculation in tolerant and susceptible cultivar, respectively (Table 5). The salt stress also reduced lipid content by 32.8% and 61.01%, whereas AMF inoculation increased its content by 150.3% and 29.2% in tolerant and susceptible cultivars, respectively (Table 5).

Discussion

Previous studies demonstrated that salt stress decrease the colonization of AMF in roots of *Sesbania sesban* (Abd_Allah *et al.,* 2015a) and *Panicum turgidum* (Hashem *et al.,* 2015). Similar results were observed in our study whereas the salt stress inhibited the number of mycelium, arbuscules and vesicles of AMF. In *Vicia faba*, Hashem *et al.* (2014a) has demonstrated that salinity reduces growth of mycelium, arbuscules and vesicles within the host plants resulting in perturbed growth. Salinity stress causes considerable reduction in photosynthetic pigments resulting in reduced photo-assimilation and hence reduced growth. Reduction in chlorophyll due salinity may be because of reduced uptake of magnesium that forms central part of chlorophyll pigments (Abd_Allah *et al.,* 2015a,b). Salinity reduces synthesis of cholorphyll proteins and also causes interruptions in the *de novo* synthesis of chlorophyll molecules. Hakim *et al.* (2014) has reported that irrigating saline waters to rice reduces cholorphyll contents and thereby causing drastic decline in growth. Moreover salinity stress hampers functioning of oxygen evolving complex resulting in altered metabolism. However, AMF inoculation not only enhanced cholorphyll pigments but also ameliorated the deleterious impacts of salinity on cholorphyll content to some extent. Our results of enhanced chlorophyll contents due to AMF inoculation and subsequent allaying of salinity induced negative effects support the results of Alqarawi *et al.* (2014a) for *Ephedra aphylla*, Hashem *et al*. (2014a) for *Vicia faba* and Abd_Allah *et al*. (2015a) for *Sesbania sesban*. AMF enhances chlorophyll because AMF inoculated plants maintain higher contents of magnesium resulting in greater synthesis of cholorphyll.

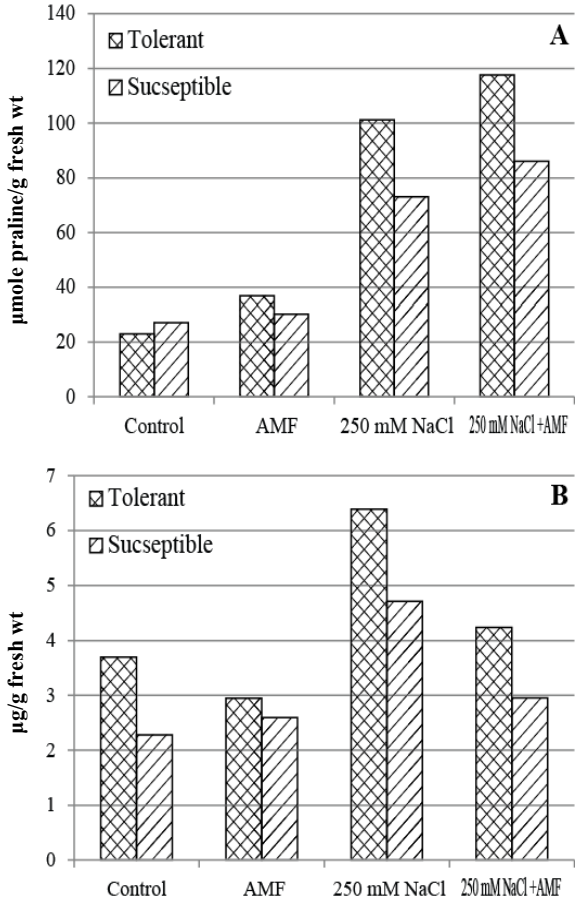

Fig. 2. A-B: Effect of salinity (250 mM NaCl) in presence and absence of AM fungi on [A] proline (µmole proline/ g fresh wt) and [B] malonaldehyde content (µg/ g fresh wt) in sweet basil (*Ocymum basilicum*) cultivars. Data presented are the means ± SE (n = 5).

The higher rate of lipid peroxidation was observed in salt stressed plants. Peroxidation of membrane lipids is one of the prominent effects of oxidative damage caused due to stress exposure. Our results of increased MDA production support our earlier results in *Ephedra aphylla* (Alqarawi *et al*., 2014a), *Ephedra alata* (Alqarawi *et al*., 2014b), *Vicia faba* (Hasheem *et al.,* 2014a), *Panicum turgidum* (Hasheem *et al.,* 2015) and *Sesbania sesban* (Abd_Allah *et al.,* 2015a). The AMF inoculated plants showed reduced production of MDA. Recently in salt stressed *Sesbania sesban*, Abd_Allah *et al*. (2015a) have demonstrated the ameliorative role of AMF in reducing the salinity triggered lipid peroxidation. Decreased lipid peroxidation in AMF inoculated plants may be because of the quick scavenging of toxic free radicals mediated by increased activities of antioxidant enzymes as well as increased production of non enzymatic antioxidants (Wu *et al.,* 2014; Abd_Allah *et al*., 2015a, c).

Salinity stressed plants showed increased accumulation of proline and AMF inoculation further enhanced the proline content. Proline accumulation in plants is considered as an important tolerance strategy. Our results of enhanced proline accumulation in salt stressed plants corroborate with the results of Benhassaini *et al.* (2012) for *Pistacia atlantica*, Unal *et al.* (2014) for barley and Abd_Allah *et al.* (2015a) for *Sesbania sesban*. Under stressed conditions proline accumulation results from the cumulative effects on the proline metabolism as well as gene expression and amino acid degradation. Plants subjected to stressed conditions show higher activity of proline synthesizing enzymes while as the activity of catabolizing enzymes is down regulated (Iqbal *et al.*, 2015; Khan *et al.*, 2015). This enzyme regulated proline metabolism results in enhanced accumulation of proline and hence imparts stress tolerance in plants. Increase in proline accumulation due to AMF inoculation may be because of its direct impact on the metabolizing machinery. Our results of increased accumulation of proline in AMF colonized plants support the findings of Abd_Allah *et al.* (2015a) for *Sesbania sesban* and Hashem *et al.* (2015) for *Panicum turgidum*. Proline is believed to protect enzyme activity and also act as an antioxidant for scavenging free radicals. Improved accumulation of proline due to AMF inoculation results in enhanced uptake of water from the soil solution by maintaining the tissue water potential less than the surrounding soil. AMF has direct effect on the root morphology and also alters both physiology and biochemistry of the host plants (Ahanger *et al.,* 2014; Wu *et al.,* 2014; Abd_Allah *et al.,* 2015b). In addition to this total soluble sugars increased considerably in salt stressed plants. AMF inoculation also enhanced the total soluble sugar content providing further strength to plant cells in maintaining the turgor dependent processes. Salt stress triggered enhanced accumulation of total soluble sugars in our present study are in confirmation with the results of Mahboobeh & Akbar (2013) for *Nicotiana plumbaginifolia* and Masood *et al.* (2013) for mustard. Plant species that have the ability to accumulate the exopolysaccharides, that play an important role in plant stress tolerance (Kawakam *et al.,* 2008; Ahanger *et al.,* 2015). Increased accumulation of carbohydrates, proline and other osmolytes impart tolerance to higher salt concentrations leading to protection of the major metabolic pathways (Hoseini, 2010). Accumulation of osmolytes protect membrane structures and reduce the damage caused by ROS by restricting their formation (Alqarawi *et al.,* 2014a, b; Ahanger *et al.,* 2015; Abd_Allah *et al.,* 2015a).

In the present study, salinity stress triggered enhancement in activities of SOD, APX and GPX corroborate with the findings of Alqarawi *et al.* (2014a), Hashem *et al.* (2014a), Hashem *et al*. (2015) and Abd_Allah *et al.* (2015a) for *Ephedra aphylla*, *Vicia faba*, *Panicum turgidum* and *Sesbania sesban*, respectively. However tolerant species maintained higher activities of SOD, APX and GPX as compared to susceptible one. In *Cicer arietinum*, Rasool *et al.* (2013) has also demonstrated that tolerant cultivars showed higher activity and expression levels of antioxidant enzymes resulting in improved salt tolerance. Inoculation of AMF not only enhanced the activities of antioxidant enzymes studied but also caused a substantial increase in plants subjected to salinity. Our results are in confirmation with the results of Tang *et al.* (2009) for *Zea mays*, Abdel Latef & Chaoxing

(2011) for tomato and Hashem *et al.* (2015) for *Panicum turgidum*. Recently Abd_Allah *et al.* (2015a) has observed that AMF inoculation to salt stressed *Sesbania sesban* resulted in better growth adaptation by inducing activities of key antioxidant enzymes. SOD, the important free radical scavenging enzyme, catalyses conversion of superoxide radicals into peroxide which is further scavenged by either APX in ascorbate-glutathione pathway (Hashem *et al.,* 2014b). Enhancement in the activities of SOD and APX in AMF inoculated plants demonstrate an ameliorative role of AMF in increasing salt tolerance of *Ocimum basilicum*. Enhanced activity of APX reduces chances of the formation of most toxic hydroxyl radical thereby reducing the chances of membrane disorganization and hence results in maintaining their integrity. Increased expression and activity of APX mediates efficient removal of free radicals and contributes to maintain the efficient electron transport (Rasool *et al.,* 2013; Acosta-Motosa *et al.,* 2015).

Exposure of *Ocimum basilicum* to salt stress reduced uptake and accumulation of essential mineral elements like potassium, phosphorous, magnesium and calcium while as increased sodium content resulting in reduced growth. Salinity mediated reduction in the essential elements in our results are in confirmation with the findings of Garg & Manchanda (2009) for *Cajanus cajan*, Kohler *et al*. (2009) for lettuce, Hashem *et al*. (2014b) for *Ochradenus baccatus* and Abd_Allah *et al*. (2015b) for *Phaseolus vulgaris*. Higher sodium shows antagonistic behavior towards essential elements like potassium at the membrane level.AMF inoculation enhanced the uptake and accumulation of essential elements analysed and also caused substantial reduction in uptake of sodium ions in both sensitive and tolerant cultivars. Improved and balanced uptakes of mineral elements mediate well regulated and maintained growth through their evident involvement in several physiological and biochemical processes like enzyme activation (Ahanger *et al.,* 2014, 2015). Earlier in *Phaseolus vulgaris,* Abd_Allah *et al*. (2015b) have also demonstrated enhanced growth in AMF colonized plants through its direct positive influence on the uptake of mineral elements. Increased magnesium content in AMF inoculated plants has positive influence on the synthesis of chlorophyll pigments resulting in enhanced synthesis of photoassimilates and hence leads to growth improvement. On the other hand reduced uptake of sodium and increased uptake of potassium in AMF inoculated plants enhance K/Na ratio significantly, an important strategy of stress tolerance (Abd_Allah *et al.,* 2015b; Hashem *et al.,* 2015). Increased accumulation of potassium improves plant growth by activating the antioxidant system resulting in quick scavenging of toxic ROS (Ahanger *et al.,* 2015) while as calcium besides bringing modulations in antioxidant enzyme activities also acts as an important signaling component for sensing the environmental cues (Xu *et al.,* 2014; Ahmad *et al.,* 2015). Improved uptake of calcium positively influences activity of nitrogen metabolizing enzymes and thereby causing an enhancement in nitrogen assimilation and increased production of amino acids and proteins (Xu *et al.,* 2014).

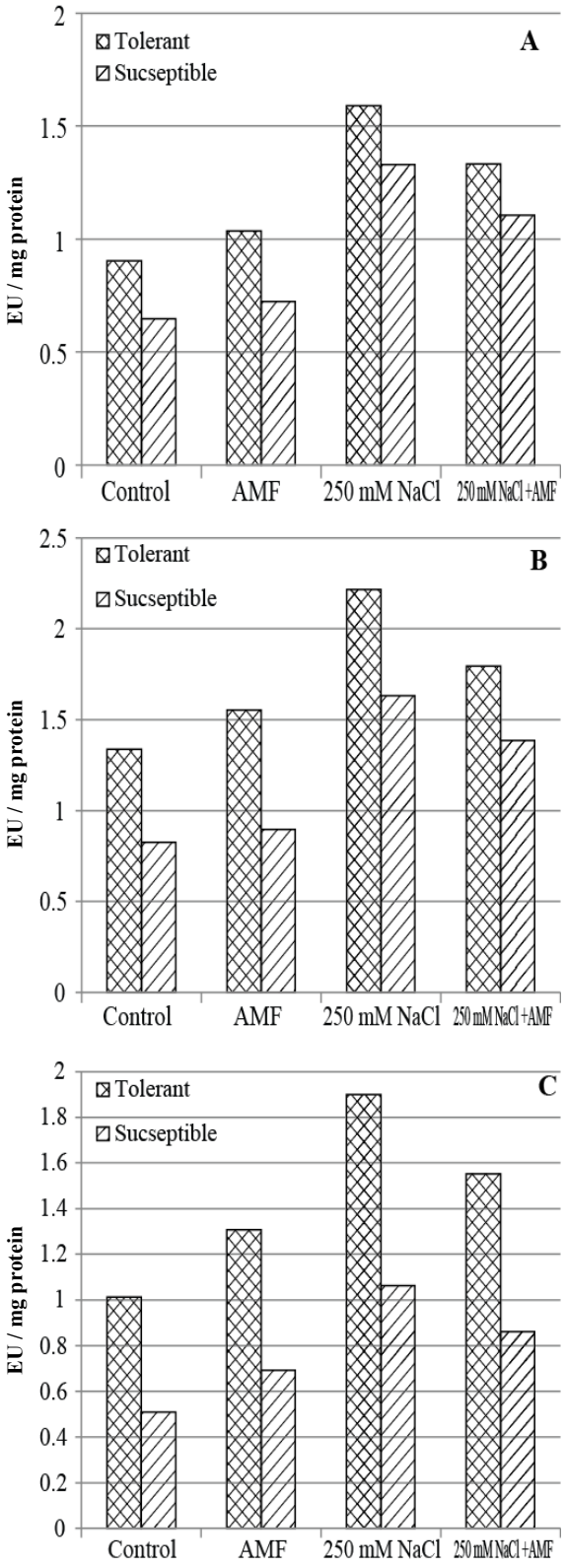

Fig. 3. A-C: Effect of salinity (250mM) in presence and absence of AM fungi on [A] guaiacol peroxidase (GPX), [B] superoxide dismutase (SOD) and [C] ascorbate peroxidase (APX) in sweet basil (*Ocymum basilicum*) cultivars. Data presented are the means ± SE (n = 5).

Table 3. Effect of salinity (250 mM NaCl) in presence and absence of AMF on soluble sugars (monosaccharides, disaccharides), polysaccharides and total sugars in sweet basil (*Ocymum basilicum*) cultivars. Data presented are the means ± SE (n = 5).

Treatments	Sugars fractions (mg/ g dry weight)						Polysaccharide		Total sugars	
	Monosaccharide		Disaccharides		Total soluble sugars					
	T	S	T	S	T	S	T	S	T	S
Control	2.7	2.7	1.7	1.3	4.4	4.0	42.4	27.8	46.9	31.9
AMF	2.1	2.6	1.9	1.5	4.0	4.1	68.0	29.8	72.0	34.0
250 mM NaCl	6.5	7.9	1.1	0.8	7.7	8.8	29.9	16.9	37.6	25.7
250 mM NaCl + AMF	3.0	5.9	1.5	1.2	4.6	7.1	39.3	21.8	43.9	29.0
LSD at: 0.05	0.27	0.06	0.7	0.05	0.12	0.03	2.14	1.08	2.07	1.27

T: Tolerant; S: Susceptible; AMF: Arbuscular mycorrhizal fungi

Table 4. Effect of salinity (250 mM NaCl) in presence and absence of AMF on elements accumulation (mg/ g dry weight) of sodium, potassium, calcium, magnesium and phosphorous in sweet basil (*Ocymum basilicum*) cultivars. Data presented are the means ± SE (n = 5).

Treatments	Elements accumulation (mg/ g dry weight)									
	Sodium		Potassium		Calcium		Magnesium		Phosphorus	
	T	S	T	S	T	S	T	S	T	S
Control	12.3	8.6	18.2	11.9	21.1	17.0	1.7	1.19	2.2	0.9
AMF	10.7	7.9	24.5	14.8	26.7	18.3	2.0	1.43	4.4	1.2
250 mM NaCl	15.2	11.6	10.5	7.3	16.1	10.9	1.1	0.63	1.8	0.4
250 mM NaCl + AMF	13.1	11.0	17.2	10.1	20.2	13.3	1.5	0.90	1.9	0.6
LSD at: 0.05	0.63	0.42	0.87	0.92	0.48	0.75	0.14	0.18	0.04	0.13

T: Tolerant; S: Susceptible; AMF: Arbuscular mycorrhizal fungi

Table 5. Effect of salinity (250 mM NaCl) in presence and absence of AMF on crude fiber, total lipids and ash content in sweet basil (*Ocymum basilicum*) cultivars. Data presented are the means ± SE (n = 5).

Treatments	Nutritional value (%)					
	Crude fiber (%)		Total lipids (%)		Ash content (%)	
	T	S	T	S	T	S
Control	33.4	29.6	0.28	0.17	9.1	8.2
AMF	30.0	27.5	0.70	0.25	11.1	9.0
250 mM NaCl	38.1	36.6	0.18	0.06	13.6	10.5
250 mM NaCl + AMF	32.8	34.9	0.57	0.11	15.0	11.5
LSD at: 0.05	0.41	1.37	0.07	0.02	1.52	0.37

T: Tolerant; S: Susceptible; AMF: Arbuscular mycorrhizal fungi

Salt stress reduced lipid content drastically, however, effect was much pronounced in sensitive variety. Our results of reduced lipid content due to salt stress are in concurrence with the results of Hashem *et al.* (2014b) for *Ochradenus baccatus*, Alqarawi *et al.* (2014c) for *Ephedra alata*. Recently Abd_Allah *et al.* (2015c) has also demonstrated reduced lipid content in *Helianthus annus* due to cadmium stress. Reduced lipid content has stern effects on the functioning of biological membranes. It has been accepted that major part of chloroplast thylakoid and its efficient functioning strongly depends on the appropriate lipid content in their membranes (Mizusawa & Wada, 2012). Exposure of plants to stresses lead to peroxidation of membrane lipids particularly the polyunsaturated ones resulting in loss of integrity and functioning. Increased loss of lipids under stressed conditions is due to the upregulation of lipoxygenase enzymes causing rapid peroxidation of lipids (Sofo *et al.,* 2004). AMF enhanced lipid content and also allayed the ravaging effect of salinity to some degree. Recently in *Helianthus annus* AMF has been reported to enhance the cadmium stress induced damage to lipids (Abd_Allah *et al.,* 2015c). Our results of enhanced lipid content and amelioration of salt induced damage in AMF inoculated plants demonstrate the role of AMF in alleviating the salt stress. Positive association of AMF with plant growth is also evident from the reduced lipid peroxidation in AMF inoculated plants. Salt stress also leads to accumulation of the crude fibre content. Plant fiber includes cellulose, gum and lignins that are mainly synthesized from the secondary metabolism pathways. During stress plants speed up synthesis of fibres to improve the mechanical strength (Wang *et al.,* 1997). Salt stress does not affect the ash content so significantly (Zhuo *et al.,* 2015). Wang *et al.* (1997) have also reported increase in ash content in salinity stressed *Atriplex prostrate*.

Conclusion

Salt stress drastically reduced the AMF colonizing potential and the growth of *Ocimum basilicum* by reducing cholorphyll synthesis and causing increased lipid peroxidation resulting in alterations in lipid content. However AMF inoculation not only enhanced the growth parameters studied but also reversed the effect of salt stress. Present investigation strongly supports the role of AMF in assuaging the negative effects of salinity stress in *Ocimum basilicum*. Improved antioxidant system concurrent with enhanced accumulation of osmotic constituents in AMF inoculated plants contribute to improved growth under salt stressed condition.

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References

Abd_Allah, E.F., Abeer Hashem, A.A. Alqarawi, A.H. Bahkali and Mona S. Alwhibi. 2015a. Enhancing growth performance and systemic acquired resistance of medicinal plant *Sesbania sesban* (L.) Merr using arbuscular mycorrhizal fungi under salt stress. *Saudi J. Bio. Sci.,* 22: 274-283.

Abd_Allah, E.F., Abeer Hashem, A.A. Alqarawi and M.S. Alwhibi. 2015b. Alleviation of adverse impact of salt in *Phaseolus vulgaris* L. by arbuscular mycorrhizal fungi. *Pak. J. Bot.,* 47(3): 1167-1176.

Abd_Allah, E.F., Abeer Hashem, A.A. Alqarawi and H.A. Alwathnani. 2015c. Alleviation of adverse impact of cadmium stress in sunflower (*Helianthus annuus* L.) by arbuscular mycorrhizal fungi. *Pak. J. Bot*., 47(2): 785-795.

Abdel Latef, A.A.H. and H. Chaoxing. 2011. Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. *Scientia Hort.,* 127: 228-233.

Acosta-Motos, J.R., P. Diaz-Vivancos, S. Alvarez, N. Fernandez-Garcia, M.J. Sanchez-Blanco and J.A. Hernandez. 2015. NaCl-induced physiological and biochemical adaptative mechanisms in the ornamental *Myrtus communis* L. plants. *J. Plant Physiol.,* 183: 41-51.

Ahanger, M.A., Abeer Hashem, E.F. Abd_Allah and P. Ahmad, 2014. Arbuscular mycorrhiza in crop improvement under environmental stress. In: (Eds.): P. Ahmad and S. Rasool. Emerging Technologies and Management of Crop Stress Tolerance, Volume 2.Pp 69-95.

Ahanger, M.A., R.M. Agarwal, N.S. Tomar and M. Shrivastava. 2015. Potassium induces positive changes in nitrogen metabolism and antioxidant system of oat (*Avena sativa* L cultivar Kent). *J. Plant Int.,* 10(1): 211-223.

Ahmad, P., M. Sarwat, N.A. Bhat, M.R. Wani, A.G. Kazi and L.S. Tran. 2015. Alleviation of cadmium toxicity in *Brassica juncea* L. (Czern. &Coss.) by calcium application involves various physiological and biochemical strategies. *PLoS ONE.,* 10(1): e0114571. doi:10.1371/journal.pone.0114571.

Alexander, M. 1982. Most probable number method for microbial populations. In: *Methods of Soil Analysis*, (Ed.): Black, C.A. (American Society of Agronomy, Madison, WI, USA). 815-820.

Alqarawi, A.A., Abeer Hashem, E.F. Abd_Allah, T.S. Alshah Rani and A.A. Huqail. 2014c. Effect of salinity on moisture content, pigment system, and lipid composition in *Ephedra alata*Decne. *Acta Biol. Hungarica.* 65(1): 61-71.

Alqarawi, A.A., E.F. Abd_Allah and Abeer Hashem. 2014a. Alleviation of salt-induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. *J. Plant Inter*., 9(1): 802-810.

Alqarawi, A.A., E.F. Abd_Allah, Abeer Hashem, A.A. Al-Huqail and A.A. Al-Sahli. 2014b. Impact of abiotic salt stress on some metabolic activities of *Ephedra alata* Decne. *J. Food Agr. Environ.,* 12(2): 620-625.

Anonymous. 1995. Association of Official Analytical Chemists "Official Methods of analysis" (15thed.). Washington, D.C.

Benhassaini, H., A. Fetati, A.K. Hocine and M. Belkhodja. 2012. Effect of salt stress on growth and accumulation of proline and soluble sugars on plantlets of *Pistacia atlantica* atlantica used as rootstocks. *Biotechnol. Agron. Soc. Environ*., 16(2): 159-165.

Castillo, F.I., I. Penel and H. Greppin. 1984. Peroxidase release induced by ozone in *Sedum album* leaves. *Plant Physiol*., 74: 846-851.

Daniels, B.A. and H.D. Skipper. 1982. Methods for the recovery and quantitative estimation of propagules from soil", In: *Methods and Principles of Mycorrhizal Research*, (Ed.): N.C. Schenck, (The American Phytopathological Society), 29-36.

Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thorpe. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot*., 32: 93-101.

Garg, N. and G. Manchanda. 2009. Role of Arbuscular Mycorrhizae in the alleviation of ionic, osmotic and oxidative stresses induced by salinity in *Cajanus cajan* (L) Millsp. (pigeon pea). *J. Agron. Crop Sci.,* 110-123.

Hakim, M.A., A.S. Juraimi, M.M. Hanafi, M.R. Ismail, M.Y. Rafii, F. Aslani and A. Selamat. 2014. The effect of salinity on chlorophyll, proline and mineral nutrients in common weeds of coastal rice fields in Malaysia. *J. Environ. Biol.,* 35(5): 855-864.

Hameed, A., D. Egamberdieva, E.F. Abd_Allah, A. Hashem, A. Kumar and P. Ahmad. 2014. Salinity stress and arbuscular mycorrhizal symbiosis in plants. *In*: M. Miransari (Ed.), Use of microbes for the alleviation of soil stresses, Vol 1, DOI: 10.1007/978-1-4614-9466-9-7, Springer Science + Business Media New York.

Hashem, Abeer, E.F. Abd_Allah, A.A. Alqarawi, A.A. Al-Huqail and D. Egamberdieva. 2014b. Alleviation of abiotic salt stress in *Ochradenus baccatus* (Del.) by *Trichoderma hamatum* (Bonord.) Bainier. *J. Plant Int.,* 9(1): 857-868.

Hashem, Abeer, E.F. Abd_Allah, A.A. Alqarawi, G. Al-Didamony, M. Al-Whibi, D. Egamberdieva and P. Ahmad. 2014a. Alleviation of adverse impact of salinity on faba bean (*Vicia faba* L.) by arbuscular mycorrhizal fungi. *Pak. J. Bot.,* 46(6): 2003-2013.

Hashem, Abeer, E.F. Abd_Allah, A.A. Alqarawi, M. Al-Whibi, M.M. Alenazi, D. Egamberdieva and P. Ahmad. 2015. Arbuscular mycorrhizal fungi mitigates NaCl adverse effects on *Solanum lycopersicum* L. *Pak. J. Bot.,* 47(1): 327-340.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chlorplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.,* 125: 189-198.

Hiscox, J.D. and G.F. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.,* 57: 1332-1334.

Hoseini, S.M. 2010. Studying effects of salinity stress on germination, proline and carbohydrate content in Thyme (*Thymus vulgaris* L) seedlings. *Int. J. Agri. Crop Sci.,* 2 (1): 34-38.

Iqbal, N., S. Umar and N.A. Khan. 2015. Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). *J. Plant Physiol*., 178: 84-91.

Kawakam, A., Y. Sato and M. Yoshida. 2008. Genetic engineering of rice capable of synthesizing fructans and enhancing chilling tolerance. *J. Exp. Bot.,* 59(4): 793-802.

Khan, M.I.R., F. Nazir, M. Asgher, T.S. Per and N.A. Khan. 2015. Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. *J. Plant Physiol.,* 173: 9-18.

Kohler, J., J.A. Hernandez, F. Caravaca and A. Roldan. 2009. Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. *Environ. Exp. Bot.,* 65: 245-252.

Mahboobeh, R. and E.A. Akbar. 2013. Effect of salinity on growth, chlorophyll, carbohydrate and protein contents of transgenic *Nicotiana plumbaginifolia* expressing P5CS gene. *J. Environ. Res. Manag.,* 4(1): 163-170.

Masood, A., N. Iqbal, M. Asgher, M.I.R. Khan, M. Fatma and N.A. Khan. 2013. Variation in carbohydrate accumulation in two cultivars of mustard and its association with salt tolerance. *J. Funct. Environ. Bot.,* 3(2): 94-102.

Mizusawa N. and H. Wada. 2012. The role of lipids in photosystem II. *Bioch. Biophys Acta-Bioenerg.,* 1817: 194-208.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.,* 22: 867-880.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.,* 55: 158-161.

Rao, M.V., G. Paliyath and D.P. Ormrod. 1996. Ultraviolet and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiol*., 110: 125-136.

Rasool, S., A. Ahmad, T.O. Siddiqi and P. Ahmad. 2013. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta. Physiol. Plant*., 35: 1039-1050.

Sadasivam, S. and A. Manickam. 1996. *Biochemical methods*. 2nd Edn., New Age International Publishers (P) Ltd., New Delhi, India. pp. 107-109.

Singh, D., M. Pal, R. Singh, C.K. Singh and A.K. Chaturvedi. 2015. Physiological and biochemical characteristics of *Vigna* species for Al stress tolerance. *Acta Physiol. Plant*., 37(4): 87- 100.

Sofo, A., B. Dichio, C. Xiloyannis and A. Masia. 2004. Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. *Physiol. Plant.,*121: 58-65.

Tang, M., H. Chen, J.C. Huang and Z.Q. Tian. 2009. AM fungi effects on the growth and physiology of *Zea mays* seedlings under diesel stress. *Soil Bio. Bioch.,* 41: 936-940.

Tewar, D., A.N. Sah, H.K. Pandey and H.S. Meena. 2012. A Review on Phytoconstituents of Ocimum (Tulsi). *Int. J. Ayurvedic Medicine*, 3(1): 1-9.

Tomar, U.S., V. Daniel, K. Shrivastava, M.S. Panwar and P. Pant. 2010. Comparative evaluation and antimicrobial activity of *Ocimum basilicum* I Linn (Labiatae). *J. Global Pharma Technol*., 2 (5): 49-53.

Unal, B.T., L.Y. Aktas and A. Guven. 2014. Effects of salinity on antioxidant enzymes and proline in leaves of barley seedlings in different growth stages. *Bul. J. Agr. Sci.,* 20(4): 883-887.

Utobo, E.B., E.N. Ogbodo and A.C. Nwogbaga. 2011. Techniques for extraction and quantification of arbuscular mycorrhizal fungi. *Libyan Agric. Res. Center. Int*., 2: 68-78.

Wang, L.W., A.M. Showalter and I.A. Ungar. 1997. Effect of salinity on growth, ion content, and cell wall chemistry in *Atriplex prostrata* (Chenopodiaceae). *Amer. J. Bot.,* 84(9): 1247-1255.

Wolf, B. 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Comm. Soil Sci. Plant Anal*., 13: 1035-1059.

Wu, Q.S., Z. Ying-Ning and E.F. Abd_Allah. 2014. Mycorrhizal Association and ROS in Plants. In: P. Ahmad (Ed): Oxidative Damage to Plants. DOI: http://dx.doi.org/10.1016/B978-0-12-799963-0.00015

Xu, Z., P. Lei, X. Feng, X. Xu, J. Liang, B. Chi and H. Xu. 2014. Calcium involved in the poly(γ-glutamic acid)-mediated promotion of Chinese cabbage nitrogen metabolism. *Plant Physiol. Biochem.,* 80: 144-152.

Zhuo, Y., Y. Zhang, G. Xie and S. Xiong. 2015. Effects of salt stress on biomass and ash composition of switchgrass (*Panicum virgatum*). *Acta Agri. Scan. Section B-Soil Plant Sci.,* 65(4): 300-309.

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