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Moringa Leaf Extracts as Biostimulants-inducing Salinity Tolerance in the Sweet Basil Plant

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HORTICULTURE plants are constantly exposed to salinity problems during growing. Biostimulants from plants origin known to protect plants growth and improve their productivity within a varied conditions of salt stress. To investigate *Moringa* plants (*Moringa oleifera*, MO and *Moringa peregrena*, MP) activity as biostimulants, sweet basil (*Ocimum basilicum* L. cv. cispum) plants grown with/or without salt stress were irrigated using aqueous leaf extracts from both species. Two hundred grams of fresh *Moringa* leaves were homogenized in one liter distilled water, filtered and the dilutions: 2.5%, 5.0%, 10% and 20% were made. In stressed basil, proline and malondialdehyde had increased then decreased significantly with both *Moringa* extracts, particularly with MP treatment. Compared to control basil, 10% was the best concentration that caused enlargements of basil leaf area by 60% during salt stress. On applying 10% MO and MP extracts to salt-stressed basil, growth parameters like shoot length were increased by 32% and 38%, shoot fresh weight by 50% and 109%, shoot dry weight by 123% and 84%, number of branches by 75% and 87%, root length by 40% and 63% and root dry weight by 142% and 225%, respectively. MO treatment led to a significant increase in anthocyanin, total carbohydrates and superoxide dismutase in basil. Alternatively, MP increased ascorbic acid oxidase actively in basil leaf. The varied chemical composition of *Moringa* species underlined the resistance strategies in basil. We hypothesized that the efficacy might even become much more potent on basil with the simultaneous irrigation using the two species of *Moringa*.

Keywords: Antioxidant enzymes, Growth parameters, Malondialdehyde, *Ocimum basilicum* L. cv. cispum, Proline, Salt stress.

Introduction

The presence of increased salt ions in irrigation water or soil often leads to soil salinization and crop plant dehydration (Zhu, 2001). Salinity is an ancient, serious abiotic problem that confers deleterious impacts on agriculture in many parts of the world (Abdel Latef, 2010). In fact, millions of hectares have annually been affected due to salinity (Sheng et al., 2008). Salinity is associated with disturbance of plant water relation ending with physiological drought and malfunctions of homeostasis (Munns, 2002 and Rady, 2011). Furthermore, it interferes with water uptake by roots, prevents solubility of necessary soil components and threatens the nutritional balance via enhancing the uptake of harmful ions from the soil instead of nutrients (Silva et al., 2008). Moreover, salinity was the main factor inducing photorespiration and reactive oxygen species (ROS). However, plant tolerance is the strategy capable for development at various levels in plants (Munns & Tester, 2008). Thereby, to ameliorate the damage caused by ROS, plants evolve defense strategy in form of production of various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbic acid oxides (ASO), peroxidase (POX), ascorbate peroxidase (APX) and polyphenol oxidase (PPO). These enzymes can prevent ROS production and action (Alscher et al., 2002).

Sweet basil belongs to lamiaceaeas aromatic ornamental plant native to tropical and subtropical regions. It can be used as a medicine for several health problems (Simon et al., 1990) and have been frequently utilized in various industries (Makri & Kintzois, 2007). Thus, basil is demanded by various resources. The famous natural oils that are found in basil are linalool, methyl cinnamate,

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methyl chavicol, eugenol and geraniol (Sajjadi, 2006). Previous studies reported that basil growth, metabolism and yield are affected by osmotic and ionic stress due to salinity (Ashraf & Harris, 2004 and Saqib et al., 2012).

In agriculture, exposed plants to mild stress using 100mM NaCl provides insights about the primary tolerance and acclimation strategies in plants. In the literature, treatment of lens plant using 100mM NaCl reduced germination, suppressed growth, diminished pigment content and dry matter, induced cell membrane instability and increased Malondialdehyde (MDA) for the overexpression of reactive oxygen species and antioxidant enzymes induction (Aydemir & Erez, 2010). MDA is the global indicator of lipid peroxidation (Wada et al., 2008) and the biomarker of membrane injury (Sharma et al., 2005). Recently, exogenous application of nitric oxide in basil alleviated salinity symptoms and raised the level of biochemical activities required for growth (Saeidnejad et al., 2013). However, basil plants develop stress adaptation strategy in form of proline induction. Proline is the interfering osmoticum with stabilizing protein and membrane (Kardpol & Rao, 1985) and involved with scavenging the free radicals (Blokhina et al., 2003).

Biostimulants were recently defined as the materials capable to promote, with their minute quantities, plant growth and facilitate nutrient uptake during abiotic stress (Colla & Rouphael, 2015 and Du Jardin, 2015). Protein hydrolysates including, betaines, amino-acids, polyamines, non-protein amino acids, hormones and other nitrogenous compounds shown to play multiple roles as biostimulants of plant growth, metabolic production and recovery during environmental stress (Vranova et al., 2011 and Colla et al., 2015). Recently discovered that the antioxidant enzymes, sterols, polyamines and phytohormones like auxins, gibberellins and cytokinins extracted from seaweeds were considered potent biostimulants in stressed plants. They activated the hormone biosynthetic genes (Craigie, 2011) and regulated endogenous stress responsive genes (Battacharyya et al., 2015).

In addition to thirteen other genera, *Moringa* belongs to family Moringaceae (Olson, 2002). Two widely grown *Moringa* species in Egypt were used in this study as biostimulants of basil agriculture. The first species: *Moringa oleifera* Lam. (MO) is a deciduous tree (7-12m height) with thick grey bark, white flowers and long green pods. MO is natively growing in Pakistan, Bangladesh, Afghanistan and India (Meneghel et al., 2013) and used in medicine to produce vitamins, antioxidants, ascorbate, zeatin, minerals, and purine adenine derivatives of cytokinin and amino acids (Siddhuraju & Becker, 2003; Basra et al., 2009; Mahmood et al., 2010; Becker, 2013 and Hussain et al., 2013). Moringa leaves have also several biological activities as immune-boosting agent and tumor-suppressive effects (Fiazi et al., 1995). The second species: *Moringa peregrina*, Forresk (MP) is cultivated in Egypt (Abd El-Wahab et al., 2004). MP is growing fast, 15m height tree and recognized by grey or green bark, long leaves and yellowish white to pink flowers. Leaves and pods of MP tree enclose nutritional components like vitamin A, B and C, minerals, calcium, protein, low fats and carbohydrates, which all considered crucial for human health and liver stock (Alkahtani & Abou-Arab, 1993 and Price, 2000).

So far, little is known about the role of Moringa plants as biostimulants. The present study devoted to underline the possible effect of leaf extracts from the two species of *Moringa* trees on stimulating stress tolerance in basil. The aim of this research is studying the effect of different concentrations of aqueous leaf extracts from the two *Moringa* species on tolerance strategies that evolved from the improved growth along with the amended metabolic profile.

Materials and Methods

Plant material, leaf extraction and experimental design

Fully expanded leaves and tender branches were collected from two cultivated *Moringa* trees (*M. oleifera* Lam and *M. peregrina*, Foressk) in El-Orman garden, El-kanater El-khairia region, Kalubiah Governorate, Egypt, during day time (28ºC, June/ 2015). Two hundred grams were extracted in 500ml H_2O using locally fabricated machine (Foidle et al., 2001). The extract was filtered through muslin cloths and centrifuged at 800xg for 15min. The supernatant was completed to one liter then dilutions were made (20%, 10%, 5%, 2.5%) and kept at 4ºC till used. The experimental design was factorial based on completely randomized design. The sweet basil seeds were purchased from Agricultural Research

Centre, Dokki, Cairo, Egypt, subjected to 5min surface sterilization using 1% sodium hypochlorite (NaClO) solution and washed thoroughly using distilled water. The greenhouse experiment was controlled for temperature (25ºC/20ºC, day/night), humidity (33%) and radiation (330µmol m-2 s-1). Cultivation of basil seeds took place in plastic pot (15cm diameter, 15cm deep) and the planting process started on 15/06/2015 for six weeks until the harvesting and sampling date on 30/07/2015. Prior planting each pot was equally filled with clay: sandy soils (2:1) and irrigated with tap water to 70% water holding capacity. After one week, thinning was carried out to leave 5 plants in each pot. Two weeks following sowing, the pots were divided into 18 groups with five replicates in each group: 1- Plants left without treatment (control), 2- Treated plants with 20% *Moringa oleifera* leaf extract (OLE), 3- Treated Plants with OLE (10%), 4- Treated plants with 5% OLE, 5- Treated plants with 2.5% OLE, 6- Treated plants with 20% *Moringa peregrena* (PLE) leaf extract, 7- Treated plants with 10% PLE, 8- Treated plants with 5% PLE, 9- Treated plants with 2.5% PLE, 10- Treated plants with 100mM NaCl, 11- Treated plants with 100mM NaCl + 20% OLE, 12- Treated plants with 100mM NaCl + 10% OLE, 13- Treated plants with 100mM NaCl + 5% OLE, 14- Treated plants with 100mM NaCl + 2.5% OLE, 15- Treated plants with 100mM NaCl + 20% PLE, 16- treated plants with 100mM NaCl + 10% PLE, 17- Treated plants with 100mM NaCl + 5% PLE and 18- Treated plants with 100mM NaCl + 2.5% PLE.

The plants were irrigated with 40ml OLE or PLE alone or in combination with 40ml NaCl (100mM) at weeks 2. 3 and 4 from sowing and the plants were harvested after 6 weeks from sowing.

Biochemical analysis of sweet basil leaf and root

The biochemical composition including the photosynthetic pigments, proline, carbohydrates, malondialdehyde and the antioxidant enzymes were analysed and determined in exposed and unexposed basil to salt stress treated with only 10% *Moringa* leaf extract.

Estimation of proline

Free proline was determined according to Bates et al. (1973). Acid ninhydrin reagent was prepared by warming 1.25g ninhydrin in 30ml glacial acetic acid and 20ml 6M phosphoric acid with agitation until dissolved; kept cool and stored at 4˚C. The reagent remains stable for 24hr.

Approximately 0.1g of macerated dried tissue was homogenized in 10ml of 3% aqueous sulfosalicylic acid, then filtered through filter paper Whatman No. 2. Two ml of the filtrate were mixed with 2ml glacial acetic acid and 2ml of the acid ninhydrin reagent in a test tube and heated for 1hr at 100˚C. The reaction mixture was extracted with 4ml toluene, mixed vigorously in a test tube for 15-20sec. The chromophore containing toluene was aspired from the aqueous phase and warmed to room temperature. The absorbance was read at 520nm using toluene as blank .The proline concentration was determined using stander curve and calculated on a dry matter basis. Free proline was measured per milligram in 100g dry weight.

Estimation of photosynthetic pigments

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined in the leaves of the investigated plant. The spectrophotometric method recommended by Metzner et al. (1965) was used. A known fresh weight of leaves was homogenized in 85% aqueous acetone for 5min. The homogenate was centrifuged and the supernatant was made up to volume with 85% aqueous acetone. The extinction was measured against a blank of pure 85% aqueous acetone at 3 wave lengths of 452.5, 644, 663nm using Spectrocolourimeter DC Tiny 25III Model TUDC12B4. Taking into consideration the dilutions made of the pigment fraction, chlorophyll a, chlorophyll b and carotenoids were determined in gram fresh weight using the following equations:

Chlorophyll a= 10.3 E663 -0.918 E644= µg/ml

Chlorophyll b= 19.7 E644 -3.87 E663= µg/ml

Carotenoids= 4.2 E425.5- (0.026 chlorophyll a + 0.426 chlorophyll b)= µg/ml

Finally, the pigment contents were calculated as µg g^{-1} dry weight of leaves.

Estimation of carbohydrates

The plant material was rapidly dried in an oven at 80˚C to a constant weight and then ground to a fine powder.

Extraction and estimation of soluble sugars: A known weight of dried tissues was homogenized with 80% ethanol then put in a boiling water bath with shaking for 15 min. After cooling, the extract was filtrated and the filtrate was oven dried at 60˚C then dissolved in a known volume of water to be

ready for determination of soluble sugars. The anthrone sulphuric acid method carried out by Whistler et al. (1962) was used for determination of soluble sugars. The anthrone reagent consists of 0.2 anthrone (Merck) and 100ml 95% H_2SO_4. These were successively mixed in a conical flask under continuous cooling. This reagent should be always freshly prepared. 0.1ml of soluble sugar solution was put in a clear pyrex test tube and mixed with 10ml of anthrone reagent. The samples were then heated at 100˚C in a water bath for exactly 7min, after which it was directly cooled under tap water. The developed blue green colour was read at a wavelength of 620nm against a blank contain only water and anthrone reagent using Spectocolourimeter DC Tiny 25III Model TUDC12B4. A calibration curve using pure glucose was carried out.

Extraction and estimation of polysaccharides: The dry residue left after extraction of soluble carbohydrate was used for the determination of polysaccharides. A known weight of dried material was added to 10ml 1.5N sulfuric acid in sugar tubes with air reflux and heated at 100˚C in a water bath for 6hr. The hydrolysate was made up to a known volume to be ready for polysaccharide determination by method of anthrone sulphuric reagent (Whistler et al., 1962). A calibration curve using pure glucose was made, from which the data were calculated as mg/g dry weight.

Total carbohydrates: Total carbohydrate contents were calculated per gram in 100g dry weight as sum of soluble sugars and polysaccharides amounts of the sample.

Extraction and estimation of lipid peroxidation product (MDA)

Lipid peroxidation was measured following the method described by Heath & Pecker (1968), referring to malondialdehyde (MDA) produced by thiobabituric acid reactions.

Extraction and assaying activity of certain antioxidant enzymes

The extraction performed according to Mukheriee & Choudhuri (1983). The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined by the method recorded by Marklund & Murklund (1974). Assays of catalase (CAT, EC 1.11.1.6), polyphenol oxidase (PPO) and peroxidase (POX) activities measured by a method described by Kar & Mishra (1976). Assay of ascorbate peroxidase (APX, EC 1.11.1.11) activity assay undertaken using a method described by Koricheva et al. (1997). Assay of ascorbate oxidase (ASO) activity determined by the method described by Diallins et al. (1997).

Statistical analysis

Data were means of five variables from three independent experiments. ±SD determined using Microsoft excel, ver. 2008. Data were analysed using simple variance analysis (ANOVA), SPSS ver. 17, and the means compared with LSD at P= 0.05 and Duncan's test.

Results

Effects of irrigation using MO and MP leaf extracts in basil culture

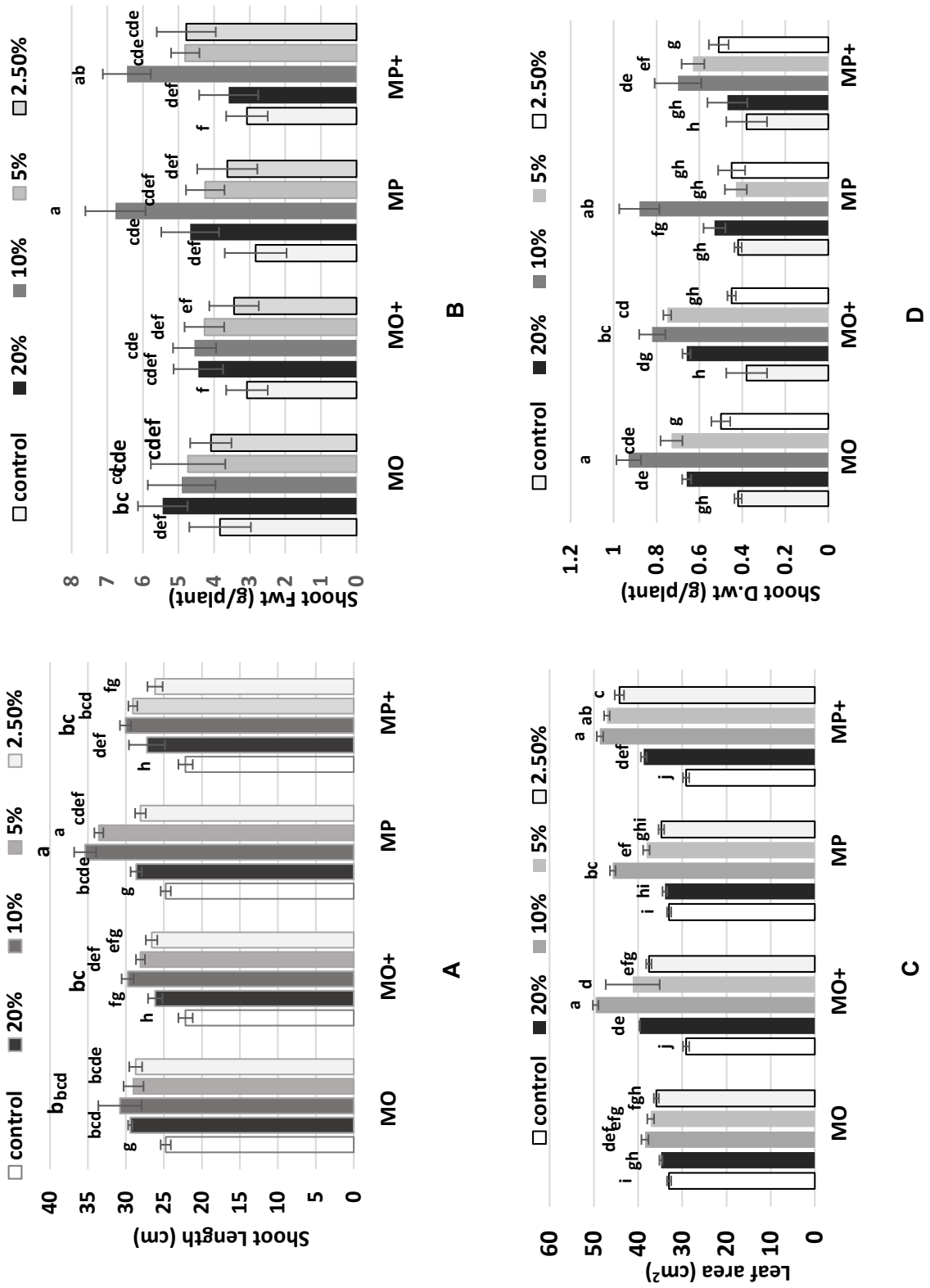
Evidence of general growth improvement has been detected upon basil plants irrigation with gradual dilutions of leaf extracts from MO and MP under normal conditions. Moreover, the growth parameters in salinized basil have significantly improved when extracts from both Moringa leaves were treated at any concentration in combination with NaCl (Fig. 1). However, 10% concentration had influenced growth much better, whereas 2.5% resulted in the lowest response.

Salt-stress experiment

Six weeks old basil length was 24.77, 28.7, 29.0, 30.78 and 28.63cm in treated basil with 0%, 2.5%, 5%, 10% and 20% leaf extract concentration from MP, respectively. On the other hand, shoot length was 26.62, 28.09, 29.77 and 27.22cm for 2.5%, 5%, 10% and 20% MO leaf extracts, respectively.

Irrigation of basil plants using 10% MO and MP leaf extracts in salt experiment resulted in basil length increase by 32% and 38% and in 50% and 109% increase of shoot fresh weight, respectively (Fig. 1 A & B).

Basil plant exhibited 60% enlargement of leaf area when irrigated with 10% MO or MP leaf extracts (Fig. 1 C). Furthermore, shoot dry mass increased by 123% and 84% with MO and MP extracts, respectively (Fig. 1 D). Number of branches increased by 75% and 87% (Fig. 1 E), root length increased by 40% and 63% and root dry weight increased by 142% and 228%, when MO and MP were applied during irrigation, respectively (Fig. 1 F & G).


control
20%
10%
5%
2.50%
MO
MO+
MP
MP+
Shoot Length (cm)
Shoot Fwt (g/plant)
Shoot D.wt (g/plant)
Leaf area (cm²)
A
B
C
D

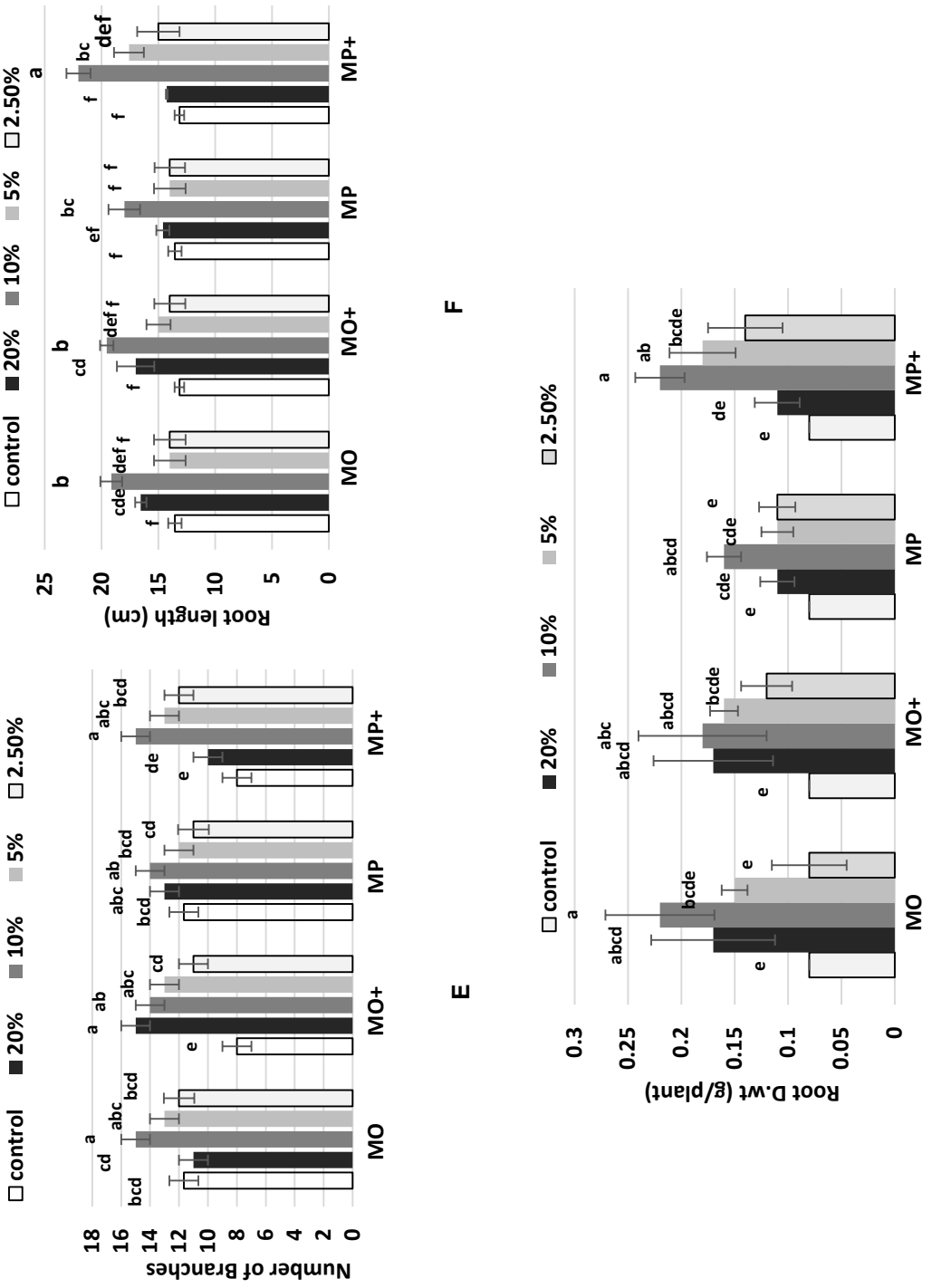


Fig. 1. Effect of *Moringa oleifera* (MO) and *Moringa peregrena* (MP) leaf extracts (20%, 10%, 5%, 2.5 %) on the growth parameters of sweet basil plant [MO and MP, Moringa treatments in salt-free basil culture, MO+ and MP+ express Moringa treatments insalinized basil cultureusing 100mM NaCl].

Salt- free experiment

Irrigating salt-free experiments using Moringa leaf extracts resulted in 33% and 46% increase of shoot length, 25% and 134% increase in shoot fresh weight, 18% and 40% enlargement of leaf area, with MO and MP treatments, respectively (Fig. 1 A-C). Furthermore, the increase of shoot dry weight exceeded 131% and 119%, number of branches increased by 25% and 16.6%, root length by 28% and 21%, and root dry weight increased by 193% and 100% upon using MO and MP extracts for irrigation, respectively (Fig. 1 D-G).

Interaction of Moringa extracts and salinity on the metabolic activity of sweet basil

The anthocyanin pigment content has increased in six weeks-old basil due to 10% MO and MP applications in salt experiments by 30% and 13.5%, respectively (Fig. 2 A). Alternatively, proline in basil leaf has decreased significantly by 57% and 60% with MO and MP, respectively (Fig. 2 B). Total carbohydrates increased by 80% and 14% (Fig. 2 C) and malondialdehyde (MDA) was reduced in basil leaf by 18.6% and 66% (Fig. 2 D) when MO and MP were applied, respectively. The activities of antioxidant enzymes increased due to salinity stress and diminished with treatments of MO and MP leaf extracts. For example, superoxide dismutase (SOD) in basil leaf reduced by 26% with MO and by 48% with MP treatment (Fig. 3 A). The catalase (CAT) enzyme activity had also been reduced with the treatments by 37% in basil leaf (Fig. 3 B). Ascorbic acid oxidase (ASO) activity was reduced significantly upon Moringa treatments by 61% with MO and up to 56% with MP extract (Fig. 3 C). The peroxidase (POX), ascorbic peroxidase (APX) and polyphenol oxidase (PPO) in basil leaf have decreased with non-significant change between both *Moringa* species. Thereby, the average reduction values were 34% for peroxidase, 31% of ascorbic peroxidase and 36% for polyphenol oxidase (Fig. 3 D-F).

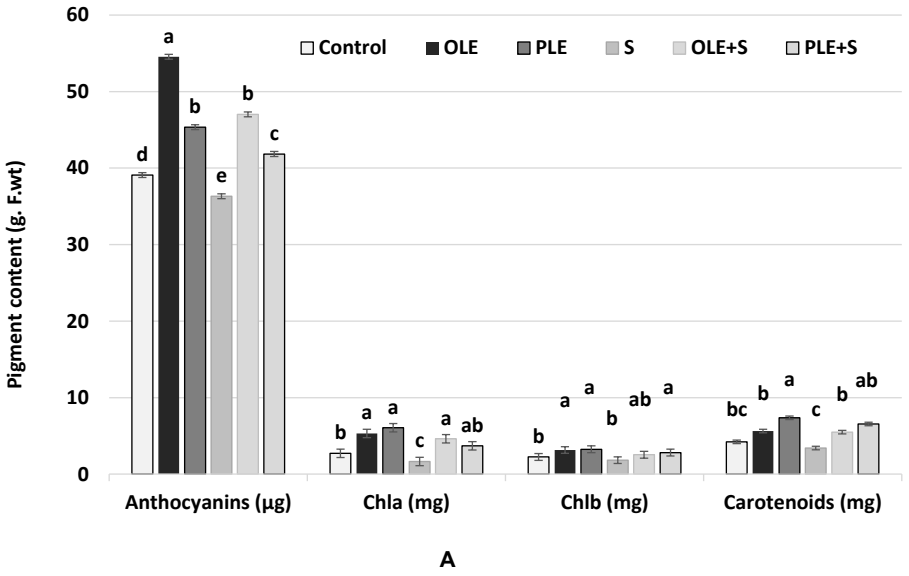
Discussion

Several reasons were responsible for the variations of plant growth and metabolic activity under salt stress such as the primary inhibitory effect of water and essential ions uptake (Marschner, 2012). The impairment of photosynthesis, the decrease of metabolic enzymes besides alterations of carbohydrates metabolism and hormonal homeostasis (Gao et al., 1998; Chartzoulakis et al., 2002; Munns, 2002 and Tabatabaei, 2006). Salinity primary effect in generating species of reactive oxygen like singlet oxygen and hydrogen peroxide and induced malondialdehyde was reported (Wildt et al., 1997). In agreement with previous findings, our data confirmed the elevation of both proline and the activity of multiple antioxidant enzymes such as: Superoxide dismutase (SOD), catalase (CAT), ascorbic acid oxides (ASO), peroxidase (POX), ascorbate peroxidase (APX) and polyphenol oxidase under salinity stress (Ben-Taarit et al., 2009; Rady et al., 2013; Yasmeen et al., 2013 and Howlader, 2014). Due to salinity, symptoms of ionic toxicity have frequently appeared in forms of malfunction of water relations (Hussein et al., 2012) and in the decreased chlorophyll content due to destruction of pigment-protein complex which subsequently led to a reduction of dry matter (Santos, 2004 and Ashraf & Harris, 2013). Furthermore, salinity effect in reducing the growth rates of leaf and root, leaf area and lateral branches (Läuchli & Grattan, 2007) could be ascribed to the known salinity effect regarding the inhibition of cell division and cell enlargement in the growing point (Kulshrestha et al., 2013).

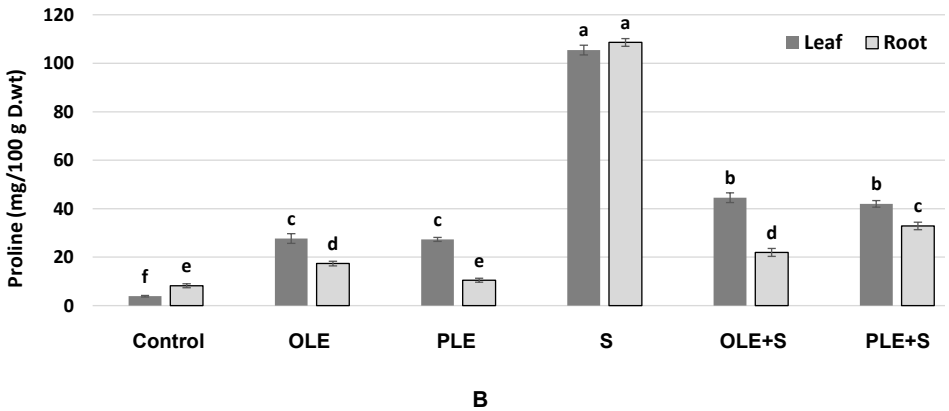
Generally, *Moringa* crude aqueous extract encloses mixture of various categories of active compounds that possess biostimulanting properties such as, carbohydrates, nitrogenous compounds, hormones and polyphenols (Talreja, 2011; Ahmed et al., 2016 and Said Al-Ahl et al., 2017). The role of MP as biostimulant was particularly important for its known richness in both ascorbic acids and reduced glutathione (Luqman et al., 2012). Every category is imposing its physiological impact either separately or simultaneously during alleviation of salinity symptoms in sweet basil. For example, the minerals effect in targeting pH regulation, homeostasis, osmosis, enzymes and signalingwas studied (Pilon-Smits et al., 2009). In a previous study, external application of calcium had ameliorated chlorophyll loss in plants (Yasmeen et al., 2013). Apart from the literature which reported that calcium content in MO was four times as much as in milk (Foidle et al., 2001), our data showed that both *Moringa* species were calcium-rich, and that MP enclosed higher amounts of calcium compared to MO (Asghari et al., 2015). The biochemical content of MP also revealed potassium richness (Asghari et al., 2015). Potassium is the element known to alleviate salinity in stressed plants through balancing Na^+:

K^+ ratio and via ameliorating stomatal conductance and turgor maintenance (Mengel & Kirkby, 2001). Compared to MO, iron was almost two folds higher in MP extract. Iron is crucial, possessing biological effect on plants, particularly during photosynthesis and DNA synthesis. Furthermore, iron is involved with chlorophyll synthesis and participates in chloroplast maintained structure and function. It is also the prosthetic groups of many antioxidant enzymes like the catalase, peroxidase, cytochromes and cytochrome oxidases and links with the last step of respiration (Rout & Sahoo, 2015).

As previously reported, the effect of *Moringa* leaf extract was concentration-dependent, and that the diluted extracts could be promotive, whereas the concentrated ones might be suppressive, likely due to the hormonal mode of action (Khan et al., 2009). Current data showed that 10% *Moringa* leaf extract was the dose seemed appropriate offering the best effect among all. Thus, we initially used 10% as a key concentration that confers the highest level of recovery. Moreover, we also confirmed that the variance in the biochemical composition of *Moringa* species had imposed varied tolerance strategies in sweet basil plant. For example, MP richness in hormones had supported length of shoot and root and improved shoot fresh weight compared to MO. Alternatively, the soluble sugars, nitrogenous compounds, amino nitrogen and total phenols of MO leaf extract have improved growth and slightly had affected shoot fresh weigh, root length, number of branches and root dry weight.



A



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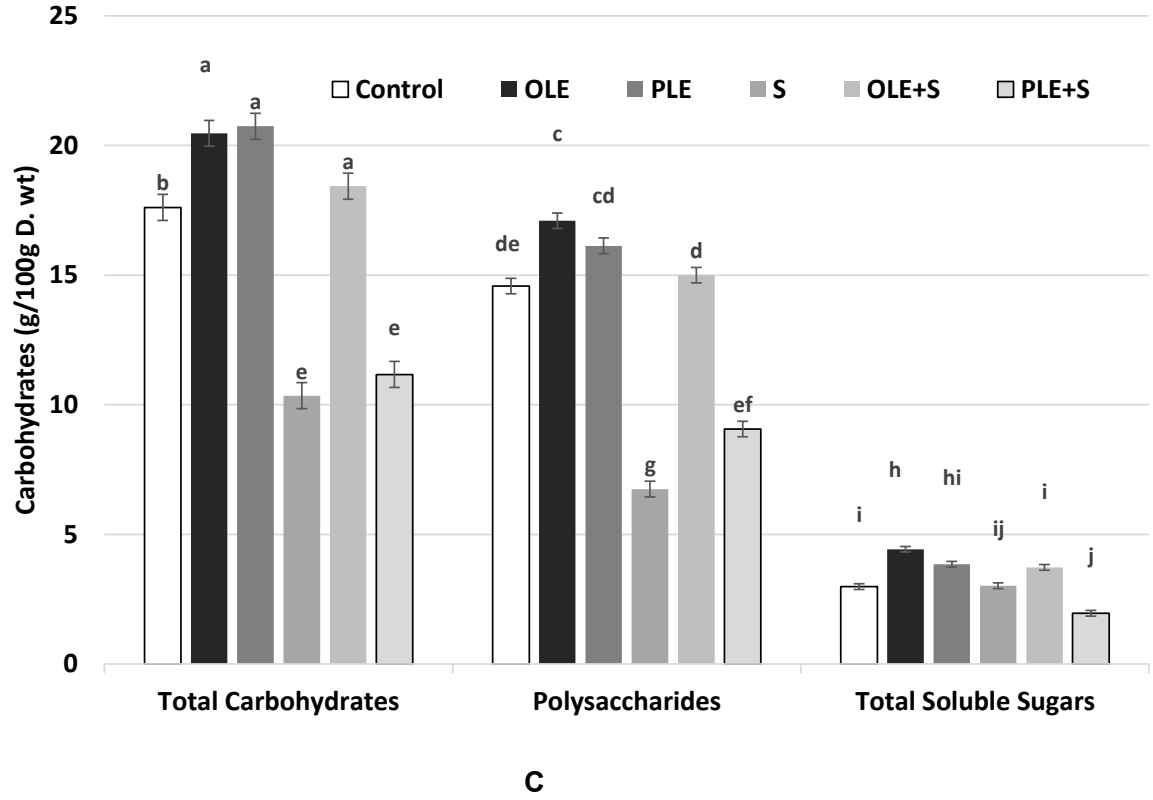


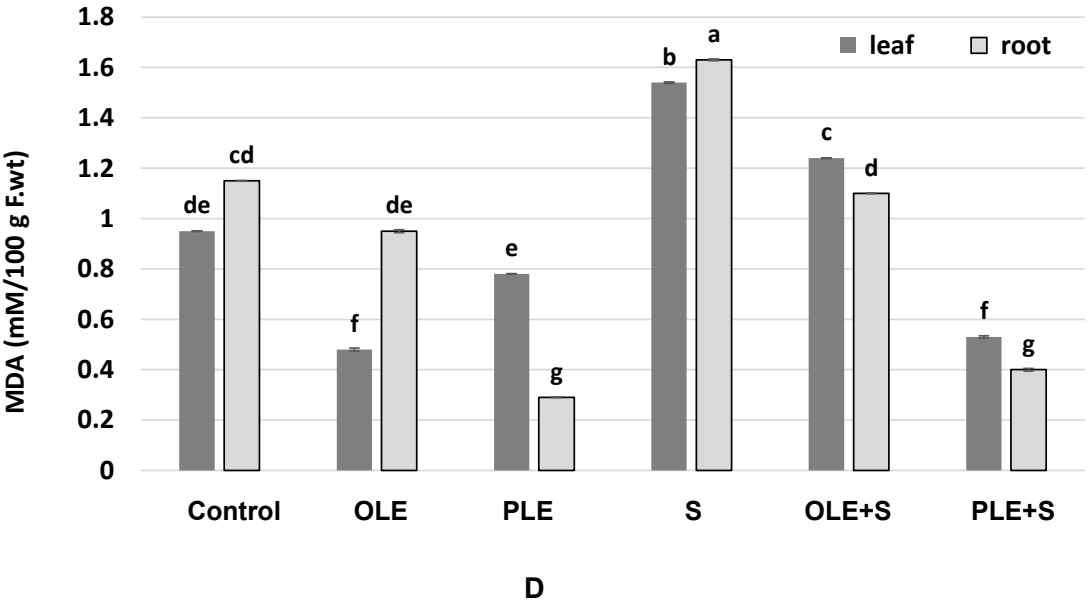
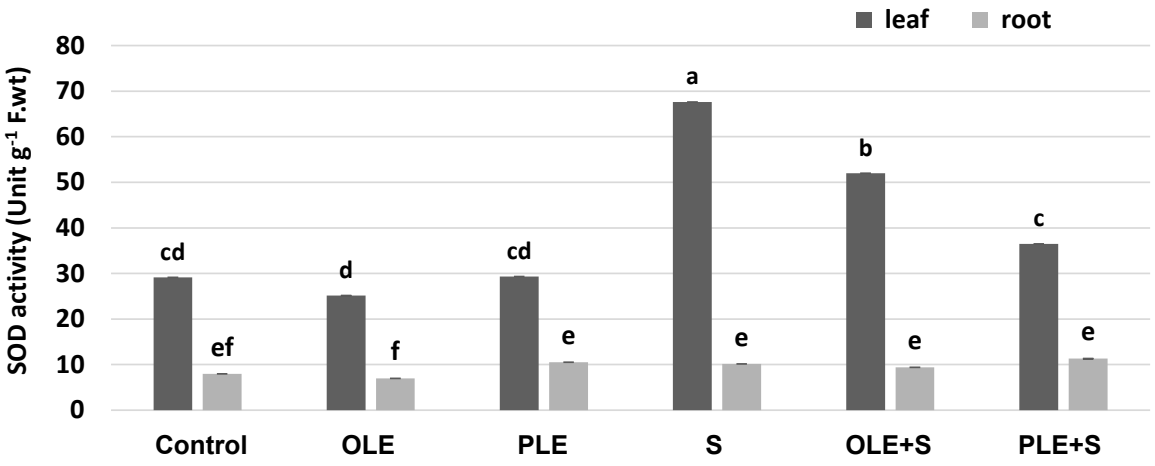
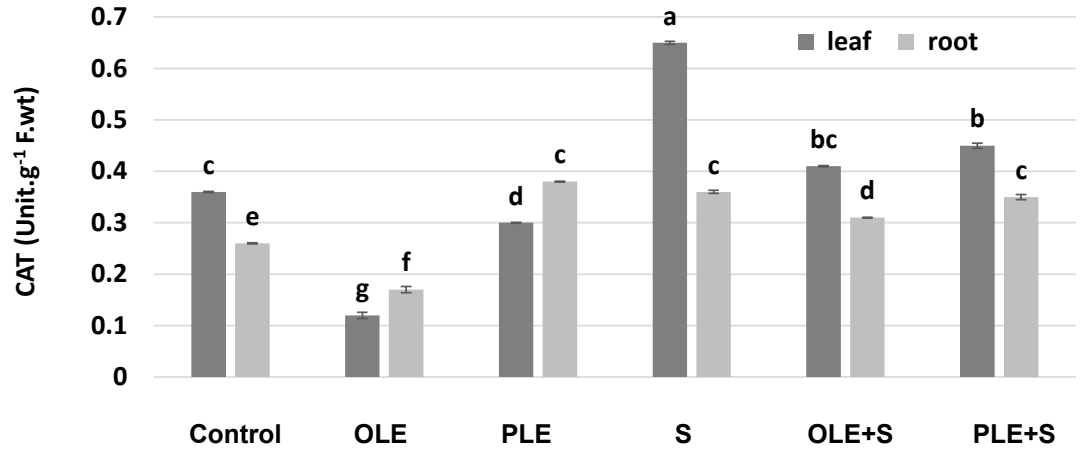


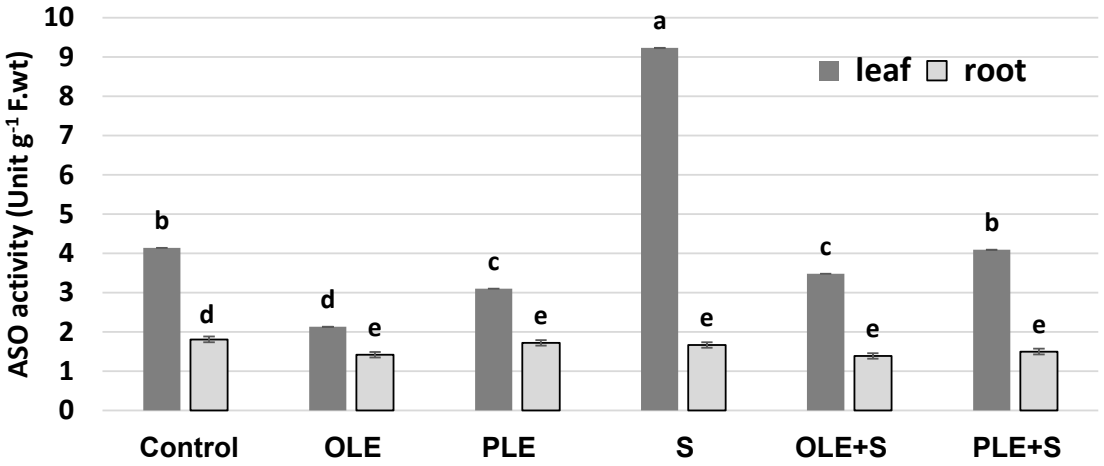
Fig. 2. Effect of leaf extracts concentration (10%) from *Moringa oleifera* (OLE) and *Moringa peregrena* (PLE) on the biochemical activities of sweet basil plant during exposure to 100mM NaCl (S). Pigment, A; proline, B; carbohydrates, C and malonyldialdehyde (MDA, D). Combined salt to *M. oleifera* extracts in basil experiment; OLE+S. Salt combined to *M. peregrena* extract; PLE+S.


leaf
root
SOD activity (Unit g-1 F.wt)
80
70
60
50
40
30
20
10
0
cd
ef
d
f
cd
e
a
e
b
e
c
e
Control
OLE
PLE
S
OLE+S
PLE+S

A


leaf
root
CAT (Unit.g-1 F.wt)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
c
e
g
f
d
c
a
c
bc
d
b
c
Control
OLE
PLE
S
OLE+S
PLE+S

B


leaf
root
ASO activity (Unit g-1 F.wt)
10
9
8
7
6
5
4
3
2
1
0
b
d
d
e
c
e
a
e
c
e
b
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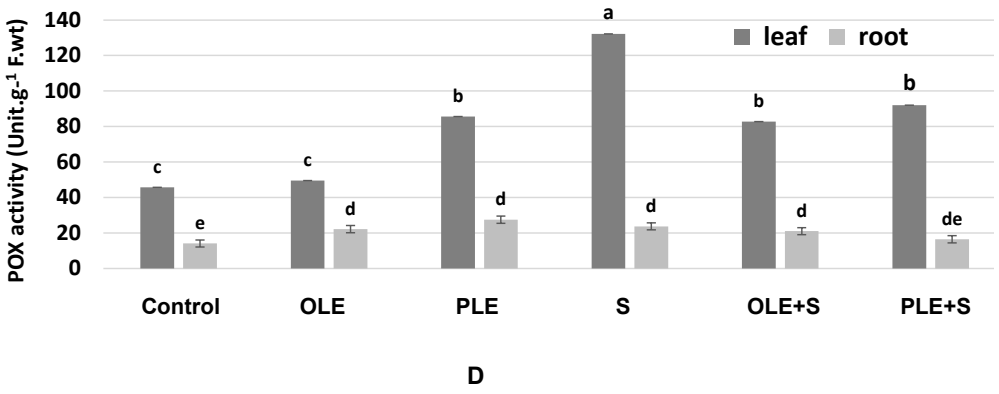
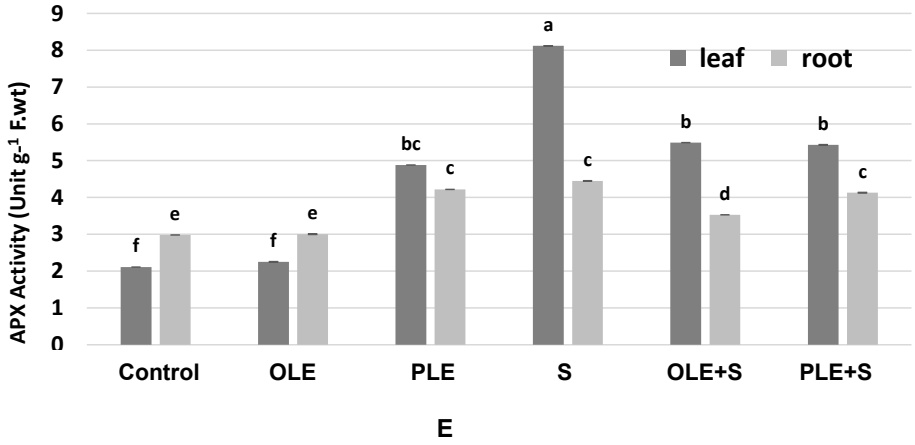
Control
OLE
PLE
S
OLE+S
PLE+S

C





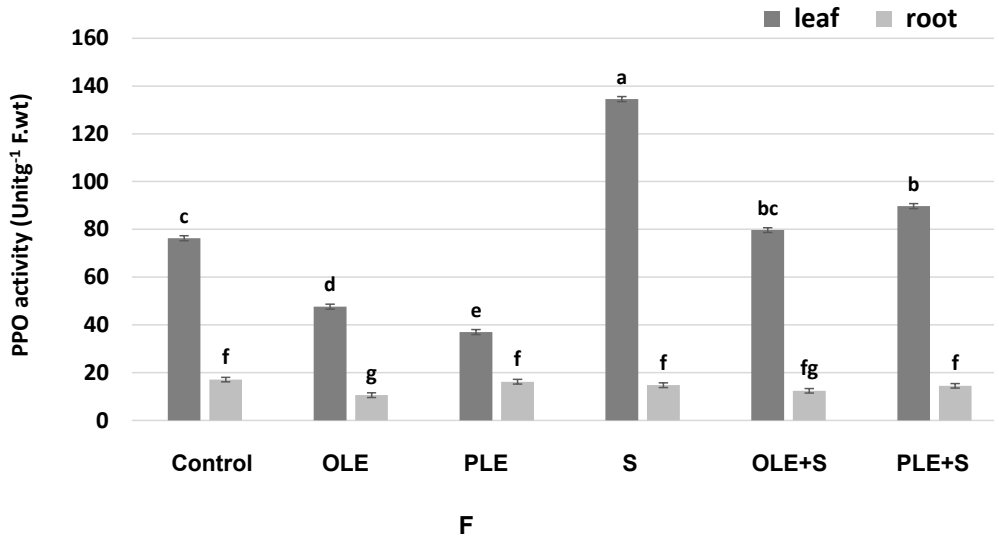


Fig. 3. Antioxidant enzymes activity of sweet basil leaf and root. Grown basil under the effect of 100mM NaCl alone; S. Salt-free basil treated with 10% leaf extracts from *Moringa oleifera* (OLE) or *Moringa peregrena* (PLE). The combined effects of 100mM NaCl and 10% *Moringa oleifera* extract on basil; OLE+S and of 100mM NaCl and 10% *Moringa peregrena* extract; PLE+S. Superoxide dismutase, A; catalase, B; ascorbic acid oxides, C; peroxidase, D; ascorbate peroxidase, E and polyphenol oxidase, F.

Some phenolic compounds of MO were absent in MP. Yet, no published data on the role of MO phenols as biostimulants were found. Apparently, basil had utilized MO overall metabolic constituents to enhance and maintain tolerance during salinity stress via raising the antioxidant enzymes activity. On the other hand, the MP defense strategy was dependent on using salicylic acid, the kind of phenols that frequently reported as crucial regulators of physiological processes the tolerance inducer of abiotic stress in many plants (Kaya et al., 2009 and Hayat et al., 2010). In addition, MP encloses hormones, elements and reduced glutathione found important for both induction and activation of cellular hydrogen peroxide antioxidant enzymes in basil like CAT and POX, the participating enzymes with metabolic processes such as, photorespiration, polymerization of phenols to lignin and in the glycolytic pathway (Romheld et al., 1984 and Abogadalla, 2010). Ascorbic acid and glutathione, the potent antioxidants of low molecular weight had relatively accumulated in substantial amounts in MP compared to MO (Blokhina et al., 2003). Ascorbic acid, on one hand, is the small metabolite that participates in the cellular detoxification of hydrogen peroxide (Noctor & Foyer, 1998) and in cell division of plant (Smirnoff, 2011). It further assists membrane protection via tocopherol regeneration (Thomas et al., 1992). In a previous report, the mitigation effect of ascorbic acid exceeded that of glutathione in olive plants subjected to salinity using 100mM NaCl (Aliniaeifard et al., 2016). Herein, ascorbic acids-rich plants like MP activate ascorbic acid oxidation (ASO) enzyme in basil. ASO is the interfering enzyme with ascorbic acids oxidation and metabolism. Moreover, the reduced glutathione had reacted during stress as non-enzymatic scavenger of hydrogen peroxide, superoxide and singlet oxygen (Larson, 1988). Furthermore, the consequent effect of increased activity of reduced glutathione on stimulating cotton plants tolerance to salt stress was also discovered (Melonia et al., 2003).

In this study, it is proposed the presence of an inversely relationship between the declined MDA level and the increased antioxidant enzymes scavenging capacity in basil with MP treatment. It can be suggested that the biochemical compounds like the reduced glutathione, ascorbic acids, hormones and minerals were actively higher regarding their efficacy as biostimulants as compared to the total phenolic content present in MO. However, each *Moringa* species had separately played the same essential role in enhancing the overall antioxidant enzymes pool and hence, basil tolerance. The abundance of MP ascorbic acids and reduced glutathione must had replenished the degraded ascorbic acid and glutathione in basil due to salinity as recorded (Valderrama et al., 2006).

Conclusion

The current study indicated that salinity in basil was the detriment that could be healthily encountered using environmental risk-free methods such as leaf extracts from MO and MP as biostimulants. Tolerance in basil was a compatible associated strategy with the biochemical profile in *Moringa* species. For future investigations, we presume that a full recovery from salt stress would be likely obtained in basil under the effect of mutual or simultaneous irrigation using low doses of *Moringa* leaf extracts.

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استخدام مستخلصات أوراق نبات المورينجا كمحفزات بيولوجية لتدعيم صفة مقاومة الأجهاد الملحي في نبات الريحان

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تتعرض نباتات البساتين مثل الريحان كغيرها من نباتات المحاصيل والخضر لمشكلة ملوحة الأراضي التي تؤثر سلبا على نموها وانتاجيتها الأقتصادية المرجوة. تم استخدام المحفزات الحيوية (البيولوجية) ذات الأصل النباتي للتغلب على مشكلات الملوحةحيث تكسبها صفة التحمل والمقاومة وبالتالي تقلل من الأثار السلبية للملوحة على النمو والإنتاجية عند معاملتها بها بطرق مختلفة. في هذه الدراسة تم الاستعانة بمستخلصات مائية بتركيزات (2.5%، 5.0%، 10% و20%) من أوراق نوعين من المورينجا هما: مورينجا اوليفيرا (البان الزيتي) ومورينجا بيريجرينا (البان الأجنبي). والمورينجا أو البان هو نوع من النباتات يتبع جنس البان من الفصيلة البانية، ويتميز بمحتواه العالي من المركبات الحيوية والأيضية. عند ري الريحان النامي في بيئة ملحية بتركيز 100 ملي مول من كلوريد الصوديوم أظهرت النتائج ارتفاعا ملحوظا بنسب البرولين والمالون داي ألدهيد(MDA) مما يدل على تأثر الريحان بالتركيز المستخدم. أما عند معاملة الريحان أثناء تعرضه للإجهاد الملحي بمستخلصات المورينجا المختلفة تبين انخفاض هذه النسب انخفاضا ملحوظا. كذلك حافظت جميع المستخلصات لاسيما تركيز 10% على الشكل الظاهري ونمو الريحان فزاد المعدل من 40-225% مقارنة بالعينة الضابطة. وقد حفز نوع الاوليفيرا في الريحان تراكم صبغ الانثوسيانين والكربوهيدرات ومضاد الأكسدة فوق أكسيد الديسميوتيز بينما ادى مستخلص نوع البيريجرينا إلى تراكم حمض الأسكوربيك أو فيتامين سي وهو أحد مضادات الأكسدة الهامة. يرجع اختلاف التأثيرين إلى اختلاف نوعي المورينجا في المحتوى الأيضي من المركبات الأيضية الهامة. نستخلص من هذه الدراسة أن مستخلصات أوراق المورينجا تعد هامة لتخفيف حدة الأجهاد الملحي بالريحان وتوصي الدراسة باستخدام النوعين معا بالتبادل لمزيد من المقاومة ولإنتاجية أفضل.