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Improving Salt Tolerance of *Helianthus annuus* (L.) Plants by *Moringa oleifera* Leaf extract

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TWo Field experiments were conducted to study the effect of seed soaking and/or foliar application with *Moringa oleifera* leaf extract (MLE; 1 extract paste: 30 tap water) on growth, physio-chemical attributes, anatomy and yield of sunflower (*Helianthus annuus* L.) plants grown on a sandy loam soil (EC = 6.42 – 6.48 dS m^{-1}). The MLE application used as seed soaking or foliar spray significantly increased growth characteristics (*i.e.,* shoot length, number and area of leaves per plant, and shoot dry weight), physio-chemical attributes (*i.e.,* Relative water content (RWC%) and membrane stability index (MSI%), concentrations of total chlorophylls, total carotenoids, total soluble sugars, free proline and ascorbic acid, contents of N, P, K and Ca, and ratios of K/Na, Ca/Na and K+Ca/Na), antioxidant enzymes (superoxide dismutase, ascorbate peroxidase and glutathione reductase), seed yield, and seed oil and protein contents, when compared with the controls (seed soaking and foliar spray using tap water). Further, the MLE application used as seed soaking in combination with foliar spray significantly increased all aforementioned parameters and improved stem anatomy compared to the control and the single (seed soaking or foliar spray) treatments with MLE. In contrast, there were significant reductions in leaf electrolyte leakage (EL%), Na% and the enzyme catalase activity. The combined treatment of seed soaking and foliar spray with MLE was found to be highly effective in improving the growth and yield of sunflower plants by alleviating the inhibitory effects of soil salinity stress.

Keywords: *Helianthus annuus* (L.), *Moringa oleifera* leaf extract, Salt stress, Growth, Anatomy and productivity, Physio-chemical attributes.

Sunflower (*Helianthus annuus* L.) is one of the most important edible oil crops worldwide, where its seeds contain a high concentration of polyunsaturated fatty acids. The production of vegetable oils in Egypt is so limited and fails to meet the increasing consumption of vegetable oil produced mainly from cotton seeds. However, the expansion of area devoted to cotton cultivation seemed to be hard due to limited cultivated area, intensive crop rotation and others. Thus, increasing production of vegetable oils must depend on the alternative oil crops such as sunflower cultivated in the newly reclaimed soils in Egypt.

Salinity stress is one of the major factors limitation to crop productivity, particularly in the arid and semiarid regions. The salt stress have adverse effects on plant growth and development by altering physiological and cellular processes (Munns, 2002; 2011 and Munns & Tester, 2008). Excessive amount of soluble salts in the root environment causes osmotic stress, which is related to uptake and utilization of essential nutrients and also in toxic ion accumulation. Salinity reduces the ability of plants to utilize water and causes a reduction in the growth and yield due to the changes in the plant metabolic processes (Munns, 2002). Plants grown under saline conditions are stressed basically in three ways; rhizosphere water deficit, phytotoxicity of Na^+ and Cl^- ions and nutrient imbalance by the reduction in the uptake and/or shoot transport (Munns & Termaat, 1986 and Marschner, 1995).

Moringa is one of the 13 species of genus *Moringa* and family Moringnance. Its leaves are potential source of vitamin A and C, iron, calcium, riboflavin, beta-carotene and phenolic acid (Nambiar *et al.,* 2005). Also, The MLE obtained from fresh moringa leaves posses' high antioxidant activity is rich in some plant secondary metabolites and osmoprotectants (Rady *et al.,* 2013). MLE having a number of plant growth promoters such as zeatin, a natural derivative of cytokinin. Also, contains mineral nutrients and vitamins in a naturally balanced composition, may be beneficial for plant growth and development. They found MLE regulated metabolic/ physiological processes of crops subjected to a biotic stress including salinity, resulting from keeping optimum tissue water status, improved membranes stability, enhanced antioxidant levels and activated plant defense system, increased levels of plant secondary metabolites, reduced uptake of undesirable Na+ and/or Cl−, and enhanced shoot or leaf K+ (Yasmeen *et al.,* 2012 and Rehman *et al.,* 2014).

The objective of the present work was to evaluate the potential effects of the exogenous application of MLE on the changes in growth, anatomy, yields, endogenous physio-chemical constituents and the antioxidant defence system of *Helianthus annuus* (L.) plants, exposed to soil salinity stress (EC = 6.42 – 6.48 dS m^{-1}) and to establish a relationship between the changes in physio-chemical constituents accompanied with the antioxidants and the degree of tolerance, in terms of improvement in growth and yield and its quality. The hypothesis tested is that exogenous applications of MLE, used as seed soaking and/or foliar spray, will elevate the level of some non-enzymatic antioxidants, antioxidant enzymes and osmoprotectants that will overcome the stress generated by salinity stress.

Materials and Methods

Soil analysis and preparation, plant material and experimental procedures

Two field experiments were conducted in two successive seasons of 2013 and 2014 at the Experimental Farm of Faculty of Agriculture, Fayoum University, Fayoum (29° 17'N; 30° 53'E), Egypt. In the first season, daily temperatures ranged from 15.4º – 26.5ºC, with an average of 20.0º ± 2.5ºC. The daily relative humidity averaged 56.0 ± 4.0 %, and ranged from 28 – 83%. In addition, the daily

temperatures ranged from 13.6º – 28.6ºC, with an average of 21.1º ± 2.6ºC, and the daily relative humidity averaged 55 ± 6.5% and ranged from 26 – 82% were for the second season.

Healthy sunflower (*Helianthus annuus* L. cv. "Sakha 53") seeds were sown on 27 April 2013, and on 23 April 2014. Seeds were obtained from the Field Crop Research Institute, Agricultural Research Centre, Giza, Egypt. Seeds were selected for uniformity by choosing those of equal size and of the same color. The selected seeds were washed with distilled water, sterilized in 1% (v/v) sodium hypochlorite for approx. 2 min, washed thoroughly again with distilled water, and left to dry at room temperature (20 ºC). Uniform, air-dried seeds were sown, after soaking in water or in *Moringa oleifera* leaf extract (MLE), in hills in rows spaced 60 cm apart. The hills were spaced about 20 cm apart in 3.0 m × 3.5 m plots. Thinning was done before the first irrigation to produce two plants per hill.

During soil preparation and plant growth, the soil was supplemented with the full dose of NPK fertilizer according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. These recommendations were for 450 kg ha^{-1} of calcium super-phosphate (15.5% P_2O_5), 200 kg ha^{-1} ammonium nitrate (33.5% N), and 60 kg ha^{-1} potassium sulphate (48% K_2O) during seed-bed preparation. An additional 200 kg ha^{-1} of ammonium nitrate and 60 kg ha^{-1} of potassium sulphate were added at the first irrigation, 2 weeks after sowing. Irrigation water was added to 100% of the reference crop evapotranspiration (ETo), values from the Fayoum Governorate Meteo Station. All other recommended agricultural practices were followed according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation.

Soil analysis of the experimental site in each season was carried out according to Black *et al.* (1965) and Jackson (1967). The results from physical and chemical analyses of the soils are shown in Table 1. Electrical conductivity (EC) was measured using a conductivity meter and an extract of each soil paste. Soil EC values were 6.42 and 6.48 dS m^{-1} for 2013 and 2014 seasons, respectively. These EC values classed the soils as being moderately saline according to Dahnke & Whitney (1988). The experiment was arranged in a randomized complete block design, with one level of each of water and MLE with three replicate plots per treatment.

Preparation, analysis and applications of moringa leaf extract (MLE)

Fresh leaves of *Moringa oleifera* were harvested from fully matured trees then air-dried, grinded and extracted. For extraction, ethyl alcohol was added to leaf powder and the mixture was put for 4 h a Rotary Shaker. Extract was purified by filtering twice through whatman No. 1 filter paper. After purification, the extract was subjected to a Rotary Evaporator to fully evaporate the alcohol. Centrifugation at 8,000 × *g* for 15 min was then conducted for supernatant. Supernatant was diluted to 30 times and used to seed soaking and foliar spray applications. The extract was analyzed and its chemical constituents are presented in Table 2.

TABLE 1. Physical and chemical properties of the experimental soil in two seasons.

Parameter	2013 season	2014 season
Clay	11.0	10.7
Silt	13.5	13.0
Sand	75.5	76.3
Soil texture	Sandy loam	
pH	7.70	7.74
EC (dS m^{-1})	6.42	6.48
Organic matter%	0.92	0.90
$CaCO_3$ (%)	5.65	5.58
CEC* ($cmol_c$ kg^{-1})	32.8	33.2
Field capacity (%)	27.6	27.2
Available water (%)	12.9	12.6
Available N (mg kg^{-1} soil)	149.5	145.2
Available P (mg kg^{-1} soil)	11.4	10.8
Available K (mg kg^{-1} soil)	141.3	135.9
Available Fe (mg kg^{-1} soil)	20.7	18.8
Available Mn (mg kg^{-1} soil)	10.5	10.9
Available Zn (mg kg^{-1} soil)	4.5	4.3

TABLE 2 . Some chemical constituents of *Moringa oleifera* leaf extract (on dry weight basis) in two seasons .

Parameter	2013 season	2014 season
Amino acids	125.9	126.8
Proline	25.1	26.9
Total soluble sugars	152.3	159.2
Ash	112.5	113.9
Calcium	8.628	8.986
Magnesium	5.987	6.098
Potassium	28.75	28.89
Phosphorus	6.285	6.231
Sodium	0.689	0.675
Iron	1.985	1.895
Manganese	0.987	1.110
Zinc	0.468	0.499
Copper	0.210	0.230
Soluble phenols	2.248	2.155
Total carotenoids	2.255	2.462
Total chlorophyll	4.724	4.989
Ascorbic acid	3.302	3.450
Phytohormones (μg g^{-1} DW):		
Indole-3-acetic acid	0.885	0.910
Gibberellins	0.807	0.833
Zeatin	0.928	0.985
Abscisic acid	0.288	0.279

For seed soaking, sunflower seeds were soaked in tap water and MLE using seed weight to solution volume ratio 1:5 for 12 h at room temperature (25 °C). After soaking, seeds were given washings with water and re-dried overnight at room temperature. At early morning, treated seeds were sown as mentioned before. Foliar spray of water or MLE was done at early morning with a sprayer (Vol. 20 L) to run-off twice, at 25 and 40 days after sowing. The duration of seed soaking, the concentration of MLE, and the number and timing of sprays were based on results from a preliminary pot trial (data not shown). To ensure optimal penetration into leaf tissues, 0.1% (v/v) Tween-20 was added to the foliar sprays as a surfactant.

Plant growth analysis and yield estimations

Fifty-day-old sunflower plants (n = 9) were removed from each of the four treatments and the number of leaves plant^{-1} were counted. Shoot lengths were measured using a meter scale, then the leaf areas were measured manually using a graph sheet, where the squares covered by the leaf were counted to note the leaf area. Shoots were then placed in an oven at 70 ºC until constant weight to record the dry weights. At the end of the experiments, all heads on plants in each plot were collected, and seeds were then extracted from their heads, air-dried and weighed to record each of 100-seed weight and seed yield per hectare. Oil (%) of sunflower seeds was determined in the air-dried seeds according to the method described by A.O.A.C. (1995) using Soxhlet apparatus and petroleum ether (60 – 80°C) as solvent. Protein (%) of seeds was calculated by multiplying N (%) × 6.25 after determining N (%) of seeds using micro-Kjeldahl method described in the A.O.A.C. (1995).

Determination of leaf pigment concentrations

Total chlorophylls and carotenoids concentrations (mg g^{-1} FW) were determined according to the procedure of Cherry (1973). Leaf discs (0.2 g from each replicate-plot of each treatment) were homogenized in 50 ml 80% (v/v) acetone and centrifuged at 10,000 × *g* for 10 min. The absorbance of each acetone extract was measured at 663, 645, and 470 nm using a UV-160A UV-visible recording spectrometer (Shimadzu, Kyoto, Japan).

Determination of membrane stability index (MSI), electrolyte leakage (EL), and relative water content (RWC)

Membrane stability index % was determined using duplicate 0.2 g samples of leaf tissue that were placed in test tubes containing 10 ml of double-distilled water (Rady, 2011). One sample was heated at 40 °C in a water bath for 30 min, and the electrical conductivity of the solution was recorded using a conductivity bridge (EC_1). The second sample was boiled at 100 °C for 10 min, and the conductivity was measured (EC_2). The MSI% was calculated using the following formula:

$$MSI\ (\%) = [1 - (EC_1/\ EC_2)] \times 100$$

The total inorganic ion leaked from leaves (EL) was determined using the method of Sullivan & Ross (1979). Twenty leaf discs were placed in a boiling

tube containing 10 ml deionized water and the electrical conductivity (EC_1) was recorded. The contents were then heated to 45°C − 55°C for 30 min each in a water bath and the electrical conductivity (EC_2) was recorded. The sample was boiled at 100 °C for 10 min and the electrical conductivity (EC_3) was recorded. EL% was calculated using the following formula:

$$EL\ (\%) = [(EC_2 - EC_1) / EC_3] \times 100$$

Excluding the midrib, fresh 2 cm-diameter fully-expanded leaf discs were used to determine the RWC% as described by Weatherly (1950) with some modifications (Osman & Rady, 2014). The discs were weighed (fresh mass; FM) and immediately floated on double-distilled water in Petri dishes for 24 hr, in the dark, to saturate them with water. Any adhering water was blotted dry and the turgid mass (TM) was measured. The dry mass (DM) was recorded after dehydrating the discs at 70°C for 48 h. The RWC% was then calculated using the following formula:

$$RWC\ (\%) = [(FM - DM) / (TM - DM)] \times 100$$

Determination of leaf concentrations of free proline, total soluble sugars and ascorbic acid

Leaf free proline concentrations (μg g^{-1} DW) were determined using the rapid colourimetric method suggested by Bates *et al.* (1973). Proline was extracted from 0.5 g of each fresh leaf sample by grinding in 10 ml 3% (v/v) sulphosalicylic acid and the mixture was then centrifuged at 10,000 × *g* for 10 min. Two ml of the supernatant was placed in a test-tube, to which 2 ml of a freshly prepared acid ninhydrin solution was added. The tubes were incubated in a water bath at 90 ºC for 30 min and the reaction was terminated in an ice bath. Each reaction mixture was extracted with 5 ml toluene and vortex-mixed for 15 sec. The tubes were allowed to stand for at least 20 min in the dark, at room temperature, to allow separation of the toluene and aqueous phases. Each toluene phase was then carefully collected into a clean test-tube and its absorbance was read at 520 nm. The free proline concentration in each sample was determined from a standard curve prepared using analytical grade proline, and expressed on a DW basis.

Total soluble sugars were extracted and determined according to Irigoyen *et al.* (1992). A 0.2 g sample of fresh leaves was homogenized in 10 ml of 96% (v/v) ethanol and washed with 5 ml 70% (v/v) ethanol. The extract was centrifuged at 3,500 × g for 10 min and the supernatant was stored at 4°C for measurement. Total soluble sugar concentrations were determined by reacting 0.1 ml of the ethanolic extract with 3 ml of freshly prepared anthrone reagent [150 mg anthrone plus 100 ml of 72% (v/v) sulphuric acid] and placed in a boiling water bath for 10 min. After cooling, the absorbance of the mixture was recorded at 625 nm using a Bausch and Lomb-2000 Spectronic Spectrophotometer.

The extraction and determination of ascorbic acid (AsA) from sunflower leaf samples were conducted following the method of Kampfenkel *et al.* (1995).

Plant leaf material (1.0 g) was obtained from each replicate-plot of each treatment, homogenized immediately in liquid N_2 and extracted with 10 ml 5% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 4°C for 5 min at 15,600 × *g*. The supernatant was transferred to a clean reaction vessel and immediately assayed for AsA content in a 1.0 ml reaction mixture containing 50 µl 10 mM DTT, 100 µl 0.2 M phosphate buffer (pH 7.4), 0.5% (v/v) Nethylmaleimide, 10% (w/v) TCA, 42% (v/v) H_3PO_4, 4% (v/v) 2,2'-dipyridyl, and 3% (w/v) $FeCl_3$.

Antioxidant enzyme activities

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assessed by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) (Giannopilitis & Ries, 1977; Beyer & Fridovicht, 1987 and Yu *et al.,* 1998). One unit of SOD activity was defined as the amount of enzyme required for the reduction of 50% NBT. The SOD activity was expressed as A_{564} min^{-1} g^{-1} protein.

Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the consumption of H_2O_2 (Nakano & Asada, 1981). The reaction mixture consisted of 25 mM Tris-acetate buffer, pH 7.0, 0.8 mM Na-EDTA and 20 mM H_2O_2. The enzyme assay was performed at 25 °C. CAT activity was expressed as A_{290} min^{-1} g^{-1} protein.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined following the method described by Rao *et al.* (1996) by recording the optical density at 290 nm and the activity was expressed as A_{290} min^{-1} g^{-1} protein.

Glutathione reductase (GR; EC 1.6.4.1) activity was measured after monitoring the oxidation of NADPH for three absorbance taken at 340 nm, and the activity was expressed as A_{340} min^{-1} mg^{-1} protein (Rao *et al.,* 1996).

Protein was estimated in crude enzyme extracts by dye binding assay (Bradford, 1976).

Determination of leaf contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and sodium (Na)

The content of N (%) in dried sunflower leaf was determined by using micro-Kjeldahl method described in the A.O.A.C. (1995). The molybdenum-reduced molybdophosphoric blue color method (Jackson, 1967), in sulphuric acid (with reduction to exclude arsenate), was used to determine P content (%). Sulphomolybdic acid (molybdenum blue), diluted sulphomolybdic acid, and 8% (w/v) sodium bisulphite-H_2SO_4 solution were used as reagents. Leaf Ca^{2+} content was determined using a Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer (Chapman & Pratt, 1961). The contents of K^+ (%) and Na^+ (%) were determined using 0.2 g of dried leaf that was digested with sulphuric acid in the presence of H_2O_2 (Wolf, 1982). The mixture was then diluted with

distilled water. The total leaf contents of Na^+ and K^+ were measured using Flame Spectrophotometry (Lachica *et al.,* 1973).

Anatomical study

During the first season at the age 35 days after sowing, samples were taken for anatomical study. Specimens (0.5 cm in length) from the middle part of the 3*rd* leaf were killed and fixed in an FAA solution (10 ml formalin + 5 ml glacial acetic acid + 50 ml ethylalcohol 95% + 35 ml distilled water) for 72 h. Thereafter, samples were washed in 50% ethyl alcohol, dehydrated, cleared in n-butyl alcohol series and embedded in paraffin wax of 56 58 °C m.p. Cross sections of 20µ thick were cut, using a rotary microtom, adhered to slides by Haupt's adhesive. Slides were then stained with the Crystal violet erythrosine combination, cleared in carbol xylene and mounted in Canada balsam. Slides were microscopically analyzed and sections were microphotographed. An average of five readings was calculated by using a micrometer eyepiece (Nassar & El-Sahhar, 1998).

Statistical analysis

The values for all parameters were subjected to statistical analysis, following the standard procedures described by Gomez & Gomez (1984). The '*F*' test was applied to assess the significance of each treatment at the 5% level of probability ($P \leq 0.05$).

Results

Growth characteristics (*i.e.,* shoot length, number of leaves per plant, leaf area per plant and shoot dry weight per plant) of salt-stressed sunflower plants were positively affected by *Moringa oleifera* leaf extract (MLE), used as seed soaking and/or foliar application over two seasons as shown in Table 3. The single MLE applications, used as seed soaking or foliar application significantly increased all growth traits compared to the controls (seed soaking or foliar spray with tap water). Further, combined MLE application (seed soaking + foliar spray) significantly increased all aforementioned growth characteristics compared to the control and the single MLE (seed soaking or foliar spray) applications. Combined MLE treatment found to be the highly effective in increasing shoot length, number of leaves per plant, leaf area per plant and shoot dry weight per plant by 21.3%, 20.7%, 21.0% and 25.8%, respectively in 2013 season and by 25.2%, 20.9%, 21.3% and 28.3%, respectively in 2014 season compared to the controls.

Application of MLE used as seed soaking and/or foliar application on plant of sunflower had enhancement effects on stem anatomy compared to the stem anatomy of tap water-sprayed control plants (Table 4 and Fig. 1). In general, the best of results was obtained from application of combined MLE application (seed soaking + foliar spray) increased stem anatomical structure, *i.e.,* stem section diameter, average number of xylem vessels, average thickness of xylem vessels, average diameter of xylem vessels, average pith diameter, average number of pith layers by 59.05%, 50.4%, 34.46%, 61.87%, 67.16%, and 15.38 %, respectively.

TABLE 3. Effect of seed soaking and/or foliar spray with moringa leaf extract (MLE) on some growth traits of sunflower (*Helianthus annuus* L. cv. "sakha 53") plants grown under moderate soil salinity in two seasons.

Treatments		Parameters			
Seed soaking	**Foliar spray**	**Shoot length (cm)**	**Number of leaves plant^{-1}**	**Leaf area plant^{-1} (dm^2)**	**Shoot dry weight (g)**
2013 season					
Tap water	Tap water	73.2c	16.4c	12.4c	29.1c
	MLE	79.5b	17.9b	13.6b	32.5b
MLE	Tap water	80.4b	18.2b	13.8b	33.0b
	MLE	88.8a	19.8a	15.0a	36.6a
2014 season					
Tap water	Tap water	71.8c	16.3c	12.7c	28.6c
	MLE	79.0b	17.7b	14.0b	32.2b
MLE	Tap water	79.8b	17.9b	14.2b	32.8b
	MLE	89.9a	19.7a	15.4a	36.7a

†Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $P \leq 0.05$ by Duncan's multiple range test.

TABLE 4. Effect of seed soaking and/or foliar spray with moringa leaf extract (MLE) on the anatomical structure of sunflower (*Helianthus annuus* L. cv. "sakha 53") stem plants grown under moderate soil salinity.

Treatments		Parameters		
Seed soaking	**Foliar spray**	**Section diameter µ**	**Average number of xylem vessels**	**Average thickness of xylem vessels µ**
Tap water	Tap water	2625.0	12.50	142.5
	MLE	3375.0	12.75	173.3
MLE	Tap water	4062.5	16.50	180.0
	MLE	4175.0	18.80	191.6
		Average diameter of xylem vessels µ	**Average pith diameter µ**	**Average number of pith layers**
Tap water	Tap water	27.80	1675.0	26
	MLE	41.25	2387.5	27
MLE	Tap water	42.50	2650.0	28
	MLE	45.00	2800.0	30

Mean values (n = 5). µ = micrometer; Measurements were made in 35- day-old plants.

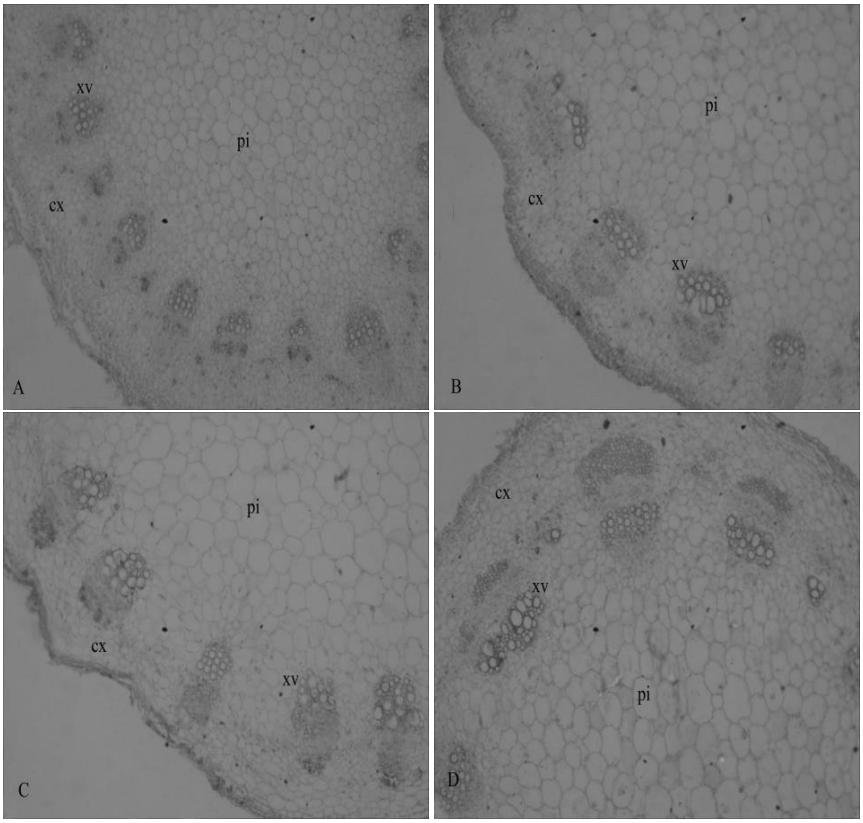

Fig. 1. Effect of seed soaking and/or foliar spray with moringa leaf extract (MLE) on the anatomical structure of sunflower (*Helianthus annuus* L. cv. "sakha 53") stem plants grown under moderate soil salinity. A: Control (tap water seed soaking+ foliar spray with tap water). B:Tap water seed soaking+ foliar spray with MLE. C: MLE seed soaking+ foliar spray with tap water . D: MLE seed soaking+ foliar spray with MLE). (cx = cortex, xv= xylem vessel, Pi= Pith). X = 89 µ.

All physio-chemical attributes shown in Table 5 (*i.e.*, concentrations of total chlorophylls and total carotenoids, RWC%, EL% and MSI%), Table 6 (*i.e.*, concentrations of total soluble sugars, free proline and ascorbic acid), Table 7 (*i.e.*, superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase) Table 8 (*i.e.*, contents of N, P, K and Ca) and Table 9 (*i.e.*, ratios of K/Na, Ca/Na and K+Ca/Na), in addition seed yield and its quality shown in Table 10 were behaved the same trend of growth characteristics. Combined MLE treatment (seed soaking + foliar spray) found to be the best, generating the best physio-chemical attributes and enzymatic activity in both 2013 and 2014 seasons.

TABLE 5. Effect of seed soaking and/or foliar spray with moringa leaf extract (MLE) on leaf photosynthetic pigments (mg g^{-1} fresh weight), relative water content (RWC %), electrolyte leakage (EL %) and membrane stability index (MSI %) of sunflower (*Helianthus annuus* L. cv. "sakha 53") plants grown under moderate soil salinity in two seasons .

Treatments		Parameters				
Seed soaking	**Foliar spray**	**Total chlorophylls**	**Total carotenoids**	**RWC (%)**	**EL (%)**	**MSI (%)**
2013 season						
Tap water	Tap water	1.63c†	0.37c	65.9c	13.15a	59.9c
	MLE	1.93b	0.48b	72.1b	10.55b	66.6b
MLE	Tap water	1.96b	0.51b	72.4b	10.31b	67.4b
	MLE	2.28a	0.60a	79.1a	7.87c	74.4a
2014 season						
Tap water	Tap water	1.68c	0.39c	67.7c	13.21a	60.5c
	MLE	1.95b	0.53b	74.6b	10.94b	67.5b
MLE	Tap water	2.01b	0.56b	75.4b	10.73b	67.6b
	MLE	2.25a	0.67a	83.1a	7.83c	76.0a

†Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $P \leq 0.05$ by Duncan's multiple range test.

TABLE 6. Effect of seed soaking and/or foliar spray with moringa leaf extract (MLE) on the shoot concentrations of total soluble sugars, free proline and ascorbic acid (AsA) of sunflower (*Helianthus annuus* L. cv. "sakha 53") plants grown under moderate soil salinity in two seasons .

Treatments		Parameters		
Seed soaking	**Foliar spray**	**Total soluble sugars (mg g^{-1} DW)**	**Free proline (µg g^{-1} DW)**	**AsA (µg g^{-1} FW)**
2013 season				
Tap water	Tap water	3.40c†	261.8c	12.4c
	MLE	3.84b	351.4b	22.7b
MLE	Tap water	3.88b	371.7b	23.1b
	MLE	4.34a	425.1a	31.8a
2014 season				
Tap water	Tap water	3.72c	283.3c	13.2c
	MLE	4.22b	374.3b	23.3b
MLE	Tap water	4.27b	395.5b	24.1b
	MLE	4.82a	450.6a	34.2a

†Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $P \leq 0.05$ by Duncan's multiple range test.

TABLE 7. Effect of seed soaking and/or foliar spray with moringa leaf extract (MLE) on superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) activities in leaves of sunflower (*Helianthus annuus* L. cv. "sakha 53") plants grown under moderate soil salinity in two seasons .

Treatments		Parameters			
Seed soaking	**Foliar spray**	**SOD (A_{564} min^{-1} g^{-1} protein)**	**CAT (A_{290} min^{-1} g^{-1} protein)**	**APX (A_{290} min^{-1} g^{-1} protein)**	**GR (A_{340} min^{-1} g^{-1} protein)**
2013 season					
Tap water	Tap water	5.20c†	99.5a	41.7c	25.9c
	MLE	7.04b	78.6b	56.0b	33.0b
MLE	Tap water	7.33b	76.3b	58.9b	36.2b
	MLE	8.85a	63.0c	89.0a	46.7a
2014 season					
Tap water	Tap water	6.28c	96.9a	51.6c	28.4c
	MLE	7.78b	84.4b	76.4b	37.4b
MLE	Tap water	8.03b	79.9b	79.9b	39.1b
	MLE	9.55a	60.3c	94.8a	57.7a

†Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $P \leq 0.05$ by Duncan's multiple range test.

TABLE 8. Effect of seed soaking and/or foliar spray with moringa leaf extract (MLE) on the contents of macro-nutrients (N, P, K and Ca) and sodium (Na) of sunflower (*Helianthus annuus* L. cv. "sakha 53") plants grown under moderate soil salinity in two seasons .

Treatments		Parameters				
Seed soaking	**Foliar spray**	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Na (%)**
2013 season						
Tap water	Tap water	2.70c†	0.29c	2.91c	1.24c	0.74a
	MLE	3.07b	0.33b	3.38b	1.40b	0.62b
MLE	Tap water	3.04b	0.35b	3.47b	1.43b	0.59b
	MLE	3.42a	0.40a	3.84a	1.55a	0.39c
2014 season						
Tap water	Tap water	2.77c	0.28c	2.85c	1.22c	0.71a
	MLE	3.08b	0.33b	3.46b	1.43b	0.58b
MLE	Tap water	3.13b	0.36b	3.57b	1.47b	0.55b
	MLE	3.43a	0.41a	3.96a	1.60a	0.35c

†Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $P \leq 0.05$ by Duncan's multiple range test.

TABLE 9. Effect of seed soaking and/or foliar spray with moringa leaf extract (MLE) on nutrient relations with Na in sunflower (*Helianthus annuus* L. cv. "sakha 53") plants grown under moderate soil salinity in two seasons .

Treatments		Parameters		
Seed soaking	**Foliar spray**	**K/Na ratio**	**Ca/Na ratio**	**K+Ca/Na ratio**
2013 season				
Tap water	Tap water	3.93c†	1.68c	5.61c
	MLE	5.45b	2.26b	7.71b
MLE	Tap water	5.88b	2.42b	8.30b
	MLE	9.85a	3.97a	13.82a
2014 season				
Tap water	Tap water	4.01c	1.72c	5.73c
	MLE	5.97b	2.47b	8.44b
MLE	Tap water	6.49b	2.67b	9.16b
	MLE	11.31a	4.57a	15.88a

†Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $P \leq 0.05$ by Duncan's multiple range test.

TABLE 10. Effect of seed soaking and/or foliar spray with moringa leaf extract (MLE) on 100-seed weight and seed yields of sunflower (*Helianthus annuus* L. cv. "Sakha 53") plants grown under moderate soil salinity in two seasons .

Treatments		Parameters			
Seed soaking	**Foliar spray**	**100-seed weight (g)**	**Seed yield (ton ha^{-1})**	**Seed oil (%)**	**Seed protein (%)**
2013 season					
Tap water	Tap water	4.15c†	1.64c	23.9c	12.7c
	MLE	5.70b	2.27b	26.8b	14.0b
MLE	Tap water	5.98b	2.33b	27.4b	14.5b
	MLE	7.33a	2.86a	29.7a	16.3a
2014 season					
Tap water	Tap water	4.28c	1.67c	25.0c	13.0c
	MLE	6.10b	2.48b	27.9b	14.8b
MLE	Tap water	6.38b	2.55b	28.5b	15.2b
	MLE	7.77a	3.03a	30.5a	16.9a

†Mean values in each column for each year followed by a different lower-case letter are significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Similarly, the combined treatment of seed soaking + foliar spray with MLE found to be the highly effective in increasing 100-seed weight, seed weight per hectare, seed oil content and seed protein content by 76.6%, 74.4%, 24.3% and 28.3%, respectively in the first season and by 81.5%, 81.4%. 22.0% and 30.0%, respectively in the second season compared to the controls. The significant improvements in the sunflower seed yield and its quality and the significant enhancements in the growth characteristics of sunflower plants were

accompanied with significant increases in the concentrations of total chlorophylls, total carotenoids, total soluble sugars, free proline and ascorbic acid by 39.9%, 62.2%, 27.6%, 62.4% and 156.5%, respectively in 2013 season and by 33.9%, 71.8%, 29.6%, 59.1% and 159.1%, respectively in 2014 season under the best combined treatment of seed soaking + foliar spray with MLE compared to the controls. In addition, this combined treatment significantly increased RWC%, MSI%, N%, P%, K%, Ca% and K+Ca/Na ratio by 20.0 %, 24.2%, 26.7%, 37.9%, 32.0%, 25.0% and 146.3%, respectively in the first season and by 22.7%, 25.6%, 23.8%, 46.4%, 38.9%, 31.1% and 177.1%, respectively in the second season compared to the controls. The activities of the tested antioxidant enzymes; SOD, APX and GR were also increased significantly by 70.2%, 100.4% and 80.3%, respectively in the first season, and by 52.1%, 83.7% and 103.7%, respectively in the second season as a result of the combined MLE treatment (seed soaking + foliar spray) compared to the controls. In contrast, the best combined treatment of seed soaking + foliar spray with MLE significantly reduced EL%, Na% and catalase activity in sunflower leaves by 40.2%, 47.3% and 36.7%, respectively in 2013 season and by 40.7%, 50.7% and 37.8%, respectively in 2014 season compared to the controls.

Discussion

Reduction in water availability to plant roots and consequently disruption in water status of plant tissues are caused due to soil salinities. Disturbances in metabolic processes and decreases in meristematic activity and cell enlargement, coupled with an increase in respiration rate due to the higher energy requirements are also some of the inhibitory effects of soil salinities on growth and productivity of plants (Kaydan & Okut, 2007 and Abdul Qados, 2015). In addition, salinity stress causes over-production of reactive oxygen species (ROS) in plant tissues which lead to destruction of plasma membrane and DNA and finally cell death, although a balance between the generation and degradation of ROS is required to avoid oxidative injury and to maintain metabolic functions under stress conditions. In plant tissues, the level of ROS is controlled by an antioxidant system that consists of antioxidant enzymes and non-enzymatic low molecular weight antioxidant molecules, including proline, ascorbic acid and carotenoids (Schutzendubel & Polle, 2002 and Semida & Rady, 2014a, 2014b). In the present study, the reduction in plant growth and yield and its quality under the adverse conditions of soil salinity (controls of Tables 3 and 10) could be attributed to the osmotic effect resulting from salt stress that causes increase of growth inhibitors (*i.e.*, abscisic acid), decrease of growth promoters (*i.e.*, indole-3-acetic acid gibberellins) and disturbance of the water balance of saline-stressed plants. These inhibitory effects of salinity lead to stomatal closure, ionic imbalance, reduction in photosynthesis, disturbance in ionic homeostasis, accumulation of toxic ions and consequently inhibition of growth (Qiu *et al.*, 2007; Rady, 2011; Rady *et al.*, 2013 and Semida & Rady, 2014b).

Seed soaking and/or foliar spray for sunflower plants with *Moringa oleifera* leaf extract (MLE; 1 extract paste: 30 water by volume) significantly improved plant growth characteristics and plant productivity and its quality as well as physio-chemical attributes under the adverse conditions of the studied soil salinity. It has been shown from the results of this study that the combined treatment of seed soaking plus foliar spray with MLE exhibited significant increments than single MLE treatments (*i.e.*, seed soaking or foliar spray).

Data shown in Table 2 is analysed of MLE and that revealed presence of essential macro- and micro-nutrients such as Ca, Mg, K, P, Fe, Mn, Cu and Zn. The MLE also contains antioxidants including proline, soluble phenolics, total carotenoids and ascorbic acid coupled with amino acids including proline, total soluble sugars and K as osmoprotectants. It is also rich in phytohormones such as indole-3-acetic acid (IAA), gibberellins (GAs) and zeatin as a cytokinin (Table 2). This diverse composition of MLE indicates that this extract can be used as a plant biostimulant. Many researches highlighted the role of MLE to improve plant growth and development in different crops (Yasmine *et al.*, 2012; Rady *et al.*, 2013 and Rehman *et al.*, 2014), which is also evident from results of the present study. Improved seedling growth traits (*i.e.*, shoot length, number and area of leaves per plant, and shoot dry weight per plant) by the MLE application might be due to the enhanced mobilization of germination related metabolites/inorganic solutes such as zeatin, ascorbic acid, Ca and K presented in the MLE (Table 2) to the growing plumule and/or the increase in amylase activity and reducing sugars, contributing to early vigor and increased plant growth (Foidl *et al.*, 2001 and Afzal *et al.*, 2012). In addition, the increased MLE content of IAA, GAs and zeatin encouraged plant growth and productivity and its quality under salt stress conditions. Seed soaking and/or seedling foliar application with MLE might provide strong and energetic start for earlier emergence and completed other phenological events well in time (Rehman *et al.*, 2014). The possible reason for this acceleration of growth might be due to the enriched content of MLE of crude proteins and growth promoting hormones, that is, auxins and cytokinins (Makkar & Becker, 1996 and Moyo *et al.*, 2011). Proteins are essential for the formation of the protoplasm, while growth hormones favored rapid cell division, cell multiplication and cell enlargement. The combined treatment of seed soaking plus foliar spray with MLE showed the best results under saline condition, ameliorating the negative effects of salt stress through preventing decreases in growth characteristics, leaf photosynthetic pigments, relative water contents, membrane stability index and nutrient elements and also by inhibiting increases in leaf electrolyte leakage (Tables 3,5,6,7,8,9).

Maintenance of green leaf area and number of leaves per plant (Table 3) maximized photosynthesizing leaves, increasing sink capacity fulfilled through supply of photo-assimilates from stayed green leaves (Thomas & Howarth, 2000) and/or re-translocation of stem reserves of the present study as a result of the application of cytokinin-rich MLE that may be induced cytokinin biosynthesis. Maximum number of photosynthetic active leaves observed from

number and area of leaves per plant indicates the delayed senescence and maintaining the chlorophylls in higher concentrations (Tables 3 and 5). Presence of zeatin-like cytokinin in MLE prevents premature leaf senescence and maintains higher leaf area for photosynthetic activity. During late stage of growth, endogenous levels of cytokinin are usually decreased and exogenously-applied cytokinin (found in MLE) can delay this process (Tetley & Thimann, 1974), possibly through activation of cytokinin dependant isopentenyl transferase (*ipt*) biosynthesis, increasing chlorophyll concentrations.

The increased concentration of chlorophylls and increased growth characteristics of sunflower plants under salt stress were positively reflected in seed yield and its quality that might be attributed to more assimilate partitioning to developing edible parts and have been correlated with cytokinin levels (Zeatin) found in MLE (Dietrich *et al.*, 1995 and Rady *et al.,* 2015).

An increase in electrical conductivity of the tested soil (Table 1) indicates elevated leakiness of ions due to a loss of membrane integrity. This is an inherent feature of plants which are exposed to stresses such as salinity (Sharma *et al.,* 2011). In this study, salt stress significantly increased leaf electrolyte leakage (EL), while the single or combined applications of MLE significantly decreased it. This reduction in EL was most effective in tolerating salt stress when MLE used as a combined application of seed soaking + foliar spray. Electrical leakage enables cell membrane injury to be assessed when plants are subject to salinity stress. Maintaining integrity of cellular membranes under salt stress is considered an integral part of salinity tolerance mechanism (Stevens *et al.,* 2006). In addition, application of MLE significantly increased RWC compared with the control, with the highest increase in RWC when using MLE seed soaking combined with MLE foliar spray (Table 5). The RWC is a useful measure of the physiological water status of plants (Gonzalez & Gonzalez-Vilar, 2001) grown under a stress condition. Water stress often results when plants are grown on saline soils. The MLE is reported to increase RWC and water potential tolerance of plants to salt stress (Rady & Mohamed, 2015). This is probably due to that MLE increased the osmoprotectant concentrations (*i.e.,* total soluble sugars and proline; Table 6), which ultimately helps in maintaining better water balance in the plants.

Soluble sugars are significantly increased in salt-stressed sunflower plants by the exogenous applications of MLE (Table 6). The MLE contributes to osmotic adjustment and can directly or indirectly modulate the expression of genes involved in metabolic processes, storage functions, and defence (Hebers & Sonnewald, 1998). It has been indicated that the oxidative damage generated during salinity stress is due to the imbalance in production of ROS and antioxidant activity alterations (Hernández *et al.,* 1993). To avoid the damage caused by oxidative stress, plants have developed many antioxidant systems; among non-enzymatic ones, the accumulation of proline is one of the most frequent changes induced by salinity or drought, although there is controversy concerning whether its accumulation is a stress resistance mechanism or a mere

indicator of the existence of stress (Thakur & Sharma, 2005). One of the substrates of the Halliwell–Asada cycle is ascorbic acid (AsA) that also act as a non-enzymatic antioxidant in an isolated way on being involved in the direct reduction of ROS during different types of stress (Del Río *et al.,* 2006) taking part in the control of the H_2O_2 levels. This situation is reflected in the total concentration of AsA in our study, which are increased either with the single treatments or combined one of MLE, and its maximum concentration was noted with the combined application of MLE seed soaking plus MLE foliar application, perhaps to overcome $O_2^{\bullet-}$ accumulation, since the AsA can directly eliminate $O_2^{\bullet-}$ and H_2O_2 in a non-enzymatic way (Foyer *et al.,* 1991). The healthy metabolic state of the stressed sunflower plants pretreated (*i.e.,* seed soaking) or treated (*i.e.,* foliar spray) with MLE resulted in the healthy plant growth (Table 3). This may be attributed to that MLE is excellent source in minerals, amino acids, soluble sugars and some antioxidants (Table 2). These increased proline and AsA antioxidants supported the antioxidant system in sunflower plants to enable them to tolerate salt stress. The combined MLE treatment (seed soaking + foliar spray) caused increases in the concentrations of proline and AsA which, in turn, protected plants against the generation of ROS and membrane injury, or may resulted in the synthesis of other substances having a protective effect on plants grown under salt stress (Xu *et al.,* 2008).

Several studies have indicated that the oxidative damage generated during salinity stress is due to the imbalance in production of ROS and antioxidant activity alterations (Hernández *et al.,* 1993). To avoid the damage caused by oxidative stress, plants have developed many antioxidant systems; among enzymatic ones, SOD constitutes the first line of defence against ROS (Alscher *et al.,* 2002) by reducing the $O_2^{\bullet-}$ radical to H_2O_2. Hydrogen peroxide can serve as a substrate for numerous enzymes such as CAT which in turn though located in the peroxysomes where the H_2O_2 concentration is very high, is absent in the cytosol and chloroplasts, and thus H_2O_2 is eliminated by peroxidases. These include APX, which is considered one of the most important enzymes in the reduction of this reactive molecule (Feierabend, 2005 and Foyer, 1996). Foyer & Noctor (2009) described the regenerating enzymes DHAR and GR as a fundamental part of the Halliwell–Asada cycle, as they formed part of the regeneration of AsA from DHA using GSH as a reducing power. In turn, the reduced glutathione (GSH) consumed can be regenerated from its oxidized form (GSSG) by the reaction of GR (Foyer *et al.,* 1991). Data of the present study show that all the treatments significantly increased the activities of SOD, APX and GR compared to the controls (Table 7); this being more pronounced in the case of the combined MLE treatment (seed soaking + foliar spray).

The increased accumulation of Na^+ ions in salt-stressed plants can disturb or upset the ionic balance, inducing a nutritional imbalance due to the blockage of other cations such as N, P, K and Ca tested in the present study or anions such as NO_3^- and thereby the induction of nutritional deficiency symptoms (Sariam *et al.,* 2002). The maintenance of the ionic homeostasis under salt stress is prerequisite to protect the plant against the build-up of toxic ions, with K^+ and

Ca^{2+} accumulating and Na^{+} reaching the minimum content in sunflower leaves (Table 8). Thus, the control of Na^{+} accumulation and therefore a high K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios (Table 9) may strengthen salinity tolerance (Cuartero & Fernández-Munoz, 1999).

In this study, salt stress tolerance in sunflower plants was improved with the mitigated antioxidant system, including non-enzymatic antioxidants (*i.e.,* carotenoids, free proline and AsA) by the application of minerals, AsA, cytokinins, GAs and IAA-containing MLE (Table 1) applied alone (seed soaking or foliar spray) or in combination (seed soaking + foliar spray). *Moringa oleifera* leaves is a rich source in zeatin (Foidl *et al.,* 2001), minerals and other phytohormones, so the effectiveness of MLE in mitigating salinity stress by better chlorophyll, antioxidants and plant growth might be due to cytokinin mediated stay green effect. The combined MLE as the best treatment supported the sunflower antioxidant defence system through increase of carotenoids, free proline and AsA concentrations, maintaining tissue water balance and ionic homeostasis.

Conclusion

Results of this study demonstrated that the negative effects of salt stress on the growth and productivity and its quality of sunflower plants could be modulated by the exogenous application of *Moringa oleifera* leaf extract (MLE) that could stimulate the plants against injuries resulted from salt stress. Soaking sunflower seeds plus to seedling foliar spray with MLE diluted up to 30 times was the most effective in stimulating sunflower plants for salt tolerance under moderate soil salinity (EC = 6.42 – 6.48). The combined MLE treatment increased accumulation of K^{+} and Ca^{2+} ions, increased concentrations of carotenoids, free proline and AsA as antioxidants and soluble sugars with free proline as osmoprotectants and reduced level of Na^{+} ions under salt stress. In addition, this best treatment improved the activity of antioxidant enzymes, reflecting in maintaining cell membrane integrity, tissue water balance and ion homeostasis, and improving plant growth, stem anatomical structure and productivity and its quality. Thus, could be used MLE combined application (seed soaking + foliar spray) as growth enhancer to stimulate growth and low productivity losses under salt stress.

References

A.O.A.C. (1995) *"Official Methods of Analysis of the Association of Official Agricultural Chemists"*, 16th ed., Washington, DC, USA.

Abdul Qados, A.M.S. (2015) Effects of salicylic acid on growth, yield and chemical contents of pepper (*Capsicum annuum* L.) plants grown under salt stress conditions. *International Journal of Agriculture and Crop Sciences,* **8** (2), 107–113.

Afzal, I., Hussain, B., Basra, S.M.A. and Rehman, H. (2012) Priming with MLE reduces imbibitional chilling injury in spring maize. *Seed Science and Technology* , **40**(2), 271–276.

Alscher, R.G., Erturk, N. and Heath, L.S. (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of Experimental Biology*, **53,** 1331–1341.

Bates, L.S., Waldeen, R.P. and Teare, I.D. (1973) Rapid determination of free proline for water stress studies. *Plant and Soil*, 3**9,** 205–207.

Beyer, W.F. and Fridovicht, I. (1987) Assaying for the superoxide dismutase activity: Some larges consequences of minor changes in conditions. *Analytical Biochemistry*, **161**, 559–566.

Black, C.A., Evans, D.D., Ensminger, L.E., White, L.L. and Clark, E. (1965) "*Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties"*. American Society of Agronomy. Madison , WI, USA, pp. 771–1569.

Bradford, M.M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72,** 248–254.

Chapman, H.D. and Pratt, P.F. (1961) *"Methods of Analysis for Soil, Plants and Water"*. University of California, Division of Agricultural Science, Berkeley, CA, USA, pp. 56–63.

Cherry, J.H. (1973) *"Molecular Biology of Plants (A Text Manual)"*. Columbia Univ. Press, New York.

Cuartero, J. and Fernández-Munoz, R. (1999) Tomato and salinity. *Scientia Horticulturae*, **78**, 83–125.

Dahnke, W.C. and Whitney, D.A. (1988) Measurement of soil salinity. In: "*Recommended Chemical Soil Test Procedures for the North Central Region"*. (Dahnke, W.C., Ed.). pp.32–34. North Central Regional Publication 221. North Dakota Agricultural Experiment Station Bulletin 499.

Del Río, L.A., Sandalio, L.M. and Corpas, F.J. (2006) Reactive oxygen species and reactive nitrogen species in peroxisomes. Production scavenging, and role in cell signaling. *Plant Physiology*, **141**, 330–335.

Dietrich, J.T., Kaminek, V., Belvins, D.G., Reinbett, T.M. and Morris, R.D. (1995) Changes in cytokinins and cytokinin oxidase activity in developing maize kernel and the effects of exogenous cytokinin on kernel development. *Plant Physiology and Biochemistry* , **33**, 327–336.

Feierabend, J. (2005) Catalases in plants: Molecular and functional properties and role in stress defense. In: *"Antioxidants and Reactives Oxygen Species in Plants"*. Smirnoff, N. (Ed.). pp. 101–140. Blackwell Publishing, London, UK.

Foidl, N., Makkar, H.P.S. and Becker, K. (2001) The potential of *Moringa oleifera* for agricultural and industrial uses. In: *Proceedings of the International Workshop What development potential for Moringa products?*, Dar-es-Salaam, Tanzania, pp. 47–67.

Foyer, C.H. (1996) Free radical processes in plants. *Biochemical Society Transactions*, **24**, 427–434.

Foyer, C.H. and Noctor, G. (2009) Redox regulation in photosynthetic organisms: Signaling, acclimation and practical implications. *Trends in Plant Science*, **6**, 486–492.

Foyer, C.H., Lelandais, M., Edwards, E.A. and Mullineaux, P.M. (1991) The role of ascorbate in plants, interactions with photosynthesis and regulatory significance. In: *"Active Oxygen/Oxidative Stress and Plant Metabolism"*. Pell, E., Steffen, K. (Ed.), pp. 131–144.American Society of Plant Physiology, Rockville, MD.

Giannopilitis, C.N. and Ries, S.K. (1977) Superoxide dismutase occurrence in higher plants. *Plant Physiology*, **59**, 309–314.

Gomez, K.A. and Gomez, A.A. (1984) *"Statistical Procedures for Agricultural Research"*, 2nd ed. p. 680. John Wiley & Sons, Singapore.

Gonzalez, L. and Gonzalez-Vilar, M. (2001) Determination of relative water content. In: *"Handbook of Plant Ecophysiology Techniques"*. Reigosa, M.J. (Ed). pp 207–212. Kluwer Academic, Dordrecht.

Hebers, K. and Sonnewald, V. (1998) Altered gene expression: Brought about by inter and pathogen interactions. *Journal of Plant Research* , **111**, 323–328.

Hernández, J.A., Francisco, F.J., Corpas, G.M., Gómez, L.A. and Del Río, F.S. (1993) Salt induced oxidative stresses mediated by activated oxygen species in pea leaves mitochondria. *Plant Physiology*, **89**, 103–110.

Irigoyen, J.J., Emerich, D.W. and Sanchez-Diaz, M. (1992) Water stress induced changes in the concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia Plantarum*, **8**, 455–460.

Jackson, M.L. (1967) *"Soil Chemical Analysis"*. Prentice Hall of India Pvt. Ltd, New Delhi, India, pp. 144–197, 326–338.

Kampfenkel, K., Van Montagu, M. and Inze, D. (1995) Extraction and determination of ascorbate and dehydroascorbate from plant tissue. *Analytical Biochemistry*, **225**, 165–167.

Kaydan, D. and Okut, M.Y. (2007) Effects of salicylic acid on the growth and some physiological characters in salt stressed wheat (*Triticum aestivum* L.). *Tarim Bİlimleri Dergisi*. **13**(2), 114–119.

Lachica, M., Aguilar, A. and Yanez, J. (1973) Analisis foliar. Métodos utilizados enla EstaciLn Experimental del Zaidin. *Anales de Edafologia y Agrobiologia*, **32**, 1033–1047.

Makkar, H.P.S. and Becker, K. (1996) Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Animal Feed Science and Technology*, **63**, 211–228.

Marschner, H. (1995) *"Mineral Nutrition of Higher Plants"*, 2nd ed. Academic Press, London.

Moyo, B., Masika, P.J., Hugo, A. and Muchenje, V. (2011) Nutritional characterization of Moringa (*Moringa oleifera* Lam) leaves. *African Journal of Biotechnology*, **10**(60), 12925–12933.

Munns, R. (2002) Comparative physiology of salt and water stress. *Plant, Cell & Environment* , **25**, 239–250.

Munns, R. (2011) Plant Adaptations to salt and water stress: Differences and commonalities. *Advances in Botanical Research*. **57**, 25-38.

Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **59**, 651- 681.

Munns, R. and Termaat, A. (1986) Whole-plant responses to salinity. *Australian Journal of Plant Physiology*, **13**, 143–160.

Nakano, Y. and Asada, K. (1981) Hydrogen peroxide scavenged by ascorbate specific peroxidase in spinach chloroplast. *Plant and Cell Physiology* , **22**, 867–880.

Nambiar, V.S., Mehta, R. and Daniel, M. (2005) Polyphenol content of three indian green leafy vegetables. *J. Food Sci. Technol.* **42**(6), 312-315.

Nassar, M. A. and El-Sahhar, K. F. (1998) "*Botanical Preparations and Microscopy (Microtechnique)".* Academic Bookshop. Dokki. Giza, Egypt. 219 pp. (In Arabic).

Osman, A.Sh. and Rady, M.M. (2014) Effect of humic acid as an additive to growing media to enhance the production of eggplant and tomato transplants. *Journal of Horticultural Science & Biotechnology*, **89** (3), 237–244.

Qiu, D.-L., Lin, P. and Guo, S.Z. (2007) Effects of salinity on leaf characteristics and CO_2/H_2O exchange of *Kandelia candel* (L.) druce seedlings. *Journal of Forest Science* , **53**, 13–19.

Rady, M.M. (2011) Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. *Scientia Horticulturae*, **129**, 232–237.

Rady, M.M., Bhavya Varma, C. and Howladar, S.M. (2013) Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. *Scientia Horticulturae* , **162**, 63–70.

Rady, M.M. and Mohamed, G.F. (2015) Modulation of salt stress effects on the growth, physio-chemical attributes and yields of *Phaseolus vulgaris* L. plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract. *Scientia Horticulturae*, **193,** 105–113.

Rady, M.M., Mohamed, G.F., Abdalla, A.M. and Ahmed, Yasmin H.M. (2015) Integrated application of salicylic acid and *Moringa oleifera* leaf extract alleviates the salt-induced adverse effects in common bean plants. *International Journal of Agricultural Technology*, **11**(7), 1595–1614.

Rao, M.V., Paliyath, G. and Ormrod, D.P., (1996) Ultraviolet-B radiation and ozone-induced biochemical changes in the antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology*, **110**, 125–136.

Rehman, H., Nawaz, M.Q., Basra, S.M.A., Afzal, I., Yasmeen, A. and Hassan, F.U. (2014) Seed priming influence on early crop growth, phenological development and yield performance of linola (*Linum usitatissimum* L.). *Journal of Integrative Agriculture*, **13**(5), 990–996.

Sairam, R.K., Rao, K.V. and Srivastava, G.C. (2002) Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science*, **163**, 1037–1046.

Schutzendubel, A. and Polle, A. (2002) Plant responses to abiotic stresses: Heavy metal induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany*, **53**, 1351–1365.

Semida, W.M. and Rady, M.M. (2014a) Pre-soaking in 24-epibrassinolide or salicylic acid improves seed germination, seedling growth, and anti-oxidant capacity in *Phaseolus vulgaris* L. grown under NaCl stress. *Journal of Horticultural Science & Biotechnology* , **89** (3), 338–344.

Semida, W.M. and Rady, M.M. (2014b) Presoaking application of propolis and maize grain extracts alleviates salinity stress in common bean (*Phaseolus vulgaris* L.). *Scientia Horticulturae*, **168**, 210–217.

Sharma, D., Dubey, A., Srivastav, M., Singh, A., Sairam, R., Pandey, R., Dahuja, A. and Kaur, C. (2011) Effect of putrescine and paclobutrazol on growth, physiochemical parameters, and nutrient acquisition of salt-sensitive citrus rootstock *Karna khatta* (*Citrus karma* Raf.) under NaCl Stress. *Journal of Plant Growth Regulation*, **30**, 301–311.

Stevens, J., Senaratna, T. and Sivasithamparam, K. (2006) Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. 'Roma'): associated changes in gas exchange, water relations and membrane stabilisation. *Plant Growth Regulation*, **49**, 77–83.

Sullivan, C.Y. and Ross, W.M. (1979) Selecting the drought and heat resistance in grain sorghum. In: *"Stress Physiology in Crop Plants"* (Mussel, H., Staples, R.C., Ed.). pp. 263–281. John Wiley & Sons, New York, USA.

Tetley, R.M. and Thimann, K.V. (1974) The metabolism of oat leaves during senescence: I. Respiration, carbohydrate metabolism, and the action of cytokinins. *Plant Physiology*, **54**, 294–303.

Thakur, M. and Sharma, A.D. (2005) Salt-stress-induced proline accumulation in germinating embryos: Evidence suggesting a role of proline in seed germination. *Journal of Arid Environments*, **62**, 517–523.

Thomas, H. and Howarth, C.J. (2000) Five ways to stay green. *Journal of Experimental Botany*, **51**, 329–337.

Weatherly, P.E. (1950) Studies in the water relations of cotton. 1. The field measurement of water deficits in leaves, *New Phytologist*, **49**, 81–97.

Wolf, B. (1982) A comprehensive system of leaf analysis and its use for diagnosing crop nutrients status. *Communications in Soil Science and Plant Analysis*, **13**, 1035–1059.

Xu, Q., Xu, X., Zhao, Y., Jiao, K., Herbert, S.J. and Hao, L. (2008) Salicylic acid, hydrogen peroxide and calcium-induced saline tolerance associated with endogenous hydrogen peroxide homeostasis in naked oat seedlings. *Plant Growth Regulation*, **54**, 249–259.

Yasmeen, A., Basra, S.M.A., Ahmad, R. and Wahid, A. (2012) Performance of late sown wheat in response to foliar application of *Moringa oleifera* Lam. leaf extract. *Chilean Journal of Agricultural Research*, **2**, 92–97.

Yu, Q., Osborne, L. and Rengel, Z. (1998) Micronutrient deficiency changes activities of superoxide dismutase and ascorbate peroxidase in tobacco plants. *Journal of Plant Nutrition*, **21**, 1427–1437.

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تحسين مقاومة الملوحة في نباتات عباد الشمس بواسطة مستخلص أوراق المورينجا

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أجريت تجربتان حقليتان في موسمين مختلفين لدراسة تأثير نقع البذور و/أو رش النباتات بمستخلص أوراق المورينجا (المخفف بنسبة ١عجينه:٣٠ ماء بالوزن) على النمو، الصفات الفسيوكيماويه، التشريح ومحصول نباتات عباد الشمس النامية على ارض طمييه رمليه (EC=٦,٤٢-٦,٤٨). أدى إستخدام مستخلص أوراق المورينجا كمحلول نقع للبذرة أو كمحلول رش للمجموع الخضري إلى زيادة خصائص النمو (إرتفاع النبات، عدد الأوراق، مساحة الأوراق/ نبات، الوزن الجاف للمجموع الخضري)، الصفات الفسيوكيماويه (المحتوى المائي النسبي، دليل ثبات الغشاء، تركيزات كل من الكلورفيلات الكلية، الكاروتينات الكلية، السكريات الذائبة، البرولين الحر، حامض الاسكوربيك، محتويات كل من النتروجين، الفوسفور، البوتاسيوم ، الكالسيوم، ونسب كل من البوتاسيوم/الصوديوم، الكالسيوم/الصوديوم، البوتاسيوم + الكالسيوم/الصوديوم) الأنزيمات المضادة للأكسدة (سوبر اكسيد ديسموتيز ، اسكوربيد بيروكسيديز، جلوتاثيون ريدكتيز)، محصول البذرة، محتويات البذرة من الزيت والبروتين بالمقارنة بنباتات الكنترول (لم يتم نقع بذورها ولم يتم رشها بمستخلص المورينجا). إضافة إلى ما سبق أدى استخدام مستخلص أوراق المورينجا للغرضين (نقع البذور+ الرش الورقي بمستخلص المورينجا) إلى زيادة معنوية في جميع الصفات سابقة الذكر بالاضافه إلى تحسين الخصائص التشريحية للساق بالمقارنة بالكنترول والمعاملات الفردية. على عكس ما سبق، وجدت انخفاضات معنوية في الإستنزاف الإلكترويتى للورقة، محتويات ايونات الصوديوم ونشاط إنزيم الكتاليز. وقد وجد من النتائج المتحصل عليها أن المعاملة المتكاملة (نقع البذرة+ رش المجموع الخضري بمستخلص أوراق المورينجا) كانت أفضل المعاملات تأثيرا في تحسن النمو و الانتاجيه لنباتات عباد الشمس من خلال تخفيف التأثيرات الضارة الناتجة عن ملوحة التربة.