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Using *Moringa olifera* Leaf Extract as a Bio-fertilizer for Drought Stress Mitigation of *Glycine max* L. Plants

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DROUGHT is one of a considerable environmental stresses limiting productivity of crops. This work was conducted to investigate the effect of foliar spray of plant leaf extract (*Moringa olifera*) to alleviate drought stress in *Glycine max*plants (cv.Giza 111). Drought caused significant decreases in growth parameters (shoot and root length, fresh and dry weight of shoots and roots) and photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments). In addition, it caused significant increasesi n non-enzymatic antioxidants (ascorbic acid, tocopherol and reduced glutathione), enzymatic antioxidants (glutathione reductase, superoxide dismutase and ascrobate peroxidase), oxidative damage (lipid peroxidation) and osmolyte compounds (proline, total soluble sugars and total phenols) in soybean plants. Moreover, foliar spray with *Moringa* leaf extract ($MLE_{(30)}$) enhanced all the above parameters as compared with either the control plants and drought stressed plants. It appeared that $MLE_{(30)}$ was able to enhance the tolerance of the studied plant to drought stress.

Keywords: Drought, *Moringa olifera*, Lipid peroxidation, Antioxidant enzymes, Phytohormones, Minerals.

Introduction

Droughtis abiotic stress limiting factors of plant growth and yields thus negatively affect production of over 25% of world agriculture. Deficit of soil water caused significant reduction in photochemical activities in some plants, reduction in the activities of enzymes responsible for some processes like respiration, translocation, hormone balance, macro and micro nutrients uptake and metabolism (Rohbakhsh, 2013). Drought give rise to excessive generation of some reactive oxygen species (ROS) (superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$) and singlet oxygen ($^1O_2$) as a result of the imbalance between the productions of these species and the production of the antioxidant defense system resulting in oxidative damages in plant cells (El-Tayeb, 2006) that represented in lipid peroxidation, chlorophyll bleaching, protein, DNA and RNA damages. On the other hand, plants tend to accumulate some osmo-protectants and antioxidant compounds such as proline, phenols and soluble sugars, as well as formation of scavenging radicals such as glutathione, ascorbate, α-tocopherol, flavonoids, carotenoids and some antioxidant enzymes such as glutathione reductase (GR), superoxide dismutase (SOD), ascorbate peroxides (APX) which play a protective role in scavenging ROS free radical species (El-Beltagi & Mohamed, 2013).

Soybean (*Glycine max*) is an important economical and nutritious crop plant, it has high contents of protein (40–42%), oils (18–22%) and has some secondary metabolic compounds such as phenols, flavonoids and saponins (Sakthivelu et al., 2008). Inorganic fertilizers are good source of plant nutrients but they are high cost. Thus, recent searches need to find alternative safe, effective and natural plant fertilizers. *Moringa oleifera*tree (Identified as Mother's Best Friend and Miracle Tree.) belongs to Moringaceae. It has great importance due to its parts (root, bark, gum, leaf, flower, fruit, seed and seeds oil) which have incredible effects of food, medication and industrial purposes (Moyo et al., 2011).*Moringa oleifera* leaves extract (MLE) is the most natural

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plant growth enhancer, has no cost and enhance the tolerance of plants under different environmental conditions like drought. It needs more attentions due to its high contents of proteins, antioxidants (ascorbic acid, flavonoids, phenolics,carotenoids), mineral ions (P, Ca, Fe, K, Cr, Cu, Mg, Mn and Zn), amino acids, vitamin A, vitamin C, B- complex and plant hormones especially cytokinins (zeatin) (Azra et al., 2012). Drought stress has destructive effect on the content of cytokinin in plant. On the other hand, the high level of zeatin makes *Moringa* leaves extract (MLE) more effective as a natural compound promoting plant tolerance under stress conditions (Zaki & Rady, 2015). Therefore, this work was designed with objective to evaluate the potential effects of the foliar application of aqueous leaves extract of *Moringa* (MLE) as a bio-organic fertilizer in alleviating the effects of water stress (drought) in Soybean (*Glycine max* L.) plants.

Material and Methods

Plant material, cultivation and drought imposition

Healthy *Glycine max* (cv. Giza 111) seeds were obtained from the Crop Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Soybean seeds were washed in distilled water then sterilized using ethanol (70%) for 2 min, then sodium hypochlorite (5%) ((v/v) for 10 min followed by rinsed with distilled water several times and left to air dried. Seeds are grown at 23-cm depth in 25 cm diameter pots containing equal amount of homogeneous loamy clay soil. The pots were arranged into two groups designed with two factors, MLE dilutions (30 times) and soil water levels (well-watered and drought stressed). After 7 weeks from sowing, drought stress (based on soil water holding capacity) was imposed. The irrigation levels were maintained at 80% (well-watered), 60% (moderate drought stressed) and 40% (severe drought stressed) field capacity. The plants were divided into six groups and treated as follows: Plants of the 1st group were left without any treatment to serve as control (well-watered). The 2nd group was well watered and sprayed with MLE_{30} extract. The 3rd group was irrigated with 60% of hold water capacity. The 4th group was irrigated with 60% of hold water capacity and sprayed with MLE_{30} extract. The 5th group was irrigated with 40% of hold water capacity. The 6th group was irrigated with 40% of hold water capacity and sprayed with MLE_{30} extract. The foliar application with MLE extract was carried out twice at the age of 7 and 10 weeks (20 ml / plant). After 14 weeks from sowing, five plants were collected to determine growth parameters (shoot length, root length, fresh and dry weights of shoots and roots), photosynthetic pigments, non-enzymatic antioxidants (tocopherol (vitamin E), ascorbic acid (vitamin C) and glutathione), lipid peroxidation, enzymatic antioxidant (glutathione reductase, superoxide dismutase and ascorbate peroxides), soluble sugars, proline, total phenols, phytohormones (IAA, GA_3 and ABA) and minerals in plant leaves.

Preparation and analysis of MLE extract

MLE was prepared according to procedures described by Azra et al. (2012). *Moringa oleifera* leaves were collected from the botanical garden of Faculty of Education, Ain Shams University. Leaves after storing in freezing temperature overnight were ground in water (1 / 10 wt/v). The mixture was shaken for 4 h using electrical stirrer, and then left in dark for 28 h at room temperature. The mixture was filtered using cheesecloth and the filtrate centrifuged at 8000 g for 15 min and diluted 30 times by using distilled water.

Biochemical analysis

Determination of photosynthetic pigments

Chlorophyll a,b and carotenoids were estimated by spectrophotometric method recommended by Lichtenthaler (1987) with Spekol spectrophotometer VEB Carl Zeiss at the recommended lengths. The pigments content were calculated as mg g^{-1} fresh weight of leaves.

Changes in non-enzymatic antioxidant

Determination of ascorbic acid (ASA) (vitamin C): ASA was estimated as detected by Mukherjee & Choudhuri (1983). The content was expressed in µg g^{-1} fresh weight. A sample of fresh weight leaves (2g) was homogonized using trichloroacetic acid (TCA) (6%). After filtration of the extract, it was centrifuged at 1000g for 20 min. and then the filtrate was adjusted to 10 ml with TCA. Four ml of the extract was added to 2 ml of acidic 2% dinitrophenyl hydrazine and few drops of 10% thiourea (in 70% ethanol). The mixture was boiled in a water bath for 15 min followed by cooling at room temperature, 5 ml of 80% H_2SO_4 (v/v) was added to the mixture in an ice bath; the obtained color was measured at 530 nm. The amount of ascorbic acid was calculated and expressed as mg g^{-1} fresh weight.

Determination of α-tocopherol (vitamin E):

α-tocopherol content was measured according to the method described by Philip et al. (1954). Five grams of fresh leaves was homogenized in

10 ml of a solution consists of petroleum ether and ethanol (2: 1.6 v/v). The extract was centrifuged at 10,000 g for 20 min. One ml of extract was mixed with 0.2 ml of 2% 2.2-dipyridyl in ethanol and kept in the dark for 5 min. The resulting color (red color) was diluted with 4 ml of distilled water, then measured at 520 nm. The α-tocopherol contents were calculated against a known standard curve using several concentrations of α-tocopherols. The content was expressed as µg/g fresh weight.

*Determination of reduced glutathione contents:*The total non-protein SH group GSH (reduced glutathione) was estimated following the method of Paradiso et al. (2008).One gram plant sample was ground with 6% meta-phosphoric acid (w/v) and 1 mM EDTA. The homogenate was centrifuged at 11.500 g for 15 min at 4°C. Then, 0.4 ml of supernatant and 0.5 M potassium phosphate buffer (pH 7.5) were added. This was incubated in an assay mixture containing 10 mM BSA, 10 mM 5,5′-dithio-bis (2 nitrobenzoic acid) (DTNB), 0.5 mM NADH at 37°C for 15 min. Absorbance was read at 412 nm. Oxidized glutathione (GSSG) was assayed in the same mixture but with addition of 2-vinyl pyrrolidine for reduced glutathione (GSH) removal and reacted in the same way as reported by Yu et al. (2003). For standard preparation synthetically glutathione, reduced (GSH) and oxidized (GSSG) was used.

Assay of antioxidant enzymes activities

The samples (0.3g) were grinded in 5.0 ml sodium phosphate buffer (pH 7.8). After filtration, the sample was centrifuged at 10.000 g for 20 min and the obtained supernatant was used for GR, SOD and APX activities. Glutathione reductase (GR) activity was determined as the rate of NADPH oxidation according to Schaedle & Bassham (1977). The absorbance was measured at 340 nm.Superoxide dismutase:SOD (EC 1.12.1.1) activity was assayed by inhibition of nitroblue tetrazolium chloride (NBT) as described by Chen & Wang (2006). Absorbance was read at 560 nm. Ascorbate peroxidase: APX(EC 1.11.1.11) activity was determined according to the method of Nakano & Asada (1987). The hydrogen peroxide was determined by the decrease in absorbance of ascorbate at 290 nm.

Determination of malondialdehyde (MDA) content

Lipid peroxidation was determined based on MDA content and measured according to the method of Heath & Packer (1968). 0.5 g fresh leaves were ground in 10 ml of 6% trichloroacetic acid (TCA) and centrifuged at 10.000 g for 15 min. 1 ml of the supernatant and 4 ml of 0.5% thiobarbitric acid were mixed well at 95° C for 30 min. Non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance. The content of MDA was calculated by using the coefficient of 155 m/M/cm and expressed as nmol MDA/g fresh weight.

Determination of soluble sugars

A known weight of dry leaves (1g) was submerged in 10 ml of 80% (v/v) ethanol at 25°C overnight with shaking, and centrifuged at 6000 g. Ethanol in the supernatant was then evaporated completely and dissolved in a known volume of distilled water to determine soluble carbohydrates (Homme et al., 1992). Total soluble sugars (TSS) were detected by reacting 0.1 ml of ethanolic extract with 3.0 ml anthrone (freshly prepared) (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for 10 min and reading the cooled samples (cooled at room temperature) at 625 using glucose standard for calibration. The soluble sugars in the sample were expressed as mg glucose/g dry weight.

Assay of free proline content

Proline content was determined using the method of Bates et al. (1973). One gram fresh leaves of soybean plants were homogenized in aqueous sulfo-salicylic acid (3%). The extract was filtered. Then the glacial acetic acid and ninhydrin solution at 100°C for 1 h followed by terminating in ice bath and mixed with toluene. The toluene phase was aspirated from the aqueous phase. The contents were determined colorimetric at 520 nm and calculated as µM g^{-1} fresh weight.

Determination of total phenolic contents (TPC)

One gram of frozen leaves was ground in 50 ml of methanol. The homogenate was shaken for 1 h at room temperature. Then the extract was filtered. The concentration of total phenols was detected according to Kim et al. (2003) using tannic acid as a standard. 1 ml of extract, deionized water (10 ml) and 1 ml of 10% Folin–Ciocalteu phenol were added. Five min later, sodium carbonate solution 20% (2.0 ml) was added. Then solution was kept in darkness, the absorbance was measured at 750 nm and expressed as mg tannic acid g^{-1} dry weight.

Determination of phytohormones

Extraction and estimation of endogenous

phytohormones were carried out following the method of Ünyayar *et al.* (1996). The frozen leaves were ground in cold 80% methanol, then, triple extraction with methanol at 0 °C for 2 h was occur. Analysis of acidic hormones, indole acetic acid (IAA), gibberellic acid (GA_3) and abscisic acid (ABA) were analyzed using GLC (Varien Vesta, 6000).

Determination of Minerals

Nitrogen (N) was determined in dried leaf tissues by using micro-Kjeldahl method after digestion in sulphuric acid according to Yemm & Willis (1956). Phosphorus (P) was determined according to the method described by Humphries (1956) by using molybdenum blue method. The total contents of cation (K) in dried leaf tissues were estimated after digestion method using perchloric acid ($HClO_4$), nitric acid (HNO_3) in a Kjeldahl digestion apparatus (AOAC, 1995). K content was measured by using flame photometer.

Statistical analysis

Results of all parameters were statistically analyzed by using ANOVA test and the mean differences were compared by the Duncan test at 5% significance level.

Results

As shown in Table 1 it shows some chemical composition of *Moringa* leaf extract. It was found that $MLE_{(30)}$ is rich in some nutrients, some antioxidants such as ascorbic acid, α- tochopherol, phenols, flavonoids, as well as phytohormones like indol acidic acid and gibberellins. Results presented in Table 2, showed that, plant growth parameters (shoot and root length, fresh and dry weights of shoots and roots) of drought stressed *Glycine max* plants were affected. Data showed that the effect of drought on soybean plant increased according to decreased water hold capacity (60% and 40% hold water capacity). It was decreased significantly with increasing drought stress as compared with control plants. Further, spraying *Moringa oleifera* leaf extract (MLE_{30}) alone caused a noticeable improvement in all of mentioned growth parameters compared to untreated plants, indicating the higher efficiency of growth, development in the presence of (MLE_{30}) supplementation. On the other hand, combined $MLE_{(30)}$ application with drought stress showed highly significant increases in growth traits as compared with either drought stressed plants or well-watered plants.Photosynthetic pigment (chlorophyll a, b and total pigments) contents in leaves of soybean plants were significantly decreased gradually with increasing level of drought stress. Data in Table 3 clearly showed that, spraying with $MLE_{(30)}$alone alleviates the drought stress and caused significant increase in pigment contents (chlorophyll a, chlorophyll b and consequently total pigments) as compared with control. Data in the same table also show that, foliar spraying of soybean plants with *Moringa oleifera* leaf extract (MLE_{30}) caused significant increases in chlorophyll a, b, carotenoids and consequently total pigments as compared with control and the corresponding drought levels (60% and 40% hold water capacity).

It is evident that drought stress caused a noticeable increase in ascorbic acid, α-tocopherol and glutathione contents in leaves of soybean plants with increasing drought levels as compared with control plants (Table 4). In addition, spraying plants with *Moringa oleifera* leaf (MLE_{30}) extract, gave significant enhancement in ascorbic acid, α-tocopherol and GSH contents of leaves of treated soybean plants above that of the corresponding controls. Pronounced increases in antioxidant compounds was observed under foliar spraying of drought stressed soybean plants with MLE_{30} as compared with control plants and the corresponding drought levels(60% and 40% hold water capacity).

Changes in the activity of antioxidant enzymes were correlated with oxidative stress. The effect of drought stress levels on GR, SOD and APX activities in leaves of soybean cv. 111 plant either without or with MLE_{30} treatment was assayed. As shown in Table 5, increasing drought stress resulted in considerable increases in the activities of GR, SOD and APX enzymes of stressed plants as compared with those of control plants. The maximum activity of the antioxidant enzymes was observed in SOD enzyme. SOD was displayed at the highest level of drought as compared with the unstressed control plants. Spraying of MLE_{30} alone or combined with drought stress caused high significant increase in SOD activity in the leaves of plants as compared with control. The same trends for GR and APX activities in the presence and absence of MLE_{30} in drought stressed plants were obtained as similar to those for SOD. Under drought stress, MDA content increased in soybean plants as compared with the control plants (Table 6). In contrast, foliar spray with MLE_{30}reduced the MDA levels by 89% and 84% with drought levels (60% and 40% hold water capacity, respectively) as compared with drought stressed plants.

TABLE 1. Some chemical components of *Moringa oleifera* leaf extract (dry weight basis).

Chemical components (mg g^{-1} DW)			
Calcium	5.17	Ascorbic acid (vitamin C)	5.3
Magnesium	4.85	Tochopherol (vitamin E)	113
Phosphorus	4.08	Total phenols	3.36
Potassium	25.5	Total flavonoids	7.12
Copper	0.38	IAA	0.746
Iron	2.4	GA_3	0.602
Total amino acids	210.5	ABA	0.176

TABLE 2. Effects of foliar spraying of $MLE_{(30)}$ on growth parameters in *Glycine max* (cv. Giza 111) plants grown under drought stress conditions.

Treatments	Shoot length (cm)	Root length (cm)	Fresh wt. of shoots (g)	Dry wt. of shoots (g)	Fresh wt. of roots (g)	Dry wt. of roots (g)
Control	45.3^{d}	31.4^{b}	5.6^{c}	1.5^{b}	1.8^{ab}	0.35^{b}
MLE (30)	52.0^{b}	32.2^{ab}	6.5^{b}	1.8^{b}	1.9^{ab}	0.45^{a}
Hold water capacity 60%	39.6^{e}	27.7^{c}	5.3^{c}	1.3^{c}	1.6^{bc}	0.32^{b}
Hold water capacity 60%+ $MLE_{(30)}$	56.2^{a}	33.5^{a}	7.2^{a}	1.9^{a}	2.1^{a}	0.41^{a}
Hold water capacity 40%	32.4^{f}	26.3^{c}	4.8^{d}	1.1^{c}	1.2^{c}	0.28^{b}
Hold water capacity 40%+ $MLE_{(30)}$	50.3^{c}	33.3^{ab}	6.1^{b}	1.6^{b}	1.9^{ab}	0.35^{b}

Means with the same letters are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

TABLE 3. Effects of foliar spraying of $MLE_{(30)}$ on photosynthetic pigment content (mg/g) in *Glycine max* (cv. Giza 111) plants grown under drought stress conditions.

Treatments	Chl a mg g^{-1} FW	Chl b mg g^{-1} FW	Carotenoids mg g^{-1} FW	Total pigments mg g^{-1} FW
Control	8.21^{c}	3.32^{b}	2.72^{ab}	14.25^{d}
$MLE_{(30)}$	9.25^{b}	3.56^{b}	2.44^{ab}	15.25^{c}
Hold water capacity 60%	7.10^{d}	2.79^{c}	2.49^{ab}	12.13^{c}
Hold water capacity 60%+ $MLE_{(30)}$	10.0^{2a}	4.47^{a}	2.99^{a}	17.51^{a}
Hold water capacity 40%	5.29^{e}	2.22^{d}	2.05^{b}	9.56^{f}
Hold water capacity 40%+ $MLE_{(30)}$	9.60^{b}	3.53^{b}	2.88^{a}	16.01^{b}

Means with the same letters are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

TABLE 4. Effect of foliar spraying of MLE $_{(30)}$ on non-enzymatic antioxidants in *Glycine max* (cv. Giza 111) plants grown under drought stress conditions

Treatments	Ascorbic acid µmol g^{-1} FW	α-tocopherol µg g^{-1} FW	Reduced glutathione (GSH) µmol g^{-1} FW µmol g^{-1} FW
Control	40.96^{e}	27.12^{d}	13.69^{d}
MLE $_{(30)}$	44.65^{d}	43.31^{c}	14.99$_{d}$
Hold water capacity 60%	48.43^{c}	44.11^{c}	25.96^{c}
Hold water capacity 60%+ MLE $_{(30)}$	49.55^{bc}	52.99^{b}	29.40^{b}
Hold water capacity 40%	51.24^{ab}	55.21^{ab}	31.88^{b}
Hold water capacity 40%+ MLE $_{(30)}$	53.33^{a}	55.99^{a}	35.37^{a}

Means with the same letters are not significantly different at P ≤ 0.05 by Duncan's multiple range test.

TABLE 5. Effect of foliar spraying of MLE $_{(30)}$ on enzymatic antioxidants in in *Glycine max* (cv. Giza 111) plants grown under drought stress conditions.

Treatments	Glutathione reductase (GR) (unit min^{-1} g^{-1} FW)	Superoxide dismutase (SOD) (unit min^{-1} g^{-1} FW)	Ascorbate peroxidase (APX) (unit min^{-1} g^{-1} FW)
Control	0.606^{a}	24.37^{d}	0.318^{d}
MLE $_{(30)}$	0.616^{a}	29.16^{c}	0.375^{d}
Hold water capacity 60%	0.631^{a}	32.22^{b}	0.388^{d}
Hold water capacity 60%+ MLE $_{(30)}$	0.634^{a}	34.69^{ab}	0.484^{c}
Hold water capacity 40%	0.716^{a}	36.24^{a}	0.615^{b}
Hold water capacity 40%+ MLE $_{(30)}$	0.818^{a}	36.53^{a}	0.760^{a}

Means with the same letters are not significantly different at P ≤ 0.05 by Duncan's multiple range test.

TABLE 6. Effect of foliar spraying of MLE $_{(30)}$ on lipid peroxidation and osmolyte compounds (soluble sugars, proline and total phenol) in Glycine max (cv. Giza 111) plants grown under drought stress conditions.

Treatments	Lipid Peroxidation (nmol MDA g^{-1} FW)	Soluble Sugars (mg g^{-1} DW)	Proline (µg g^{-1} FW)	Total phenol (mg tannic acid g^{-1} FW)
Control	35.22^{e}	65.5^{e}	20.2^{d}	2.5^{d}
MLE $_{(30)}$	34.89^{e}	70.5^{d}	22.4^{d}	3.1^{cd}
Hold water capacity 60%	51.16^{b}	79.9^{c}	32.8^{c}	3.8^{cd}
Hold water capacity 60%+ MLE $_{(30)}$	45.79^{d}	83.4^{bc}	36.2^{b}	4.5^{bc}
Hold water capacity 40%	56.42^{a}	86.6$^{a\,b}$	43.9^{a}	5.8^{b}
Hold water capacity 40%+ MLE $_{(30)}$	47.64^{c}	87.6^{b}	46.6^{a}	7.8^{a}

Means with the same letters are not significantly different at P ≤ 0.05 by Duncan's multiple range test.

The osmolyte compounds (total soluble sugars, proline and total phenol) accumulated under drought stresses may be contributed as scavenges of reactive oxygen species (ROS). As shown in Table 6, high significant increases were observed with increasing drought stress levels. In addition, application of $MLE_{(30)}$caused highly significant increases in soluble sugars, proline and total phenol as compared with control plants. A markedly increases in antioxidant compounds was observed under foliar spraying of drought stressed soybean plants with $MLE_{(30)}$ as compared with control plants and the corresponding drought levels.The changes in the growth regulating hormones (auxins, gibberellins and abscisic acid) levels regulate the protective responses of plants against biotic and abiotic stresses. Results presented in Table 7 showed the changes in growth regulators extracted from soybean plants sprayed with $MLE_{(30)}$ and stressed plants in the presence and absence of MLE_{30}.

TABLE 7. Effect of foliar spraying MLE $_{(30)}$ on phytohormone contents (mg/100g) and mineral contents (%) in *Glycine max* (cv. Giza 111) plants grown under drought stress conditions

Treatments	phytohormone contents (mg/100g)			Chemical contents (%)		
	IAA	GA_3	ABA	N	P	K
Control	10.59^{c}	13.52^{bc}	3.27^{c}	3.2^{c}	0.64^{c}	1.3^{b}
MLE $_{(30)}$	12.65^{a}	16.01^{a}	2.07^{d}	5.1^{a}	0.80^{a}	1.4^{b}
Hold water capacity 60%	8.69^{d}	12.43^{c}	4.50^{b}	2.6^{d}	0.42^{e}	0.84^{c}
Hold water capacity 60%+ MLE $_{(30)}$	11.82^{b}	14.01^{b}	3.97^{c}	4.3^{b}	0.69^{b}	1.7^{a}
Hold water capacity 40%	7.52^{e}	10.08^{d}	7.02^{a}	2.6^{d}	0.34^{f}	0.69^{c}
Hold water capacity 40%+ MLE $_{(30)}$	11.01^{b}	13.97^{b}	2.64^{d}	3.4^{c}	0.52^{d}	0.86^{c}

Means with the same letters are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

The obtained results showed that IAA and GA_3 levels were reduced in soybean plants with increasing levels of drought stress (60% and 40% hold water capacity) as compared with those of the control. In contrast and proportional to drought stress, ABA content was significantly enhanced in the stressed plants over control.The foliar application of MLE_{30} alone or in combination with drought stress caused highly significant increase in both IAA and GA_3 contents. On the other hand, ABA content showed highly significant decrease as compared with control plants. From the results obtained in Table 7, it was revealed that a high significant decrease in nutrients content under drought levels was observed as compared with control plants. In contrast of this result, there were positive increases in the accumulation of (N, P and K) with spraying *Moringa* leaf extract. Uptake and accumulation of these mineral ions in soybean plants were significantly higher due to the enhancingeffect of *Moringa* leaf extract.

Discussion

Water soil deficit causes a directand observable reduction in plant growth of soybean plants. Drought caused significant reductions in the shoot and root lengths, fresh and dry weight of shoots and roots of stressed soybean cv. Giza 111 as compared with the control plant. These results are agreement with the results obtained by Assaha et al. (2016) who reported that water deficit markedly inhibited shoots and roots growth of huckleberry (*Solanum scabrum* Mill.) plant. The suppression of plant growth under drought stress might be attributed to the metabolic disorders induced by stress, generation of ROS that causes a reduction in division and elongation of cells, meristematic divisions, cell turgor, cell volume and eventually cell growth, decrease in photosynthetic capacity of plant leaves and/or blocking up the translocation vessels thus it hindering any movement of water or nutrients through it (Banon *et al*., 2006). On the other hand, foliar spray with *Moringa oleifera* leaf extract (MLE_{30}) in drought stressed soybean plants significantly improved plant growth parametersas well as physio-chemical attributes under the adverse conditions of water deficit. These results are in harmony with Ali *et al*. (2011) who reported that the plant growth parameters of *Zea mays* (shoot and root length, fresh and dry weights of shoots and roots) decreased significantly with increasing drought stress as compared with control plants.The observed slight reduction in growth could be attributed to its role as growth enhancer because its enriched with protein contents that essential for the formation of the protoplasm,vitamin C, essential nutrients such as potassium, calcium and magnesium that act as good source of natural antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids that make it excellent plant growth enhancer. Also, it has promoting hormones such as auxins and cytokinins (especially zeatin) that have growth promoting capabilities in enhancing the cell division, multiplication, enlargement and inducing chlorophyll biosynthesis.(Rady *et al*., 2015).

Chlorophylls content must be maintained under water stressed to support photosynthetic capacity in plants. In the present study, chlorophylls (chlorophyll a, b) carotenoids and total pigment contents are drastically reduced under drought stress as compared with control. These result is agree with Jomo *et al*. (2016) who found that, soil water deficit significantly reduced chlorophyll contents of amaranth species with increasing the level of drought stress.This reduction can be considered as a typical symptom of oxidative stress. This reduction may be

attributed to the photo-oxidation or chlorophyll degradation by the formation of proteolytic enzymes like chlorophyllase, deterioration in chloroplast, stomatal closure. On the other hand, a possible reduction in stomata conductance leading to a decreased photosynthetic level which is attributed to the inhibitory effect of decreased water content in leaves (Vurayai et al., 2011). Whilst MLE_{30} foliar application improved these contents in *Glycine max* leaves. Azra et al. (2013) also found an increase in chlorophyll contents when MLE was applied to wheat plants under drought conditions. The foliar application of *Moringa* extract could stimulate the production of phyto-regulator cytokinins in plant or correlated with cytokinin levels (Zeatin) found in MLE which prevents leaf senescence and reduction in chlorophylls content. Moreover, *Moringa* leaves have high content of nutritional potentialities of different macro elements such as Mg, a constituent of chlorophyll that would responsible for induction in the amounts of chlorophyll a,b in *Glycine max* plants. Also, its appreciable contents of carotenoids (α, β carotene, xanthin and lutein) that have potent antioxidant properties (Zaki & Rady, 2015).

Many studies have indicated that, to avoid the damage caused by water deficit stress, plants have developed some non-enzymatic antioxidant substances that having low molecular weights and generated in the direct elimination of ROS during stress. Some of these substrates are ascorbic acid (AsA), α-tocopherol and reduced glutathione (GSH). Drought stress exhibit significant increases in ascorbic acid, α-tocopherol and glutathione in soybean plants (cv. Giza 111) as compared with control plant. These are in agreement with the result of Abdul Jaleel (2009) who reported high levels of ascorbic acid, tocopherol and glutathione contents in *Withania somnifera* plants under drought stress as compared with control plant.

Moreover, Hasanuzzaman & Fujita (2011) stated that under drought stress rapeseed seedlings showed an enhancement in GSH content. Ascorbic acid acts as a powerful that can donate electrons to the enzymatic and non-enzymatic antioxidant systems leading to regulation of cell elongation, protection of proteins, lipids and thus protected against oxidative stress (Hasanuzzaman & Fujita, 2011). The induced levels of tocopherol under water deficit stress may be due to gene activities responsible for tochopherol synthesis. Glutathione (a substrate for GPX) has vital roles in some metabolic processes like cell division, activity of enzymes, biosynthesis of proteins and expression of genes. Moreover, itprotects cells from H_2O_2toxicity, organic peroxides, proteins and membranes from oxidation (Mullineaux & Rausch, 2005). These increases in the levels of reduced glutathione in soybean plants under drought stress may be attributed to the induction in glutathione reductase (GR) activity as well as higher rate of GSH synthesis. On the other hand, spraying plants with $MLE_{(30)}$ showed significant increase in GSH, ascorbic acid and α- tocopherol contents of soybean plants above that of the corresponding controls. Because of $MLE_{(30)}$ is good source of nutrients, amino acids, phenolics, soluble sugars and some antioxidant such as free proline and ascorbate, all of this induced the content of ascorbic acid (ASA), α-tocopherol and reduced glutathione contentsas antioxidants and consequently enable soybean plants to tolerate drought stress.

Moreover, the high significant increase in the activities of the antioxidant enzymes (GR, SOD and APX) was also reported in drought stressed soybean (cv. Giza 111) as compared with untreated plants. Similar results obtained by (Mirzaee et al., 2013) who revealed higher SOD and APX activities were observed in Canola (*Brassica napus* L.)subjected to drought stress. Moreover, Hasanuzzaman & Fujita (2011) revealed that GR activity showed significant enhancement in rapeseed seedlings under mild drought stress, but not changed with severe drought.Application of *Moringa* leave extract caused high significant accumulation in SOD activity in soybean followed by GR and APX respectively. Zaki & Rady (2015) reported that $MLE_{(30)}$ application used as seed soaking or foliar spray were caused significant increase in the antioxidant enzymes glutathione reductase (GR), superoxide dismutase (SOD) and ascorbate peroxidase (APX) in common bean (*Phaseolus vulgaris* L.) plants.This extract of *Moringa*can prevent oxidative effect and afford significant protection against oxidative damage by modulate the gene expression responsible for metabolic processes and defense system. Lipid peroxidation (MDA content) is accumulated in leaves of soybean plants under drought stress. These results are in accordance with Saruhan et al. (2012) who found that drought increased the content of MDA in two maize genotypes. Lipid peroxidation is being a first indicator of stresses measured as malondialdehyde (MDA) content (Jain et al., 2001).It considered a major type of

oxidative damage. It decreases cell membrane fluidity, ion channels, membrane proteins and enzyme activities and induce the leakiness of the cell membrane (Shehab et al., 2010). On the other hand, foliar spray with MLE_{30} extract significantly reduced MDA level in leaves of soybean plants when compared to drought stressed plants and control plants. *Moringa olifera* extract contains a significant level of calcium which can preventing injurious and leakage of membrane as well as stabilizing membrane structure under adverse drought conditions.

Soluble sugars and proline existence has a vital contribution for osmotic adjustment in plant cell (Hayashi et al., 1997), there were a significantly increased in both drought-stressed soybean plants and treated plants by $MLE_{(30)}$ (Table 6). Stress caused a depletion of starch and accumulation of soluble sugars in plant leaves. These accumulated sugars act as osmolytes to maintain proteins, cell turgor and membrane stability from damage (Kaplan & Guy, 2004).

On the other hand the accumulation of proline is considered one of the most frequent changes induced during stress damages.These results are in accordance with Abass & Mohamed (2011) who showed that the drought condition caused significant increase in the soluble sugar and proline contents in shoot of common bean (*Phaseolus vulgaris* L.) plants. Proline and soluble sugar levels are significantly over those induced in soybean plants when leaves sprayed with $MLE_{(30)}$ as compared with stressed plants and untreated plants. These results are in accordance with Foidl et al. (2001) who revealed that spraying of plant with *Moringa* extract caused induction in total soluble sugar contents. The accumulation of total sugars in soybean plants treated with $MLE_{(30)}$ may be due to the high sugar content which makes it of great scientific and agricultural interest (Yameogo et al., 2011). Also, Rady et al. (2015) who detected that the integrated application of $MLE_{(30)}$ improved the accumulation of antioxidant compounds in common bean plants via the increase in the contents of proline. In this respect, the protection effect of exogenous application of *Moringa oleifera* leaves extract (MLE_{30}) that containing mineral ions, ascorbates, cytokinins (zeatin) and phenolics (Azra, 2011) on soybean plants against drought stress is believed to be caused indirectly by proline accumulation that has a defensive roles in modulation of mitochondrial functions, maintaining cell water, ionic balance and thus plant development under drought stress conditions.

Phenols are one of the largest groups of plants secondary metabolite. They are significantly increased under water deficit conditions. They directly react with super-oxide anions and lipid peroxyl radicals that consequently reduce or breakdown the chain of lipid peroxidation (Rajanandh & Kavitha, 2010). The present investigation showed a significant increase in phenols content under drought stress (Table 6), over the control plant. Application of $MLE_{(30)}$ by a foliar spraying have more stimulatory effects on total phenolic levels in drought stressed soybean plants as compared with control and corresponding drought levels.

These results are in harmony with Basra et al. (2011) who reported that phenolic contents induced in maize seedlings when the seeds were treated with MLE_{30}. This enhancement of phenolic contents due to MLE application might be attributed to its higher content of phenols. Plants under stress conditions revealed some modification such as higher or lower production of growth regulators (Mok & Mok, 2001). This modification via phytohormone production levels which is influenced by the change of enzyme that participates in phytohormones synthesis or that involved in the degradation of xenobiotics (Vaseva-Gemisheva et al., 2005). On the other hand, the content of growth hormones (auxins and gibberellins) increased significantly in response to MLE_{30}, while ABA levels decreased. These results are harmony with Abdalla (2013) who reported that treated rocket (*Eruca vesicaria*) plants with *Moringa* extract increased the levels of phytohormones (Table 7).

Inorganic ions such as nitrogen (N), phosphorus (P), potassium (K) have important roles in plant mechanisms. The decline in soil water actually reduces nutrient ions uptake and nutrient transport in plants (Sardans & Penuelas, 2012).The inhibition in nutrient uptake by plants under water stress (Table 7) is related to reduction in transpiration process, impaired active transport and membrane permeability (Tanguilig *et al*., 1987). Leaf extract of *Moringa oleifera* plants has been reported to be a rich source of many minerals such as Ca, P, Na, Mg, K, Fe and others that can be valorized for nutrition balance in plants (Moyo et al., 2011). The uptake and accumulation of nutrients N, P, K in soybean plants were significantly induced due to the effect of the

Moringa leaf extract fertilization. Sivakumar & Ponnusami (2011) realized the increased uptake and accumulations of some nutritive elements as N, K, Ca, Mg and P as well as Fe in roots and shoots of several plants by using *Moringa* leaf extract. MLE is supposed to accelerate the nutrient uptake and translocation through the soybean plant by increasing the root membranes permeability for electrolytes, preventing nutrients fixation and increasing its mobility in soil.

Conclusion

The present results suggest that *Moringa* leaf extract (MLE_{30}) could trigger the activation of physiological compounds persist in plants to alleviate the oxidative damage causing by drought, leading to improvements in physiological and biochemical aspects for the plant growth under drought conditions. *Moringa* leaves extracts can be used to alleviate the adverse effect of water deficit stress.

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استخدام مستخلص أوراق مورينغا أوليفيرا كأسمدة بيولوجية لتخفيف إجهاد الجفاف لنباتات فول الصويا (جلايسين ماكس)

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الجفاف هو أحد الإجهادات البيئية الكبيرة والذى يحد من إنتاجية المحاصيل. أجري هذا العمل للتحقق من آثار الرش الورقى بمستخلص ورق نلنبات (مورينجا أوليفيرا) لتخفيف إجهاد الجفاف الواقع على نبات فول الصويا (جيزة 111). سبب الجفاف انخفاضا كبيرا في مقاييس النمو (طول المجموع الخضرى و الجذرى، الوزن الرطب والجاف من المجموع الخضري والجذري) وأصباغ البناءالضوئي (كلوروفيل أ،كلوروفيل ب، الكاروتينات والاصباغ الكليه). بالإضافة إلى ذلك، سبب زيادة كبيرة في مضادات الأكسدة غير الأنزيمية (حمض الاسكوربيك، توكوفيرول والجلوتاثيون المختزل)، ومضادات الأكسدة الأنزيمية (الجلوتاثيون ريدكتيز ، سوبر اوكسيد ديسميوتيز و الاسكوربات بيروكسيدز)، والضرر التأكسدي (أكسدة الليبيدات) والمركبات الاسموزيه (البرولين، السكريات الكلية الذائبة والفينول الكلى) في نباتات فول الصويا. وعلاوة على ذلك، فأن الرش الورقي بمستخلص أوراق المورينجا(إم إل إى$_{30}$) يعزز جميع المعاملات السابقة مقارنة بكلٌ من النباتات الضابطة والنباتات الواقعة تحت ضغط الجفاف. وظهر أن (إم إل إى$_{30}$) قادرٌ على تعزيز مقاومة نبات الدراسة لإجهاد الجفاف.